Endocrinology
Series Editor: Andrea Lenzi
Series Co-Editor: Emmanuele A. Jannini

SPRINGER
REFERENCE

Felipe F. Casanueva
Ezio Ghigo *Editors*

Hypothalamic-Pituitary Diseases

Springer

Endocrinology

Series Editor
Andrea Lenzi
Department of Experimental Medicine
Section of Medical Pathophysiology
Food Science and Endocrinology
Sapienza University of Rome
Rome, Italy

Series Co-Editor
Emmanuele A. Jannini
Department of Systems Medicine
University of Rome Tor Vergata
Rome, Italy

Within the health sciences, Endocrinology has an unique and pivotal role. This old, but continuously new science is the study of the various hormones and their actions and disorders in the body. The matter of Endocrinology are the glands, i.e., the organs that produce hormones, active on the metabolism, reproduction, food absorption and utilization, growth and development, behavior control, and several other complex functions of the organisms. Since hormones interact, affect, regulate, and control virtually all body functions, Endocrinology not only is a very complex science, multidisciplinary in nature, but is one with the highest scientific turnover. Knowledge in the Endocrinological sciences is continuously changing and growing. In fact, the field of Endocrinology and Metabolism is one where the highest number of scientific publications continuously flourishes. The number of scientific journals dealing with hormones and the regulation of body chemistry is dramatically high. Furthermore, Endocrinology is directly related to genetics, neurology, immunology, rheumatology, gastroenterology, nephrology, orthopedics, cardiology, oncology, gland surgery, psychology, psychiatry, internal medicine, and basic sciences. All these fields are interested in updates in Endocrinology. The aim of the MRW in Endocrinology is to update the Endocrinological matter using the knowledge of the best experts in each section of Endocrinology: basic endocrinology, neuroendocrinology, endocrinological oncology, pancreas with diabetes and other metabolic disorders, thyroid, parathyroid and bone metabolism, adrenals and endocrine hypertension, sexuality, reproduction, and behavior.

More information about this series at http://www.springer.com/series/14021

Felipe F. Casanueva • Ezio Ghigo
Editors

Hypothalamic-Pituitary Diseases

With 54 Figures and 41 Tables

Springer

Editors
Felipe F. Casanueva
Faculty of Medicine
Biochemistry and Molecular Biology Department
University of Santiago de Compostela
Santiago de Compostela, Spain

Ezio Ghigo
Department of Medical Sciences
University of Torino
Torino, Italy

ISBN 978-3-319-44443-7 ISBN 978-3-319-44444-4 (eBook)
ISBN 978-3-319-44445-1 (print and electronic bundle)
https://doi.org/10.1007/978-3-319-44444-4

Library of Congress Control Number: 2018932558

Printed on acid-free paper

This Springer imprint is published by the registered company Springer International Publishing AG, part of Springer Nature.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Series Preface

Is there an unmet need for a new MRW series in Endocrinology and Metabolism? It might not seem so! The vast number of existing textbooks, monographs and scientific journals suggest that the field of hormones (from genetic, molecular, biochemical and translational to physiological, behavioral, and clinical aspects) is one of the largest in biomedicine, producing a simply huge scientific output. However, we are sure that this new Series will be of interest for scientists, academics, students, physicians and specialists alike.

The knowledge in Endocrinology and Metabolism almost limited to the two main (from an epidemiological perspective) diseases, namely hypo/hyperthyroidism and diabetes mellitus, now seems outdated and closer to the interests of the general practitioner than to those of the specialist. This has led to endocrinology and metabolism being increasingly considered as a subsection of internal medicine rather than an autonomous specialization. But endocrinology is much more than this.

We are proposing this series as the *manifesto* for "**Endocrinology 2.0**", embracing the fields of medicine in which hormones play a major part but which, for various historical and cultural reasons, have thus far been "ignored" by endocrinologists. Hence, this MRW comprises "traditional" (but no less important or investigated) topics: from the molecular actions of hormones to the pathophysiology and management of pituitary, thyroid, adrenal, pancreatic and gonadal diseases, as well as less common arguments. Endocrinology 2.0 is, in fact, the science of hormones, but it is also the medicine of sexuality and reproduction, the medicine of gender differences and the medicine of wellbeing. These aspects of Endocrinology have to date been considered of little interest, as they are young and relatively unexplored sciences. But this is no longer the case. The large scientific production in these fields coupled with the impressive social interest of patients in these topics is stimulating a new and fascinating challenge for Endocrinology.

The aim of the **MRW in Endocrinology** is thus to update the subject with the knowledge of the best experts in each field: basic endocrinology, neuroendocrinology, endocrinological oncology, pancreatic disorders, diabetes and other metabolic disorders, thyroid, parathyroid and bone metabolism, adrenal and endocrine

hypertension, sexuality, reproduction and behavior. We are sure that this ambitious aim, covering for the first time the whole spectrum of Endocrinology 2.0, will be fulfilled in this vast Springer MRW in Endocrinology Series.

Andrea Lenzi, M.D.
Series Editor
Emmanuele A. Jannini, M.D.
Series Co-Editor

Volume Preface

Hypothalamic-pituitary diseases are a common although underestimated and underdiagnosed condition, with relevant clinical implications. They can be associated both with hormone hyper- and hyposecretion and so the management of hypothalamic-pituitary diseases is one of the most difficult tasks for the endocrinologist.

The most frequent hypothalamic-pituitary disease is a functioning or non-functioning tumor of the hypothalamic-pituitary region, usually associated with panhypopituitarism secondary to tumor growth or to its treatment with surgery or irradiation. Less commonly, hypopituitarism is due to nontumoral disorders including infiltrative lesions, infective processes, vascular alterations, traumatic brain injury, empty sella, or genetic disorders. Finally, hypothalamic-pituitary dysfunctions may occur as functional consequences of systemic disorders or as a consequence of long-term administration of drugs.

It is essential to perform validated diagnostic procedures in order to promptly diagnose hypothalamic-pituitary dysfunctions so as to prevent long-term consequences. At the same time, diagnosis is complex as no single test has sufficient sensitivity to identify all patients with hypothalamic-pituitary disorder. Imaging and genetic tests are becoming essential tools – in association with hormonal assays – for the diagnosis and management of hypothalamic-pituitary disorders.

In this volume, we have tried to provide the most up-to-date informations on the pathophysiology, diagnosis, and treatment of both main organic and functional hypothalamic-pituitary dysfunctions.

Some chapters are also dedicated to the complex interactions that the hypothalamus-pituitary system has with energy, glucose, and bone metabolism.

We believe we have included in this volume many of the most scientifically exciting and clinically relevant areas in contemporary neuroendocrinology.

A fascinating update on selected issues in basic and clinical research, this book is of great interest to both neuroendocrinologists and endocrinologists working on pituitary diseases and related issues. We hope this volume will be a valid tool for the broader scientific community dealing with the diagnosis and management of hypothalamic-pituitary diseases.

Felipe F. Casanueva
Ezio Ghigo

Contents

About the Editors

Felipe F. Casanueva

- *Professor of Medicine – Universidad de Santiago de Compostela (USC)*
- *Head of Department of Endocrinology and Nutrition – Hospital Universitario de Santiago (CHUS)*

Has been President of the following scientific societies:

- Sociedad Gallega de Endocrinologia y Nutricion (SGENM), 1986–1989
- Sociedad Española de Endocrinologia y Nutricion (SEEN), 1993–1996
- *European Federation of Endocrine Societies* (EFES), 1998–2001
- *The Pituitary Society*, 2008–2009
- *International Society of Endocrinology (ISE)*, 2010–2014
- Sociedad Española para el estudio de la Obesidad (SEEDO), 2011–2016

Has written 48 chapters on international books and textbooks and delivered more than 200 lectures by invitation at International Congresses. His current H-index is 79. Dr. Casanueva has published more than 600 papers in international journals with a cumulative Impact Factor of 1981,76.

He has received several AWARDS for RESEARCH at national and international levels, such as:

- *Xunta de Galicia de Investigacion* (1992)
- *Sociedad Española de Endocrinologia y Nutricion*, to his Professional Career (2002)
- *Jose Varela Montes* to the Health Science Area Research (2005)

- *Rey Jaime I* to the Medical Research (2005)
- *Geoffrey Harris Prize* of Neuroendocrinology (2006)
- Novoa Santos (2008)
- *Fundacion Lilly* of Biomedical Research Clinic (2012)
- *Fundacion Danone* – Professional Career – *Dr. Carlos Martí Hennberg* (2015)
- *European Hormone Medal – European Society for Endocrinology* (2016)

And, he has been named

- Honorary Doctorate in Medicine of the:
  - Universidad de Lódz (Poland, 2008)
  - Universidad de Erciyes (Turkey, 2013)
  - Universidad de Belgrado (Serbia, 2014)
- Honorary Member of the *European Society of Endocrinology (ESE),* Poland 2014
- Full member of the "Real Academia de Medicina y Cirugia de Galicia" (RAMYCGA), A Coruña, 2013
- Honorary Member of the "Agencia Española de Nutricion y Ciencias de la Alimentacion" (AEN), A Coruña, 2015

Ezio Ghigo

- Full Professor of Endocrinology and Metabolism, School of Medicine, University of Turin – since 1999
- Chairman of the Department of Medicine, University Hospital – A.O.U. Città della Salute e della Scienza di Torino – Presidio Molinette – Turin – since 2008
- Dean of the School of Medicine – University of Turin – since 2010
- Vice Rector for Health Care – University of Turin – since 2010
- Chairman of Postgraduate School of Sport Medicine – School of Medicine – University of Turin

He is member of the following scientific societies:

- Italian Society of Endocrinology (President 2011–2013)
- Italian Society of Diabetes
- Italian Society of Andrology

- European Endocrine Society (Vice President 2005–2009)
- American Endocrine Society
- European Neuroendocrine Association
- Growth Hormone Research Society
- International Society of Gynecological Endocrinology

He has written more than 250 original articles published on international journals with high impact factor, more than 100 publications including reviews, book and textbook chapters, and letters to the editor.

He has delivered more than 500 lectures by invitation at National and International Congresses, including those of the European Endocrine Society, American Endocrine Society, International Society of Endocrinology, and Italian Society of Endocrinology.

He has received several AWARDS for RESEARCH at national and international levels, such as:

- The Italian Society of Endocrinology award for young investigators (1986)
- The Italian Society of Endocrinology award for his Scientific Career (2001)
- "Gold Medal Lecture" award of the British Society for Endocrinology (2004)
- "Geoffrey Harris Prize" of the European Endocrine Society (2011)

Contributors

Athanasios Antoniou-Tsigkos Endocrine Unit, ARETAIEION University Hospital, National and Kapodistrian University of Athens, Athens, Greece

Emanuela Arvat Division of Endocrinological Oncology, Città della Salute e della Scienza di Torino and Department of Medical Science, University of Torino, Torino, Italy

Paolo Beck-Peccoz University of Milan, Milan, Italy

Enrico Brignardello Transition Unit for Childhood Cancer Survivors, Department of Oncology, Città della Salute e della Scienza di Torino, Torino, Italy

Frédéric Castinetti Service d'Endocrinologie, Université Aix Marseille, Hôpital de la Conception, Marseille, France

Philippe Chanson Service d'Endocrinologie et des Maladies de la Reproduction and Centre de Référence des Maladies Hypophysaires Rares, Hôpital de Bicêtre, Hôpitaux Universitaires Paris-Sud, Assistance Publique-Hôpitaux de Paris, Le Kremlin Bicêtre, France

Faculté de Médecine Paris-Sud, Univ Paris-Sud, UMR S1185, Le Kremlin-Bicêtre, France

INSERM U1185, Le Kremlin-Bicêtre, France

Annamaria Colao Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II di Napoli, Naples, Italy

Christine Cortet-Rudelli Service d'Endocrinologie-Métabolisme, Centre Hospitalier et Universitaire de Lille, Lille, France

Martin Cuesta Academic Department of Endocrinology, Beaumont Hospital/ RCSI Medical School, Dublin, Ireland

Marília Duarte Dalmolin Neuroendocrinology Research Center, Hospital Universitário Clementino Fraga Filho of Universidade Federal do Rio de Janeiro (HUCFF/UFRJ), Rio de Janeiro, BJ, Brazil

Maria Cristina De Martino Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II di Napoli, Naples, Italy

Carlos Dieguez CIBER Fisiopatología Obesidad y Nutrición (CIBEROBN), Instituto Salud Carlos III, Majadahonda, Spain

Department of Physiology, CIMUS, University of Santiago de Compostela-Instituto de Investigación Sanitaria, Santiago de Compostela, Spain

Mauro Doga San Raffaele Vita-Salute University, Milan, Italy

Francesco Felicetti Transition Unit for Childhood Cancer Survivors, Department of Oncology, Città della Salute e della Scienza di Torino and Department of Medical Science, University of Torino, Torino, Italy

Annamaria Formenti Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

Stefano Frara San Raffaele Vita-Salute University, Milan, Italy

Mônica Gadelha Neuroendocrinology Research Center, Hospital Universitário Clementino Fraga Filho of Universidade Federal do Rio de Janeiro (HUCFF/UFRJ), Rio de Janeiro, BJ, Brazil

Neuroendocrinology Unit and Molecular Genetics Laboratory, Instituto Estadual do Cérebro Paulo Niemeyer (IECPN), Rio de Janeiro, BJ, Brazil

Françoise Galland Service d'Endocrinologie, Diabétologie, Nutrition, CHU Rennes, Hôpital Sud, Rennes, France

Silvia Garelli Endocrinology Unit and Center for Applied Biomedical Research, Department of Medical and Surgical Sciences, University of Bologna – S. Orsola-Malpighi Hospital, Bologna, Italy

Aoife Garrahy Academic Department of Endocrinology, Beaumont Hospital/RCSI Medical School, Dublin, Ireland

Valentina Gasco Department of Medical Science, Division of Endocrinology, Diabetes and Metabolism, Turin, Italy

Claudia Giavoli Endocrinology and Metabolic Diseases Unit, Department of Clinical Sciences and Community Health, University of Milan, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy

Andrea Giustina San Raffaele Vita-Salute University, Milan, Italy

Silvia Grottoli Department of Medical Science, Division of Endocrinology, Diabetes and Metabolism, Turin, Italy

Davide Iacuaniello Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II di Napoli, Naples, Italy

Leandro Kasuki Neuroendocrinology Research Center, Hospital Universitário Clementino Fraga Filho of Universidade Federal do Rio de Janeiro (HUCFF/UFRJ), Rio de Janeiro, BJ, Brazil

Neuroendocrinology Unit and Molecular Genetics Laboratory, Instituto Estadual do Cérebro Paulo Niemeyer (IECPN), Rio de Janeiro, BJ, Brazil

Fahrettin Kelestimur Department of Endocrinology and Metabolism, Erciyes University Medical School, Kayseri, Turkey

Andrea Lania Department of Biomedical Sciences, Humanitas University and Endocrinology Unit, Humanitas Research Hospital, Rozzano, Italy

Anne-Lise Lecoq Service d'Endocrinologie et des Maladies de la Reproduction and Centre de Référence des Maladies Hypophysaires Rares, Hôpital de Bicêtre, Hôpitaux Universitaires Paris-Sud, Assistance Publique-Hôpitaux de Paris, Le Kremlin Bicêtre, France

Faculté de Médecine Paris-Sud, Univ Paris-Sud, UMR S1185, Le Kremlin-Bicêtre, France

INSERM U1185, Le Kremlin-Bicêtre, France

Andrzej Lewiński Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, Polish Mother's Memorial Hospital Research Institute, Lodz, Poland

Valentina Lo Preiato Endocrinology Unit and Center for Applied Biomedical Research, Department of Medical and Surgical Sciences, University of Bologna – S. Orsola-Malpighi Hospital, Bologna, Italy

Djuro Macut Clinic of Endocrinology, Diabetes and Metabolic Diseases, University of Belgrade, Belgrade, Serbia

Filippo Maffezzoni Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

Mónica Marazuela Department of Endocrinology, Hospital Universitario La Princesa, Instituto de Investigación Princesa, Universidad Autónoma, Madrid, Spain

George Mastorakos Endocrine Unit, ARETAIEION University Hospital, National and Kapodistrian University of Athens, Athens, Greece

Gherardo Mazziotti Endocrine Unit, Department of Medicine, A.S.S.T. Carlo Poma, Mantova, Italy

Dragana Miljic Clinic for Endocrinology, Diabetes and Metabolic Diseases, Neuroendocrine Unit, Clinical Centre of Serbia, Belgrade, Serbia

University of Belgrade, School of Medicine, Belgrade, Serbia

Sebastian J. Neggers Department of Internal Medicine, Erasmus University MC, Rotterdam, The Netherlands

Eberhard Nieschlag Center of Reproductive Medicine and Andrology, University of Münster, Münster, Germany

Uberto Pagotto Endocrinology Unit and Center for Applied Biomedical Research, Department of Medical and Surgical Sciences, University of Bologna – S. Orsola-Malpighi Hospital, Bologna, Italy

Sandra Pekic Clinic for Endocrinology, Diabetes and Metabolic Diseases, Neuroendocrine Unit, Clinical Centre of Serbia, Belgrade, Serbia

University of Belgrade, School of Medicine, Belgrade, Serbia

Rosario Pivonello Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II di Napoli, Naples, Italy

Vera Popovic University of Belgrade, School of Medicine, Belgrade, Serbia

Nunzia Prencipe Department of Medical Science, Division of Endocrinology, Diabetology and Metabolism, Città della Salute e della Scienza di Torino, University of Torino, Torino, Italy

Ana M. Ramos-Leví Department of Endocrinology, Hospital Universitario La Princesa, Instituto de Investigación Princesa, Universidad Autónoma, Madrid, Spain

Gerald Raverot Fédération d'Endocrinologie, Groupement Hospitalier Est, Hospices Civils de Lyon, Lyon, France

INSERM U1028; CNRS UMR5292; Centre de Recherche Lyon Neuroscience, Université de Lyon, Lyon, France

Giulia Rodari Endocrinology and Metabolic Diseases Unit, Department of Clinical Sciences and Community Health, University of Milan, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy

Julia Rohayem Center of Reproductive Medicine and Andrology, University of Münster, Münster, Germany

Sylvie Salenave Service d'Endocrinologie et des Maladies de la Reproduction and Centre de Référence des Maladies Hypophysaires Rares, Hôpital de Bicêtre, Hôpitaux Universitaires Paris-Sud, Assistance Publique-Hôpitaux de Paris, Le Kremlin Bicêtre, France

Luisa Maria Seoane Grupo Fisiopatología Endocrina, IDIS, Complejo Hospitalario Universitario de Santiago de Compostela (CHUS/SERGAS), Santiago de Compostela, Spain

CIBER Fisiopatología Obesidad y Nutrición (CIBEROBN), Instituto Salud Carlos III, Majadahonda, Spain

Chiara Simeoli Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II di Napoli, Naples, Italy

Magdalena Stasiak Department of Endocrinology and Metabolic Diseases, Polish Mother's Memorial Hospital Research Institute, Lodz, Poland

Marko Stojanovic Clinic for Endocrinology, Diabetes and Metabolic Diseases, Neuroendocrine Unit, Clinical Centre of Serbia, Belgrade, Serbia

University of Belgrade, School of Medicine, Belgrade, Serbia

Fatih Tanriverdi Department of Endocrinology and Metabolism, Erciyes University Medical School, Kayseri, Turkey

Christopher J. Thompson Academic Department of Endocrinology, Beaumont Hospital/RCSI Medical School, Dublin, Ireland

Sulay Tovar CIBER Fisiopatología Obesidad y Nutrición (CIBEROBN), Instituto Salud Carlos III, Majadahonda, Spain

Department of Physiology, CIMUS, University of Santiago de Compostela-Instituto de Investigación Sanitaria, Santiago de Compostela, Spain

Aart J. van der Lely Department of Internal Medicine, Erasmus University MC, Rotterdam, The Netherlands

Valentina Vicennati Endocrinology Unit and Center for Applied Biomedical Research, Department of Medical and Surgical Sciences, University of Bologna – S. Orsola-Malpighi Hospital, Bologna, Italy

Physiology of the Hypothalamus Pituitary Unit

1

Luisa Maria Seoane, Sulay Tovar, and Carlos Dieguez

Contents

L. M. Seoane (✉)
Grupo Fisiopatología Endocrina, IDIS, Complejo Hospitalario Universitario de Santiago de Compostela (CHUS/SERGAS), Santiago de Compostela, Spain

CIBER Fisiopatología Obesidad y Nutrición (CIBEROBN), Instituto Salud Carlos III, Majadahonda, Spain
e-mail: luisamaria.seoane@usc.es

S. Tovar · C. Dieguez
CIBER Fisiopatología Obesidad y Nutrición (CIBEROBN), Instituto Salud Carlos III, Majadahonda, Spain

Department of Physiology, CIMUS, University of Santiago de Compostela-Instituto de Investigación Sanitaria, Santiago de Compostela, Spain
e-mail: carlos.dieguez@usc.es

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_1

Abstract

The hypothalamic-pituitary unit constitutes the main regulator of the physiological functions in the organism. In the last years, the classical view about the regulation and the function of the main hormonal axis somatotroph, thyrotrophic, lactotroph, and gonadotroph has changed by the inclusion of new signals and organs acting in the hypothalamic-pituitary unit allowing a communication between metabolic status and hormonal system in order to regulate energy balance and physiological functions such as growth, lactation, adiposity, and reproduction.

Keywords

Hypothalamus · Pituitary · Growth hormone · Thyroid · Prolactin · Gonadal

List of Abbreviations

ACTH	Adrenocorticotropic hormone
ADH	Anti-diuretic hormone
AMPK	AMP-activated protein kinase
aMSH	Alpha-melanocyte stimulating hormone
ARC	Arcuate nucleus
BAT	Brown adipose tissue
BBB	Blood brain barrier
BMI	Body mass index
CART	Cocaine and amphetamine regulated transcript neuropeptides
CB1	Cannabinoid receptor 1
CCK	Cholecystokinin
CRH	Corticotroph releasing hormone
CRH	Corticotropin releasing hormone
D1	Deiodinase type 1
D3	Deiodinase type 3
DMH	Dorsomedial hypothalamus
FSH	Follicle-stimulating hormone
FSH-RH	Follicle-stimulating hormone–releasing hormone
G6PC	Glucose-6-phosphatese
GH	Growth hormone
GHR	Growth hormone binds at specific transmembrane receptor
GHRH	Growth hormone releasing hormone
GHSR	Growth hormone secretagogue receptor
GLP-1	Glucagon-like peptide-1
GnIH	Gonadotropin Inhibiting Hormone
GnRH	Gonadotropin-releasing hormone
GOAT	Ghrelin O-acyltransferase
GPCR	Seven-transmembrane G-protein coupled receptor
IGF1R	Receptor for IGF1
IGFBB	IGF1 binding proteins

JAK2	Janus kinase 2
KNDy	Kisspeptin/neurokinin B/dynorphin neurons.
LDL-C	Low-density lipoprotein cholesterol
LH	Luteinizing hormone
LHRH	Luteinizing hormone–releasing hormone
MAPK	Mitogen-activated protein kinase
MCT-8	Monocarboxylate transporter 8
NAFLD	Nonalcoholic fatty liver disease
NKA	Neurokinin A
NKB	Neurokinin B
NPY/AgRP	Orexigenic peptides Neuropeptide Y and Agouty-related protein
OATP1C1	Organic anion-transporting polypeptide 1C1
PACAP	Pituitary adenylate cyclase-activating polypeptide
PCK1	Phosphoenolpyruvate carboxykinase
PI3K/AKT	Phosphotidyl inositol 3-kinase
Pit-1	Pituitary transcription factor
POMC/CART	Pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript
PRL	Prolactin
PVH	Paraventricular hypothalamus nucleus
SP	Substance P
SS	Somatostatin
SSTR	Somatostatin receptor
STAT5	Signal transducer and activator of transcription 5
TH	Thyroid hormones T3 and T4
TRH/TRF	Thyrotropin-releasing hormone/thyrotropin-releasing factor
TSH	Thyrotropin/thyroid-stimulating hormone
VIP	Vasoactive intestinal peptide
VMH	Ventromedial hypothalamus.
Δ9THC	Δ9Tetrahydrocannabinol

Introduction

The endocrine system releasing several hormonal signals that act in target organs to regulate many physiological functions of the body, such as development, growth, energy metabolism, and reproduction.

Among the components of the endocrine system, the hypothalamus-pituitary unit is a key factor into regulation the physiology of the human body. The unit is composed by several axes connecting the two main central structures, i.e., hypothalamus and pituitary, with peripheral organs such as thyroid gland, the adrenal gland, and the gonads in order to influence and control its physiological functions.

Hypothalamus

The hypothalamus, the main organ of the endocrine system, is located under the thalamus over the brainstem and connected with different regions of the central nervous system such as limbic structures and different areas of the autonomous nervous system. The hypothalamus in response to messages received from the body and also the central nervous system delivers different hypothalamic neurohormones to the adenopituitary through the hypophyseal portal system. The hormones released from the hypothalamus exert a tropic action on the pituitary inducing the release in the pituitary gland of different hormones that influence the main endocrine axis.

The hypothalamus is composed by neuronal clusters which led to a network of neurons that has been shown to regulate appetite and energy homeostasis. The neurons in the hypothalamus are grouped into so-called nucleus: arcuate (ARC) paraventricular (PVH), dorsomedial (DMH), lateral (LH), and ventromedial (VMH) hypothalamus.

The ARC is considered the main nucleus regulating food intake and it is located in the basal hypothalamus just on the median eminence. In this area hardly exists blood brain barrier (BBB) allowing numerous substances crossing from the blood to the brain and quickly responding to nutrients and hormones fluctuations. The neuronal groups constituting the ARC are highly connected with different brain areas and express receptors for an elevated number of hormones which indicate its role in hormonal regulation. Two different neuronal population are found in the ARC: one with orexigenic actions by liberating the orexigenic peptides Neuropeptide Y and Agouty-related protein (NPY/AgRP), while the second population exert the anorexigenic effect by expressing Pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript (POMC/CART), two peptides which rapidly respond to changes in nutritional status (Cone et al. 2001) (Fig. 1).

The DMH nucleus received inputs from other areas such as the medium and lateral hypothalamus, as well as NPY/AgRP from the ARC (Elmquist 1998). This nucleus is related with glucocorticoid secretion, growth, body temperature, sleep, circadian cycle, and locomotor activity (Gooley et al. 2006).

The paraventricular nucleus (PVH) is located in the anterior hypothalamus, close to the third ventricle. It is the main nucleus producing corticotropin releasing hormone (CRH) and thyrotropin releasing hormone (TRH). The projections from the NPY/AgRP neurons of the ARC, melanocortins, and orexins convey information to the PVH and with the nucleus of tractus solitary. The PVH is very sensitive to peripheral peptides involved in energy balance such as cholecystokinin (CCK), NPY, GHRELIN, or glucagon-like peptide-1 (GLP-1). All together indicates that the PVH nucleus integrate nutritional signals with different endocrine axis (Gooley et al. 2006).

The lateral hypothalamus (LH) synthesized two types of peptides: the hypocretins (orexins) and the melanin concentrating hormone (MCH). These peptides are characterized by send projections to a wide number of areas in the central nervous system such as the cortex, thalamus, or limbic system. The nucleus regulates functions among them, such as learning, memory, emotion, and motor response to the energetic status, through the action of different hormones such as ghrelin and leptin.

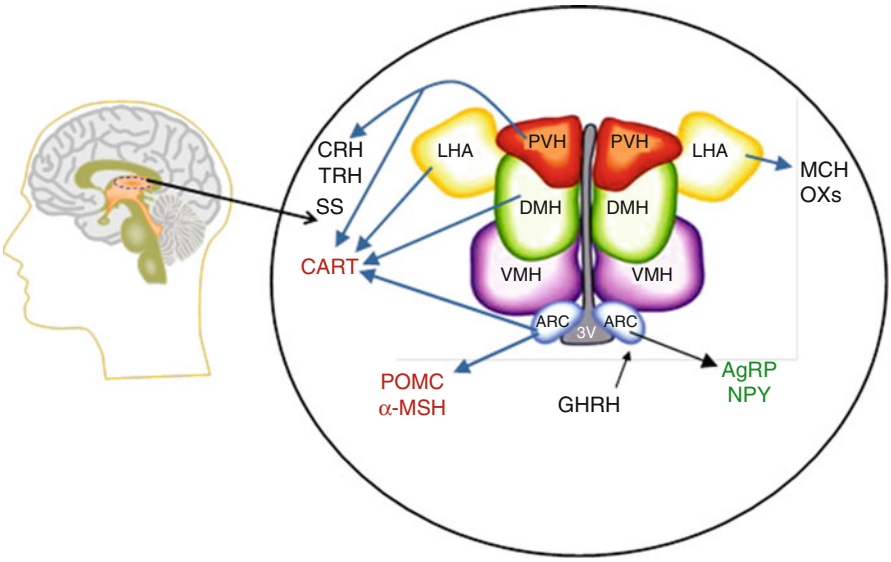


Fig. 1 The main hypothalamic nuclei involved in the regulation of hyptothalamic-pituitary axis (see Fig. 4 for the gonadal axis) (Martinez de Moretin et al. 2016)

The main hypothalamic hormones that regulate pituitary secretion comprise:

GHRH (growth hormone releasing hormone): is the hypothalamic hormone that stimulates the growth hormone (GH) secretion by the pituitary. Its effect is counterbalanced by somatostatin.

CRH (corticotroph releasing hormone): is a peptide hormone and neurotransmitter involved in the stress response belonging to corticotropin-releasing factor family.

TRH (thyrotropin-releasing hormone): also called thyrotropin-releasing factor (TRF) or thyroliberin, is a releasing hormone, produced by the hypothalamus, which stimulates the release of thyrotropin (thyroid-stimulating hormone or TSH) and prolactin from the anterior pituitary.

GnRH (gonadotropin-releasing hormone): also known as follicle-stimulating hormone–releasing hormone (FSH-RH), luteinizing hormone–releasing hormone (LHRH), is a releasing hormone responsible for the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary.

Pituitary

The pituitary gland is a key component of the hypothalamus-pituitary axis. The gland is located under the hypothalamus and connected with it through the pituitary stalk. The hypothalamus signals pituitary to stimulate or release pituitary hormones.

Anatomical level of the pituitary is composed of two well-differentiated parts: the anterior and posterior pituitary.

The anterior pituitary or adenohypophysis receives the hypothalamic peptides released in the median eminence and stimulates or inhibits the release of a set of hormones in response to the information received from the hypothalamus. The pituitary hormones act in the target organs to maintain the physiological balance. The classical view of the AP-gland is that it is composed of five secretory cell types, namely, somatotrophs, lactotrophs, corticotrophs, gonadotrophs, and thyrotrops.

Data gleaned in recent years have shown the existence of an additional cell population, stem cells, which is particularly relevant to pituitary function and pituitary disease. For many years, one intriguing and unanswered question was related to why to pituitary plasticity allowing either hypoplasia or hyperplasia during physiological changes such as puberty, pregnancy, and lactation. The recent characterization of pituitary stem cells may also allow uncovering the mechanisms involved in pituitary cell renewal or tumorigenesis. These cells appear to form a functional niche of adult precursor cells, termed GPS (**G**FRa2+, **P**rop1+, **S**tem) cells, exhibit the following main features: (a) these are organized in a single-cell layer around the cleft originated from the Rathke's pouch; and (b) these niche cells display a clear expression of the pituitary specific homebox protein Prophet of Pit1 (Prop1), a transcription factor required for pituitary development and mutated in pituitary disease. In addition, these niche cells also express well-established stem cell markers such as Oct4, Sox2, Sox9 (Garcia-Lavandeira et al. 2009, 2015).

Among the hormones produced in the anterior lobe of the pituitary, we can find:

- Adrenocorticotropic hormone (ACTH): is secreted from the corticotroph cells in the adenohypophysis and stimulates secretion of glucocorticoids from the adrenal glands in response to stress.
- Follicle-stimulating hormone (FSH): is secreted from the gonadotroph cells in the pituitary and induces the hormonal release from the gonads: secretion of estrogen in females and stimulation of sperm production in the testes in males to ensure normal reproductive function.
- Luteinizing hormone (LH): LH together with FSH is released from the gonadotroph cells and acts in ovary and testes to ensure the reproductive function and stimulates secretion of estrogen and progesterone in females and testosterone in male.
- Growth hormone (GH): GH is a key hormone that involves in development and maintenance of healthy body composition and function. It is released by the somatotroph cells of the pituitary and acts in different peripheral tissues: liver, muscles, and bones, to regulate growth, protein synthesis, and metabolism.
- Prolactin: It is released from the lactotroph cells in the pituitary, acts on the mammary gland, and is crucial to pregnancy and lactation.
- Thyroid-stimulating hormone (TSH): It is released in the thyrotroph cells of the pituitary in response to the stimulating effect of TRH. TSH stimulates the thyroid gland to produce thyroid hormones.

The posterior pituitary or neurohypophysis contains the axons ends of nerve cells coming from the different hypothalamic nucleus. Through the hypophyseal artery

and hypophyseal veins, the pituitary releases its hormones directly into the systemic circulation.

The hormones released from the neurohypophysis are:

- Antidiuretic hormone (ADH): This hormone prompts the kidneys to increase water absorption in the blood.
- Oxytocin: Oxytocin is involved in a variety of processes such as contracting the uterus during childbirth and stimulating breast milk production.

Among the main hypothalamic-pituitary axis at the present chapter, we carefully review the somatotroph, prolactin, thyrotroph, gonadotroph, and corticotroph axis.

The Somatotroph Axis

The somatotroph axis is composed by three different locations: hypothalamus, pituitary, and peripheral target organs. At hypothalamic level, the main peptides released to the hypophyseal blood are growth hormone releasing hormone (GHRH) with stimulatory action on pituitary hormonal release and somatostatin (SS) an inhibitor peptide of the growth hormone secretion from the pituitary. From 1999 a new peptide derived from the gastrointestinal tract was discovered as the endogenous ligand for growth hormone releasing peptide (GHSR), ghrelin (Kojima et al. 1999) which showed a potent effect in releasing growth hormone secretion and was proposed as a part of the somatotroph axis.

Components of the GH Axis

Growth Hormone Releasing Hormone (GHRH)

GHRH was first isolated in 1982 from two patients with pancreatic tumors developed acromegaly (Guillemin et al. 1982). It is generated by a proteolytic process from the precursor proGHRH. At hypothalamic level, it is mainly expressed in neurons in the ARC and medium eminence, although at lower levels expression was also described in the VMH and DMH (Mayo et al. 1983). GHRH has been involved in the stimulation of GH release and accordingly lesions in the ventromedial nucleus of the hypothalamus have been associated with a deficiency in GH production. Accordingly GHRH was used to treat GH deficiency in children (Sassolas 2000) although it was not found always effective because of the different etiology of GH deficiency and it was recently used as a factor for diagnosis of GH deficiency (Ghigo et al. 1996).

Somatostatin

Somatostatin (SS) was first proposed as a regulation of GH production in 1973 by its inhibitory action on GH secretion in rats and humans (Brazeau et al. 1973; Siler et al. 1973). It was proposed as a useful factor to treat the excess in growth hormone

secretion characteristic of acromegaly (Reichlin et al. 1976). The cloning of the somatostatin receptor (SSTR) showed the existence of five different isoforms as differently expressed in different tissues indicating a great variety of actions of SS in peripheral organs (Patel et al. 1995). Accordingly, in addition to the effect of SS on GH regulation, an effect of treating diabetes for its action on insulin and glucagon secretion was also attributed to SS (Koerker et al. 1974).

Insulin-Like Growth Factor 1 (IGF1)

IGF1 is a protein of 7,649 Daltons molecular weight constituted by 70 amino acids with three disulfide bridges (Rinderknecht and Humbel 1978). It is produced mainly by the liver and its production is regulated through life with the highest production in the pubertal period. IGF1 is produced as a result of the GHR activation by its binding to GH and circulates to bind IGF1-binding proteins (IGFBB). The receptor for IGF1 (IGF1R) is produced in a great variety of tissues in the organism and present activity tyrosine kinase (Frasca et al. 2008). The intracellular pathways that are activated by IGF1R include the phosphotidyl inositol 3-kinase (PI3K/AKT) that is directly involved in growth and proliferation and mitogen-activated protein kinase (MAPK). In addition to IGF1R, it has been shown that IGF1 is also able to bind insulin receptor albeit to a much lower affinity.

IGF1 is involved in multiple actions related to growth in several tissues such as cartilage bone and lungs (Scarth 2006).

Growth Hormone

The growth hormone (GH) represents the 10% of the total pituitary hormonal production. It was first identified in 1945 from a pituitary bovine extracts (Daughaday 1989). GH is a globular protein constituted by 191 aminoacids with to disulfur bridges, the most abundant form in plasma presents a molecular weight of 21,800 daltons and is the 22 K growth hormone form. Additionally, other forms of 5, 17.5, and 20 kDa were also identified (Baumann 1991; Kopchick et al. 2002). At genomic level, several subtypes of genes were found derived from a common precursor expressing GH, GH-N, GH-V, GH-A, GH-B, and GH-L. It was described that the GH-N is the gene who coded the 22 kDa GH form (Kopchick et al. 2002).

Growth hormone binds at specific transmembrane receptor (GHR) that is dimerized after its union to GH. The mentioned dimerization induces the phosphorylation of Janus kinase 2 (JAK2). The activation of GHR acts through the signal transducer and activator of transcription 5 (STAT5) signaling pathway (Lanning and Carter-Su 2006). In addition to the STAT5/JACK2 pathway, the RAS/MAP kinase and phosphatidylinositol 3′ kinase (PI3K/Akt) pathways have also been involved in GH actions at different levels (Savastano et al. 2014).

Growth Hormone Regulation

GH is secreted with a pulsatile pattern. This pulsatile secretion of GH is at least in part due to the stimulatory action of the hypothalamic GHRH and the inhibitory

action of SS. However, another relevant factor different of GHRH and SS has also been involved in GH regulation. IGF-1 is produced in the liver by GH action and by a feedback mechanism that inhibits GH secretion by two different mechanism: the first includes the direct action of IGF1 in the somatotroph cell and the second one is mediated by the stimulation of SS and the inhibition of GHRH at hypothalamic level (Ohlsson et al. 2009; Romero et al. 2010) (Fig. 2).

It was also shown that pulsatile GH secretion is tightly regulated by different external stimuli such as nutritional status, age, gender, and body composition (Veldhuis et al. 2008). It has been described a negative correlation between body mass index and GH levels that reduces the production of GH in those individuals with elevated body mass index (BMI) (Iranmanesh et al. 1991). These findings were supported by animal experiments showing that a $GHR^{-/-}$ mice show elevated adiposity (Berryman et al. 2004). In the last years, a wide variety of studies have tried to identify the mechanism behind the inverse relation between GH and adiposity; however, the exact mechanisms are yet unclear. The variations in the circulating levels of free fatty acids (FFA) and insulin in response to changes in BMI have been proposed, at least in part, as responsible for the alterations in GH (Cordido et al. 1996; Luque et al. 2006; Cornford et al. 2011; Karpe et al. 2011).

In addition, growth hormone gene is under the control of different hormonal axis such as glucocorticoids, thyroid hormones, and pituitary transcription factor (Pit-1) (Kopchick et al. 2002) (Fig. 3). Another adipokines such as leptin (Carro et al. 1997b) and adiponectin (Nilsson et al. 2005) have been also proposed as mediators of the relation of adiposity and GH in animal studies; however, due to the complexity of human obesity pathology the exact role has not fully elucidated.

Age and sexual dimorphism have also been described in the bibliography as key factors involved in GH regulation (Fig. 2). In keeping the pulsatile pattern of GH has been found different in males and females and these finding led the community to find if gonadal steroids might be involved in the differential regulation of growth hormone. It was found that testosterone might act in the brain to increase GH secretion but also at peripheral level, in the liver to induce the secretion of IGF-1 (Veldhuis et al. 1997). Estrogens has also been associated to GH regulation although at this moment it is not known if this sexual hormones might be regulating GH by a direct action of somatotrophs, since the existence of estrogen receptors in somatotrophs was described (Avtanski et al. 2014) of if the effect is produced at peripheral level and mediated by IGF-1(Meinhardt and Ho 2006). At central level it was proposed that estrogens and testosterone act in the hypothalamus to modulate the release of GHRH and SS in response to ghrelin (Norman et al. 2014). Interestingly, it was also proposed that estrogen and testosterone might modulate the gastric production of ghrelin (Seoane et al. 2007a).

Ghrelin

The discovery of ghrelin in 1999 as the endogenous ligand for growth hormone secretagogue receptor (GHSR) was proposed for the first time the implication of the gastrointestinal tract in the somatotroph axis. Ghrelin is a 28 aminoacid peptide mainly secreted from the gastrointestinal tract (Kojima et al. 1999; Ariyasu et al. 2001).

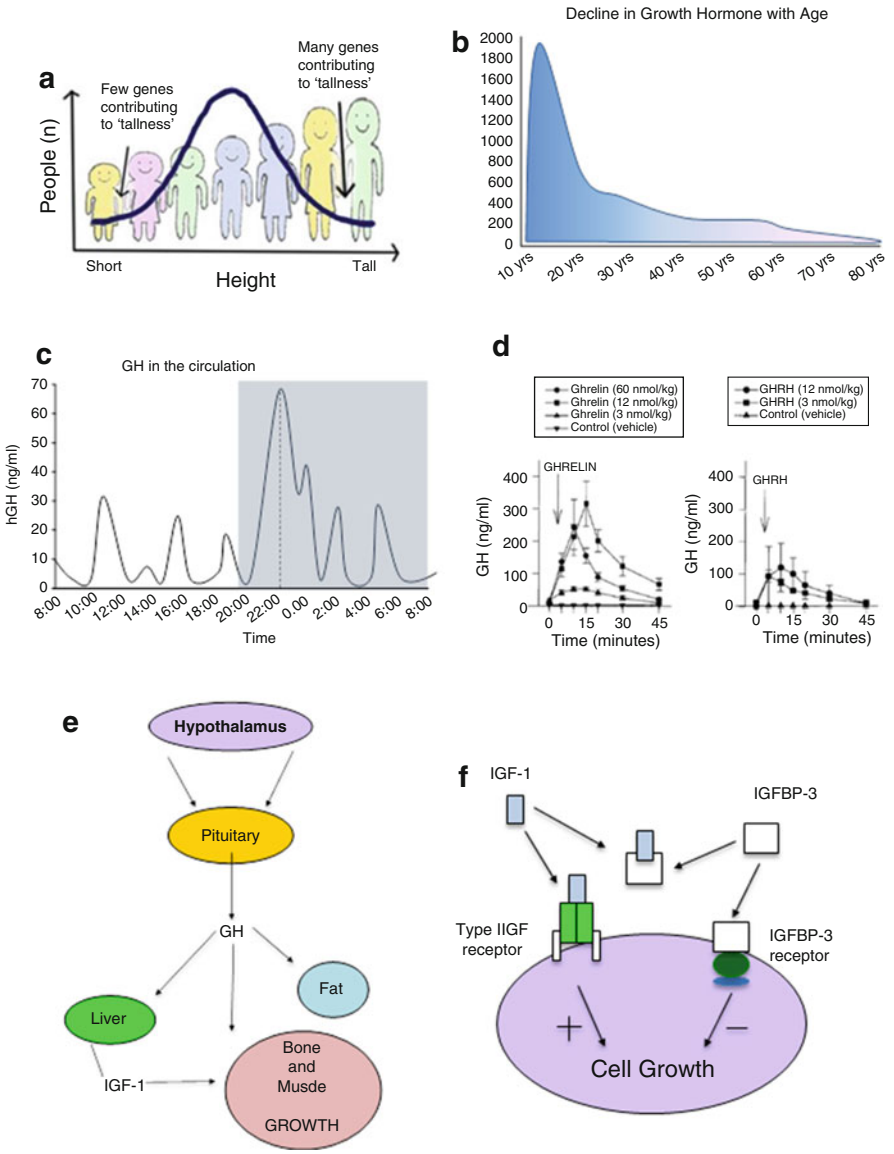


Fig. 2 Growth, growth hormone and IGF-1. (**a**) A large number of genes are implicated in achieving final height. (**b**) Circulating levels of GH are age-dependent. (**c**) GH secretion is episodic and quite unpredictable in normal human subjects. (**d**) Episodic GH administration in rodents elicits a greater effect on wight gain than the same dose administered in a continuous infusion (Seoane et al. 2000). (**e**) The hypothalamus regulates pituitary GH production which exerts several effects in different tissues and organs directly or indirectly through the hepatic IGF-1. (**f**) The effect of GH in the liver includes an increase in IGF-1 and its binding protein IGFBP3 in circulation

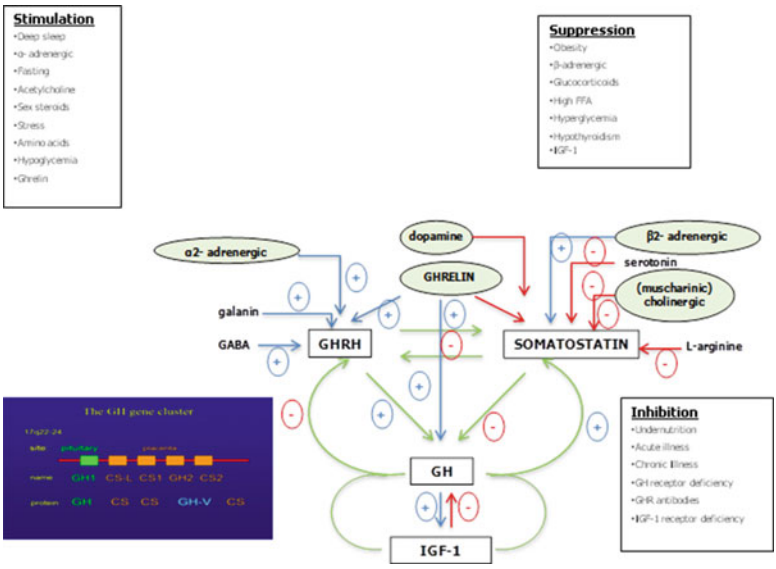


Fig. 3 GH secretion is regulated by different physiological factors

The active form of ghrelin able to bind the GHSR is the acetylated ghrelin with an n-octanoic acid in at Ser3 and its acylation is produced by the ghrelin O-acyltransferase (GOAT).

Ghrelin production by the stomach is tightly regulated by nutritional status and its regulation reflected in ghrelin circulating levels; thus, under fasting conditions, ghrelin circulating levels are increased returning to basal conditions immediately after food intake (Tschop et al. 2000; Cummings et al. 2002). Moreover, the sensorial stimuli related with food but without real food ingestion are also able to reduce the increased levels of gastric ghrelin production and circulating levels in fasted animals, although this is a transient effect compared to the decrease induced by true feed and this effect is mediated by the parasympathetic nervous system (Seoane et al. 2007b).

Multiple functions were attributed to ghrelin and related to energy balance regulation such as the regulation of appetite and food intake, gastric motility, gastric acid secretion, endocrine and exocrine pancreatic secretions, cell proliferation, glucose and lipid metabolism, and cardiovascular and immunologic processes (Seoane et al. 2004). Between the pleiotropic effects of ghrelin, a relevant neuroendocrine function has been described based on the effect of exogenous administration of the peptide on GH secretion, as well as its impact on the corticotroph axis and prolactin secretion (Wren et al. 2000). In this context, defective ghrelin signaling from the stomach could contribute to abnormalities in energy balance, growth, and associated gastrointestinal and neuroendocrine functions.

Supporting the implication of ghrelin as a part of the somatotroph axis, it has been showed that its production from the stomach is directly regulated by several components of the somatotroph axis, especially GH and SS. In gastric mucosa, SS receptors are expressed (Patel 1999) and the SS producing neurons are in direct contact with ghrelin producing neurons (Di Vito et al. 2002). Accordingly, in gastric explants, SS treatment directly induces a decrease in ghrelin secretion from the gastric tissue (Seoane et al. 2007a). In humans, it has been shown that SS treatment also decreases ghrelin circulating levels (Broglio et al. 2002). With respect to the effect of GH on ghrelin secretion from the stomach, the first suspect was based on the fact that GHR is expressed in the stomach (Nagano et al. 1995). Taking advantage of a model of gastric explants, an inhibitory effect of GH treatment was described in ghrelin secretion from the gastric tissue (Seoane et al. 2007a).

The most powerful action of ghrelin into secreting GH was described in vitro. It was shown a releasing effect of ghrelin on pituitary cell cultures with a similar potency to the obtained after GHRH treatment (Kojima et al. 1999). Accordingly, it was described in vivo that ghrelin administration to different doses induces a relevant increase in plasma GH levels in freely moving rats (Seoane et al. 2000) even major that those secretory effect influence by GHRH administration (Seoane et al. 2000) (Fig. 2). In addition, it was also found that not only the pituitary but also the hypothalamus is a key organ involved in the GH-releasing effects of ghrelin in vitro since the effect is higher in hypothalamic-pituitary unit that when is only administrated to pituitary cells (Popovic et al. 2003). Several evidences in vivo have supported the necessity of an intact hypothalamic-pituitary unit to ghrelin eject its GH secretagogue action since the effect was decreased in animals with lesions in the pituitary stalk (van der Lely et al. 2004) and humans under traumatic brain injuries (Pavlovic et al. 2010).

Regarding to the mechanism of ghrelin to induce GH secretion, several evidences showed a relation between ghrelin and GHRH in growth hormone secretion, in fact at pituitary level GHRH administration induces an increase in ghrelin expression (Kamegai et al. 1999) and the GHRH system should be operative in order to ghrelin act on GH secretion (Bowers 2001). With respect to the relation between SS and ghrelin to induce GH secretion several times, it was found that the effect of ghrelin secreting GH is independent of hypothalamic SS (Tannenbaum et al. 2003). In animal studies, the hypothalamic levels of GHRH and SS were not found altered after ghrelin administration, but it is not clear if truly ghrelin does not affect the expression of this hypothalamic neuropeptides or if the increased levels of GH exhibited after ghrelin administration is responsible for a feed back that would masking the hypothalamic effects of ghrelin (Seoane et al. 2003).

In addition to the GH releasing effects of ghrelin mediated by pituitary activation of the GHSR in somatotroph cells, recently a novel mechanism involving central nervous system in the regulation of growth hormone production by ghrelin has been described.

The main source of ghrelin in the organism is constituted by the gastrointestinal tract and specially the stomach. The vagus nerve is the principal connector between

the brain and the periphery, and it was found that this nerve is crucial to ghrelin induce growth hormone secretion (Popovic et al. 2003). Ghrelin when secreted from the stomach interact with the vagal pathway to produce these central effects on growth hormone secretion. Accordingly, the vagal blockade in experimental animals after surgical vagotomy induces a decrease in the basal GH and IGF-1 levels, but also in the GH secretion induced in response to ghrelin administration (Al-Massadi et al. 2011). Moreover, in the experimental animals with the vagal blockade, it was shown the hypothalamic levels of GHRH are widely decreased. At pituitary level, the disruption of the vagus nerve also affects to the somatotroph axis since the receptors for GHRH and the GHS-R are down-regulated (Al-Massadi et al. 2011).

However, due to the initial enthusiasm about the relevant role of ghrelin as a component of the somatotroph axis regulating GH secretion, several studies found that either ghrelin knock-out animals or GHS-R knock-out animals in spite of the lack of ghrelin effect do not present alterations in body growth or GH levels (Sun et al. 2003; Wortley et al. 2004; Zigman et al. 2005). The enzyme responsible for ghrelin acylation, GOAT, was discovered in 2008 (Kirchner et al. 2009). It was reported that KO animals for GOAT do not present relevant alterations in growth under normal conditions, however when subjected to severe caloric restriction the glucose levels decrease as leading to death of the animals, while in wild type animals after an initial decrease in glucose associated to food restriction the glucose levels stabilize (Yi et al. 2012). This fact was related with the decrease in GH levels under food restriction conditions that was transient in wild-type animals but maintained in the GOAT KO. These findings propose a role for endogenous ghrelin to maintain GH secretion under conditions of negative energy balance.

Ghrelin Regulates GH Secretion Through Cannabinoid System

A novel peripheral mechanism involved in ghrelin regulation of somatotroph axis includes the action of the cannabinoid system. For several years, a direct connection between GH and cannabinoid system was proposed in rodents (Kokka and Garcia 1974; Dalterio et al. 1981; Martin-Calderon et al. 1998) and humans (Benowitz et al. 1976).

It was showed that the active compound of marihuana Δ9Tetrahydrocannabinol (Δ9TH) induces an inhibitory action on GH secretion mediated by the activation of the SS levels in the hypothalamus (Rettori et al. 1990). Recently it was described a mechanism regulating GH secretion by the somatotroph axis elicited by the interaction between ghrelin and the cannabinoid receptor 1 (CB1) in the stomach. It was described that the pharmacological blockade of gastric CB1 decreases ghrelin secretion but also is reflected in a diminution of the pulsatile GH and the GH response to ghrelin. Moreover, this peripheral antagonism in the CB1 is reflected at central level by an inhibition in GHRH expression in the hypothalamus and this effect is mediated by the parasympathetic nervous system. Taken together, this mechanism reveals that ghrelin acts as a link between nutritional status and somatotroph axis.

Lactotrope Axis

Prolactin (PRL) is the hormone which is in charge to initiate and maintain lactation. The lactotroph cells of the adenohypophysis produce this hormone which presents a very similar structure with growth hormone and lactogeno placentario.

Regulation of Lactotroph Axis

The physiological control of prolactin levels is coordinated by the hypothalamic-pituitary lactotroph axis and presents several particularities, which differentiate it from the other known hypothalamic-pituitary axis. Differently to the other endocrine axis, PRL is the only pituitary hormone subjected to a negative control by the hypothalamus. It was shown that hypothalamic lesions induce a partial atrophy of pituitary cells which led to a reduction in the secretion of the pituitary hormones with the exception of PRL which is increased after median eminence lesions or pituitary stalk disruption (Kanematsu et al. 1963; Arimura et al. 1972). It is also the only hypothalamic hormone which regulator is not a hypothalamic peptide but it is a neurotransmitter, the dopamine.

For more than 40 years, it is known that dopamine antagonist induces an increase in pituitary release of prolactin. This fact was also found in in vitro experiments by using isolated pituitary glands where dopamine administration was able to decrease prolactin secretion (MacLeod et al. 1970). More recently it has been also found that dopamine receptor is expressed in lactotroph cells and the knockout mice for dopamine that presents increased levels of prolactin. Therefore, PRL secretion is mostly regulated by hypothalamic variations in dopamine levels, which inhibit PRL releases through the specific receptors in the lactotroph cell.

Prolactin (PRL)

For many years, the study of the physiological regulation of prolactin production by the pituitary has attracted great interest; however, its mechanism of production is actually not completely elucidated. As described for other hypothalamic-pituitary axis, a feed-back mechanism between target organs and hypothalamic peptides releasing-hormones governs the production of the main pituitary hormones. However, prolactin is completely different to the other hormones at this point, since it has no target tissue to induce this feedback mechanism. Prolactin release is controlled by a short loop feedback where it acts at central level to regulate its own production. PRL in plasma is able to cross the blood brain barrier through a transporter-mediated mechanism (Walsh et al. 1987) activating the prolactin receptors localized in the dopaminergic neurons which stimulate dopamine release and therefore inhibiting prolactin secretion (Hokfelt and Fuxe 1972; Gudelsky and Porter 1979). Under physiological changes of status that is produced during lactation or pregnancy where high levels of circulating prolactin are needed in order to promote milk

production, it was proposed that a decrease in the sensitivity to this feedback mechanism is responsible for the hyperprolactinemic state (Grattan and Kokay 2008). So a decrease in the activity of dopamine neurons was reported in late pregnancy (Andrews et al. 2001). In lactation, dopamine production remains also low with increased levels of PRL secretion, which is mandatory to milk production. Moreover, releases of prolactin during lactation are drive by the suckling reflects and it is coordinated by several molecular changes produced downstream on the PRL receptor (Feher et al. 2010; Romano et al. 2013).

The PRL release is produced in a pulsatile pattern through the day and especially at the beginning of the nocturnal phase and is not related to the cycle of sleep. The stimulation of PRL release is induced by the administration of TRH; during hypoglycemic state the release of PRL is also induced by insulin and after treatment with dopamine antagonist drugs. Under stress conditions, PRL production was also found increased. The estrogens increase PRL release and also induce lactotroph cells hyperplasia, which explains the increase in the pituitary size in pregnancy (Fink 1988). Lactation in woman and hypothyroidism in both sex increase the circulating levels of PRL, which is the clinical parameter. Other factors, in addition to TRH, have been proposed to regulate prolactin secretion such as the opioid peptides, which release prolactin through the inhibition of dopamine neurons. Other peptides directly related with an increase in PRL production are pituitary adenylate cyclase-activating polypeptide (PACAP) and the vasoactive intestinal peptide (VIP) (Horseman 1995).

All the mentioned factors regulating prolactin secretion act through the modulation of dopamine levels or directly in the lactotroph cells. However, although several evidences have shown the existence of a physiological peptide releasing prolactin, similar to the ones governing the secretion of other pituitary hormones, the efforts to find this factor were unsuccessful.

Prolactin Physiological Actions

The physiological actions of prolactin are classically only considered relevant in women under pregnancy of lactation status. However, the prolactin receptor is widely expressed in several tissues including the brain, the reproductive tract, and the gut (Goffin et al. 2002). Taking into account that pregnancy is a very complex process which requires an extensive range of adaptive changes, it would be plausive to think that the elevated levels of prolactin found during pregnancy might be involved in different tissues to facilitate the adaptive changes. Several changes that prolactin induces in order to adapt the organism to pregnancy have been recorded in the bibliography including the role of prolactin in maintaining the pregnancy by stimulating the function of the corpus luteum in rodents (Bachelot et al. 2009) and the regulation of the oxytocin release mediated by prolactin receptors in oxytocin neurons (Sapsford et al. 2012).

At central level, several actions have been proposed for prolactin all aimed to facilitate pregnancy and maternal life. It was showed that prolactin levels are directly

involved in maternal behavior, in fact a model of genetic mice with mutations in prolactin receptor showed a clear impairment in this behavior (Lucas et al. 1998).

At metabolic level, pregnancy and also lactation are physiological states that require very high-energy availability and specific metabolic requirements. Regarding adipose tissue metabolism, the lipid metabolism is severely affected in pregnancy and lactation and the utilization of lipids by the mammary gland in order to facilitate breast feeding induces the fat mobilization from the adipose stores (Barber et al. 1992). PRL might be involved in this adaptation at adipose level, since it has been showed that prolactin receptor is expressed in adipose tissue, which participates in both adipogenesis and adipocyte differentiation with the aim of modulating lipid metabolism. PRL levels have also showed to regulate the production of different adipokines such as leptin and adiponectin (Ben-Jonathan et al. 2006; Carre and Binart 2014).

It was also widely report that during pregnancy glucose homeostasis should be regulated in order to attend the requirements of this physiological state. The demands of insulin from the maternal tissues increase during pregnancy in order to provide glucose to the fetal tissues. Moreover, an state of insulin resistance was described in pregnancy (Herrera 2000) The mentioned changes induced during pregnancy in glucose homeostasis would be also mediated by prolactin since prolactin receptor is expressed in the pancreas, concretely in beta-cells, and its expression is induced in pregnancy (Moldrup et al. 1993).

Central control of food intake is a complex process involving different brain areas. The main center controlling appetite at central level is the hypothalamus and the hypothalamic nucleus involved in food intake regulation also expresses prolactin receptors (Bakowska and Morrell 1997; Pi and Grattan 1998; Brown et al. 2010). It was found that PRL administration induces food intake in different species (Moore et al. 1986) and the associated mechanism would be an increase in the leptin-resistant state during pregnancy induced by prolactin (Grattan et al. 2007).

The increased food intake and appetite characteristic of the pregnancy might be also mediated in part by prolactin levels since it was showed that prolactin stimulates food intake in nonpregnant animals.

The total physiological actions of the prolactin are not completely known, but its pathological effects were described. So the lacking of PRL prevents lactation and the hypersecretion produces hipogonadism in both sexes since it suppress the hypothalamic secretion of GnRH. Hiperprolactinemic states have been associated to infertility mediated by a suppression of LHRH release (Weber et al. 1983) which indicates a potential role of prolactin in regulating gonadal axis and reproductive function. Recently, the kisspeptin system has been proposed as a potent regulator of puberty in humans (de Roux et al. 2003); a crosstalk mechanism between prolactin and kisspeptin is one of the most novel mechanisms proposed for puberty development (Sonigo et al. 2012).

PRL prepare the organism for lactation and after delivery in a breast under the adequate stimulation of estrogens and progesterone, stimulate the synthesis of the specific proteins in the breast milk. After the delivery and during lactation, the suckling stimulus on the nipple induces a neural signal transmitted via spinal to the

hypothalamus that provokes an inhibition of the dopamine secretion and the subsequent release of prolactin. This stimulus provokes also a release of oxytocin that contracts the mammary follicles to its ejection. At the end of the breastfeeding period, the system returns to the conditions existing before delivering. Under this context is clear that an elevated prolactinemic tone is needed to the development of the mammary gland and the production of milk during lactation (Hovey et al. 2001; Trott et al. 2012).

Thyrotrophic Axis

The thyrotrophic axis is composed by three different hormonal steps previous to its biological action on peripheral tissues. The first step consists of the release of TRH from the hypothalamus to the portal hypophyseal vessels; these hypothalamic peptide acts on the pituitary and specially at the thyrotroph cells inducing TSH release (Shupnik et al. 1986; Martinez de Mena et al. 2010). In addition to TSH release, TRH also exert its hormonal releasing effect in other cellular types as prolactin producing cells and somatotroph cells inducing prolactin and GH secretion, respectively. TRH not only induce TSH release; moreover, it regulates its glycosylation pattern to increase its biological activity and half life (Weintraub et al. 1989). TSH through the circulating reach the thyroid gland where stimulate the thyroid follicles to induce the secretion of the thyroid hormones (TH) T3 and T4. Thyroid hormones act in the peripheral tissues to exert their physiological functions. The major form of circulating TH is T4. Three different enzymes are in charge of converting T4 in T3. Deiodinase type 1 (D1) is expressed in liver, kidney, pituitary, and thyroid and converts T4 to T3. The second discovered deiodinase type 2 (D2) is expressed in brain, pituitary, thyroid, BAT, and heart and converts also T4 to T3. Deiodinase type 3 (D3) is expressed in placenta en skin and inactivates T4 and T3. These three enzymes regulate the levels of T3 by cell-specific mechanisms.

Regulation of Thyrotrophic Axis

TSH is released in a circadian pattern with peak in the night. The thyrotroph axis is regulated by a feed-back mechanism, so when T3 and T4 increased they act at pituitary level to decrease the TSH production by the pituitary reaching a new set point on its production. The feed-back mechanism in charge of thyroid axis regulation acts at different levels: TRH and TSH synthesis and release or modulating the activity of the deiodinase enzymes. Another relevant role in this axis has been attributed to specific thyroid hormones transporters through the blood brain barrier. The main discovered thyroid hormones transporters are: monocarboxylate transporter 8 (MCT-8) and organic anion-transporting polypeptide 1C1 (OATP1C1).

The thyroid hormone production is negatively regulated by different factors such as glucocorticoids and somatostatin. Oppositely, estrogens induce a positive regulation of TSH secretion from the pituitary.

In addition, nutritional status severely regulates thyroid axis function, indeed fasting and food deprivation inhibited TH circulating levels (Reichlin 1957; Palmblad et al. 1977; Harris et al. 1978). The main components of the hypothalamus pituitary thyrotrophic axis are involved in the inhibitory effect of fasting in the thyroid hormone levels. As an example, the mRNA levels of TRH in the paraventricular nucleus in the hypothalamus are decreased in fasting state. Taking into account that fasting is a hypoleptinaemic state and considering the relevant effect of leptin on metabolism (Hardie et al. 1996), several authors proposed that leptin might be mediating the regulation of thyroid hormone production by nutritional status. As a prove, it was reported that leptin administration in fasting state is able to prevent the decrease in TRH expression in the paraventricular nucleus (Legradi et al. 1997). Between the main hypothalamic nucleus involve in appetite and food intake regulation, the arcuate nucleus consist of two different neural populations. One of this populations produce orexigenic responses mainly through the expression of neuropeptide Y (NPY)/and agouty-related peptide (AgRP) peptides; and a second neural population in the arcuate is responsible for anorexigenic responses mediated by proopiomelanocortin (POMC), precursor of alpha-melanocyte stimulating hormone (aMSH), and cocaine and amphetamine regulated transcript (CART) neuropeptides. Several peripheral hormones act at central level in order to regulate these hypothalamic neuropeptides and induce the orexigenic/anorexigenic responses with the aim to get the energy balance. TRH neurons in the hypothalamus are connected with aMSH, NPY, and AgRP neurons. The anorexigenic a MSH stimulates TRH production, while the anorexigenic NPY and AgRP inhibits the TRH expression in the hypothalamus (Fekete and Lechan 2014). These mechanisms govern the regulation of thyropthoph axis by nutritional status.

Individuals under hypothyroidism are more prone to gain weight and accumulate adiposity (Strata et al. 1978). Obese animals with obesity induced by diet, which are characterized by increased leptin concentrations, present an overstimulation of thyrotrophic axis (Xia et al. 2015).

Physiological Actions of Thyroid Hormones

For years the role of thyroid hormone in regulating energy metabolism was studied by different authors and a wide variety of physiological functions have been identified as potential targets for thyroid hormone effects among them growth, development, and metabolic rate (Hollenberg and Forrest 2008; Cheng et al. 2010) (Table 1). It was widely known that thyroid pathologies such as hypothyroidism and hyperthyroidism are associated to noticeable alterations in energy balance affecting body composition and food intake (Pearce 2012). Accordingly, patients with hyperthyroidism are characterized by an increase in the circulating levels of T3 and T4, present decrease in body weight in spite of the increase in food intake which is due to an increased metabolic rate (Silva 2006; Laurberg et al. 2012). Opposite, the individuals suffering of hypothyroid status are more prone to gain body weight

Table 1 Physiological functions of thyroid hormones

Physiological functions/organs	Effects
Growth	Thyroid hormones promote growth, an effect in part mediate by GH
Development	Hypothyroidism retards development; hyperthyroidism accelerates development
Cardiovascular system	Increase in heart rate, cardiac contractility, and cardiac output
Central nervous system	Thyroid hormones are essential to the development of CNS. Altered levels induce mental alterations
Reproductive system	Thyroid hormones are required for normal reproductive behavior
Skeletal muscle	Thyroid hormones increase contraction and relaxation rates. Hypo- and hyperthyroidism are associated to negative effects on skeletal muscle

despite of the decreased food intake with a decreased metabolic rate (Klieverik et al. 2009a; Mullur et al. 2014).

The classical physiology has attributed the effects of thyroid hormones on metabolism to its direct action on targets organs such as skeletal muscle, adipose tissue and the liver.

For years the thyrotrophic axis has been associated to the regulation of fatty acids, carbohydrates and glucose metabolism. Hypothyroid patients are more prompt to develop nonalcoholic fatty liver disease (NAFLD) which is associated to obesity and insulin resistance (Chung et al. 2012). Among the several mechanism related to the TH effects on the liver are an increase in the free fatty acids uptake for the liver through this action on the fatty acid transporters at hepatic level (Klieverik et al. 2009a), increasing the novo lipogenesis through the modulation of the transcription of different enzymes involved in this process (Liu and Brent 2010; Hashimoto et al. 2013), or promoting FA oxidation (Oppenheimer et al. 1991; Liu and Czaja 2013).

By the other way, patients with hyperthyroidism present increased levels of glucose production, insulin resistance in the liver, and hyperglycemia (Dimitriadis and Raptis 2001). It was reported that TH act directly in the liver to regulate crucial genes involved in glucose metabolism such as Sirt1 (Thakran et al. 2013), phospho-enolpyruvate carboxykinase (PCK1) (Park et al. 1999), and glucose-6-phosphatese (G6PC) (Suh et al. 2013).

In addition to the peripheral actions of TH in the liver, the most recent studies have proposed a central effect of TH at the hypothalamus that governs the hepatic glucose and lipid homeostasis. It was shown that the central administration of T3 in the PVH nucleus of the hypothalamus increases glucose production in the liver and this effect is mediated by the sympathetic nervous system (Klieverik et al. 2009b).

The thyrotrophic axis has classically associated with the pattern of food intake with a stimulatory effect of these hormones on appetite. The hypothalamus is the key organ involved in the orexigenic effects of TH drive by its interaction with key neuropeptides regulating appetite such as AGRP and NPY (Lopez et al. 2002) and the anorectic POMC in the arcuate nucleus (Lopez et al. 2010). Recent evidences

indicate the involvement of mTOR/S6K hypothalamic pathway in this effect. (Varela et al. 2012).

In the last years, a great amount of studies tried to get insight on the role of thyroid hormone in energy metabolism regulation with special interest in the mechanism that activates thermogenesis in brown adipose tissue. It is widely known that BAT express the TH receptors α and β and it was described as T3 induced uncoupling protein 1 (UCP-1) expression in BAT through the activation of TRβ which induces the thermogenic program (Ribeiro et al. 2010; Fekete and Lechan 2014). Moreover, it was showed that the thermogenesis induced in the brown adipose tissue (BAT) is mediated by sympathetic tone which is modulated by TH (Cannon and Nedergaard 2004; Silva 2006). The most recently findings indicate that central administration of thyroid hormones induces the brown adipose tissue thermogenesis, and this effect is mediated by the hypothalamic AMP-activated protein kinase AMPK (Lopez et al. 2010).

In addition to the described effects of thyroid hormones on brown adipose tissue, the effects of these hormones over white adipose tissue and lipid metabolism have been known for several years. A negative correlation was described between thyroid hormones and low-density lipoprotein cholesterol (LDL-C) levels (Coppola et al. 2007). Accordingly, hypothyroidism was classically associated to atherogenic events. However, the collateral effects at cardiovascular level of thyroid hormones administration widely limits its therapeutically use in lowering cholesterol.

Gonadal Axis

The hypothalamic-pituitary-gonadal axis is the main endocrine axis regulating crucial physiological functions mainly sexual maturation and reproduction. This axis is constituted at hypothalamic level by the neurohormone GnRH that acts in the pituitary releasing from the gonadotroph cells the follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones act on the gonads exerting a large number of processes including the secretion of peptidic and nonpeptidic gonadal hormones. The pulsatile pattern of GnRH is variable and its activity is subjected to feedback mechanism leaded by gonadal hormones. The pulsatile release of GnRH is reflected in a pulsatile liberation of FSH and LH from the pituitary and finally in a pulsatile pattern in the gonads. The gonadotropin concentrations remain very low in infantile age and become pulsatile during the night in the pubertal period and later in age during the entire day. Noteworthy, new actors have appeared in the scenario of the hypothalamic-pituitary gonadal axis such as the RFamides peptides, gonadotropin-inhibiting hormone (GnIH), and the related peptides Kisspeptins.

Gonadotrope Regulation

For several years, it was intensively searched the main hypothalamic factor able to be released in the portal blood and acting at pituitary level involved in the liberation of

the gonadotropins (FSH and LH). Finally, LHRH was first isolated in 1971 by two different groups from bovine and ovine brain (Amoss et al. 1971; Matsuo et al. 1971). LHRH is encoded by the GNRH1 gene and released from the hypothalamus to the hypophyseal portal circulation in charge of regulate the synthesis and secretion of the gonadotropin FSH and LH (Seeburg and Adelman 1984).

GnRH acts through the activation of its specific receptor which is a 60 kDa member of the seven-transmembrane G-protein coupled receptor (GPCR) family. It is expressed on the pituitary gonadotroph cells, but it was also found expression of this receptor in another tissues such as lymphocytes, breast, ovary, and prostate (Millar 2005).

In women, Gn-RH pulsatile releases control the switch off/on mechanism of the reproductive system. An increased exposition to Gn-RH presents a paradoxical effect, since it is able of induce a desensitization of the pituitary receptors for these gonadotropin and block the LH and FSH release. This action is used as a therapy to induce a chemical castration reversible after the treatment with high doses of LH.

LH and FSH pulses are very relevant in women. During the follicular phase, the LH pulses are following by a pulse of estrogens and during the medium and advance period of the luteum phase the pulses of LH stimulates progesterone en estradiol secretion from the follicles (Plant 2015). Estradiol together with progesterone and inhibin normally exerts a negative feed-back on LH secretion, but it changes to induce an stimulant effect in this phase of the cycle inducing a LH discharge that provokes the ovulation (Plant 2015). Data gleaned in recent years have put forward a new model of activation of the HPG axis. In this model, the activation of the GnRH pulse generator is dependent on a particular subset of neurons in the arcuate nucleus. Their main feature is that they exhibited the coexpression of the neuropeptides kisspeptin (encoded by *Kiss1*), dynorphin A (encoded by *Dyn*), and neurokinin B (NKB; encoded by *Tac2*) which belong to the family of tachykinins. This peptide family is comprised of the related peptides, substance P (SP), neurokinin A (NKA), and neurokinin B (NKB). NKB has emerged as regulator of kisspeptin release in the arcuate nucleus (ARC), whereas the roles of SP and NKA in reproduction remain unknown. These neurons (NKB) have been named kisspeptin/neurokinin B/dynorphin (KNDy) neurons. According to this model, the pulses are created by the release of kisspeptin, acting on GnRH neurons via the kisspeptin receptor, Kiss1R. The release of kisspeptin is in turn under the dynamic interplay of two major forces: (1) NKB autostimulation to elicit kisspeptin secretion and (2) pulse termination by the inhibitory actions of dynorphin A, thereby creating a dynamic activation-inactivation oscillator (Overgaard et al. 2014).

Finally, discovery of the gonadotropin-inhibitory hormone (GnIH) in birds, and its mammalian homolog, RFRP, opened up the exciting possibility that this inhibitory signal might operate centrally to suppress, directly or indirectly, GnRH/gonadotropin secretion, thus reciprocally cooperating with other stimulatory inputs in the dynamic regulation of the reproductive hypothalamic-pituitary unit. Pharmacological studies administering RFRP-agonists indicate that they inhibit basal and stimulated LH secretion. In keeping administration of an antagonist, RF9, led in most instances to an increase in LH levels although there are doubts regarding the receptor specificity of this pharmacological tool. Nevertheless the issue is now much clearer with the

assessment of gonadal function in genetic models with deletion of the receptor (NPFF1 $R^{-/-}$). In contrast to WT and as expected in light of their putative inhibitory effect, NPFF1R-deficient male and female mice had preserved fertility. In fact, the mean size of litters from NPFF1R KO pairs was significantly higher than that of WT breeders. Pubertal analyses of null animals evidenced that KO males, but not females, displayed constitutively elevated LH levels before and during puberty, whereas FSH levels were similar between genotypes during the pubertal transition. Pubertal progression was not apparently altered by the congenital lack of NPFF1R, as evidenced by similar mean ages of occurrence of external signs of puberty: balano-preputial separation in males and vaginal opening in females (Leon and Tena-Sempere 2015).

The LH discharge is not produced in the male; in this gender the only feed-back induced by estradiol on GnRH is negative. Testosterone and its active metabolite, Dihydrotestosterone, inhibit LH and FSH release through the pituitary and hypothalamic mechanisms. The negative feedback exerted by the gonad on FSH secretion is mediated by a peptidic hormone, inhibin, which is synthesized in the ovarian granulosa and in the Sertoli's cells. On the contrary gonadal production of other hormone, activin, stimulates the liberation of FSH by the pituitary.

A novel crucial factor regulating gonadotroph axis is the kisspeptin system. Kisspeptin(s) originally identified and termed as metastatin is the endogenous ligand of GPR54. The interest in the kisspeptin-GPR54 system arose following the finding of mutations in the gene coding GPR54 in some patients suffering from idiopathic (de Roux et al. 2003; Seminara et al. 2003). Kisspeptin has showed to induce an increase in LH release in almost all the animal models studied, including humans, following their administration via different central and peripheral routes. The stimulatory effect of kisspeptins is exerted in both male and female animals, at almost every stage of life (neonatal to late adulthood) with clear-cut increases in LH and FSH levels although the elevation in FSH requires the administration of much larger doses of kisspeptins (Dhillo et al. 2005; Tovar et al. 2006; George et al. 2011; Chan et al. 2012; Pinilla et al. 2012).

Although it is widely assumed that the most relevant actions of kisspeptins in the HPG axis are exerted at central level, some noncentral actions are also known. Interestingly, it has been reported the presence of the kisspeptin peptide in the hypothalamic-pituitary portal circulation. In addition, both the ligand and the receptor appear to be expressed in pituitary cells. Since it was reported several in vitro effects of kisspeptins at the pituitary, it is generally believed that they influence the gonadal axis by a variety of autocrine, paracrine, and endocrine effects (Gahete et al. 2016).

Further support for this concept comes out from data demonstrating that Kiss1r haplo-insufficiency induces a state of primary ovarian failure, which was not due to impaired gonadotropin secretion. In fact they showed that the failure of follicular development and ovulation linked to the absence of Kiss1r could not be rescued by gonadotropin replacement. These findings suggest a direct ovarian role of kisspeptin signaling in the regulation of the gonadal axis (Gaytan et al. 2014).

Finally, one of the main features in this axis is the crosstalk between gonadal hormones with the pituitary and the hypothalamus. In addition to the effects exerted

by peptidic and nonpeptidic gonadal hormones at pituitary level above described, it is well known that they also influence the hypothalamic control of this axis. In this regard, it should be emphasized that the nature of the crosstalk is related to gender and age. It may also be species-specific in some instances although this issue is much unclear. In any event it is now beyond doubt that gonadal steroids during fetal and neonatal periods play a key role in ensuring an adequate development of kisspeptins neuron. In terms of Kiss1 mRNA levels and their fiber density, being shown that these neurones are very sensitive to changes in sex steroid milieu during critical periods of sexual maturation and believe to be at the roots on how some of endocrine disruptors influence this axis. The effects of gonadal are (mainly) exerted via ER-alpha, which is expressed in almost all Kiss1 neurones with its effects being nucleus-specific. Accordingly, in rodents, estrogens inhibit the Kiss1 mRNA levels in the arcuate thereby inhibiting LH secretion. On the opposite, they increase Kiss1 mRNA levels at the AVPV/RP3V. This later finding implies the involvement of these neurones in the positive-feedback actions of estrogens to generate the preovulatory surge of gonadotropins. However, this AVPV kisspeptin neuronal population is not conserved in other species including nonhuman or human primates. It is likely therefore the existence of other populations in other locations such as the mediobasal hypothalamus where they may exert a similar function.

The Link Between Metabolism and Reproduction

The hypothalamic control of LH and FSH is very sensitive to environmental conditions such as stress nutritional changes or energy homeostasis alterations. The mental stress or a reduction in caloric intake inhibits the GnRH secretion, which is translated in a reduction of LH and FSH and its pulsatility and is manifest as amenorrhea. In male the mental stress leads to important reduction of testosterone levels.

The hypothalamic neurons in charge of release GnRH integrate the information from different signals such as neurotransmitters, environmental clues, and nutritional factors to respond regulating the pituitary release of LH and FSH (Wierman et al. 2011). In the last years, it has been revealed that metabolic status and reproductive function are directly related. The knowledge about different peripheral signals such as ghrelin and leptin has highlighted the possibility of a communication between metabolic state and gonadal axis mediated by these signals. In this sense, leptin is a hormone released from the adipose tissue that plays an important role in the initiation of puberty, as it was first shown in 1997 by the fact that the administration of leptin antiserum to rats led to a rapid change from regular 4-day cycles to anestrus (Carro et al. 1997a). Accordingly, it was also found that under food deprivation conditions which is a hypoleptinemic state the estrous cycle of the rat is severely deregulated (Knuth and Friesen 1983). Supporting the role of leptin in gonadal axis regulation, it was also found that exogenous leptin administration increases basal LH levels and ovarian and uterine weights and also corrects the sterility defect in female ob/ob mice (a genetic model of leptin deficiency) (Barash et al. 1996; Chehab et al. 1996). In male, leptin administration induces a increase in FSH levels, testicular and

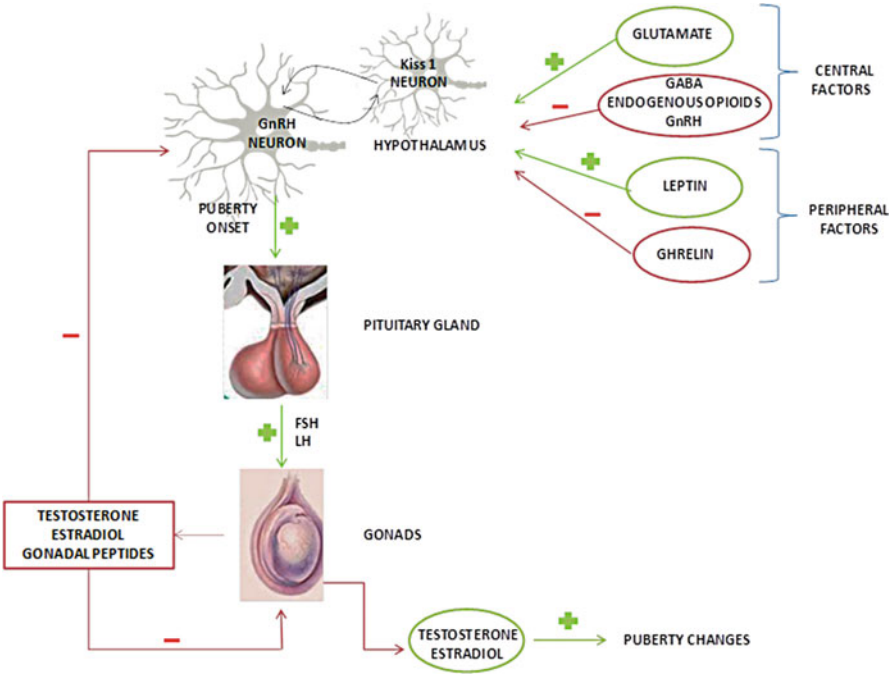


Fig. 4 Hypothalamic pituitary axis is regulated by central and peripheral factors in order to regulate the normal pubertal development

seminal vesicle weights, and sperm counts in male ob/ob mice (Mounzih et al. 1997). It has been proposed that leptin might act as a signal to inform the brain that the critical adipose stores are achieved and this allows the LHRH production in order to initiate the reproductive function by the activation of the hypothalamic pituitary gonadal axis. A central mechanism of action behind leptin regulation of gonadal axis was described. The stimulatory effect of leptin on GnRH is produced at hypothalamic level regulating neuronal and secretory activity of the GnRH neurons (Watanobe 2002) through its regulation of different neuropeptides such as neuropeptide Y, proopiomelanocortin, and kisspeptin (Roa 2013). It was also proposed that leptin is able to affect the sensitivity of the pituitary to GnRH and is also able to act directly on the female gonads to affect the different cycle phases (Elias and Purohit 2013).

Another hormone related with nutritional status regulating reproductive function is the gastric derived peptide ghrelin. In the rat, it was found that ghrelin modulates hypothalamic-pituitary gonadal axis by acting at the hypothalamus but also at pituitary level regulating GnRH production (Fernandez-Fernandez et al. 2007). Ghrelin administration induces the pulse of GnRH from the hypothalamus mediated by its action on NPY neurons (Lebrethon et al. 2007). At pituitary level, ghrelin suppresses the LH pulse characteristic of the ovulation; however, this action might

occur through the suppression of the hypothalamic kiss1 expression (Forbes et al. 2009). In addition to these central effects of ghrelin on hypothalamic-pituitary gonadal axis, a direct effect of ghrelin on the gonads regulating reproduction function was also proposed. Accordingly ghrelin and ghrelin receptors are expressed in both ovary, which vary depending of the cycle phase (Du et al. 2010) and testis especially in Leydig and Sertoli cells (Tena-Sempere et al. 2002).

In conclusion, the hypothalamus pituitary axis regulation is more complex that firstly supposed. It is exerted at different levels, hypothalamus, pituitary, and gonads, but also the signals from another relevant organs involved in metabolism are relevant in the regulation of the gonadal axis to ensure the reproduction (Fig. 4).

Summary

The main physiological functions such as growth, lactation, adiposity, and reproduction are regulated by the hypothalamic-pituitary unit. Throughout this chapter, the classical functions of this relevant system are revised together with the new signals and organs recently included in the hypothalamic-pituitary unit by the newest research published.

Cross-References

▶ Neuroendocrinology of Energy Homeostasis
▶ Physiopathology, Diagnosis, and Treatment of GH Deficiency
▶ Physiopathology, Diagnosis, and Treatment of Secondary Hypothyroidism

References

Al-Massadi O, Trujillo ML, Senaris R, Pardo M, Castelao C, Casanueva FF, Seoane LM. The vagus nerve as a regulator of growth hormone secretion. Regul Pept. 2011;166:3–8.

Amoss M, Burgus R, Blackwell R, Vale W, Fellows R, Guillemin R. Purification, amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. Biochem Biophys Res Commun. 1971;44:205–10.

Andrews ZB, Kokay IC, Grattan DR. Dissociation of prolactin secretion from tuberoinfundibular dopamine activity in late pregnant rats. Endocrinology. 2001;142:2719–24.

Arimura A, Dunn JD, Schally AV. Effect of infusion of hypothalamic extracts on serum prolactin levels in rats treated with nembutal, CNS depressants or bearing hypothalamic lesions. Endocrinology. 1972;90:378–83.

Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab. 2001;86:4753–8.

Avtanski D, Novaira HJ, Wu S, Romero CJ, Kineman R, Luque RM, Wondisford F, Radovick S. Both estrogen receptor alpha and beta stimulate pituitary GH gene expression. Mol Endocrinol. 2014;28:40–52.

Bachelot A, Beaufaron J, Servel N, Kedzia C, Monget P, Kelly PA, Gibori G, Binart N. Prolactin independent rescue of mouse corpus luteum life span: identification of prolactin and luteinizing hormone target genes. Am J Physiol Endocrinol Metab. 2009;297:E676–84.

Bakowska JC, Morrell JI. Atlas of the neurons that express mRNA for the long form of the prolactin receptor in the forebrain of the female rat. J Comp Neurol. 1997;386:161–77.

Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK, Steiner RA. Leptin is a metabolic signal to the reproductive system. Endocrinology. 1996;137:3144–7.

Barber MC, Clegg RA, Finley E, Vernon RG, Flint DJ. The role of growth hormone, prolactin and insulin-like growth factors in the regulation of rat mammary gland and adipose tissue metabolism during lactation. J Endocrinol. 1992;135:195–202.

Baumann G. Metabolism of growth hormone (GH) and different molecular forms of GH in biological fluids. Horm Res. 1991;36(Suppl 1):5–10.

Ben-Jonathan N, Hugo ER, Brandebourg TD, LaPensee CR. Focus on prolactin as a metabolic hormone. Trends Endocrinol Metab. 2006;17:110–6.

Benowitz NL, Jones RT, Lerner CB. Depression of growth hormone and cortisol response to insulin-induced hypoglycemia after prolonged oral delta-9-tetrahydrocannabinol administration in man. J Clin Endocrinol Metab. 1976;42:938–41.

Berryman DE, List EO, Coschigano KT, Behar K, Kim JK, Kopchick JJ. Comparing adiposity profiles in three mouse models with altered GH signaling. Growth Horm IGF Res. 2004;14:309–18.

Bowers CY. Unnatural growth hormone-releasing peptide begets natural ghrelin. J Clin Endocrinol Metab. 2001;86:1464–9.

Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science. 1973;179:77–9.

Broglio F, Koetsveld Pv P, Benso A, Gottero C, Prodam F, Papotti M, Muccioli G, Gauna C, Hofland L, Deghenghi R, Arvat E, Van Der Lely AJ, Ghigo E. Ghrelin secretion is inhibited by either somatostatin or cortistatin in humans. J Clin Endocrinol Metab. 2002;87:4829–32.

Brown RS, Kokay IC, Herbison AE, Grattan DR. Distribution of prolactin-responsive neurons in the mouse forebrain. J Comp Neurol. 2010;518:92–102.

Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev. 2004;84:277–359.

Carre N, Binart N. Prolactin and adipose tissue. Biochimie. 2014;97:16–21.

Carro E, Pinilla L, Seoane LM, Considine RV, Aguilar E, Casanueva FF, Dieguez C. Influence of endogenous leptin tone on the estrous cycle and luteinizing hormone pulsatility in female rats. Neuroendocrinology. 1997a;66:375–7.

Carro E, Senaris R, Considine RV, Casanueva FF, Dieguez C. Regulation of in vivo growth hormone secretion by leptin. Endocrinology. 1997b;138:2203–6.

Chan YM, Butler JP, Sidhoum VF, Pinnell NE, Seminara SB. Kisspeptin administration to women: a window into endogenous kisspeptin secretion and GnRH responsiveness across the menstrual cycle. J Clin Endocrinol Metab. 2012;97:E1458–67.

Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet. 1996;12:318–20.

Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. Endocr Rev. 2010;31:139–70.

Chung GE, Kim D, Kim W, Yim JY, Park MJ, Kim YJ, Yoon JH, Lee HS. Non-alcoholic fatty liver disease across the spectrum of hypothyroidism. J Hepatol. 2012;57:150–6.

Cone RD, Cowley MA, Butler AA, Fan W, Marks DL, Low MJ. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. Int J Obes Relat Metab Disord. 2001;25(Suppl 5):S63–7.

Coppola A, Liu ZW, Andrews ZB, Paradis E, Roy MC, Friedman JM, Ricquier D, Richard D, Horvath TL, Gao XB, Diano S. A central thermogenic-like mechanism in feeding regulation: an interplay between arcuate nucleus T3 and UCP2. Cell Metab. 2007;5:21–33.

Cordido F, Peino R, Penalva A, Alvarez CV, Casanueva FF, Dieguez C. Impaired growth hormone secretion in obese subjects is partially reversed by acipimox-mediated plasma free fatty acid depression. J Clin Endocrinol Metab. 1996;81:914–8.

Cornford AS, Barkan AL, Horowitz JF. Rapid suppression of growth hormone concentration by overeating: potential mediation by hyperinsulinemia. J Clin Endocrinol Metab. 2011;96:824–30.

Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med. 2002;346:1623–30.

Dalterio SL, Michael SD, Macmillan BT, Bartke A. Differential effects of cannabinoid exposure and stress on plasma prolactin, growth hormone and corticosterone levels in male mice. Life Sci. 1981;28:761–6.

Daughaday WH. A personal history of the origin of the somatomedin hypothesis and recent challenges to its validity. Perspect Biol Med. 1989;32:194–211.

de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A. 2003;100:10972–6.

Dhillo WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, McGowan BM, Amber V, Patel S, Ghatei MA, Bloom SR. Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. J Clin Endocrinol Metab. 2005;90:6609–15.

Di Vito L, Broglio F, Benso A, Gottero C, Prodam F, Papotti M, Muccioli G, Dieguez C, Casanueva FF, Deghenghi R, Ghigo E, Arvat E. The GH-releasing effect of ghrelin, a natural GH secretagogue, is only blunted by the infusion of exogenous somatostatin in humans. Clin Endocrinol. 2002;56:643–8.

Dimitriadis GD, Raptis SA. Thyroid hormone excess and glucose intolerance. Exp Clin Endocrinol Diabetes. 2001;**109**(Suppl 2):S225–39.

Du C, Li H, Cao G, Xilingaowa, Wang C, Li C. Expression of the orexigenic peptide ghrelin and the type 1a growth hormone secretagogue receptor in sheep oocytes and pre-implantation embryos produced in vitro. Reprod Domest Anim. 2010;45:92–8.

Elias CF, Purohit D. Leptin signaling and circuits in puberty and fertility. Cell Mol Life Sci. 2013;70:841–62.

Elmquist JK. CNS regulation of energy balance and body weight: insights from rodent models. Lab Anim Sci. 1998;48:630–7.

Feher P, Olah M, Bodnar I, Hechtl D, Bacskay I, Juhasz B, Nagy GM, Vecsernyes M. Dephosphorylation/inactivation of tyrosine hydroxylase at the median eminence of the hypothalamus is required for suckling-induced prolactin and adrenocorticotrop hormone responses. Brain Res Bull. 2010;82:141–5.

Fekete C, Lechan RM. Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions. Endocr Rev. 2014;35:159–94.

Fernandez-Fernandez R, Tena-Sempere M, Roa J, Castellano JM, Navarro VM, Aguilar E, Pinilla L. Direct stimulatory effect of ghrelin on pituitary release of LH through a nitric oxide-dependent mechanism that is modulated by estrogen. Reproduction. 2007;133:1223–32.

Fink G. Oestrogen and progesterone interactions in the control of gonadotrophin and prolactin secretion. J Steroid Biochem. 1988;30:169–78.

Forbes S, Li XF, Kinsey-Jones J, O'Byrne K. Effects of ghrelin on kisspeptin mRNA expression in the hypothalamic medial preoptic area and pulsatile luteinising hormone secretion in the female rat. Neurosci Lett. 2009;460:143–7.

Frasca F, Pandini G, Sciacca L, Pezzino V, Squatrito S, Belfiore A, Vigneri R. The role of insulin receptors and IGF-I receptors in cancer and other diseases. Arch Physiol Biochem. 2008;114:23–37.

Gahete MD, Vazquez-Borrego MC, Martinez-Fuentes AJ, Tena-Sempere M, Castano JP, Luque RM. Role of the Kiss1/Kiss1r system in the regulation of pituitary cell function. Mol Cell Endocrinol. 2016;438:100–6.

Garcia-Lavandeira M, Quereda V, Flores I, Saez C, Diaz-Rodriguez E, Japon MA, Ryan AK, Blasco MA, Dieguez C, Malumbres M, Alvarez CV. A GRFa2/Prop1/stem (GPS) cell niche in the pituitary. PLoS One. 2009;4:e4815.

Garcia-Lavandeira M, Diaz-Rodriguez E, Bahar D, Garcia-Rendueles AR, Rodrigues JS, Dieguez C, Alvarez CV. Pituitary cell turnover: from adult stem cell recruitment through differentiation to death. Neuroendocrinology. 2015;101:175–92.

Gaytan F, Garcia-Galiano D, Dorfman MD, Manfredi-Lozano M, Castellano JM, Dissen GA, Ojeda SR, Tena-Sempere M. Kisspeptin receptor haplo-insufficiency causes premature ovarian failure despite preserved gonadotropin secretion. Endocrinology. 2014;155:3088–97.

George JT, Veldhuis JD, Roseweir AK, Newton CL, Faccenda E, Millar RP, Anderson RA. Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. J Clin Endocrinol Metab. 2011;96:E1228–36.

Ghigo E, Aimaretti G, Gianotti L, Bellone J, Arvat E, Camanni F. New approach to the diagnosis of growth hormone deficiency in adults. Eur J Endocrinol. 1996;134:352–6.

Goffin V, Binart N, Touraine P, Kelly PA. Prolactin: the new biology of an old hormone. Annu Rev Physiol. 2002;64:47–67.

Gooley JJ, Schomer A, Saper CB. The dorsomedial hypothalamic nucleus is critical for the expression of food-entrainable circadian rhythms. Nat Neurosci. 2006;9:398–407.

Grattan DR, Kokay IC. Prolactin: a pleiotropic neuroendocrine hormone. J Neuroendocrinol. 2008;20:752–63.

Grattan DR, Ladyman SR, Augustine RA. Hormonal induction of leptin resistance during pregnancy. Physiol Behav. 2007;91:366–74.

Gudelsky GA, Porter JC. Release of newly synthesized dopamine into the hypophysial portal vasculature of the rat. Endocrinology. 1979;104:583–7.

Guillemin R, Brazeau P, Bohlen P, Esch F, Ling N, Wehrenberg WB. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science. 1982;218:585–7.

Hardie LJ, Rayner DV, Holmes S, Trayhurn P. Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA. Biochem Biophys Res Commun. 1996;223:660–5.

Harris AR, Fang SL, Azizi F, Lipworth L, Vagenakis AG, Barverman LE. Effect of starvation on hypothalamic-pituitary-thyroid function in the rat. Metabolism. 1978;27:1074–83.

Hashimoto K, Ishida E, Miura A, Ozawa A, Shibusawa N, Satoh T, Okada S, Yamada M, Mori M. Human stearoyl-CoA desaturase 1 (SCD-1) gene expression is negatively regulated by thyroid hormone without direct binding of thyroid hormone receptor to the gene promoter. Endocrinology. 2013;154:537–49.

Herrera E. Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. Eur J Clin Nutr. 2000;54(Suppl 1):S47–51.

Hokfelt T, Fuxe K. Effects of prolactin and ergot alkaloids on the tubero-infundibular dopamine (DA) neurons. Neuroendocrinology. 1972;9:100–22.

Hollenberg AN, Forrest D. The thyroid and metabolism: the action continues. Cell Metab. 2008;8:10–2.

Horseman ND. Prolactin, proliferation, and protooncogenes. Endocrinology. 1995;136:5249–51.

Hovey RC, Trott JF, Ginsburg E, Goldhar A, Sasaki MM, Fountain SJ, Sundararajan K, Vonderhaar BK. Transcriptional and spatiotemporal regulation of prolactin receptor mRNA and cooperativity with progesterone receptor function during ductal branch growth in the mammary gland. Dev Dyn. 2001;222:192–205.

Iranmanesh A, Lizarralde G, Veldhuis JD. Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men. J Clin Endocrinol Metab. 1991;73:1081–8.

Kamegai J, Wakabayashi I, Kineman RD, Frohman LA. Growth hormone-releasing hormone receptor (GHRH-R) and growth hormone secretagogue receptor (GHS-R) mRNA levels during postnatal development in male and female rats. J Neuroendocrinol. 1999;11:299–306.

Kanematsu S, Hilliard J, Sawyer CH. Effect of reserpine on pituitary prolactin content and its hypothalamic site of action in the rabbit. Acta Endocrinol. 1963;44:467–74.

Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. Diabetes. 2011;60:2441–9.

Kirchner H, Gutierrez JA, Solenberg PJ, Pfluger PT, Czyzyk TA, Willency JA, Schurmann A, Joost HG, Jandacek RJ, Hale JE, Heiman ML, Tschop MH. GOAT links dietary lipids with the endocrine control of energy balance. Nat Med. 2009;15:741–5.

Klieverik LP, Coomans CP, Endert E, Sauerwein HP, Havekes LM, Voshol PJ, Rensen PC, Romijn JA, Kalsbeek A, Fliers E. Thyroid hormone effects on whole-body energy homeostasis and tissue-specific fatty acid uptake in vivo. Endocrinology. 2009a;150:5639–48.

Klieverik LP, Janssen SF, van Riel A, Foppen E, Bisschop PH, Serlie MJ, Boelen A, Ackermans MT, Sauerwein HP, Fliers E, Kalsbeek A. Thyroid hormone modulates glucose production via a sympathetic pathway from the hypothalamic paraventricular nucleus to the liver. Proc Natl Acad Sci U S A. 2009b;106:5966–71.

Knuth UA, Friesen HG. Starvation induced anoestrus: effect of chronic food restriction on body weight, its influence on oestrous cycle and gonadotrophin secretion in rats. Acta Endocrinol. 1983;104:402–9.

Koerker DJ, Ruch W, Chideckel E, Palmer J, Goodner CJ, Ensinck J, Gale CC. Somatostatin: hypothalamic inhibitor of the endocrine pancreas. Science. 1974;184:482–4.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999;402:656–60.

Kokka N, Garcia JF. Effects of delta 9-THC on growth hormone and ACTH secretion in rats. Life Sci. 1974;15:329–38.

Kopchick JJ, Parkinson C, Stevens EC, Trainer PJ. Growth hormone receptor antagonists: discovery, development, and use in patients with acromegaly. Endocr Rev. 2002;23:623–46.

Lanning NJ, Carter-Su C. Recent advances in growth hormone signaling. Rev Endocr Metab Disord. 2006;7:225–35.

Laurberg P, Knudsen N, Andersen S, Carle A, Pedersen IB, Karmisholt J. Thyroid function and obesity. Eur Thyroid J. 2012;1:159–67.

Lebrethon MC, Aganina A, Fournier M, Gerard A, Parent AS, Bourguignon JP. Effects of in vivo and in vitro administration of ghrelin, leptin and neuropeptide mediators on pulsatile gonadotrophin-releasing hormone secretion from male rat hypothalamus before and after puberty. J Neuroendocrinol. 2007;19:181–8.

Legradi G, Emerson CH, Ahima RS, Flier JS, Lechan RM. Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. Endocrinology. 1997;138:2569–76.

Leon S, Tena-Sempere M. Dissecting the roles of gonadotropin-inhibitory hormone in mammals: studies using pharmacological tools and genetically modified mouse models. Front Endocrinol. 2015;6:189.

Liu YY, Brent GA. Thyroid hormone crosstalk with nuclear receptor signaling in metabolic regulation. Trends Endocrinol Metab. 2010;21:166–73.

Liu K, Czaja MJ. Regulation of lipid stores and metabolism by lipophagy. Cell Death Differ. 2013;20:3–11.

Lopez M, Seoane L, Tovar S, Senaris RM, Dieguez C. Thyroid status regulates CART but not AgRP mRNA levels in the rat hypothalamus. Neuroreport. 2002;13:1775–9.

Lopez M, Varela L, Vazquez MJ, Rodriguez-Cuenca S, Gonzalez CR, Velagapudi VR, Morgan DA, Schoenmakers E, Agassandian K, Lage R, Martinez de Morentin PB, Tovar S, Nogueiras R, Carling D, Lelliott C, Gallego R, Oresic M, Chatterjee K, Saha AK, Rahmouni K, Dieguez C, Vidal-Puig A. Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. Nat Med. 2010;16:1001–8.

Lucas BK, Ormandy CJ, Binart N, Bridges RS, Kelly PA. Null mutation of the prolactin receptor gene produces a defect in maternal behavior. Endocrinology. 1998;139:4102–7.

Luque RM, Gahete MD, Valentine RJ, Kineman RD. Examination of the direct effects of metabolic factors on somatotrope function in a non-human primate model, *Papio anubis*. J Mol Endocrinol. 2006;37:25–38.

MacLeod RM, Fontham EH, Lehmeyer JE. Prolactin and growth hormone production as influenced by catecholamines and agents that affect brain catecholamines. Neuroendocrinology. 1970;6:283–94.

Martin-Calderon JL, Munoz RM, Villanua MA, del Arco I, Moreno JL, de Fonseca FR, Navarro M. Characterization of the acute endocrine actions of (-)-11-hydroxy-delta8-tetrahydrocannabinol-dimethylheptyl (HU-210), a potent synthetic cannabinoid in rats. Eur J Pharmacol. 1998;344:77–86.

Martinez de Mena R, Scanlan TS, Obregon MJ. The T3 receptor beta1 isoform regulates UCP1 and D2 deiodinase in rat brown adipocytes. Endocrinology. 2010;151:5074–83.

Matsuo H, Baba Y, Nair RM, Arimura A, Schally AV. Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem Biophys Res Commun. 1971;43:1334–9.

Mayo KE, Vale W, Rivier J, Rosenfeld MG, Evans RM. Expression-cloning and sequence of a cDNA encoding human growth hormone-releasing factor. Nature. 1983;306:86–8.

Meinhardt UJ, Ho KK. Modulation of growth hormone action by sex steroids. Clin Endocrinol. 2006;65:413–22.

Millar RP. GnRHs and GnRH receptors. Anim Reprod Sci. 2005;88:5–28.

Moldrup A, Petersen ED, Nielsen JH. Effects of sex and pregnancy hormones on growth hormone and prolactin receptor gene expression in insulin-producing cells. Endocrinology. 1993;133:1165–72.

Moore BJ, Gerardo-Gettens T, Horwitz BA, Stern JS. Hyperprolactinemia stimulates food intake in the female rat. Brain Res Bull. 1986;17:563–9.

Mounzih K, Lu R, Chehab FF. Leptin treatment rescues the sterility of genetically obese ob/ob males. Endocrinology. 1997;138:1190–3.

Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. Physiol Rev. 2014;94:355–82.

Nagano M, Chastre E, Choquet A, Bara J, Gespach C, Kelly PA. Expression of prolactin and growth hormone receptor genes and their isoforms in the gastrointestinal tract. Am J Physiol. 1995;268: G431–42.

Nilsson L, Binart N, Bohlooly YM, Bramnert M, Egecioglu E, Kindblom J, Kelly PA, Kopchick JJ, Ormandy CJ, Ling C, Billig H. Prolactin and growth hormone regulate adiponectin secretion and receptor expression in adipose tissue. Biochem Biophys Res Commun. 2005;331:1120–6.

Norman C, Rollene N, Weist SM, Wigham JR, Erickson D, Miles JM, Bowers CY, Veldhuis JD. Short-term estradiol supplementation potentiates low-dose ghrelin action in the presence of GHRH or somatostatin in older women. J Clin Endocrinol Metab. 2014;99:E73–80.

Ohlsson C, Mohan S, Sjogren K, Tivesten A, Isgaard J, Isaksson O, Jansson JO, Svensson J. The role of liver-derived insulin-like growth factor-I. Endocr Rev. 2009;30:494–535.

Oppenheimer JH, Schwartz HL, Lane JT, Thompson MP. Functional relationship of thyroid hormone-induced lipogenesis, lipolysis, and thermogenesis in the rat. J Clin Invest. 1991;87:125–32.

Overgaard A, Ruiz-Pino F, Castellano JM, Tena-Sempere M, Mikkelsen JD. Disparate changes in kisspeptin and neurokinin B expression in the arcuate nucleus after sex steroid manipulation reveal differential regulation of the two KNDy peptides in rats. Endocrinology. 2014;155:3945–55.

Palmblad J, Levi L, Burger A, Melander A, Westgren U, von Schenck H, Skude G. Effects of total energy withdrawal (fasting) on the levels of growth hormone, thyrotropin, cortisol, adrenaline, noradrenaline, T4, T3, and rT3 in healthy males. Acta Med Scand. 1977;201:15–22.

Park EA, Song S, Vinson C, Roesler WJ. Role of CCAAT enhancer-binding protein beta in the thyroid hormone and cAMP induction of phosphoenolpyruvate carboxykinase gene transcription. J Biol Chem. 1999;274:211–7.

Patel YC. Somatostatin and its receptor family. Front Neuroendocrinol. 1999;20:157–98.

Patel YC, Greenwood MT, Panetta R, Demchyshyn L, Niznik H, Srikant CB. The somatostatin receptor family. Life Sci. 1995;57:1249–65.

Pavlovic D, Pekic S, Stojanovic M, Zivkovic V, Djurovic B, Jovanovic V, Miljic N, Medic-Stojanoska M, Doknic M, Miljic D, Djurovic M, Casanueva F, Popovic V. Chronic cognitive sequelae after traumatic brain injury are not related to growth hormone deficiency in adults. Eur J Neurol. 2010;17:696–702.

Pearce EN. Thyroid hormone and obesity. Curr Opin Endocrinol Diabetes Obes. 2012;19:408–13.

Pi XJ, Grattan DR. Distribution of prolactin receptor immunoreactivity in the brain of estrogen-treated, ovariectomized rats. J Comp Neurol. 1998;394:462–74.

Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev. 2012;92:1235–316.

Plant TM. 60 YEARS OF NEUROENDOCRINOLOGY: the hypothalamo-pituitary-gonadal axis. J Endocrinol. 2015;226:T41–54.

Popovic V, Miljic D, Micic D, Damjanovic S, Arvat E, Ghigo E, Dieguez C, Casanueva FF. Ghrelin main action on the regulation of growth hormone release is exerted at hypothalamic level. J Clin Endocrinol Metab. 2003;88:3450–3.

Reichlin S. The effect of dehydration, starvation, and pitressin injections on thyroid activity in the rat. Endocrinology. 1957;60:470–87.

Reichlin S, Saperstein R, Jackson IM, Boyd AE 3rd, Patel Y. Hypothalamic hormones. Annu Rev Physiol. 1976;38:389–424.

Rettori V, Aguila MC, Gimeno MF, Franchi AM, McCann SM. In vitro effect of delta 9-tetrahydrocannabinol to stimulate somatostatin release and block that of luteinizing hormone-releasing hormone by suppression of the release of prostaglandin E2. Proc Natl Acad Sci U S A. 1990;87:10063–6.

Ribeiro MO, Bianco SD, Kaneshige M, Schultz JJ, Cheng SY, Bianco AC, Brent GA. Expression of uncoupling protein 1 in mouse brown adipose tissue is thyroid hormone receptor-beta isoform specific and required for adaptive thermogenesis. Endocrinology. 2010;151:432–40.

Rinderknecht E, Humbel RE. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. J Biol Chem. 1978;253:2769–76.

Roa J. Role of GnRH neurons and their neuronal afferents as key integrators between food intake regulatory signals and the control of reproduction. Int J Endocrinol. 2013;2013:518046.

Romano N, Yip SH, Hodson DJ, Guillou A, Parnaudeau S, Kirk S, Tronche F, Bonnefont X, Le Tissier P, Bunn SJ, Grattan DR, Mollard P, Martin AO. Plasticity of hypothalamic dopamine neurons during lactation results in dissociation of electrical activity and release. J Neurosci. 2013;33:4424–33.

Romero CJ, Ng Y, Luque RM, Kineman RD, Koch L, Bruning JC, Radovick S. Targeted deletion of somatotroph insulin-like growth factor-I signaling in a cell-specific knockout mouse model. Mol Endocrinol. 2010;24:1077–89.

Sapsford TJ, Kokay IC, Ostberg L, Bridges RS, Grattan DR. Differential sensitivity of specific neuronal populations of the rat hypothalamus to prolactin action. J Comp Neurol. 2012;520:1062–77.

Sassolas G. Growth hormone-releasing hormone: past and present. Horm Res. 2000;53(Suppl 3):88–92.

Savastano S, Di Somma C, Barrea L, Colao A. The complex relationship between obesity and the somatropic axis: the long and winding road. Growth Horm IGF Res. 2014;24:221–6.

Scarth JP. Modulation of the growth hormone-insulin-like growth factor (GH-IGF) axis by pharmaceutical, nutraceutical and environmental xenobiotics: an emerging role for xenobiotic-metabolizing enzymes and the transcription factors regulating their expression. A review. Xenobiotica. 2006;36:119–218.

Seeburg PH, Adelman JP. Characterization of cDNA for precursor of human luteinizing hormone releasing hormone. Nature. 1984;311:666–8.

Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA,

Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH. The GPR54 gene as a regulator of puberty. N Engl J Med. 2003;349:1614–27.
Seoane LM, Tovar S, Baldelli R, Arvat E, Ghigo E, Casanueva FF, Dieguez C. Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. Eur J Endocrinol. 2000;143:R7–9.
Seoane LM, Lopez M, Tovar S, Casanueva FF, Senaris R, Dieguez C. Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. Endocrinology. 2003;144:544–51.
Seoane LM, Al-Massadi O, Lage M, Dieguez C, Casanueva FF. Ghrelin: from a GH-secretagogue to the regulation of food intake, sleep and anxiety. Pediatr Endocrinol Rev. 2004;1(Suppl 3):432–7.
Seoane LM, Al-Massadi O, Barreiro F, Dieguez C, Casanueva FF. Growth hormone and somatostatin directly inhibit gastric ghrelin secretion. An in vitro organ culture system. J Endocrinol Invest. 2007a;30:RC22–5.
Seoane LM, Al-Massadi O, Caminos JE, Tovar SA, Dieguez C, Casanueva FF. Sensory stimuli directly acting at the central nervous system regulate gastric ghrelin secretion. An ex vivo organ culture study. Endocrinology. 2007b;148:3998–4006.
Shupnik MA, Ardisson LJ, Meskell MJ, Bornstein J, Ridgway EC. Triiodothyronine (T3) regulation of thyrotropin subunit gene transcription is proportional to T3 nuclear receptor occupancy. Endocrinology. 1986;118:367–71.
Siler TM, VandenBerg G, Yen SS, Brazeau P, Vale W, Guillemin R. Inhibition of growth hormone release in humans by somatostatin. J Clin Endocrinol Metab. 1973;37:632–4.
Silva JE. Thermogenic mechanisms and their hormonal regulation. Physiol Rev. 2006;86:435–64.
Sonigo C, Bouilly J, Carre N, Tolle V, Caraty A, Tello J, Simony-Conesa FJ, Millar R, Young J, Binart N. Hyperprolactinemia-induced ovarian acyclicity is reversed by kisspeptin administration. J Clin Invest. 2012;122:3791–5.
Strata A, Ugolotti G, Contini C, Magnati G, Pugnoli C, Tirelli F, Zuliani U. Thyroid and obesity: survey of some function tests in a large obese population. Int J Obes. 1978;2:333–40.
Suh JH, Sieglaff DH, Zhang A, Xia X, Cvoro A, Winnier GE, Webb P. SIRT1 is a direct coactivator of thyroid hormone receptor beta1 with gene-specific actions. PLoS One. 2013;8:e70097.
Sun Y, Ahmed S, Smith RG. Deletion of ghrelin impairs neither growth nor appetite. Mol Cell Biol. 2003;23:7973–81.
Tannenbaum GS, Epelbaum J, Bowers CY. Interrelationship between the novel peptide ghrelin and somatostatin/growth hormone-releasing hormone in regulation of pulsatile growth hormone secretion. Endocrinology. 2003;144:967–74.
Tena-Sempere M, Barreiro ML, Gonzalez LC, Gaytan F, Zhang FP, Caminos JE, Pinilla L, Casanueva FF, Dieguez C, Aguilar E. Novel expression and functional role of ghrelin in rat testis. Endocrinology. 2002;143:717–25.
Thakran S, Sharma P, Attia RR, Hori RT, Deng X, Elam MB, Park EA. Role of sirtuin 1 in the regulation of hepatic gene expression by thyroid hormone. J Biol Chem. 2013;288:807–18.
Tovar S, Vazquez MJ, Navarro VM, Fernandez-Fernandez R, Castellano JM, Vigo E, Roa J, Casanueva FF, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M. Effects of single or repeated intravenous administration of kisspeptin upon dynamic LH secretion in conscious male rats. Endocrinology. 2006;147:2696–704.
Trott JF, Schennink A, Petrie WK, Manjarin R, VanKlompenberg MK, Hovey RC. Triennial lactation symposium: prolactin: the multifaceted potentiator of mammary growth and function. J Anim Sci. 2012;90:1674–86.
Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature. 2000;407:908–13.
van der Lely AJ, Tschop M, Heiman ML, Ghigo E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. Endocr Rev. 2004;25:426–57.
Varela L, Martinez-Sanchez N, Gallego R, Vazquez MJ, Roa J, Gandara M, Schoenmakers E, Nogueiras R, Chatterjee K, Tena-Sempere M, Dieguez C, Lopez M. Hypothalamic mTOR

pathway mediates thyroid hormone-induced hyperphagia in hyperthyroidism. J Pathol. 2012;227:209–22.
Veldhuis JD, Metzger DL, Martha PM Jr, Mauras N, Kerrigan JR, Keenan B, Rogol AD, Pincus SM. Estrogen and testosterone, but not a nonaromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. J Clin Endocrinol Metab. 1997;82:3414–20.
Veldhuis JD, Keenan DM, Pincus SM. Motivations and methods for analyzing pulsatile hormone secretion. Endocr Rev. 2008;29:823–64.
Walsh RJ, Slaby FJ, Posner BI. A receptor-mediated mechanism for the transport of prolactin from blood to cerebrospinal fluid. Endocrinology. 1987;120:1846–50.
Watanobe H. Leptin directly acts within the hypothalamus to stimulate gonadotropin-releasing hormone secretion in vivo in rats. J Physiol. 2002;545:255–68.
Weber RF, de Greef WJ, de Koning J, Vreeburg JT. LH-RH and dopamine levels in hypophysial stalk plasma and their relationship to plasma gonadotrophins and prolactin levels in male rats bearing a prolactin- and adrenocorticotrophin-secreting pituitary tumor. Neuroendocrinology. 1983;36:205–10.
Weintraub BD, Gesundheit N, Taylor T, Gyves PW. Effect of TRH on TSH glycosylation and biological action. Ann N Y Acad Sci. 1989;553:205–13.
Wierman ME, Kiseljak-Vassiliades K, Tobet S. Gonadotropin-releasing hormone (GnRH) neuron migration: initiation, maintenance and cessation as critical steps to ensure normal reproductive function. Front Neuroendocrinol. 2011;32:43–52.
Wortley KE, Anderson KD, Garcia K, Murray JD, Malinova L, Liu R, Moncrieffe M, Thabet K, Cox HJ, Yancopoulos GD, Wiegand SJ, Sleeman MW. Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. Proc Natl Acad Sci U S A. 2004;101:8227–32.
Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA, Bloom SR. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology. 2000;141:4325–8.
Xia SF, Duan XM, Hao LY, Li LT, Cheng XR, Xie ZX, Qiao Y, Li LR, Tang X, Shi YH, Le GW. Role of thyroid hormone homeostasis in obesity-prone and obesity-resistant mice fed a high-fat diet. Metab Clin Exp. 2015;64:566–79.
Yi CX, Heppner KM, Kirchner H, Tong J, Bielohuby M, Gaylinn BD, Muller TD, Bartley E, Davis HW, Zhao Y, Joseph A, Kruthaupt T, Ottaway N, Kabra D, Habegger KM, Benoit SC, Bidlingmaier M, Thorner MO, Perez-Tilve D, Tschop MH, Pfluger PT. The GOAT-ghrelin system is not essential for hypoglycemia prevention during prolonged calorie restriction. PLoS One. 2012;7:e32100.
Zigman JM, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, Jones JE, Deysher AE, Waxman AR, White RD, Williams TD, Lachey JL, Seeley RJ, Lowell BB, Elmquist JK. Mice lacking ghrelin receptors resist the development of diet-induced obesity. J Clin Invest. 2005;115:3564–72.

Physiopathology, Diagnosis, and Treatment of GH Deficiency

2

Fatih Tanriverdi and Fahrettin Kelestimur

Contents

Abstract

Growth hormone deficiency (GHD) in adults arises due to decreased secretion of GH from the pituitary gland. Until two decades ago, GHD in adults was not accepted as a clinical syndrome, and it was supposed that GH has little physiologic effects after adolescence. However after the advent of recombinant GH, physiologic role of GH becomes clearer in the adult life, and the studies during the last two decades revealed GHD as a real disease associated with a plenty of clinical manifestations. GHD is one of the most common hormonal deficiencies in adults with hypopituitarism due to variety of causes. In the present chapter,

F. Tanriverdi (✉) · F. Kelestimur
Department of Endocrinology and Metabolism, Erciyes University Medical School, Kayseri, Turkey
e-mail: fatihtan@erciyes.edu.tr; fktimur@erciyes.edu.tr

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_2

acquired causes, pathophysiology, diagnosis, and treatment of GHD in adults will be discussed based on the recent advances in the literature.

Keywords
Growth hormone deficiency (GHD) · Growth hormone replacement therapy (GHRT) · Hypopituitarism · Recombinant GH (rhGH) · Traumatic brain injury (TBI) · Sheehan's syndrome · Pituitary · Hypothalamus

Introduction

Growth hormone deficiency (GHD) in adults arises as a result of decreased secretion of GH from the pituitary gland. Until two decades ago, GHD in adults was not accepted as a clinical syndrome, and it was assumed that GH has little physiologic effects after cessation of growth. After the advent of recombinant GH, the physiologic role of GH becomes clearer in the adult life. GH continues to be produced until older ages and is the most abundant hormone in the adult pituitary gland.

Epidemiology, Causes, and Pathophysiology of GHD in Adults

The pathophysiology of GHD is closely related to the causes of GHD. Therefore they are discussed under the same subtitle. Growth hormone deficiency (GHD) can develop due to a variety of conditions and may occur either as an isolated hormone deficiency or as multiple pituitary hormone deficiencies. GH deficiency is one of the most common hormonal deficiencies in hypopituitary adults. Moreover, GH is typically the first hormone to become clearly deficient in the majority of the different causes of hypopituitarism (Arafah 1986; Schneider et al. 2007a; Tanriverdi et al. 2015; Vance 1994).

Adults with GHD can be grouped into those who had GHD occurring in childhood (childhood-onset GHD, CO-GHD) and those who acquired GHD in adult life (adult-onset GHD, AO-GHD). In the KIMS database (Pfizer International Metabolic Database), which includes nearly 13,000 adults with GHD, nearly 25% of the patients were reported as having CO-GHD (Brabant et al. 2009). Congenital causes including some GH-gene-related mutations, congenital structural defects of the hypothalamo-pituitary region, and several acquired causes including perinatal insults are some of the causes of CO-GHD (Molitch et al. 2011). In this chapter the acquired causes of GHD in adults will be discussed.

Classical Causes

The most frequent classical causes of GHD in adults are pituitary adenomas and/or their treatment (Bates et al. 1996; Vance 1994). Vance et al. previously reported that the most common etiology of hypopituitarism was pituitary tumors and/or their

treatment. In that etiological classification, head trauma was accepted as an uncommon cause of hypopituitarism, and Sheehan's syndrome was not even listed, probably due to the fact that the data were largely derived from Western population studies (Vance 1994). To date, two population-based epidemiological studies have been published investigating the rates and causes of hypopituitarism (Fernandez-Rodriguez et al. 2013; Regal et al. 2001). Moreover three registry-based studies were published, and two of them specifically investigated the rates and causes of GHD in adults (Sassolas et al. 1999; Stochholm et al. 2006; Tanriverdi et al. 2014a).

In the population-based studies, the prevalence and incidence of hypopituitarism were reported to range 37–45 cases per 100,000 inhabitants and 2.1–4.2 cases per 100,000 per year, respectively (Fernandez-Rodriguez et al. 2013; Regal et al. 2001). In the first epidemiological study, the most common cause of hypopituitarism were pituitary tumors (nonsecretory adenomas were the most common) and/or their treatment, accounting for at least 60% of all cases. Non-tumoral causes were reported as 30% of all cases, and among them the idiopathic group (11% of all cases) and Sheehan's syndrome (6% of all cases; 2.6 per 100,000 women) were the first two leading etiologies. Additionally, 61% of all the patients had GHD (Regal et al. 2001). In the second epidemiological study at the same region, the most common cause of hypopituitarism was pituitary tumors (45.7%), and peri-pituitary tumors (craniopharyngioma, chordoma, meningioma) were seen in about 9% of the patients. Infiltrative diseases were reported in 5.3% of the patients, but Sheehan's syndrome was not reported, and traumatic brain injury (TBI) was reported as a rare cause of hypopituitarism in 1.4% of all patients (Fernandez-Rodriguez et al. 2013). In a recent registry study including 773 adult patients with hypopituitarism, the etiology of hypopituitarism was investigated in tertiary care institutions in Turkish population. In contrast to previous epidemiological studies, the most common etiology of hypopituitarism was non-tumoral causes (49.2%, including Sheehan's syndrome, idiopathic causes, empty sella, TBI, lymphocytic hypophysitis, apoplexia, subarachnoid hemorrhage, histiocytosis, acute meningitis). Pituitary tumors and/or treatment was found to cause hypopituitarism in 43.6% of all patients. However, when the causes were analyzed according to gender, the most common cause of hypopituitarism in males was pituitary tumors (nonsecretory pituitary adenomas were the most common, 20.9% among all patients); however one of the most common causes of hypopituitarism in females was Sheehan's syndrome (13.8% of all patients) (Tanriverdi et al. 2014a).

In the nationwide registry study conducted between 1980 and 1999 in Denmark, the incidence and causes of GHD were specifically investigated among 1823 patients with GHD. The average incidence rate of AO-GHD was reported as 3.3 per 100,000 per year. The most common causes of AO-GHD were as follows: pituitary adenomas and/or treatment (nonfunctioning adenomas were the leading cause), craniopharyngioma, peri-pituitary tumors, and apoplexy. Sheehan's syndrome, TBI, and meningitis/encephalitis were reported as very rare causes of GHD (less than 2% of all patients) (Stochholm et al. 2006). In a retrospective (data were collected during 1994–1995) registry study from France, 1652 adult patients with GHD were enrolled. The annual incidence of AO-GHD was reported as 1.2 per

100,000. The causes of GHD were not reported in detail, but the most common cause was pituitary tumors (78% of all patients), and non-tumoral pathologies were found in 8% of the patients (Sassolas et al. 1999). These two registry studies imply that by the year 2000, classical causes of GHD were predominant, such as pituitary and peri-pituitary tumors and/or their treatment. However, the studies published in the last decade clearly show that the etiological patterns of AO-GHD have slightly changed to non-tumoral causes, probably due to increasing awareness after the approval of GH replacement therapy in adults and/or due to accumulating data from different populations, which will be discussed in the next section.

Based on the KIMS pharmaco-epidemiological database, Abs. et al. analyzed the demographic and clinical characteristics of 1034 adult patients with GHD. The first three common etiologies of GHD were reported as pituitary tumors and/or their treatment (53.9%), craniopharyngioma (12.3%), and idiopathic causes (10.2%). Moreover, cranial tumors far from the pituitary and their treatment by surgery or cranial irradiation caused GHD in 7% of the patients (Abs et al. 1999). In one of the recent reanalysis of the KIMS database investigating the etiologies of GHD in 13,167 adult patients, the three most common causes of GHD were pituitary adenomas (44%), idiopathic causes (16%), and craniopharyngioma (11%) (Brabant et al. 2009). In the KIMS database, during 10 years' time, although pituitary adenomas were still the most common cause of adult GHD, the rate of tumoral causes was substantially decreased, and non-tumoral causes including idiopathic causes increased (Abs et al. 1999; Brabant et al. 2009). However it is important to mention that the diagnostic criteria for the idiopathic GHD were not standardized and stringent in these database studies. Therefore the reported relatively high frequency of idiopathic causes is controversial. Detailed history, including head trauma and contact sports and central nervous system infections, and stringent diagnostic criteria (section "Who to Test") are warranted for precise diagnosis of idiopathic GHD (Melmed 2013; Molitch et al. 2011). Based on the current data, substantial amount of the adult patients with the diagnosis of idiopathic GHD might have overlooked TBI history (section "Nonclassical Causes").

The pathophysiology of hypopituitarism due to pituitary adenomas is mainly associated with size of the tumor. Microadenomas (<1 cm) very rarely cause hypopituitarism. Rates of hypopituitarism (particularly GH is the most common pituitary hormone deficit) increase with tumor size; pituitary dysfunction was present in all patients with tumors >4 cm in diameter (Nomikos et al. 2004). Nearly 50% of patients with macroadenomas evaluated before surgery already had GHD; after surgery about 80% of the patients had GHD. Moreover, it was found that GHD developed after 5 years in 100% of the patients who had received radiotherapy after surgery (Littley et al. 1989). Macroadenomas cause pituitary hormone deficiencies by compressing the portal vessels in the pituitary stalk, either directly due to an expanding tumor mass or indirectly by increasing intrasellar pressure (Arafah et al. 2000). The risk of hypopituitarism due to pituitary surgery depends on the size of the original adenoma, the degree of infiltration, and the experience of the surgeon. However patients undergoing transsphenoidal surgery less likely develop pituitary hormone deficiencies than transcranial surgical approach. Pituitary functions may

also recover after pituitary surgery. Since, surgical debulking of an adenoma may reduce pressure on the portal vessels and normal pituitary tissue (Arafah et al. 2000). The possible mechanisms by which cranial irradiation causes hypopituitarism are not fully understood. It is thought that ionizing radiation causes direct neuronal damage and consequent degeneration (Darzy 2013). Moreover somatotropes are the most sensitive pituitary cells to radiation damage and the only cells affected by radiation doses lower than 20 Gy (Littley et al. 1989). Therefore most of the acquired classical causes of GHD lead to attenuated GH synthesis and secretion via several mechanisms including somatotroph impingement, compression, inflammation, or vascular insult (Melmed 2013). The acquired causes of GHD in adults are summarized in Table 1. The common classical causes of GHD are as follows: pituitary adenomas and/or their treatment including surgery or cranial irradiation, idiopathic causes, craniopharyngioma, cranial tumors far from the pituitary, and their treatment including surgery or cranial irradiation. The rare classical causes of GHD are as follows: peri-pituitary tumors (meningiomas, gliomas, metastases) and/or their treatment, lymphocytic hypophysitis, empty sella, apoplexia, and infiltrative/granulomatous diseases (Langerhans cell histiocytosis, sarcoidosis, tuberculosis, hemochromatosis).

Table 1 Acquired causes of adult-onset GH deficiency

Pituitary adenomas and/or their treatment[a]
Brain injury[a]
Traumatic brain injury
Sports-related head trauma (including chronic repetitive head trauma)
Subarachnoid hemorrhage
Blast injury
Sheehan's syndrome (postpartum pituitary necrosis)[b]
Peri-pituitary tumors
Craniopharyngiomas, meningiomas, gliomas, chordomas, metastases, etc.
Cranial tumors far from the pituitary and their treatment (surgery or cranial irradiation)
Infarction
Apoplexia
Infections
Acute viral or bacterial meningitis/meningoencephalitis, abscess, fungal infections
Autoimmune disorders
Lymphocytic hypophysitis
Infiltrative/granulomatous diseases
Tuberculosis, histiocytosis, sarcoidosis, hemochromatosis, etc.
Empty sella
Idiopathic causes

[a]Although pituitary adenomas are the most common classical cause of GHD, an increasing number of recent studies and estimated prevalence have revealed that brain injury might outnumber pituitary adenomas in causing GHD

[b]Sheehan's syndrome is still an important problem in developing and underdeveloped countries and not a very rare cause of GHD, as previously known

Nonclassical Causes

Under the title of nonclassical causes of GHD, some of the discussed causes (such as sports-related head trauma and blast injury) which appeared in the current literature are novel; however, some of the other discussed causes (such as TBI, Sheehan's syndrome, subarachnoid hemorrhage, and acute viral or bacterial meningitis/meningoencephalitis) were known previously but accepted as very rare causes of GHD in adults.

Traumatic Brain Injury (TBI) and Sports-Related Head Trauma

Traumatic brain injury (TBI) is a worldwide public health problem which affects mainly the young or middle-aged adult population. Although it is commonly stated in textbooks as one of the rare causes of hypopituitarism, after the year 2000, substantial amount of studies showed that head trauma-mediated hypopituitarism could actually be more frequent. In a meta-analysis including 1015 adult TBI patients, from 10 cross-sectional and 4 12-month prospective studies, the pooled prevalence of anterior hypopituitarism was reported as 27.5% (95% CI, 22.8- 28.9). The pooled prevalences of hypopituitarism in mild, moderate, and severe TBI (severity is classified according to Glasgow Coma Scale) were estimated as 16.8%, 10.9%, and 35.3%, respectively. These data clearly demonstrated that although the risk of developing hypopituitarism is highest in severe TBI, the risk is substantially high in mild TBI and is even comparable with moderate TBI. In addition, the most common pituitary hormone deficiency after TBI was GHD, and isolated GHD was found to be more frequent than multiple hormone deficiencies (Schneider et al. 2007b). The reported variations in the frequency of GHD after TBI are in part caused by the heterogeneity of the dynamic endocrine tests and/or diagnostic criteria for GHD (Kokshoorn et al. 2010). Moreover, the variations in inclusion criteria for mild, moderate, and severe TBI patients and genetic background in different populations could be other reasons for the different prevalences of GHD reported in studies (Tanriverdi et al. 2008a, 2015; Tanriverdi and Kelestimur 2015).

Reanalysis of hypopituitarism in the chronic phase after TBI was performed in a recent systematic review including 1203 adult patients. Prospective studies performed 3 and 5 years after TBI were also included (Tanriverdi et al. 2008b, 2013) in this analysis. By using conservative criteria, the overall rate of persistent GHD after TBI was 12%, which was significantly more common than in the normal population (Tanriverdi et al. 2015). The incidence of head trauma is nearly 40-fold higher than that of pituitary adenomas, and at least 10–15% of these TBI patients have GHD. Therefore these findings suggest that TBI-induced hypopituitarism could be one of the most common causes of adult GHD (Schneider et al. 2007a) (Table 1). However future epidemiological studies are warranted to confirm this estimated rate. Nevertheless, the main problem found in this field is that the clinicians who treat TBI patients have very little awareness of the risk of GHD after TBI, and most of these patients were not referred to an endocrinologist.

Sports, including contact/combative sports (boxing, kickboxing, soccer, football, ice hockey, rugby, etc.), are common around the world, especially among the younger generation. Sports-related head trauma (including mainly sports-related chronic repetitive head trauma) is an important public health problem that is associated with increased risk of TBI. In a previous study, 21% of all TBIs, which equate to an incidence rate of 170 per 100,000, were identified as resulting from a sports-related activity (Theadom et al. 2014). The International Kickboxing Federation estimated that about one million participants around the world are involved in kickboxing. Like boxing, kickboxing is characterized by chronic repetitive head trauma, and the head is one of the most frequently injured organs (Zazryn et al. 2003). Although the relationship between boxing and kickboxing TBI has been known for a long time, pituitary functions have not been routinely investigated until recently. Current data clearly showed that nearly 10–20% of active boxers or kickboxer and nearly 40% of retired athletes have GHD, and most of them have isolated GHD (Kelestimur 2005; Tanriverdi et al. 2007, 2008c). Recently, the risk of TBI-induced hypopituitarism in football players has been investigated in a cohort of retired US professional football players. GH deficiency (19.1%) was reported as the most common pituitary dysfunctions after repetitive sports-related head trauma in football players (Kelly et al. 2014). These abovementioned studies clearly demonstrated that sports-related TBI is a novel cause of GHD in adults. Although pituitary dysfunction has been shown in sports-related head trauma, large-scale screening studies are warranted to understand the real burden of GHD among active and retired athletes.

The exact pathophysiology of pituitary dysfunction after TBI and sports-related head trauma is not well understood, but multiple pathological mechanisms have been proposed, including compression of the pituitary gland and hypothalamic nuclei or interruption of the long hypophyseal portal vessels by edema; direct mechanical trauma to the pituitary gland, stalk, and/or hypothalamus; increased intracranial pressure; hemorrhage; skull fracture; any cause of ischemic/hypoxic insult; genetic predisposition (Apo E polymorphisms); persistent neuroinflammation; and autoimmunity caused by antipituitary and/or antihypothalamus antibodies (Dusick et al. 2012; Tanriverdi et al. 2015).

Blast Injury

Blast TBI from explosive devices is mainly seen in soldiers. However, unfortunately, due to increased war injuries all over the world, many civilians are also exposed to blast injury. Recently, the relationship between blast injury (blast TBI) and pituitary dysfunction was investigated in two studies including small number of soldiers. The first study included 26 male soldiers whose blast exposure was at least 1 year prior to the evaluation. In 42% of the participants with blast injury, deficiencies in one or more pituitary axis were reported. The most common pituitary hormone deficits were GH and FSH/LH deficiencies (Wilkinson et al. 2012). The second study included 19 male soldiers who had blast TBI and 39 male controls that had non-blast TBI. The authors demonstrated that 32% of the soldiers had anterior pituitary dysfunction (10.5% had

GHD), which was significantly more frequent than in the non-blast TBI group (Baxter et al. 2013). These two studies imply that blast injury may cause hypopituitarism at least 12 months after the event. The literature data in this area are too premature to accept blast injury as a frequent cause of GHD, but it is a new cause of GHD.

Sheehan's Syndrome

Sheehan's syndrome was first described by HL Sheehan and classically refers to postpartum pituitary dysfunction due to pituitary necrosis occurring after severe hypotension and shock secondary to massive bleeding during delivery (Kelestimur 2003; Sheehan 1937). In the developed regions and countries of the world, Sheehan's syndrome was reported to be a very rare cause of hypopituitarism due to the advent of modern obstetric care (Rosen and Bengtsson 1990; Sheehan 1965; Toogood et al. 1995). However, in the KIMS pharmaco-epidemiological database, 1034 GH-deficient patients, mainly from different parts of European countries, were analyzed, and Sheehan's syndrome was found as the sixth most common cause of GHD (3.1% of all patients) (Abs et al. 1999). In a recent population-based retrospective study from Iceland, the prevalence of Sheehan's syndrome (5.1 per 100,000 women) was found to be higher than expected (Kristjansdottir et al. 2011). This prevalence is clearly higher than in two previous epidemiological studies from Spain (Fernandez-Rodriguez et al. 2013; Regal et al. 2001). These recent findings suggest that in Western countries, Sheehan's syndrome seems to be not a very rare etiology of hypopituitarism and GHD as previously thought, probably due to increased migration from underdeveloped countries or to less improved obstetric care in the rural parts of the developed countries.

The World Health Organization estimated that nearly 100,000 women died yearly due to Sheehan's syndrome and approximately more than three million women were suffering from Sheehan's syndrome. In India, in the Kashmir region, Sheehan's syndrome frequency was reported as 3.1% among the female population, and two-thirds of women were still giving birth at home (Zargar et al. 2005). In a retrospective study from the Philippines, the data of 143 patients with hypopituitarism at a tertiary care institution were analyzed. Sheehan's syndrome was reported in 8% of all patients, which is the third most frequent etiology of hypopituitarism, suggesting a higher occurrence of Sheehan's syndrome than in the Western population (Elumir- Mamba et al. 2010). In a recent registry study, 773 adult patients with hypopituitarism who were admitted to tertiary care institutions in a Turkish population were included. The frequency of Sheehan's syndrome among all patients was found as 13.8%, which is the second most common cause of hypopituitarism. Only 17% of the patients with Sheehan's syndrome were less than 40 years old, implying the tendency of decreasing frequency over time probably due to the decrease in home deliveries and the improvement of obstetric care in Turkey (Tanriverdi et al. 2014a). It was previously demonstrated that patients with Sheehan's syndrome have more severe pituitary hormone deficiencies than in the patients with hypopituitarism due to other causes, and almost all patients have severe GHD (Kelestimur et al. 2005; Tanriverdi et al. 2005).

Therefore, current literature data imply that Sheehan's syndrome is a nonclassical cause of GHD in developed countries, but due to the reemergence of Sheehan's

syndrome in the Western world, it is not a rare cause of GHD, as previously thought. However, in underdeveloped and developing countries, Sheehan's syndrome still seems to be one of the leading causes of GHD.

The certain pathophysiology of GHD in Sheehan's syndrome is not clearly understood. It is well-known that the pituitary gland is physiologically enlarged during pregnancy which makes it vulnerable to ischemia. The basic process is infarction secondary to arrest of blood flow to the anterior lobe of the pituitary gland, and it may be due to massive postpartum uterine hemorrhage, vasospasm, thrombosis, or vascular compression. The size of the sella may play a role in the pathogenesis of Sheehan's syndrome. A relatively small sella size, hypercoagulation, genetic factors, and pituitary autoimmunity were also suggested as risk factors for the development of Sheehan's syndrome (Diri et al. 2016; Kelestimur 2003; Kovacs 2003).

Other Nonclassical Causes of GHD

Several studies after the year 2000 have demonstrated that aneurysmal subarachnoid hemorrhage (Karaca et al. 2013; Kelly et al. 2000; Kronvall et al. 2015) and acute viral or bacterial meningitis/meningoencephalitis (Schaefer et al. 2008; Tanriverdi et al. 2012; Tsiakalos et al. 2010) may cause substantial frequency of GHD which is in contrast to previous knowledge.

Unlike TBI and Sheehan's syndrome, the current literature data are not consistent or sufficient to draw a clear conclusion regarding the real frequencies of GHD in these disorders. However, based on the current findings, it is tempting to speculate that subarachnoid hemorrhage and acute viral or bacterial meningitis/meningoencephalitis are nonclassical etiologies of AO-GHD which cause higher frequencies of hypopituitarism than previously known.

Based on the classical literature knowledge and current data, the acquired causes of GHD in adults are listed in Table 1.

Diagnosis of GHD in Adults

Clinical Features of GHD in Adults

GHD in adults results in a clinical syndrome characterized by symptoms and signs summarized in Table 2. Adults with GHD manifest a range of body compositional, physical, and psychological abnormalities. The functions of several organ systems are directly altered by the loss of or decreased effects of GH.

GHD in adults manifest decreased lean body mass and increased body fat mass, particularly in the visceral compartment. Therefore these patients are mainly overweight or obese and display a disproportionate increase in abdominal fat. Body composition abnormalities are mainly due to the loss of lipolytic and anabolic actions of GH. Together these alterations contribute to the development of metabolic syndrome (Attanasio et al. 2010; Carroll et al. 1998; Hoffman et al. 1995; Moller and Jorgensen 2009).

Table 2 Symptoms and signs of adult GHD

Increased body fat mass (patients are overweight mainly with abdominal adiposity)
Reduced muscle bulk (decreased lean body mass)
Reduced strength and exercise performance
Reduced sweating (thin and dry skin)
Psychological problems and decreased quality of life (depression, anxiety, fatigue, impaired sleep, increased social isolation, memory problems, and impaired cognitive functions)

Exercise capacity is reduced in patients with GHD, and patients show significant impairment in physical performance and muscle strength. Exercise performance could be dependent on several factors including neuromuscular and cardiorespiratory functions. Furthermore, a mild reduction in body sodium levels and water volume has been described in adult patients with GHD. This decrease in tissue hydration could partially be responsible for reduced lean body mass measurements and reduced exercise performance (Amato et al. 1993; Cuneo et al. 1992). GH and/or IGF-I has an important role in skin physiology, and GHD deficiency in adults causes structural and functional changes in the skin and its appendages. The eccrine sweat glands are hypoplastic causing decreased sweat secretion rate, and skin thickness is decreased (Lange et al. 2001; Tanriverdi et al. 2014b).

Impaired quality of life (QoL) and decreased psychological well-being are well-established clinical features in adult patients with GHD. Decreased energy and vitality, depressed mood, increased anxiety, social isolation, impaired cognitive functions, and deficits in memory and concentration are commonly reported in these patients (Golgeli et al. 2004; McGauley et al. 1990). Additionally decreased in sleep quality and impaired sleep physiology may also contribute to the decreased QoL (Ismailogullari et al. 2009; Tanriverdi et al. 2014b).

GHD in adults is difficult to diagnose based on clinical features because the signs and symptoms of GHD are nonspecific. Therefore cause-specific history (Table 1) becomes more important for clinical suspicion and selecting patients for diagnostic testing.

Who to Test?

To select appropriate candidates for GH replacement therapy (GHRT), the principles of the evaluation of patients for diagnostic testing are as follows (Ho 2007; Molitch et al. 2011):

1. The adult patients with structural hypothalamic/pituitary disease (surgery or irradiation in these areas), history of TBI, or evidence of other pituitary hormone deficiencies should be considered for testing for acquired GHD.
   Because TBI is an important cause of hypopituitarism and at least 10–15% of patients have GHD, it is tempting to suggest that the first approach should be testing all patients with a history of head trauma. However, this strategy is not

cost-effective and would result in unnecessary health resource consumption for a community. Those patients (regardless of the severity of TBI) who need hospitalization for at least 24 h and who need ICU monitoring, in particular, should be screened for GHD during the acute phase and prospectively (yearly at least 5 years after TBI). Those with a history of complicated mild TBI, moderate TBI, and severe TBI at any time after the event are good candidates for biochemical testing for GHD. But mild TBI patients who are discharged from emergency units and/or who have no loss of consciousness or who have posttraumatic amnesia of less than 30 min are not recommended for GHD testing (Tanriverdi et al. 2015).

2. Patients with CO-GHD should be retested as adults after achievement of adult height (except cases with specific proven mutations, congenital lesions causing multiple hormone deficiencies, irreversible structural hypothalamic-pituitary lesions/damage).
3. To enhance the diagnostic precision of the idiopathic acquired GHD in adults, stringent criteria are necessary. Exhaustive history for an overlooked organic cause of GHD such as head trauma, meningitis, contact sports, etc. is warranted (Melmed 2013). In the absence of suggestive clinical circumstances, there is a significant false-positive error rate in response to a single dynamic test. Therefore two GH stimulation tests are recommended for the diagnosis of idiopathic GHD in adults.
4. Serum insulin-like growth factor-1 (IGF-I) concentrations are useful only when age-adjusted normal ranges are used. Normal IGF-I levels does not exclude the diagnosis of GHD but makes dynamic tests mandatory. There is considerable overlap in serum IGF-I levels between individuals with and without GHD. However, a low age-matched IGF-I levels, in the absence of liver disease, poorly controlled diabetes, malnutrition, and oral estrogen therapy, are strong evidence for GHD and may be useful in identifying patients who may benefit from GH replacement therapy (GHRT) and therefore require dynamic testing for GHD.

Additionally adult patients with three or more other pituitary hormone deficiencies with an IGF-I level below age-matched reference range have a likelihood of GHD more than 95% (Diri et al. 2015; Hartman et al. 2002). Therefore in these patients, performing GH stimulation test for the diagnosis of GHD is not necessary or could be performed optional (Molitch et al. 2011).

In summary, owing to false-positive rates of current diagnostic dynamic tests, diagnostic testing for GHD should be considered in patients with high pretest probability of hypothalamic-pituitary disorders who fulfill the abovementioned criteria. In all these cases, an intention to treat needs to be presented before testing.

Dynamic Tests and Diagnostic Criteria

GH is secreted episodically; therefore measurement of a low basal or random plasma GH level is not diagnostic, and as mentioned above measurement of IGF-I level is of limited utility in diagnosing GHD. So, for the diagnosis of adult GHD, the GH-IGF-I

axis should be evaluated by dynamic tests (stimulation tests), unless all other pituitary axes are deficient and age-matched IGF-1 is low (Molitch et al. 2011; Schneider et al. 2007a). Except idiopathic GHD, one stimulation test is sufficient for the diagnosis of GHD in patients who are selected according to appropriate criteria (section "Who to Test?"). Moreover patients have to be adequately replaced with other deficient hormones before any GH stimulation test is performed.

For the diagnosis of adult GHD, the insulin tolerance test (ITT) and GHRH + arginine tests are now considered as the tests of choice with similar accuracy (Molitch et al. 2011; Schneider et al. 2007a).

The ITT explores the integrity of the hypothalamic-pituitary function and is considered the gold standard for the evaluation of GH axis (Schneider et al. 2007a); however, it cannot be performed in patients with severe cardiovascular morbidity and epileptic seizures. For the ITT, peak GH response levels lower than 3 mcg/l indicate severe GH deficiency in adults (if hypoglycemia is adequately reached) (Molitch et al. 2011; Schneider et al. 2007a). For patients in the transition phase between puberty and early adulthood, cutoff levels of 6.1 (Maghnie et al. 2005) or 5 μg/l (Clayton et al. 2005) have been suggested.

The GH-releasing hormone plus arginine test (GHRH + ARG, 1 mcg/kg GHRH i.v. as a bolus plus 30 g arginine as an infusion over 30 min) is easy to perform, is well tolerated, and has been shown to reliably detect severe GH deficiency in a lean adult population when a cutoff of 9 mcg/l is used (Aimaretti et al. 1998; Ghigo et al. 1996). However, the GH response to GHRH + ARG, and to all known GH provocative stimuli, significantly declines with increasing body mass index in adults (Bonert et al. 2004; Qu et al. 2005); thus, the use of a non-BMI-related cutoff in obese subjects causes a high percentage of false-positive results (Schneider et al. 2006). Therefore appropriate BMI-adjusted cutoff levels for diagnosing GHD are proposed as follows: 11 mcg/l for those with a BMI less than 25 kg/m^2, 8 mcg /l for those with a BMI of 25–30 kg/m^2, and 4 mcg/l for those with a BMI higher than 30 kg/m^2 (Biller et al. 2002; Corneli et al. 2005).

Another potent and validated provocative test is the GHRH+ GH-releasing peptide-6 test (Kelestimur et al. 2006; Popovic et al. 2000). It has a BMI-dependent cutoff (10 and 5 mcg /l for lean and obese with BMI $>$ 35 kg/m^2, respectively) and possesses great accuracy in distinguishing normal subjects from patients with GH deficiency (Kelestimur et al. 2006; Popovic et al. 2000).

As mentioned above, the GHRH + arginine test is a good and reliable alternative if ITT is contraindicated. However, in the recent Endocrine Society guidelines, the glucagon stimulation test (GST) is accepted as a good alternative test for the diagnosis of GH deficiency when GHRH is unavailable or there is contraindication for ITT (Molitch et al. 2011). The GST (1 mg glucagon im [1.5 mg for patients $>$90 kg], GH measurements every 30 min until 240 min after administration) has been proposed as an alternative diagnostic because it is able to differentiate between GH-deficient and healthy subjects with an acceptable sensitivity and specificity if a peak GH of 3 μg/l is considered (Gomez et al. 2002; Molitch et al. 2011). However, like the other tests, it is age- and BMI-dependent (Gomez et al. 2002; Molitch et al. 2011), and it is more time-consuming than other stimulation tests (Gomez et al.

Table 3 Dynamic tests for the diagnosis of adult GHD

Stimulation test	GH diagnostic cutoff value	Advantages	Disadvantages
Insulin tolerance test (ITT)	3 mcg/l	Gold standard test; simultaneous assessment of ACTH reserve	Contraindicated in ischemic heart disease and history of seizures; due to risk adverse effects, continuous presence of physician is required
GHRH plus arginine (GHRH + ARG)	BMI <25 kg/m^2: 11 mcg/l BMI 25–30 kg/m^2: 8 mcg/l BMI >30 kg/m^2: 4 mcg/l	Low risk of adverse effects; good accuracy; test of choice if ITT is contraindicated	GHRH is not commercially available in most countries
GHRH plus GHRP-6	10 mcg/l (5 mcg/l if BMI >35 kg/m^2)	Good accuracy and tolerability	GHRH is not commercially available in most countries
Glucagon stimulation test (GST)	1 mcg/l[a], 3 mcg/l[b]	Low risk of severe adverse effects; recommended if ITT is contraindicated and GHRH is unavailable; good accuracy by using appropriate cutoff levels	Adverse effects (nausea, vomiting, headache, and rarely late hypoglycemia), more time-consuming than other stimulation tests

GHRH growth hormone-releasing hormone, *GHRP* GH-releasing peptide, *BMI* body mass index
[a]Diri et al. 2015; Hamrahian et al. 2016
[b]Molitch et al. 2011

2002; Molitch et al. 2011). Recent studies clearly demonstrated that GST with cutoff level 3 μg/l has considerable false-positive rates. Therefore peak GH cutoff point decreased to 1 μg/l with a significantly high diagnostic accuracy (Diri et al. 2015; Hamrahian et al. 2016). By using appropriate cutoff values, the reliability and efficacy of GST and ITT could be similar (Simsek et al. 2014).

The recommended stimulation tests for the diagnosis of adult GHD and the diagnostic cutoff values are summarized in Table 3.

Treatment of GHD in Adults

Effects of GHD and GHRT

The effects of GHD and GH replacement therapy (GHRT) are summarized in Table 4. It is important to mention that GH-deficient adult patients with isolated GHD and multiple pituitary hormone deficiencies have similar clinical presentation and respond equally well to GHRT (Abs et al. 2005; Klose et al. 2009).

Table 4 Summary of the effects of adult GHD and the response to GHRT

Untreated GHD	Effects of GHRT	Comments
Adverse effects on body composition and exercise capacity	**Reversal of adverse changes**	
↓ LBM and muscle bulk	↑ LBM and muscle bulk	
↑ Body fat mass and WHR	↓ Body fat mass (mainly visceral) and WHR	GHRT has sustained effects (10 years) on lean body mass and muscle strength but not on body fat
↓ Exercise performance and skeletal muscle strength	↑ Exercise performance and skeletal muscle strength	
Increased CVS risk factors and premature atherosclerosis	**Improvement of CVS risk factors**	
↑ Total and LDL-C	↓ Total and LDL-C	
↓ HDL-C	↑ HDL-C	Although estimated risks of cardiovascular events or cardiovascular mortality decreased after GHRT, there is no study demonstrating the increased longevity due to GHRT
↑ Pro-inflammatory markers (CRP and IL-6)	↓ Pro-inflammatory markers	
↑ Carotid IMT and arterial stiffness	↓ Carotid IMT and arterial stiffness	
↓ LV mass and diameter, ↓ EF (mainly in CO-GHD)	Myocardial changes commonly improve	
Increased risk of osteoporosis	**Reversal of adverse changes**	
↓ BMD ↑Fracture rate	↑BMD ↓Fracture rate	In short-term GHRT, the BMD generally does not increase; however after 18–24 months of the treatment, the bone effects are seen
Impaired QoL and decreased psychological well-being	**Sustained long-term improvement in affected QoL areas**	Ideally assessed with specifically designed standardized questionnaire such as the AGHDA

Abbreviations: *GHD* growth hormone deficiency, *GHRT* growth hormone replacement therapy, *BMD* bone mineral density, *CVS* cardiovascular, *QoL* quality of life, *AGHDA* assessment of GHD in adults, *LBM* lean body mass, *WHR* waist-to-hip ratio, *IMT* intima-media thickness

Body Composition Parameters and Exercise Capacity

GHD in adults is characterized by decreased lean body mass and muscle bulk and increased body fat mass (increased waist-to-hip ratio). These changes are independent from the etiology of the GHD (Attanasio et al. 2010; Carroll et al. 1998; Kelestimur et al. 2005; Moller and Jorgensen 2009; Tanriverdi et al. 2005). Moreover exercise capacity and muscle strength are reduced in adult patients with GHD, and patients show significant impairment in physical performance (Amato et al. 1993; Cuneo et al. 1992; Johannsson et al. 1997). GHRT induces important changes in body composition. Most of the studies clearly demonstrated significant reduction in body fat and increase in lean body mass. The greatest reduction in body

fat occurs in visceral and abdominal fat. Long-term, 10 years duration, studies showed that GHRT has sustained effects on lean body mass but not on body fat (Gotherstrom et al. 2009; Hoffman et al. 2004). The changes in body composition generally occurred without a significant change in body weight.

Untreated GHD in adults has reduced exercise performance and isometric muscle strength. GHRT has beneficial effects on muscle strength and exercise performance. The increase in exercise capacity is in parallel with an increase in maximal oxygen uptake, and the oxygen consumption increased progressively over a 5-year period of GHRT (Cenci et al. 2009). In a long-term study, GHRT increased muscle strength (isometric knee flexor and hand grip strength) gradually over the first 5 years and thereafter protects against the age-decline and normalize muscle strength over a 10-year period (Gotherstrom et al. 2009). Therefore GHRT of adults with GHD offers substantial clinical benefits in exercise capacity and body composition (Molitch et al. 2011).

Cardiovascular Risk Factors and Mortality

Most of the cardiovascular risk of GHD in adults appears to be related to dyslipidemia, inflammation, and impaired myocardial function.

GH affects lipoprotein metabolism, and increased total and LDL cholesterol, decreased HDL cholesterol, and increased triglyceride levels were reported in adults with GHD (Cuneo et al. 1998; Rosen et al. 1993; Sesmilo et al. 2000; Tanriverdi et al. 2005). GHRT was shown to decrease total cholesterol levels significantly. Most, but not all, studies have shown decreases in LDL cholesterol and increases in HDL cholesterol after institution of GHRT. It seems that GHRT has little effect on triglyceride levels (Maison et al. 2004; Sesmilo et al. 2000). It was recently demonstrated that the favorable effects of GHRT in improving dyslipidemia maintained for 2 years (Abs et al. 2006). Additionally pro-inflammatory markers including CRP and IL-6 are elevated in GHD, and administration of GHRT significantly decreases the inflammatory markers which are related with cardiovascular risk (Bollerslev et al. 2006; Sesmilo et al. 2000).

Epidemiological data revealed that increases in carotid intima-media thickness (IMT) may predict the development of symptomatic coronary artery disease nearly 8 years after the initial measurement (Hodis et al. 1998). Increased carotid IMT and arterial stiffness have been documented in adults with GHD. Several short- and long-term studies showed that GHRT reduces carotid IMT and improves flow-mediated dilatation (Borson-Chazot et al. 1999; Gibney et al. 1999). Moreover myocardial function may also be significantly impaired in adult patients with GHD. Some echocardiography studies revealed a reduced left ventricular (LV) mass and intraventricular septal thickness and decreased LV diameter in adult patients with GHD. But these myocardial changes and decreased ejection fraction are more prominent in childhood-onset GHD (Colao et al. 2001; Sartorio et al. 1997). Analysis of several treatment studies has demonstrated that most consistent increases after GHRT were LV mass, longitudinal myocardial velocities, stroke volumes, and LV end-diastolic volumes (Maison and Chanson 2003; Ozdogru et al. 2007).

Hypopituitarism is associated with significantly increased mortality when compared with age- and gender-matched populations. Although GHD is not the only independent predictor, it has a significant contribution to the increased mortality in adult patients with hypopituitarism (Rosen and Bengtsson 1990; Sherlock et al. 2010). The most important causes of premature mortality in adult patients with GHD are cerebrovascular and cardiovascular diseases. In KIMS database it has been demonstrated that in 10 years, estimated risks of cardiovascular events or cardiovascular mortality calculated from different score algorithms are significantly increased in adult patients with GHD. Moreover after 4 years of GHRT, these estimated cardiovascular risks were returned to baseline level in these patients (Schneider et al. 2011). However to date it has not yet been shown that GHRT significantly improves the mortality and increases longevity. But two recent meta-analyses clearly demonstrated that short- and long-term GHRT does not increase cardiovascular mortality (Deodati et al. 2014; Stochholm and Johannsson 2015).

In summary GHRT in adults significantly improves several cardiovascular surrogate outcomes including lipid profile, inflammatory cardiovascular biomarkers, carotid IMT, and some aspects of myocardial function.

Bone Mineral Density

Several studies have shown that in adult patients with GHD, bone mineral density (BMD) is decreased ranging from osteopenia to osteoporosis (Holmes et al. 1994; Tanriverdi et al. 2005). Moreover osteoporotic fracture rate was also increased due to GHD (Wuster et al. 2001). Nearly 35% of CO-GHD patients and 20% of AO-GHD patients have osteoporosis (T scores less than −2.5 SD), and the age of onset of GHD significantly affects the severity of the bone mass loss (Lissett and Shalet 2000). GHRT has an overall anabolic effect on bone tissue, but its effects are biphasic. After the short-term GHRT (before 12 months of treatment), the BMD generally does not increase and may even show a decrease (Biller et al. 2000). However after 18–24 months of GHRT, most studies have shown significant increase in BMD, mainly with greater effects at vertebral sites (Biller et al. 2000; Shalet et al. 2003). Data regarding the effects of GHRT on fracture rates are limited. In one study GHRT has shown to be decreasing the rate of radiological vertebral fractures (Mazziotti et al. 2006).

Quality of Life

Impaired quality of life (QoL) and decreased psychological well-being are common in adults with GHD, but QoL evaluations in GHD patients have shown high degree variability. QoL is usually assessed via self-administered questionnaires and ideally assessed with specifically designed standardized questionnaire such as the AGHDA (Assessment of GHD in Adults) (Koltowska-Haggstrom et al. 2009). Significant impairment of QoL was more frequently observed in adults with AO-GHD than in those with CO-GHD (Attanasio et al. 1997). Several studies have demonstrated that

GHRT improves QoL in most of the patients and this improvement sustained for long term (Koltowska-Haggstrom et al. 2009; Murray et al. 1999).

Treatment Strategies of GHD and Monitoring

Evidence supporting beneficial effects of recombinant GH (rhGH) in adults with GHD led to the extensive approval of GHRT especially in patients with severe GHD. In adults initially weight-based dosing regimen was used, leading to supraphysiological doses and increased incidence of adverse effects (mainly fluid retention). It therefore is recommended in recent years that GH-dosing regimens should be individualized rather than weight-based, and starting low dose (0.2–0.3 mg/day between the age 30 and 60 years) and titration according to clinical response, side effects, and IGF-I levels is essential (Cook et al. 2009; Ho 2007; Molitch et al. 2011). GH secretion is higher in younger individuals than in older ones and in women than in men. Estrogen inhibits GH action in the liver, and in women usually more GH is required to achieve the same IGF-I response (Birzniece et al. 2009). Therefore while rhGH dosing, age, gender, and estrogen status need to be taken into consideration. 0.4–0.5 mg/day and 0.1–0.2 mg/day starting doses are recommended in younger age (<30 years) and in older age (>60 years) and patients with diabetes, respectively (Cook et al. 2009).

After starting GHRT patients should be monitored at 1- to 2-month intervals during dose titration, and dose increments are suggested as 0.1–0.2 mg/day based on IGF-I levels, clinical response, and side effects. Target for IGF-I level is the upper half of the age-adjusted reference range. After maintenance doses have been achieved, monitoring is generally recommended at 6-month intervals. Clinical evaluation (blood pressure, weight, waist circumference, BMI, etc.) and assessment for side effects along with IGF-I level measurements should be performed during semiannual monitoring. Fasting glucose and fasting lipid profile annually and after any GH dose increase. But if the patient is diabetic or susceptible to glucose intolerance, fasting glucose and HbA1c monitoring could be more frequent. Optimally QoL measures need to be assessed at baseline and then at least annually. If the initial bone DEXA scan is abnormal, repeat evaluations are recommended at 1.5- to 2-year intervals (Cook et al. 2009; Molitch et al. 2011).

Some studies have shown that GHRT causes a decrease of serum tree T4 levels. Additionally GHRT has also been found to cause lowering effect on serum cortisol levels due to reversal of the increased conversion of cortisone to cortisol during GHD (Giavoli et al. 2004; Porretti et al. 2002). Thus, thyroid and adrenal functions need to be monitored, and if the patients are on thyroid and/or steroid replacement therapy, drug doses should be adjusted during GHRT.

The optimal duration of GHRT is unclear. If benefits are achieved, continuation of the treatment is recommended. In the literature 10–15 years of safety and benefits of the GHRT in adults have been demonstrated (Appelman-Dijkstra et al. 2013; Elbornsson et al. 2013). On the other hand, if there are no obvious or objective benefits of the treatment after at least 1 year, discontinuing may be appropriate.

Side Effects and Long-Term Safety of GHRT

The most common side effects, occurring in approximately 5–18% of patients, are related to fluid retention and paresthesia, peripheral edema, joint stiffness, myalgia, and arthralgia. Carpal tunnel syndrome occurs almost 2% due to GHRT (Holmes and Shalet 1995). Most of these adverse effects are transient and improve with dose reduction.

Retinopathy and benign intracranial hypertension are extremely rare complications of GHRT and reported as case reports in adults (Koller et al. 1998; Malozowski et al. 1993). However presence of benign intracranial hypertension and proliferative retinopathy are accepted as contraindications of GHRT (Table 5). Pregnancy is not a contraindication for GHRT, but treatment should be discontinued in the second trimester, as GH is produced by the placenta (Ho 2007; Karaca et al. 2010).

Because GH and IGF-I stimulate the growth of tissues, there has been concern that GHRT may increase the risk of pituitary adenoma recurrence or development of neoplasia. Analysis of extensive pediatric experience demonstrated that GHRT is not linked to the development of malignancy or tumor recurrence (Bell et al. 2010; Darendeliler et al. 2006). Moreover GHRT does not increase tumor recurrence, malignancy rates, and malignancy-related mortality in adult patients (Burman et al. 2013; van Bunderen et al. 2014). Although there are substantial numbers of studies showing that GHRT is not linked to increased cancer risk or tumor recurrence, it is recommended that GHRT should not be used in patients with active malignancy (Table 5) (Molitch et al. 2011).

The overall effect of GHRT on insulin resistance is controversial. GHRT decreases fat mass and increasing IGF-I improves insulin sensitivity. But, GH also

Table 5 Recommendations for adult GHRT and monitoring

Starting dose
0.2–0.3 mg/day between the age 30 and 60 years
0.4–0.5 mg/day in younger age (<30 years)
0.1–0.2 mg/day in older age (>60 years) and in patients with diabetes
Dose titration
Patient should be monitored at 1- to 2-month intervals during dose titration, and dose increments are suggested as 0.1–0.2 mg/day based on IGF-I levels, clinical response, and side effects
Goal and monitorization
Target for IGF-I level is the upper half of the age-adjusted reference range
After maintenance doses are achieved, monitoring is generally recommended at 6-month intervals
If the patients are on thyroid and/or steroid replacement therapy, drug doses should be adjusted during GHRT
Women may require more rhGH doses than men, and dose requirements are greater with oral than with transdermal estrogen therapy in women
If there are no obvious or objective benefits of the treatment after at least 1 year, discontinuing GHRT may be appropriate
Contraindications
Presence of active malignancy, benign intracranial hypertension, and proliferative retinopathy

directly antagonizes insulin action in the liver and other tissues (Clemmons 2004). A meta-analysis of placebo-controlled studies revealed that GHRT was associated with a slight increase in both fasting insulin levels and fasting glucose (Maison et al. 2004). Therefore GHRT is not contraindicated in patients with diabetes mellitus, but lower starting rhGH doses (Table 5) and adjustment of antidiabetic medications are required (Molitch et al. 2011).

In a position statement published recently, it was concluded that GH currently has a good safety record when used for approved indications and at recommended doses, but continued surveillance of the GHD patients exposed to rhGH treatment is required to address the long-term safety (Allen et al. 2016).

Summary

It has been previously demonstrated that GH is one of the most frequent hormonal deficiencies in adult patients with hypopituitarism. The most common classical causes of AO-GHD are pituitary adenomas and/or their treatment. However, during the last decade, an increasing number of studies from different parts of the world have revealed that non-tumoral causes of hypopituitarism such as traumatic brain injury and Sheehan's syndrome are more common than previously known.

GHD in adults is difficult to diagnose based on clinical features because the signs and symptoms of GHD are nonspecific. Therefore cause-specific history becomes more important for clinical suspicion and selecting patients for diagnostic testing. The adult patients with structural hypothalamic/pituitary disease (surgery or irradiation in these areas), history of TBI, or evidence of other pituitary hormone deficiencies should be considered for testing for acquired GHD. For the diagnosis of adult GHD, the insulin tolerance test and GHRH + arginine tests are now considered as the tests of choice with similar accuracy. However, the glucagon stimulation test is accepted as a good alternative test for the diagnosis of GH deficiency when GHRH is unavailable or there is contraindication for insulin tolerance test.

GHD in adults is associated with impaired body composition and exercise capacity, increased cardiovascular risk factors and premature atherosclerosis, increased risk of osteoporosis, impaired QoL, and decreased psychological well-being. Short- and long-term studies clearly demonstrated that after physiological dose of GHRT, most of these adverse changes are normalized in adult patients with GHD. rhGH currently has a good safety record when used for approved indications and at recommended doses.

Cross-References

► Neuroendocrine Control of Carbohydrate Metabolism
► Neuroendocrinology of Bone Metabolism

References

Abs R, Bengtsson BA, Hernberg-Stahl E, Monson JP, Tauber JP, Wilton P, Wuster C. GH replacement in 1034 growth hormone deficient hypopituitary adults: demographic and clinical characteristics, dosing and safety. Clin Endocrinol. 1999;50:703.

Abs R, Mattsson AF, Bengtsson BA, Feldt-Rasmussen U, Goth MI, Koltowska-Haggstrom M, Monson JP, Verhelst J, Wilton P. Isolated growth hormone (GH) deficiency in adult patients: baseline clinical characteristics and responses to GH replacement in comparison with hypopituitary patients. A sub-analysis of the KIMS database. Growth Horm IGF Res. 2005;15:349.

Abs R, Feldt-Rasmussen U, Mattsson AF, Monson JP, Bengtsson BA, Goth MI, Wilton P, Koltowska-Haggstrom M. Determinants of cardiovascular risk in 2589 hypopituitary GH-deficient adults – a KIMS database analysis. Eur J Endocrinol. 2006;155:79.

Aimaretti G, Corneli G, Razzore P, Bellone S, Baffoni C, Arvat E, Camanni F, Ghigo E. Comparison between insulin-induced hypoglycemia and growth hormone (GH)-releasing hormone + arginine as provocative tests for the diagnosis of GH deficiency in adults. J Clin Endocrinol Metab. 1998;83:1615.

Allen DB, Backeljauw P, Bidlingmaier M, Biller BM, Boguszewski M, Burman P, Butler G, Chihara K, Christiansen J, Cianfarani S, Clayton P, Clemmons D, Cohen P, Darendeliler F, Deal C, Dunger D, Erfurth EM, Fuqua JS, Grimberg A, Haymond M, Higham C, Ho K, Hoffman AR, Hokken-Koelega A, Johannsson G, Juul A, Kopchick J, Lee P, Pollak M, Radovick S, Robison L, Rosenfeld R, Ross RJ, Savendahl L, Saenger P, Toft SH, Stochholm K, Strasburger C, Swerdlow A, Thorner M. GH safety workshop position paper: a critical appraisal of recombinant human GH therapy in children and adults. Eur J Endocrinol. 2016;174:1.

Amato G, Carella C, Fazio S, La Montagna G, Cittadini A, Sabatini D, Marciano-Mone C, Sacca L, Bellastella A. Body composition, bone metabolism, and heart structure and function in growth hormone (GH)-deficient adults before and after GH replacement therapy at low doses. J Clin Endocrinol Metab. 1993;77:1671.

Appelman-Dijkstra NM, Claessen KM, Roelfsema F, Pereira AM, Biermasz NR. Long-term effects of recombinant human GH replacement in adults with GH deficiency: a systematic review. Eur J Endocrinol. 2013;169:R1.

Arafah BM. Reversible hypopituitarism in patients with large nonfunctioning pituitary adenomas. J Clin Endocrinol Metab. 1986;62:1173.

Arafah BM, Prunty D, Ybarra J, Hlavin ML, Selman WR. The dominant role of increased intrasellar pressure in the pathogenesis of hypopituitarism, hyperprolactinemia, and headaches in patients with pituitary adenomas. J Clin Endocrinol Metab. 2000;85:1789.

Attanasio AF, Lamberts SW, Matranga AM, Birkett MA, Bates PC, Valk NK, Hilsted J, Bengtsson BA, Strasburger CJ. Adult growth hormone (GH)-deficient patients demonstrate heterogeneity between childhood onset and adult onset before and during human GH treatment. Adult Growth Hormone Deficiency Study Group. J Clin Endocrinol Metab. 1997;82:82.

Attanasio AF, Mo D, Erfurth EM, Tan M, Ho KY, Kleinberg D, Zimmermann AG, Chanson P. Prevalence of metabolic syndrome in adult hypopituitary growth hormone (GH)-deficient patients before and after GH replacement. J Clin Endocrinol Metab. 2010;95:74.

Bates AS, Van't Hoff W, Jones PJ, Clayton RN. The effect of hypopituitarism on life expectancy. J Clin Endocrinol Metab. 1996;81:1169.

Baxter D, Sharp DJ, Feeney C, Papadopoulou D, Ham TE, Jilka S, Hellyer PJ, Patel MC, Bennett AN, Mistlin A, McGilloway E, Midwinter M, Goldstone AP. Pituitary dysfunction after blast traumatic brain injury: the UK BIOSAP study. Ann Neurol. 2013;74:527.

Bell J, Parker KL, Swinford RD, Hoffman AR, Maneatis T, Lippe B. Long-term safety of recombinant human growth hormone in children. J Clin Endocrinol Metab. 2010;95:167.

Biller BM, Sesmilo G, Baum HB, Hayden D, Schoenfeld D, Klibanski A. Withdrawal of long-term physiological growth hormone (GH) administration: differential effects on bone density

and body composition in men with adult-onset GH deficiency. J Clin Endocrinol Metab. 2000;85:970.

Biller BM, Samuels MH, Zagar A, Cook DM, Arafah BM, Bonert V, Stavrou S, Kleinberg DL, Chipman JJ, Hartman ML. Sensitivity and specificity of six tests for the diagnosis of adult GH deficiency. J Clin Endocrinol Metab. 2002;87:2067.

Birzniece V, Sata A, Ho KK. Growth hormone receptor modulators. Rev Endocr Metab Disord. 2009;10:145.

Bollerslev J, Ueland T, Jorgensen AP, Fougner KJ, Wergeland R, Schreiner T, Burman P. Positive effects of a physiological dose of GH on markers of atherogenesis: a placebo-controlled study in patients with adult-onset GH deficiency. Eur J Endocrinol. 2006;154:537.

Bonert VS, Elashoff JD, Barnett P, Melmed S. Body mass index determines evoked growth hormone (GH) responsiveness in normal healthy male subjects: diagnostic caveat for adult GH deficiency. J Clin Endocrinol Metab. 2004;89:3397.

Borson-Chazot F, Serusclat A, Kalfallah Y, Ducottet X, Sassolas G, Bernard S, Labrousse F, Pastene J, Sassolas A, Roux Y, Berthezene F. Decrease in carotid intima-media thickness after one year growth hormone (GH) treatment in adults with GH deficiency. J Clin Endocrinol Metab. 1999;84:1329.

Brabant G, Poll EM, Jonsson P, Polydorou D, Kreitschmann-Andermahr I. Etiology, baseline characteristics, and biochemical diagnosis of GH deficiency in the adult: are there regional variations? Eur J Endocrinol. 2009;161(Suppl 1):S25.

Burman P, Mattsson AF, Johannsson G, Hoybye C, Holmer H, Dahlqvist P, Berinder K, Engstrom BE, Ekman B, Erfurth EM, Svensson J, Wahlberg J, Karlsson FA. Deaths among adult patients with hypopituitarism: hypocortisolism during acute stress, and de novo malignant brain tumors contribute to an increased mortality. J Clin Endocrinol Metab. 2013;98:1466.

Carroll PV, Christ ER, Bengtsson BA, Carlsson L, Christiansen JS, Clemmons D, Hintz R, Ho K, Laron Z, Sizonenko P, Sonksen PH, Tanaka T, Thorne M. Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. Growth Hormone Research Society Scientific Committee. J Clin Endocrinol Metab. 1998;83:382.

Cenci MC, Soares DV, Spina LD, de Lima Oliveira Brasil RR, Lobo PM, Mansur VA, Gold J, Michmacher E, Vaisman M, Conceicao FL. Effects of 5 years of growth hormone (GH) replacement therapy on cardiac parameters and physical performance in adults with GH deficiency. Pituitary. 2009;12:322.

Clayton PE, Cuneo RC, Juul A, Monson JP, Shalet SM, Tauber M. Consensus statement on the management of the GH-treated adolescent in the transition to adult care. Eur J Endocrinol. 2005;152:165.

Clemmons DR. The relative roles of growth hormone and IGF-1 in controlling insulin sensitivity. J Clin Invest. 2004;113:25.

Colao A, Marzullo P, Di Somma C, Lombardi G. Growth hormone and the heart. Clin Endocrinol. 2001;54:137.

Cook DM, Yuen KC, Biller BM, Kemp SF, Vance ML. American Association of Clinical Endocrinologists medical guidelines for clinical practice for growth hormone use in growth hormone-deficient adults and transition patients – 2009 update. Endocr Pract. 2009;15(Suppl 2):1.

Corneli G, Di Somma C, Baldelli R, Rovere S, Gasco V, Croce CG, Grottoli S, MacCario M, Colao A, Lombardi G, Ghigo E, Camanni F, Aimaretti G. The cut-off limits of the GH response to GH-releasing hormone-arginine test related to body mass index. Eur J Endocrinol. 2005;153:257.

Cuneo RC, Salomon F, McGauley GA, Sonksen PH. The growth hormone deficiency syndrome in adults. Clin Endocrinol (Oxf). 1992;37:387.

Cuneo RC, Judd S, Wallace JD, Perry-Keene D, Burger H, Lim-Tio S, Strauss B, Stockigt J, Topliss D, Alford F, Hew L, Bode H, Conway A, Handelsman D, Dunn S, Boyages S, Cheung NW, Hurley D. The Australian multicenter trial of growth hormone (GH) treatment in GH-deficient adults. J Clin Endocrinol Metab. 1998;83:107.

Darendeliler F, Karagiannis G, Wilton P, Ranke MB, Albertsson-Wikland K, Anthony PD, On Behalf of the Kigs International Board. Recurrence of brain tumours in patients treated with

growth hormone: analysis of KIGS (Pfizer International Growth Database). Acta Paediatr. 2006;95:1284.
Darzy KH. Radiation-induced hypopituitarism. Curr Opin Endocrinol Diabetes Obes. 2013;20:342.
Deodati A, Ferroli BB, Cianfarani S. Association between growth hormone therapy and mortality, cancer and cardiovascular risk: systematic review and meta-analysis. Growth Horm IGF Res. 2014;24:105.
Diri H, Karaca Z, Simsek Y, Tanriverdi F, Unluhizarci K, Kelestimur F. Can a glucagon stimulation test characterized by lower GH cut-off value be used for the diagnosis of growth hormone deficiency in adults? Pituitary. 2015;18:884.
Diri H, Karaca Z, Tanriverdi F, Unluhizarci K, Kelestimur F. Sheehan's syndrome: new insights into an old disease. Endocrine. 2016;51:22.
Dusick JR, Wang C, Cohan P, Swerdloff R, Kelly DF. Pathophysiology of hypopituitarism in the setting of brain injury. Pituitary. 2012;15:2.
Elbornsson M, Gotherstrom G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. Fifteen years of GH replacement improves body composition and cardiovascular risk factors. Eur J Endocrinol. 2013;168:745.
Elumir- Mamba LAS, Andag-Silva AA, Fonte JS, Mercado-Asis LB. Clinical profile and etiology of hypopituitarism at the University of Santo Tomas Hospital. Philipp J Intern Med. 2010;48:23.
Fernandez-Rodriguez E, Lopez-Raton M, Andujar P, Martinez-Silva IM, Cadarso-Suarez C, Casanueva FF, Bernabeu I. Epidemiology, mortality rate and survival in a homogeneous population of hypopituitary patients. Clin Endocrinol (Oxf). 2013;78:278.
Ghigo E, Aimaretti G, Gianotti L, Bellone J, Arvat E, Camanni F. New approach to the diagnosis of growth hormone deficiency in adults. Eur J Endocrinol. 1996;134:352.
Giavoli C, Libe R, Corbetta S, Ferrante E, Lania A, Arosio M, Spada A, Beck-Peccoz P. Effect of recombinant human growth hormone (GH) replacement on the hypothalamic-pituitary-adrenal axis in adult GH-deficient patients. J Clin Endocrinol Metab. 2004;89:5397.
Gibney J, Wallace JD, Spinks T, Schnorr L, Ranicar A, Cuneo RC, Lockhart S, Burnand KG, Salomon F, Sonksen PH, Russell-Jones D. The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. J Clin Endocrinol Metab. 1999;84:2596.
Golgeli A, Tanriverdi F, Suer C, Gokce C, Ozesmi C, Bayram F, Kelestimur F. Utility of P300 auditory event related potential latency in detecting cognitive dysfunction in growth hormone (GH) deficient patients with Sheehan's syndrome and effects of GH replacement therapy. Eur J Endocrinol. 2004;150:153.
Gomez JM, Espadero RM, Escobar-Jimenez F, Hawkins F, Pico A, Herrera-Pombo JL, Vilardell E, Duran A, Mesa J, Faure E, Sanmarti A. Growth hormone release after glucagon as a reliable test of growth hormone assessment in adults. Clin Endocrinol. 2002;56:329.
Gotherstrom G, Elbornsson M, Stibrant-Sunnerhagen K, Bengtsson BA, Johannsson G, Svensson J. Ten years of growth hormone (GH) replacement normalizes muscle strength in GH-deficient adults. J Clin Endocrinol Metab. 2009;94:809.
Hamrahian AH, Yuen KC, Gordon MB, Pulaski-Liebert KJ, Bena J, Biller BM. Revised GH and cortisol cut-points for the glucagon stimulation test in the evaluation of GH and hypothalamic-pituitary-adrenal axes in adults: results from a prospective randomized multicenter study. Pituitary. 2016;19:332.
Hartman ML, Crowe BJ, Biller BM, Ho KK, Clemmons DR, Chipman JJ. Which patients do not require a GH stimulation test for the diagnosis of adult GH deficiency? J Clin Endocrinol Metab. 2002;87:477.
Ho KK. Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. Eur J Endocrinol. 2007;157:695.
Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, Azen SP. The role of carotid arterial intima-media thickness in predicting clinical coronary events. Ann Intern Med. 1998;128:262.
Hoffman DM, O'Sullivan AJ, Freund J, Ho KK. Adults with growth hormone deficiency have abnormal body composition but normal energy metabolism. J Clin Endocrinol Metab. 1995;80:72.

Hoffman AR, Kuntze JE, Baptista J, Baum HB, Baumann GP, Biller BM, Clark RV, Cook D, Inzucchi SE, Kleinberg D, Klibanski A, Phillips LS, Ridgway EC, Robbins RJ, Schlechte J, Sharma M, Thorner MO, Vance ML. Growth hormone (GH) replacement therapy in adult-onset gh deficiency: effects on body composition in men and women in a double-blind, randomized, placebo-controlled trial. J Clin Endocrinol Metab. 2004;89:2048.

Holmes SJ, Shalet SM. Which adults develop side-effects of growth hormone replacement? Clin Endocrinol (Oxf). 1995;43:143.

Holmes SJ, Economou G, Whitehouse RW, Adams JE, Shalet SM. Reduced bone mineral density in patients with adult onset growth hormone deficiency. J Clin Endocrinol Metab. 1994;78:669.

Ismailogullari S, Tanriverdi F, Kelestimur F, Aksu M. Sleep architecture in Sheehan's syndrome before and 6 months after growth hormone replacement therapy. Psychoneuroendocrinology. 2009;34:212.

Johannsson G, Grimby G, Sunnerhagen KS, Bengtsson BA. Two years of growth hormone (GH) treatment increase isometric and isokinetic muscle strength in GH-deficient adults. J Clin Endocrinol Metab. 1997;82:2877.

Karaca Z, Tanriverdi F, Unluhizarci K, Kelestimur F. Pregnancy and pituitary disorders. Eur J Endocrinol. 2010;162:453.

Karaca Z, Tanriverdi F, Dagli AT, Selcuklu A, Casanueva FF, Unluhizarci K, Kelestimur F. Three years prospective investigation of pituitary functions following subarachnoid haemorrhage. Pituitary. 2013;16:76.

Kelestimur F. Sheehan's syndrome. Pituitary. 2003;6:181.

Kelestimur F. Chronic trauma in sports as a cause of hypopituitarism. Pituitary. 2005;8:259.

Kelestimur F, Jonsson P, Molvalilar S, Gomez JM, Auernhammer CJ, Colak R, Koltowska-Haggstrom M, Goth MI. Sheehan's syndrome: baseline characteristics and effect of 2 years of growth hormone replacement therapy in 91 patients in K. Eur J Endocrinol. 2005;152:581.

Kelestimur F, Popovic V, Leal A, Van Dam PS, Torres E, Perez Mendez LF, Greenman Y, Koppeschaar HP, Dieguez C, Casanueva FF. Effect of obesity and morbid obesity on the growth hormone (GH) secretion elicited by the combined GHRH + GHRP-6 test. Clin Endocrinol (Oxf). 2006;64:667.

Kelly DF, Gonzalo IT, Cohan P, Berman N, Swerdloff R, Wang C. Hypopituitarism following traumatic brain injury and aneurysmal subarachnoid hemorrhage: a preliminary report. J Neurosurg. 2000;93:743.

Kelly DF, Chaloner C, Evans D, Mathews A, Cohan P, Wang C, Swerdloff R, Sim MS, Lee J, Wright MJ, Kernan C, Barkhoudarian G, Yuen KC, Guskiewicz K. Prevalence of pituitary hormone dysfunction, metabolic syndrome, and impaired quality of life in retired professional football players: a prospective study. J Neurotrauma. 2014;31:1161.

Klose M, Jonsson B, Abs R, Popovic V, Koltowska-Haggstrom M, Saller B, Feldt-Rasmussen U, Kourides I. From isolated GH deficiency to multiple pituitary hormone deficiency: an evolving continuum – a KIMS analysis. Eur J Endocrinol. 2009;161(Suppl 1):S75.

Kokshoorn NE, Wassenaar MJ, Biermasz NR, Roelfsema F, Smit JW, Romijn JA, Pereira AM. Hypopituitarism following traumatic brain injury: prevalence is affected by the use of different dynamic tests and different normal values. Eur J Endocrinol. 2010;162:11.

Koller EA, Green L, Gertner JM, Bost M, Malozowski SN. Retinal changes mimicking diabetic retinopathy in two nondiabetic, growth hormone-treated patients. J Clin Endocrinol Metab. 1998;83:2380.

Koltowska-Haggstrom M, Mattsson AF, Shalet SM. Assessment of quality of life in adult patients with GH deficiency: KIMS contribution to clinical practice and pharmacoeconomic evaluations. Eur J Endocrinol. 2009;161(Suppl 1):S51.

Kovacs K. Sheehan syndrome. Lancet. 2003;361:520.

Kristjansdottir HL, Bodvarsdottir SP, Sigurjonsdottir HA. Sheehan's syndrome in modern times: a nationwide retrospective study in Iceland. Eur J Endocrinol. 2011;164:349.

Kronvall E, Valdemarsson S, Saveland H, Nilsson OG. High prevalence of pituitary dysfunction after aneurysmal subarachnoid hemorrhage: a long-term prospective study using dynamic endocrine testing. World Neurosurg. 2015;83:574.

Lange M, Thulesen J, Feldt-Rasmussen U, Skakkebaek NE, Vahl N, Jorgensen JO, Christiansen JS, Poulsen SS, Sneppen SB, Juul A. Skin morphological changes in growth hormone deficiency and acromegaly. Eur J Endocrinol. 2001;145:147.

Lissett CA, Shalet SM. Effects of growth hormone on bone and muscle. Growth Horm IGF Res. 2000;10(Suppl B):S95.

Littley MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML. Hypopituitarism following external radiotherapy for pituitary tumours in adults. Q J Med. 1989;70:145.

Maghnie M, Aimaretti G, Bellone S, Bona G, Bellone J, Baldelli R, de Sanctis C, Gargantini L, Gastaldi R, Ghizzoni L, Secco A, Tinelli C, Ghigo E. Diagnosis of GH deficiency in the transition period: accuracy of insulin tolerance test and insulin-like growth factor-I measurement. Eur J Endocrinol. 2005;152:589.

Maison P, Chanson P. Cardiac effects of growth hormone in adults with growth hormone deficiency: a meta-analysis. Circulation. 2003;108:2648.

Maison P, Griffin S, Beglah-Nicoue M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a metaanalysis of blinded, randomized, placebo-controlled trials. J Clin Endocrinol Metab. 2004;89:2192.

Malozowski S, Tanner LA, Wysowski D, Fleming GA. Growth hormone, insulin-like growth factor I, and benign intracranial hypertension. N Engl J Med. 1993;329:665.

Mazziotti G, Bianchi A, Bonadonna S, Nuzzo M, Cimino V, Fusco A, De Marinis L, Giustina A. Increased prevalence of radiological spinal deformities in adult patients with GH deficiency: influence of GH replacement therapy. J Bone Miner Res. 2006;21:520.

McGauley GA, Cuneo RC, Salomon F, Sonksen PH. Psychological well-being before and after growth hormone treatment in adults with growth hormone deficiency. Horm Res. 1990;33(Suppl 4):52.

Melmed S. Idiopathic adult growth hormone deficiency. J Clin Endocrinol Metab. 2013;98:2187.

Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Vance ML. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1587.

Moller N, Jorgensen JO. Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. Endocr Rev. 2009;30:152.

Murray RD, Skillicorn CJ, Howell SJ, Lissett CA, Rahim A, Smethurst LE, Shalet SM. Influences on quality of life in GH deficient adults and their effect on response to treatment. Clin Endocrinol (Oxf). 1999;51:565.

Nomikos P, Ladar C, Fahlbusch R, Buchfelder M. Impact of primary surgery on pituitary function in patients with non-functioning pituitary adenomas – a study on 721 patients. Acta Neurochir (Wien). 2004;146:27.

Ozdogru I, Tanriverdi F, Dogan A, Kaya MG, Tugrul IM, Kalay N, Topsakal R, Unluhizarci K, Atmaca H, Kemal EN, Kelestimur F. Impaired longitudinal myocardial velocities in patients with growth hormone deficiency improves after hormone replacement therapy. J Am Soc Echocardiogr. 2007;20:1093.

Popovic V, Leal A, Micic D, Koppeschaar HP, Torres E, Paramo C, Obradovic S, Dieguez C, Casanueva FF. GH-releasing hormone and GH-releasing peptide-6 for diagnostic testing in GH-deficient adults. Lancet. 2000;356:1137.

Porretti S, Giavoli C, Ronchi C, Lombardi G, Zaccaria M, Valle D, Arosio M, Beck-Peccoz P. Recombinant human GH replacement therapy and thyroid function in a large group of adult GH-deficient patients: when does L-T(4) therapy become mandatory? J Clin Endocrinol Metab. 2002;87:2042.

Qu XD, Gaw GI, Al Sayed MY, Cohan P, Christenson PD, Swerdloff RS, Kelly DF, Wang C. Influence of body mass index and gender on growth hormone (GH) responses to GH-releasing hormone plus arginine and insulin tolerance tests. J Clin Endocrinol Metab. 2005;90:1563.

Regal M, Paramo C, Sierra SM, Garcia-Mayor RV. Prevalence and incidence of hypopituitarism in an adult Caucasian population in northwestern Spain. Clin Endocrinol (Oxf). 2001;55:735.

Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. Lancet. 1990;336:285.

Rosen T, Eden S, Larson G, Wilhelmsen L, Bengtsson BA. Cardiovascular risk factors in adult patients with growth hormone deficiency. Acta Endocrinol (Copenh). 1993;129:195.

Sartorio A, Ferrero S, Conti A, Bragato R, Malfatto G, Leonetti G, Faglia G. Adults with childhood-onset growth hormone deficiency: effects of growth hormone treatment on cardiac structure. J Intern Med. 1997;241:515.

Sassolas G, Chazot FB, Jaquet P, Bachelot I, Chanson P, Rudelli CC, Tauber JP, Allannic H, Bringer J, Roudaut N, Rohmer V, Roger P, Latapie JL, Reville P, Leutenegger M. GH deficiency in adults: an epidemiological approach. Eur J Endocrinol. 1999;141:595.

Schaefer S, Boegershausen N, Meyer S, Ivan D, Schepelmann K, Kann PH. Hypothalamic-pituitary insufficiency following infectious diseases of the central nervous system. Eur J Endocrinol. 2008;158:3.

Schneider HJ, Herrmann BL, Schneider M, Sievers C, Schaaf L, Stalla GK. Discrepant results in the diagnosis of GH deficiency with the insulin-tolerance test and the GHRH plus arginine test in patients with traumatic brain injury. Eur J Endocrinol. 2006;155:553.

Schneider HJ, Aimaretti G, Kreitschmann-Andermahr I, Stalla GK, Ghigo E. Hypopituitarism. Lancet. 2007a;369:1461.

Schneider HJ, Kreitschmann-Andermahr I, Ghigo E, Stalla GK, Agha A. Hypothalamopituitary dysfunction following traumatic brain injury and aneurysmal subarachnoid hemorrhage: a systematic review. JAMA. 2007b;298:1429.

Schneider HJ, Klotsche J, Wittchen HU, Stalla GK, Schopohl J, Kann PH, Kreitschmann-Andermahr I, Wallaschofski H. Effects of growth hormone replacement within the KIMS survey on estimated cardiovascular risk and predictors of risk reduction in patients with growth hormone deficiency. Clin Endocrinol (Oxf). 2011;75:825.

Sesmilo G, Biller BM, Llevadot J, Hayden D, Hanson G, Rifai N, Klibanski A. Effects of growth hormone administration on inflammatory and other cardiovascular risk markers in men with growth hormone deficiency. A randomized, controlled clinical trial. Ann Intern Med. 2000;133:111.

Shalet SM, Shavrikova E, Cromer M, Child CJ, Keller E, Zapletalova J, Moshang T, Blum WF, Chipman JJ, Quigley CA, Attanasio AF. Effect of growth hormone (GH) treatment on bone in postpubertal GH-deficient patients: a 2-year randomized, controlled, dose-ranging study. J Clin Endocrinol Metab. 2003;88:4124.

Sheehan HL. Postpartum necrosis of the anterior pituitary. J Pathol. 1937;45:189.

Sheehan HL. The frequency of post-partum hypopıtuıtarısm. J Obstet Gynaecol Br Commonw. 1965;72:103.

Sherlock M, Ayuk J, Tomlinson JW, Toogood AA, Aragon-Alonso A, Sheppard MC, Bates AS, Stewart PM. Mortality in patients with pituitary disease. Endocr Rev. 2010;31:301.

Simsek Y, Karaca Z, Tanriverdi F, Unluhizarci K, Selcuklu A, Kelestimur F. A comparison of low-dose ACTH, glucagon stimulation and insulin tolerance test in patients with pituitary disorders. Clin Endocrinol (Oxf). 2014;82:45.

Stochholm K, Johannsson G. Reviewing the safety of GH replacement therapy in adults. Growth Horm IGF Res. 2015;25:149.

Stochholm K, Gravholt CH, Laursen T, Jorgensen JO, Laurberg P, Andersen M, Kristensen LO, Feldt-Rasmussen U, Christiansen JS, Frydenberg M, Green A. Incidence of GH deficiency – a nationwide study. Eur J Endocrinol. 2006;155:61.

Tanriverdi F, Kelestimur F. Pituitary dysfunction following traumatic brain injury: clinical perspectives. Neuropsychiatr Dis Treat. 2015;11:1835.

Tanriverdi F, Unluhizarci K, Kula M, Guven M, Bayram F, Kelestimur F. Effects of 18-month of growth hormone (GH) replacement therapy in patients with Sheehan's syndrome. Growth Horm IGF Res. 2005;15:231.

Tanriverdi F, Unluhizarci K, Coksevim B, Selcuklu A, Casanueva FF, Kelestimur F. Kickboxing sport as a new cause of traumatic brain injury-mediated hypopituitarism. Clin Endocrinol (Oxf). 2007;66:360.

Tanriverdi F, Taheri S, Ulutabanca H, Caglayan AO, Ozkul Y, Dundar M, Selcuklu A, Unluhizarci K, Casanueva FF, Kelestimur F. Apolipoprotein E3/E3 genotype decreases the risk of pituitary

dysfunction after traumatic brain injury due to various causes: preliminary data. J Neurotrauma. 2008a;25:1071.

Tanriverdi F, Ulutabanca H, Unluhizarci K, Selcuklu A, Casanueva FF, Kelestimur F. Three years prospective investigation of anterior pituitary function after traumatic brain injury: a pilot study. Clin Endocrinol. 2008b;68:573.

Tanriverdi F, Unluhizarci K, Kocyigit I, Tuna IS, Karaca Z, Durak AC, Selcuklu A, Casanueva FF, Kelestimur F. Brief communication: pituitary volume and function in competing and retired male boxers. Ann Intern Med. 2008c;148:827.

Tanriverdi F, De Bellis A, Teksahin H, Alp E, Bizzarro A, Sinisi AA, Bellastella G, Paglionico VA, Bellastella A, Unluhizarci K, Doganay M, Kelestimur F. Prospective investigation of pituitary functions in patients with acute infectious meningitis: is acute meningitis induced pituitary dysfunction associated with autoimmunity? Pituitary. 2012;15:579.

Tanriverdi F, De Bellis A, Ulutabanca H, Bizzarro A, Sinisi AA, Bellastella G, Amoresano PV, Dalla ML, Selcuklu A, Unluhizarci K, Casanueva FF, Kelestimur F. A five year prospective investigation of anterior pituitary function after traumatic brain injury: is hypopituitarism long-term after head trauma associated with autoimmunity? J Neurotrauma. 2013;30:1426.

Tanriverdi F, Dokmetas HS, Kebapci N, Kilicli F, Atmaca H, Yarman S, Ertorer ME, Erturk E, Bayram F, Tugrul A, Culha C, Cakir M, Mert M, Aydin H, Taskale M, Ersoz N, Canturk Z, Anaforoglu I, Ozkaya M, Oruk G, Hekimsoy Z, Kelestimur F, Erbas T. Etiology of hypopituitarism in tertiary care institutions in Turkish population: analysis of 773 patients from Pituitary Study Group database. Endocrine. 2014a;47:198.

Tanriverdi F, Karaca Z, Unluhizarci K, Kelestimur F. Unusual effects of GH deficiency in adults: a review about the effects of GH on skin, sleep, and coagulation. Endocrine. 2014b;47:679–89.

Tanriverdi F, Schneider HJ, Aimaretti G, Masel BE, Casanueva FF, Kelestimur F. Pituitary dysfunction after traumatic brain injury: a clinical and pathophysiological approach. Endocr Rev. 2015;36:305.

Theadom A, Starkey NJ, Dowell T, Hume PA, Kahan M, McPherson K, Feigin V. Sports-related brain injury in the general population: an epidemiological study. J Sci Med Sport. 2014;17:591.

Toogood AA, Ryder WD, Beardwell CG, Shalet SM. The evolution of radiation-induced growth hormone deficiency in adults is determined by the baseline growth hormone status. Clin Endocrinol (Oxf). 1995;43:97.

Tsiakalos A, Xynos ID, Sipsas NV, Kaltsas G. Pituitary insufficiency after infectious meningitis: a prospective study. J Clin Endocrinol Metab. 2010;95:3277.

van Bunderen CC, van den Dries CJ, Heymans MW, Franken AA, Koppeschaar HP, van der Lely AJ, Drent ML. Effect of long-term GH replacement therapy on cardiovascular outcomes in isolated GH deficiency compared with multiple pituitary hormone deficiencies: a sub-analysis from the Dutch National Registry of Growth Hormone Treatment in Adults. Eur J Endocrinol. 2014;171:151.

Vance ML. Hypopituitarism. N Engl J Med. 1994;330:1651.

Wilkinson CW, Pagulayan KF, Petrie EC, Mayer CL, Colasurdo EA, Shofer JB, Hart KL, Hoff D, Tarabochia MA, Peskind ER. High prevalence of chronic pituitary and target-organ hormone abnormalities after blast-related mild traumatic brain injury. Front Neurol. 2012;3:11.

Wuster C, Abs R, Bengtsson BA, Bennmarker H, Feldt-Rasmussen U, Hernberg-Stahl E, Monson JP, Westberg B, Wilton P. The influence of growth hormone deficiency, growth hormone replacement therapy, and other aspects of hypopituitarism on fracture rate and bone mineral density. J Bone Miner Res. 2001;16:398.

Zargar AH, Singh B, Laway BA, Masoodi SR, Wani AI, Bashir MI. Epidemiologic aspects of postpartum pituitary hypofunction (Sheehan's syndrome). Fertil Steril. 2005;84:523.

Zazryn TR, Finch CF, McCrory P. A 16 year study of injuries to professional kickboxers in the state of Victoria, Australia. Br J Sports Med. 2003;37:448.

Physiopathology, Diagnosis, and Treatment of GH Hypersecretion

3

Leandro Kasuki, Marília Duarte Dalmolin, and Mônica Gadelha

Contents

Abstract

Growth hormone excess leads to acromegaly or gigantism, multisystemic diseases associated with great morbidity, and increased mortality when not early recognized and adequately treated. It is caused, in the vast majority of the cases, by a pituitary adenoma (somatotropinoma) that can occur sporadically or

L. Kasuki (✉) · M. Gadelha
Neuroendocrinology Research Center, Hospital Universitário Clementino Fraga Filho of Universidade Federal do Rio de Janeiro (HUCFF/UFRJ), Rio de Janeiro, BJ, Brazil

Neuroendocrinology Unit and Molecular Genetics Laboratory, Instituto Estadual do Cérebro Paulo Niemeyer (IECPN), Rio de Janeiro, BJ, Brazil
e-mail: lkasuki@yahoo.com; mgadelha@hucff.ufrj.br

M. D. Dalmolin
Neuroendocrinology Research Center, Hospital Universitário Clementino Fraga Filho of Universidade Federal do Rio de Janeiro (HUCFF/UFRJ), Rio de Janeiro, BJ, Brazil
e-mail: mariliadalmolin@hotmail.com

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_3

associated with some familial diseases. In this chapter the physiopathology, the diagnosis, and the management of GH excess, according to the current knowledge, will be discussed.

Keywords
Growth hormone · Insulin-like growth factor type I · Acromegaly · Gigantism

Introduction

Growth hormone (GH) has its importance recognized since the late nineteenth century (Murray et al. 2015). Knowledge about the pathophysiological processes that involve GH is well established in contemporary medicine, although in constant evolution. Growth hormone has receptors in multiple organs and systems, influencing many processes as, for example, the postnatal longitudinal growth, as well as the lipids, proteins, and carbohydrates metabolism. Conditions that lead to excess of GH production result in gigantism if they occur before epiphyseal fusion or in acromegaly if they occur after this time.

Excess of GH production is mostly due to a pituitary adenoma (somatotropinoma) (Ben-Shlomo and Melmed 2008). This is a rare condition but, although described in 1886 (Marie 1886), it is still underdiagnosed (Pendleton et al. 2013). Its recognition is important as soon as its cardinal signs manifest, so that the appropriate therapeutic approach can be initiated.

In this chapter we will briefly review physiological aspects of GH secretion and relevant points about its excessive production. Establishing a correct diagnostic approach and implementing an effective and safe treatment allows reduction of the morbimortality associated with this hormonal disorder and normalization of the associated high mortality rate.

Growth Hormone: Secretion Control and Physiological Aspects

Growth hormone is a polypeptide hormone with anabolic function, synthesized and secreted by the somatotroph cells in the pituitary gland (Baumann 1991). The gene that encodes GH is localized on the long arm of chromosome 17, leading to the synthesis of mainly two distinct GH molecules (22 kDa and 20 kDa) by alternative splicing (Baumann 1991). The most prevalent form, the 22 kDa GH, is a 191 amino acid single chain protein with a molecular weight of 22,000 (Kato et al. 2002).

Growth hormone secretion occurs under the main control of two hypothalamic peptides: the growth hormone releasing hormone (GHRH), that acts to stimulate GH release, and somatostatin, also known as somatotropin release-inhibiting factor (SRIF), that exerts inhibitory effects on its release, attenuating both the timing and amplitude of GH secretory pulses (Kato et al. 2002).

Growth hormone releasing hormone is composed of 44 amino acids, the first 27 amino acids from the N terminus are essential for its activity (Murray et al. 2015). The major site where GHRH is expressed is the arcuate nucleus of the hypothalamus (Baumann 1991). After being released into portal venous system, GHRH directly stimulates GH transcription and secretion from the pituitary somatotrophs by activating the corresponding GHRH seven transmembrane G protein-coupled receptors via a pathway that involves activation of adenylyl cyclase and increased cyclic adenosine monophosphate (cAMP) production (Murray et al. 2015). In addition, animal studies have shown the effect of GHRH on the proliferation of pituitary somatotrophs and patients with ectopic GHRH secretion present pituitary somatotroph hyperplasia (Garby et al. 2012). The concentration of GHRH in the blood is insignificant, since it is rapidly degraded (Murray et al. 2015).

Somatostatin-producing neurons, concentrated in the periventricular nucleus, also project to the portal venous system where they release SRIF to inhibit GH release via a seven transmembrane G protein-coupled receptor. Various somatostatin receptors (SSTR) were identified (SSTR1–5), of which SSTR2 and SSTR5 exert greater inhibition on GH secretion (Ferone et al. 2009; Kato et al. 2002).

Ghrelin is another potent GH secretagogue, derived from gastrointestinal tract and from the central nervous system, with the strongest concentrations in the stomach, that operates mainly in the hypothalamus and signals through ghrelin secretagogue receptor type Ia (GHS-RIa) to induce GH secretion in synergy with GHRH (Muller et al. 2015). Ghrelin expression in the stomach is reduced in the postprandial period and increased in fasting, which helps to explain the increase of GH during sleep (Muller et al. 2015).

The result of stimulatory actions of GHRH and ghrelin and the inhibitory effect of SRIF on GH secretion is a pulsatile pattern of secretion, characterized by sporadic discharge pulses, occurring in about 4–11 times in 24 h, most often at the second half of the night, with minimal basal secretion occurring between pulses (Murray et al. 2015).

Various physiological and pathological factors can influence this axis: sleep (slow waves), puberty, malnutrition, fasting, stress, exercise, amino acids, glucose and lipids, uncontrolled diabetes mellitus, and liver cirrhosis are factors that increase GH secretion, while aging, hypothyroidism, glucocorticoids, and increased body mass index (BMI) attenuate GH secretion (Melmed 2009; Murray et al. 2015).

Growth hormone exerts its effects by binding to the GH receptor (GHR), a 620-amino-acid, single chain glycoprotein with a single transmembrane domain and an extracellular domain, which is widely distributed in most tissues, especially liver, fat, and muscle (Melmed 2009). Activation of the receptor-associated Janus kinase (Jak 2) is the initial step in GH signaling, triggering ligand-receptor complex signaling, including signal transduction and activators of transcription (STAT) proteins, which initiate transcription of target proteins in the nucleus activating predominantly proliferative cell responses (Melmed 2009). Suppressor proteins, on the other hand, attenuate this signaling cascade, especially the suppressors of cytokine signaling (SOCS) (Melmed 2009).

Growth hormone acts directly through its own receptors, but most of its effects occur indirectly through the induced production of insulin-like growth factor type I (IGF-I) (Baumann 1991). IGF-I is a peptide of 70 amino acids, produced mainly in the liver and found in circulation almost 100% linked to transport proteins, known as IGF-binding protein (IGFBP), including IGF-binding protein 3 (IGFBP3), the most abundant form (Melmed 2009). The "IGFBP 3-IGF I" complex is stabilized by another protein, called acid labile subunit (ALS), prolonging its half-life to approximately 16 h (Schilbach and Bidlingmaier 2015). Thus, in addition to mediating most GH actions, IGF-I serves as a more stable marker of its secretion. It negatively regulates GH secretion through a feedback loop. In addition to promoting longitudinal growth and somatic maturation, IGF-I has other important actions in protein, lipid, and carbohydrate metabolism (Melmed 2009).

GH promotes anabolic effects in muscles and has counter insulin actions in other tissues, leading to enhanced liver production of glucose and reduction of its use. In addition, it increases fat mobilization and reduces fat deposition by activating hormone-sensitive lipase, resulting in increased hydrolysis of triglycerides to free fatty acids and glycerol (lipolysis), as well as decreased re-esterification of fatty acids. In the skeletal and cardiac muscles, it increases the uptake of glucose and amino acids, increasing protein synthesis and inhibiting its catabolism. Increased fat utilization, associated with increased protein synthesis, leads to an increase in lean mass. (Gunawardane et al. 2000). As previously reported, it also stimulates the production of IGF-I that mediates most of the anabolic effects of GH, as well as its effect on linear growth (bone formation, protein synthesis, glucose uptake by the cells, myelin synthesis, etc.) (Laron 2001).

Acromegaly and Gigantism

Epidemiology

The annual incidence of acromegaly ranges from 3–4 cases/million inhabitants and the prevalence varies between 40–120 cases/million inhabitants. The disease equally affects both sexes, with a peak incidence between 30–50 years of age (Bengtsson et al. 1988; Daly et al. 2006; Fernandez et al. 2010). The disease activity is associated with increased mortality of about 1.7 times that of the normal population, and effective treatment with normalization of GH and IGF-I allows normalization of mortality (Dekkers et al. 2008; Holdaway et al. 2008). Thus, the early recognition and proper treatment of acromegaly is essential.

Etiology

A pituitary adenoma originated from somatotroph cells (somatotropinoma) is the cause of acromegaly in about 98% of the cases (Ben-Shlomo and Melmed 2008). The remaining cases are caused mostly by eutopic (hypothalamic tumors, like

hamartoma, glioma, gangliocytoma) or ectopic secretion of GHRH, mainly by neuroendocrine tumors of the pancreas or lung carcinoids (Ben-Shlomo and Melmed 2008; Vieira Neto et al. 2007). Very rarely the cause can be the ectopic secretion of GH (Kayano et al. 1995; Melmed et al. 1985).

Somatotropinomas are monoclonal benign tumors, which can secrete only GH or co-secrete prolactin (40% of cases) (Herman et al. 1990). More than 70% of the somatotropinomas are macroadenomas ($\geq$ 10 mm) at diagnosis (Ben-Shlomo and Melmed 2008). They can be classified into five types according to the 2004 World Health Organization classification (Lloyd et al. 2004): densely granulated, sparsely granulated, mixed, mammosomatotrophic, and acidophilic stem cell. The first two are adenomas purely composed of cells producing GH, which differentiates them from the other types that co-secrete prolactin. Very rarely, distant metastases can be present, configuring a GH-secreting carcinoma.

Densely granulated adenomas are composed of medium or large acidophilic cells that have diffuse and intense immunostaining for GH (Obari et al. 2008). These are tumors with slow overall growth and nonaggressive behavior occurring in a higher age group (Obari et al. 2008). Sparsely granulated adenomas are characterized by the presence of few secretory granules and weak immunostaining for GH. These tumors are more aggressive, with poor response to first-generation somatostatin analogs (SA) treatment and are usually observed in younger patients (Fougner et al. 2012). The differentiation between these adenomas can be made through evaluation of the expression of cytokeratins 7 and 8 by immunohistochemistry with the antibody CAM5.2 (Lloyd et al. 2004).

The mixed adenomas are composed of two distinct cell types, one secreting GH and the other one secreting prolactin, while the mammosomatotrophic adenomas are composed of cells that can secrete both GH and prolactin. The acidophilic stem cell adenomas are bi-hormonal monomorphic tumors that predominantly secrets prolactin and present more rapid growth and increased aggressiveness (Lloyd et al. 2004).

Very rarely, the tumor can be multihormonal, co-expressing other pituitary hormones as well as GH and prolactin (Lloyd et al. 2004) (Table 1).

Acromegaly generally occurs sporadically, but about 5% of the cases can be part of a familial disease (Gadelha et al. 2013b), including Multiple Endocrine Neoplasia type 1 (MEN-1) and type 4 (MEN-4), Carney Complex (CNC), acromegaly and paraganglioma/pheochromocytoma syndrome (3PAs), and Familial Isolated Pituitary Adenomas Syndrome (FIPA), including Isolated Familial Somatotropinoma (IFS) and X-linked acrogigantism (X-LAG) (Gadelha et al. 2017a). These and other causes of acromegaly are listed in Table 1.

In MEN-1, an autosomal dominant condition, anterior pituitary adenomas, when present, are associated with parathyroid tumors or hyperplasia and pancreatic islet tumors (Thakker 2014). The clinical diagnosis of MEN-1 is confirmed if at least two of the three major components of the syndrome are present (Thakker et al. 2012). Other tumors with no endocrine activity, such as lipomas and angiofibromas, are often seen in affected patients (Thakker 2014). The gene that causes this condition is identified as *MEN-1*, located on chromosome region 11q13, and encodes a nuclear protein called menin that has a large network of interactions and, the presence of

Table 1 Causes of acromegaly

Etiology	Frequency
GH hypersecretion	
Pituitary origin	98%
Pure adenoma of somatotrophic cells	60%
Mixed cells adenoma (GH and PRL)	25%
Mammosomatotrophic cells adenoma (GH and PRL)	10%
Plurihormonal adenoma (GH, PRL, ACTH, TSH, and α subunit)	<5%
Acidophilic stem cells adenoma	Rare
Somatotrophic carcinoma	Rare
MEN 1 or MEN 4 (adenoma/somatotrophic cell hyperplasia)	Rare
Carney complex (adenoma/somatotrophic cell hyperplasia)	Rare
FIPA (adenoma)	Rare
Acromegaly and paraganglioma/pheochromocytoma (adenoma)	Rare
McCune Albright syndrome (adenoma)	Rare
Ectopic GH secretion	Very rare
Pancreatic islets tumor, non-Hodgkin's lymphoma	
GHRH hypersecretion	
Eutopic	<1%
Hypothalamic hamartoma, glioma and gangliocytoma	
Ectopic	1%
Carcinoids (bronchus, GTI / pancreas), neuroendocrine tumors of the pancreas, small cell lung carcinoma, medullary thyroid carcinoma, and pheochromocytoma	

GH growth hormone, *PRL* prolactin, *ACTH* adrenocorticotrophic hormone, *TSH* thyroid-stimulating hormone, *GHRH* growth hormone releasing hormone, *GIT* gastrointestinal tract, *MEN1* multiple endocrine neoplasia type 1, MEN4 multiple endocrine neoplasia type 4, FIPA familial isolated pituitary adenomas

inactivating mutations, leads to the phenotypic manifestations of the disease (Concolino et al. 2016). About 40% of patients with MEN-1 have a pituitary adenoma, being the first manifestation of the disease in only 17% of cases (Thakker 2014). The frequency of the different subtypes of pituitary adenomas are in agreement with that observed in sporadic cases, with somatotropinomas accounting for about 10% of these adenomas (Gadelha et al. 2017a).

MEN-4 has a similar phenotype to that of MEN-1. However, it is due to mutations in the cyclin-dependent kinase inhibitor 1B gene (*CDKN1B* 1B), which is located on chromosome region 12p13. The condition was observed in approximately 3% of *MEN-1* mutation-negative patients fulfilling clinical criteria (Thakker 2014).

Carney complex, a syndrome with autosomal dominant inheritance, occurs much less frequently than MEN-1 and is characterized by the presence of cardiac myxomas, pigmented skin spots, schwannomas, and adrenal, testicular, and pituitary tumors, of which up to 21% are somatotropinomas. The primary genetic cause in most cases is an inactivating mutation of the gene that codes for the regulatory subunit of protein kinase A1 (*PRKAR1A*) on chromosome region 17q22–24 (Correa et al. 2015).

The coexistence in patients with the diagnosis of acromegaly of paragangliomas or pheochromocytomas led to the search for mutations in genes of the succinate dehydrogenase (SDH) complex that are known causes of the latter tumors. In some cases a mutation in these genes has been found, with cases presenting mutations in SDHA, SDHB, and SDHD already described (Denes et al. 2015; Gadelha et al. 2017a).

Pituitary adenomas of any subtype, including nonfunctioning adenomas, when with familial aggregation, in the absence of diagnostic criteria for MEN-1, MEN-4, 3PAs, and CNC, are known as familial isolated pituitary adenoma (FIPA) (Gadelha et al. 2000, 2013b; Hernandez-Ramirez et al. 2015). This syndrome is characterized by the presence of two or more cases of pituitary adenomas in the same family in the absence of other familial syndromes, with somatotropinomas occurring in about 30% of the cases (Gadelha et al. 2013b). Isolated familial somatotropinoma is characterized by the presence of two or more cases of acromegaly and/or gigantism in the same family in which the diagnosis of MEN-1, MEN-4, 3PAs, and CNC were excluded (Gadelha et al. 2000, 2013b).

In 2000, Gadelha et al. (2000) established a linkage between isolated familial somatotropinomas (IFS) and the 11q1.3 locus and, in 2006, Vierimaa et al. (2006) described, in this same locus, nonsense germline mutations in the coding region of the aryl hydrocarbon receptor interacting protein (*AIP*) gene in two Finnish families with FIPA. After these initial studies, a large number of *AIP* mutations have been described (Hernandez-Ramirez et al. 2015). *AIP* is a tumor suppressor gene and its mutations are found in 20% of the FIPA families and in 50% of the IFS families (Hernandez-Ramirez et al. 2015). Patients with this mutation are younger and have more invasive and larger tumors (97% are macroadenomas) than patients without the mutation, and are less responsive to different treatment modalities (Daly et al. 2010). Although familial adenomas are uncommon, it is increasingly recognized that some patients with apparently sporadic adenomas, especially the younger ones, may carry *AIP* germline mutations, since the mutation may have low penetrance (Cazabat et al. 2012).

Recently, a subtype of FIPA was described in families that present cases of very-young onset gigantism (Trivellin et al. 2014). In these patients a microduplication of the Xq26.3 region was observed, leading to increased expression of the G protein-coupled receptor 101 gene (*GPR101*). It was first described in 13 patients (nine apparently sporadic cases and four patients belonging to two unrelated kindred) (Trivellin et al. 2014). These patients presented a median age of disease onset of 1 year, which was lower than that of patients with gigantism not presenting the microduplication (16 years). Interestingly, it has been described posteriorly in male patients that a somatic Xq26.3 microduplication can be present as a mosaicism (Iacovazzo et al. 2016).

Clinical Diagnosis

The disease is insidious and its manifestations often precede about 4–10 years the discovery of the disease and this delay in diagnosis does not seem to have changed in the last 20 years (Reid et al. 2010). Usually the phenotypic changes lead to diagnosis.

Patients with mild disease or recent onset may not have exuberant manifestations justifying in part the delay in diagnosis (Reid et al. 2010).

The GH and IGF-I receptors are present in many organs and therefore acromegaly and gigantism are multisystem diseases (Colao et al. 2004).

Growth hormone hypersecretion leads to phenotypic changes and systemic manifestations that can lead to significant morbidity (Ben-Shlomo and Melmed 2008; Katznelson et al. 2011). The overgrowth compromises face, hands, and feet, and is present in 98% of cases (Melmed 2009). Patients may present front protrusion, diastema, skin thickening, and macroglossia that lead to facial coarsening. The skin has increased oiliness, hypertrichosis, excessive sweating, skin tags, and *acanthosis nigricans* (Ben-Shlomo and Melmed 2008). The extremities (hands and feet) are increased and the fingers are thickened. Patients usually refer the need to change rings and an increase in the number of the shoes. When GH hypersecretion manifests before epiphyseal plate closure, there is accelerated linear growth, characterizing the gigantism (Katznelson et al. 2011).

The osteoarticular involvement is present in about 70% of the cases at diagnosis, being the main cause of morbidity (Kropf et al. 2013; Melmed et al. 2013). The most common clinical manifestation is arthralgia. Arthropathy is usually mechanical but may present signs of osteoarthritis. Peripheral arthropathy affects multiple joints such as shoulder, hand, wrist, hip, and particularly knee (70%) (Killinger et al. 2010). The temporomandibular joint may also be compromised. There is a greater incidence of vertebral fractures even with normal bone mass due to the increase of the vertebrae, which can lead to a measurement error of the bone mass (Madeira et al. 2013a, b). Bone loss occurs mainly in the spinal column, since there is loss of trabecular bone, while there is an increase in cortical bone (Madeira et al. 2010, 2013a). The diagnosis of vertebral fractures can be made through the thoracic and lumbar spine X-rays or alternatively, by examining vertebral fracture assessment with the use of the dual energy X-ray absorptiometry technique (DXA) (Madeira et al. 2013b).

The nerves may be compromised and symmetrical sensory and motor peripheral neuropathy can occur. Carpal tunnel syndrome occurs in 30–50% of the cases (Killinger et al. 2010).

Fatigue is a common complaint and possible etiologies are: sleep apnea, systolic cardiomyopathy, hypopituitarism, depressed mood, diabetes mellitus (DM), and myopathy (Katznelson et al. 2011).

Headache is observed in 55% of the cases and may be severe (Katznelson et al. 2011). The precise mechanism is still unknown. An episode of sudden headache with high intensity may be due to apoplexy of the adenoma that can occur in about 3.5% of the cases or due to the rupture of a brain aneurysm that has a higher incidence in the acromegaly patients, due to the change in the synthesis of vascular collagen wall (Katznelson et al. 2011; Manara et al. 2011).

Hypertrophy of the liver, spleen, kidney, prostate, tongue, heart, colon, vocal cords, and thyroid can occur.

Obstructive sleep apnea affects 60–87% of patients, being more common in males (Davi et al. 2008; van Haute et al. 2008). Structural changes of face, larynx, and

hypopharynx lead to this condition (Melmed et al. 2013). Clinical manifestations are snoring, morning headaches, and daytime sleepiness. About 33% of patients also have central sleep apnea (Melmed et al. 2013). The presence of sleep apnea can be evaluated clinically by the Epworth sleepiness scale and polysomnography is the test used for the definite diagnosis (Melmed et al. 2013).

Hypertension occurs in about 30–50% of cases and its prevalence increases proportionally to the duration of the disease, age, and GH levels (Colao et al. 2004).

Cardiac involvement has long been considered the leading cause of mortality in acromegaly, although recent studies show different results (Colao et al. 2004; Mercado et al. 2014). The cardiomyopathy is specific of the disease and occurs even in the absence of risk factors such as arterial hypertension, DM, advanced age, and long-term illness (Colao et al. 2004). The myocyte hypertrophy is the main change of acromegaly cardiomyopathy and it is usually biventricular and concentrical, mainly affecting the left ventricle in echocardiography studies (Casini et al. 2006). Recently, in a study applying the gold-standard method cardiac magnetic resonance imaging, the frequency of left ventricular hypertrophy was shown to be lower than when evaluated by echocardiography in a contemporary cohort of patients (dos Santos Silva et al. 2015a). Also, in another recent study from our group that evaluated the left ventricular strain (a marker of early ventricular dysfunction) by the speckle tracking echocardiography technic the acromegaly patients were not different from a control group (Volschan et al. 2017). Cardiac arrhythmia may also occur in a higher frequency in these patients although the majority of the studies are small and have a short-term follow-up (Colao et al. 2004, 2009a). The largest prospective study evaluating cardiac arrhythmia did not show difference in the frequency of arrhythmias in the acromegaly patients in comparison with the control group (Warszawski et al. 2016). Heart valve disease is observed in advanced stages of the disease and mainly affects the aortic and mitral valves (Colao et al. 2003). Increased diameter of the aortic root may be detected, which can lead to aortic regurgitation (Casini et al. 2011).

Acromegaly is associated with several factors that increase cardiovascular risk, like insulin resistance, DM, endothelial dysfunction, dyslipidemia (mainly hypertriglyceridemia), increased fibrinogen and plasminogen activator factor, microalbuminuria, and sleep apnea; however, the risk of coronary heart disease does not seem to be increased (Colao et al. 2004; Dos Santos Silva et al. 2015b).

The prevalence of impaired glucose tolerance is up to 46% and of DM ranges from 19 to 56% (Chanson et al. 2009; Correa et al. 2008). Insulin resistance is the main factor associated with the change of glucose metabolism (Baldelli et al. 2003; Chanson et al. 2009).

There is an increase in the reported incidence of some malignant tumors in acromegaly, especially colon and thyroid, but it is not established if there is increased mortality from malignant neoplasm (Melmed et al. 2013; Rokkas et al. 2008). Excess GH alter the progression of preexisting neoplasias, especially colorectal, which is the most common malignancy associated with acromegaly (Melmed et al. 2013). There is also a higher incidence of adenomatous intestinal polyps and of

diverticular disease of the colon (Wassenaar et al. 2010). A colonoscopy is indicated at diagnosis for all patients (Katznelson et al. 2014). The follow-up depends on the result of the first colonoscopy and if acromegaly activity persists or not. There is also a greater incidence of thyroid nodules (25–90%) and an increased risk of differentiated thyroid carcinoma (Uchoa et al. 2013). The prevalence of other neoplasms appears to be similar to the general population (Colao et al. 2004).

Hyperprolactinemia occurs in 30% of cases, leading to galactorrhea, menstrual disorders, and hypogonadism and may be due to compression of the pituitary stalk or prolactin co-secretion by the adenoma (Ben-Shlomo and Melmed 2008). Hypopituitarism can be found in 40% of patients as a consequence of compression of the pituitary stalk or the normal pituitary tissue by the adenoma (Melmed et al. 2013). Also, the compression of adjacent structures by the adenoma may lead to visual field loss or hydrocephalus (compression of the third ventricle). Therefore, visual perimetry should be performed in case of optic chiasm compression and pituitary function should be assessed (Katznelson et al. 2014). In the case of invasion of the cavernous sinus, ptosis and impaired eye mobility with variable clinical features may occur. Rhinorrhea by cerebrospinal fluid (CSF) leak can be observed when there is invasion of the sphenoid sinus with floor destruction.

Laboratory and Image Diagnosis

The initial assessment is performed with the measurement of serum IGF-I, as recommended in the last Endocrine Society guideline (Katznelson et al. 2014). In the presence of an elevated IGF-I level for age, an oral glucose tolerance test (OGTT) must be performed with administration of 75 g of anhydrous glucose and GH measurement every 30 minutes for 2 h. Failure to suppress GH levels below 1 μg/L confirms the diagnosis. Attention should be given to the factors that might induce errors in the interpretation of the test (Table 2). Stimulatory tests with thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), or GHRH to show anomalous GH response are not currently employed and do not provide additional benefit (Giustina et al. 2010).

After the laboratory diagnosis, a magnetic resonance imaging (MRI) of the sella turcica should be requested to allow the identification and characterization of the adenoma. Features such as location, size, extrasellar invasion, and relationship with structures adjacent to the sella (optic chiasm, cavernous sinus) are important for the therapeutic management (Katznelson et al. 2014; Melmed et al. 2009). Computed tomography of the sella turcica provides less accurate data, but should be used in case of contraindication of MRI, such as use of a pacemaker. The suspected ectopic production of GHRH occurs in the presence of laboratory diagnosis of acromegaly and an MRI suggestive of pituitary hyperplasia (Vieira Neto et al. 2011). In such cases, the measurement of serum GHRH can be useful.

The biochemical diagnosis of gigantism can be difficult during puberty, as serum IGF-I levels are increased during this period and lack of GH suppression on the

Table 2 Factors that can influence GH and IGF-I levels

False positive (GH measurement)	False positive (IGF-I measurement)	False negative (IGF-I measurement)
Uncontrolled DM	Hyperthyroidism	Uncontrolled DM
Malnutrition	Pregnancy	Malnutrition
Liver failure	Puberty	Liver failure
Renal failure		Renal failure
Anorexia nervosa		Anorexia nervosa
Oral estrogen therapy		Oral estrogen therapy
Hyperthyroidism		Hypothyroidism
Puberty		
Pregnancy		

GH growth hormone, *IGF-I* insulin-like growth factor type I, *DM* diabetes *mellitus*

OGTT may occur. In addition, pituitary hyperplasia may be seen during puberty and it may be difficult to differentiate hyperplasia from an adenoma on MRI. Clinical judgment is, therefore, essential in these challenging cases.

Histopathological Diagnosis

After surgery the surgical specimen is stained with hematoxylin-eosin to confirm the presence of an adenoma or, in very rare cases, of somatotroph hyperplasia. In addition, immunostaining for all pituitary hormones is performed to confirm the GH positivity and to check if the tumor co-secretes prolactin and, more rarely, other hormones (Lloyd et al. 2004). In addition, the evaluation of the Ki-67 and p53 expression is recommended in all pituitary adenomas, as a Ki-67 labeling index higher than 3% and an extensive p53 staining may indicate tumors with more aggressive behavior (Lloyd et al. 2004).

Another important analysis in the histopathological diagnosis is the granulation pattern, that allows classifying the tumors in densely or sparsely granulated tumors, which has clinical implications, as previously mentioned. This classification is done by the evaluation of the cytokeratins 7 and 8 expression pattern (perinuclear for the densely granulated tumors and "dot-like" for the sparsely granulated tumors), analyzed by staining with the CAM5.2 antibody (Obari et al. 2008).

In some centers, additional analysis can be done, like the expression of the SSTRs and of the AIP protein that can help guide the medical treatment. The expression of SSTRs can also be evaluated by molecular biology (quantitative real-time polymerase chain reaction – QPCR).

Genetic Diagnosis

Although the pathogenesis of somatotropinomas is unknown in the majority of the cases, in the presence of a clinical diagnosis of MEN-1, CNC, or 3PAs, the genetic search for the specific mutations described in the Sect. Etiology should be

performed. In the absence of a syndromic phenotype, a genetic screening for *AIP* mutation is recommended in the presence of a familial disease or in young patients with apparently sporadic disease (Korbonits et al. 2012). Mutations in the *AIP* gene should be screened in all patients diagnosed below the age of 18 (therefore in all patients with gigantism) and also in patients diagnosed below 30 years of age, who harbor a macroadenoma (Korbonits et al. 2012). In those patients with very-early onset gigantism, the search for the Xq26.3 microduplication should be performed (Trivellin et al. 2014).

Treatment

The goals of the treatment are to achieve safe GH and normal IGF-I levels according to age, to control the tumor mass effect, and to maintain a normal pituitary function, in order to reduce the morbidity and to normalize the mortality associated with the disease (Katznelson et al. 2014).

Also important, is the control of the diverse comorbidities associated with GH excess, as it can impact in the quality of life and in the survival of these patients (Melmed et al. 2013).

Three therapeutic modalities are available for the treatment of acromegaly and gigantism: surgery, medical therapy, and radiotherapy (Melmed et al. 2009). Considering the high complexity of the treatment, with various treatment options and the rarity of the disease, the management of patients with acromegaly and gigantism should be performed in reference centers and by a multidisciplinary team of neuroendocrinologists, neurosurgeons, neuropathologists, neuroradiologists, and radiotherapists.

Surgery

Surgery is the only treatment of acromegaly that allows immediate cure. When performed by experienced neurosurgeons (those who perform at least 50 pituitary surgeries/year), it has also low complication rates (Katznelson et al. 2011; Nomikos et al. 2005). Thus, it is considered the first choice therapy in the treatment of acromegaly in the majority of the cases (Katznelson et al. 2014). Another advantage of the surgery is to provide tumor tissue for histological and molecular analysis, which allows a better characterization of the tumor and hence more appropriate adjuvant treatment when cure is not achieved (Katznelson et al. 2014).

Surgery is usually performed via transsphenoidal (microscopic or endoscopic), but the transcranial approach can be rarely necessary, especially in large tumors with significant suprasellar expansion. In young onset gigantism, the lack of a sphenoid sinus aeration may difficult surgery via transsphenoidal route (Flitsch et al. 2000). The use of modern techniques, such as neuronavigation, increases the safety of the procedure (Buchfelder and Schlaffer 2010). Although endoscopic surgery allows a better view of the sellar region, theoretically allowing more extensive resections of

tumors that spread to the cavernous sinus, comparative studies with the microscopic technique did not show higher cure rates to date (Fathalla et al. 2015; Starke et al. 2013).

Indications

- Primary treatment for all patients except in the presence of:
  - Unacceptable surgical risk
  - Patient refusal
  - Unresectable adenoma (almost all tumor located within the cavernous sinus)

Efficacy

In reference centers, the cure rates for microadenomas and macroadenomas range from 80% to 95% and 40% to 74%, respectively (Melmed et al. 2009). Lesions larger than 2 cm, invasion of surrounding structures (mostly cavernous sinus), and serum GH levels above 50 μg/L appear to be associated with lower success rates (Barkan et al. 2010). Moreover, the chance of cure is proportional to the experience of the neurosurgeon (Barker et al. 2003; Melmed et al. 2009). It is interesting to note that even larger lesions and those invading nearby structures are amenable to complete resection, even though in a smaller proportion of patients (Nomikos et al. 2005).

Until recently, in patients with a low chance of surgical cure, primary treatment with first-generation SA [octreotide LAR (OCT-LAR) or lanreotide autogel (LAN-ATG)] was considered an alternative to surgical treatment (Melmed et al. 2009), since in a prospective multicenter study in which 104 treatment-naïve patients were randomized to surgery or OCT-LAR treatment, there was no difference in GH (<2.5 μg/L) and IGF-I normalization rates between the groups (39% and 27% by surgery and OCT-LAR, respectively, $p = 0.39$) after 48 weeks of treatment (Colao et al. 2009b). However, in a recent meta-analysis of the literature, comparing studies including surgery as primary treatment and those that used first-generation SA as the primary treatment, the control rate (GH < 1.0 μg/L and normal IGF-I level for age) with surgery was higher than with drug treatment (67% vs. 45%, respectively, $p = 0.02$) (Abu Dabrh et al. 2014). In addition, control rates with first-generation SA are higher in adjuvant treatment than in primary treatment (Katznelson et al. 2014). Based on these data, the last Endocrine Society guideline does not recommend surgery as primary treatment only in cases that almost all the tumor is unresectable or for patients who do not accept surgery or have unacceptable surgical risk (Katznelson et al. 2014).

The use of first-generation SA for a short period of time (3–6 months) before surgery has been proposed in an attempt to increase the chance of surgical cure (Carlsen et al. 2008, 2011). Although some short-term studies showed benefit, this was not confirmed in long-term studies and seems to be true only at centers whose surgical cure rates are lower in relation to that described at reference centers (Carlsen et al. 2008; Fougner et al. 2014; Pita-Gutierrez et al. 2013). Considering these data, the preoperative use of first-generation SA should not be recommended routinely as

it delays the surgery and increases treatment costs without having a well-established benefit (Katznelson et al. 2014). Preoperative treatment could be considered in clinically decompensated patients, to improve the clinical conditions for surgery (e.g., reduce swelling of airways to facilitate intubation), but this is not yet fully established in the literature (Annamalai et al. 2013).

In the presence of GH nadir on OGTT less than 1.0 μg/L and normal IGF-I levels for age 1 month after surgery, the patient is considered cured (Katznelson et al. 2014). If IGF-I levels persist elevated, a reevaluation after 3 months should be made, since IGF-I normalization can take this time (Giustina et al. 2010). Cured patients should be reassessed at 3 and 6 months and annually thereafter as recurrence can occur up to 10–15 years, although it is very rare (0.4%) (Barkan et al. 2010). MRI of the sellar region should be performed 3 months after surgery (Katznelson et al. 2014).

Complications of Surgery

Considering the transsphenoidal surgeries, postoperative mortality is low (<1%) and complications are inversely correlated with the neurosurgeon's experience; the most common are transient diabetes *insipidus* (DI) (20–30%) or permanent DI (2–7%), syndrome of inappropriate secretion of antidiuretic hormone (SIADH) (10–20%), sinusitis (8%), CSF leak (5%), epistaxis (2%), meningitis (1%), oculomotor palsy, visual loss, and carotid injury (rare) (Fathalla et al. 2015; Nomikos et al. 2005).

It is necessary to monitor the water balance up to 2 weeks after surgery, as SIADH can occur in up to 20% of patients and transient DI in up to 30% of patients (Jahangiri et al. 2013; Kristof et al. 2009). The syndrome of inappropriate secretion of antidiuretic hormone is manifested by hyponatremia, whose clinical picture is nonspecific and may be confused with adrenal insufficiency (appetite loss, nausea, dizziness, decreased level of consciousness). Hyponatremia is most common in the first 48 h after surgery, but a second peak of incidence of symptomatic hyponatremia occurs 7–8 days after surgery (Jahangiri et al. 2013).

Medical Treatment

Three drug classes are available for treatment of acromegaly: SA, dopamine agonists (AD), and GH receptor antagonists. The drugs in each class and the doses used are described in Table 3.

Somatostatin Analogs

First-Generation Somatostatin Analogs

The first-generation SA (OCT-LAR and LAN-ATG) bind with greater affinity to SSTR2 and are considered the mainstay of medical treatment (Katznelson et al. 2014; Taboada et al. 2008; Wildemberg et al. 2013).

Table 3 Drug classes available for treatment of acromegaly

Drug class	Drugs	Presentations	Doses (initial – maximum)
First-generation somatostatin analogs	Octreotide LAR	10, 20, and 30 mg for intramuscular administration	20–30[a] mg 4/4 weeks
	Lanreotide autogel	60, 90, and 120 mg for deep subcutaneous administration	90–120 mg 4/4 weeks
Next-generation somatostatin analogs	Pasireotide LAR	20, 40, and 60 mg for intramuscular administration	40–60 mg 4/4 weeks
Dopamine agonists	Cabergoline	0.5 mg pills	1.5–3.5 mg/week
GH antagonist	Pegvisomant	10, 15, and 20 mg for subcutaneous administration	10–30 mg/day

[a]In some countries the maximal approved dosage is 40 mg

Indications

- Adjuvant therapy in patients not cured by surgery
- Primary therapy in patients in whom complete surgical resection is highly unlikely (almost all tumor located in the parasellar region) in the absence of neuro-ophthalmological symptoms
- Patients with high surgical risk and/or who refuse surgery

Efficacy

The biggest meta-analysis of the literature show control rates for GH (<2.5 μg/L) and for IGF-I of 57% and 67%, respectively, in 612 patients using OCT-LAR (Freda et al. 2005). However, about 80% of the patients included in the analyzed studies were preselected based on the response to previous treatment with subcutaneous octreotide. Therefore, it is possible that a selection bias of included studies have influenced the outcome, overestimating the percentage of control (Colao et al. 2016). This is corroborated by the data found in prospective multicenter studies in which acromegaly control rate was approximately 20–30% using first-generation SA (Colao et al. 2009b; Mercado et al. 2007). There is no major difference in the efficacy between the two long-acting SA (OCT-LAR and LAN-ATG) considering the literature until the present date (Tutuncu et al. 2012).

In patients who were treated primarily with SA but not controlled, a surgical debulking of the resectable tumor may allow disease control in patients with partial response to SA and is a therapeutic option in these cases (Jallad et al. 2007).

Reduction of tumor volume larger than 20% is observed in 66% of patients treated with OCT-LAR, with an average reduction of 51% and 63% of patients treated with LAN-ATG with an average reduction of 27% (Caron et al. 2014; Giustina et al. 2012). The assessment of tumor volume should be performed every 6–12 months during treatment, with increased interval (2–3 years) in patients with controlled disease.

In patients with gigantism, a higher frequency of *AIP* mutations is observed (Rostomyan et al. 2015). These patients have a lower chance of disease control with first-generation SA (Daly et al. 2010).

Doses and Methods of Administration

The initial dose of OCT-LAR is 20 mg intramuscularly and of LAN-ATG is 90 mg by deep subcutaneous (SC) route, every 4 weeks, with the first reevaluation of treatment being performed after three drug applications (immediately before the fourth application). With LAN-ATG some studies demonstrated the possibility of self-administration or application by patient's relatives without loss of efficacy (Bevan et al. 2008; Salvatori et al. 2010).

Treatment control should be done only with the measurement of random GH and IGF-I levels. The measurement of GH levels during OGTT is not recommended because discordant results compared to IGF-I are found in about half of the patients and because glucose suppression of GH release is somatostatin mediated (Carmichael et al. 2009).

If disease control is not obtained, the dose should be increased to 30 mg in the case of OCT-LAR and 120 mg in the case of LAN-ATG. In patients controlled after the first evaluation, the initial dose can be maintained and in those whose IGF-I levels are below the lower limit of normal range, the dose of OCT-LAR can be reduced to 10 mg and that of LAN-ATG to 60 mg. Alternatively, the interval between applications of OCT-LAR and LAN-ATG can be extended to 6 or 8 weeks (Lorcy et al. 2000; Neggers et al. 2015).

Although 30 mg every 4 weeks is conventionally the maximum dose of OCT-LAR, few studies have evaluated the use of higher doses or smaller intervals between applications (Colao et al. 2007; Giustina et al. 2009). The 40 mg dose of OCT-LAR is authorized in some countries. One study has evaluated larger doses (60 mg) showing higher efficacy than the 30 mg dose, while there was no benefit in the shortest interval (3 weeks) group (Giustina et al. 2009). However, despite these results, the use of higher SA doses is not recommended and requires more data in the literature.

Some patients may have discordant GH and IGF-I levels during follow-up (up to 35% of the sample in some series) (Machado et al. 2008). Performing the measurements always with the same methodology and standardization of laboratory values are actions that help to reduce this finding. Alternatively, a GH profile may be performed (measurements every 30 minutes for 2 h) with a mean GH value below 1.0 μg/L indicating biochemical control. If the discordant values are still present, it is important to base the conduct in the clinical evaluation of the patient (Melmed et al. 2009).

Treatment with SA in principle should be maintained indefinitely, but in some recent studies, the possibility of SA withdrawal was evaluated in patients with controlled disease, especially with low doses of medication and approximately 22% of the patients remained in remission after drug suspension (Hatipoglu et al. 2015; Ramirez et al. 2012; Ronchi et al. 2008; Vilar et al. 2014). However, in a prospective multicenter study the remission rate was only 5% (Casagrande et al.

2017) and, therefore, there is no evidence to support a systematic suspension of the SA in controlled patients.

Side Effects

Adverse effects are generally mild and transient. Gastrointestinal disorders (flatulence, increased intestinal transit, nausea) are the most frequent, occurring in approximately 50% of the patients. Asymptomatic cholelithiasis occurs in approximately 15% of the patients. An upper abdominal ultrasound should be performed before starting treatment, which should be repeated in case of symptoms suggestive of biliary disease (Katznelson et al. 2014). Other side effects that are also described are transient hair loss, pain at the injection site, central hypothyroidism, and asymptomatic sinus bradycardia (Fatti et al. 2006; Mercado et al. 2007).

A deleterious effect on glucose metabolism can also occur through inhibition of pancreatic insulin secretion (Baldelli et al. 2003). In up to 15% of the patients alterations in glucose metabolism are observed; however, these are unpredictable because many patients benefit from the reduction of GH levels, with reduction of blood glucose levels (Correa et al. 2008).

Next-Generation Somatostatin Analogs

Pasireotide is a next-generation SA with binding potential for all SSTR, except SSTR4 (Hofland et al. 2004). Its long-acting formulation (pasireotide LAR) is approved for acromegaly treatment in many countries.

Indications

– Resistance to first-generation SA treatment (the indication may change to primary adjuvant therapy in the future with the development of robust predictors of response to first-generation SA and pasireotide)

Efficacy

Two large prospective multicenter studies evaluated treatment with pasireotide-LAR in acromegaly (Colao et al. 2014; Gadelha et al. 2014). In the first study, 358 drug-naive patients were randomized to treatment with pasireotide LAR 40 mg or OCT-LAR 20 mg, with the possibility of pasireotide LAR dose adjustment to 60 mg and of OCT-LAR to 30 mg (Colao et al. 2014). After 12 months of treatment, 36% of the patients in the pasireotide group had disease control compared to only 21% of the OCT-LAR group ($p = 0.007$) (Colao et al. 2014).

A second study included 198 patients resistant to treatment with first-generation SA, which were randomized into three groups: one group continued treatment with first-generation SA and two other groups were treated with pasireotide LAR 40 or 60 mg (Gadelha et al. 2014). After 24 weeks of treatment, control rates were 0%, 15%, and 20% for the first-generation SA, pasireotide LAR 40 mg and 60 mg groups, respectively (Gadelha et al. 2014). Thus, it was shown that pasireotide LAR may be an option for patients resistant to treatment with first-generation SA.

Tumor volume reduction greater than 20% in naive patients was observed in 81% of patients prospectively followed up for 12 months (Colao et al. 2014). No patient had significant tumor growth in the same study.

Doses and Methods of Administration

Pasireotide LAR should be administered intramuscularly and the starting dose is 40 mg every 4 weeks. Control of treatment should be done after the third application (immediately before the fourth application). In case of no disease control, the dose should be increased to 60 mg every 4 weeks. If there is a reduction in IGF-I levels to below the lower limit of normal range for the age group, the dose may be reduced to 20 mg.

Side Effects

Pasireotide LAR has the same side effects profile of first-generation SA, the only exception being the effect on glucose metabolism (Colao et al. 2014; Gadelha et al. 2017b).

Studies in healthy volunteers have shown that pasireotide inhibited insulin secretion with higher potency than the first-generation SA without altering its sensitivity, but also has an important effect on reducing incretin secretion and has less potency in inhibiting glucagon secretion (Henry et al. 2013). These effects were responsible for a higher incidence and severity of hyperglycemia in clinical studies with pasireotide LAR than previously observed with first-generation SA. In the study that included drug-naive patients, 57.3% of patients had side effects related to hyperglycemia in the pasireotide LAR group and 21.7% in the OCT-LAR group (Colao et al. 2014). Nine patients had to discontinue treatment with pasireotide LAR due to hyperglycemia. However, although it can be severe, increased blood glucose in most cases are mild to moderate, with an increase in HbA1c compared to baseline in this study of 0.87%, 0.64%, and 0.75% in patients previously diabetic, prediabetic, and those who had normal blood glucose levels, respectively (Colao et al. 2014).

Considering these data, it is recommended to monitor blood glucose, even in patients with normal blood glucose levels prior to treatment, especially in the first 3 months, because most of the patients that present hyperglycemia will present it in the beginning of the treatment. In the case of elevated blood glucose, treatment with metformin is started, as acromegaly is associated with increased insulin resistance, and if the blood glucose remains high, the best option would be to add a dipeptidyl peptidase-4 (DPP-4) inhibitor or a glucagon-like peptide 1 (GLP-1) analog (Wildemberg and Gadelha 2016).

Dopamine Agonists

From the currently available DA, only cabergoline (CAB) should be used in the treatment of acromegaly due to the low efficacy of disease control with bromocriptine (normalization of IGF-I in less than 10% of the patients) (Katznelson et al. 2014). Cabergoline has a longer half-life, more stable serum levels, and higher affinity to the dopamine receptor 2 (DR2) (Sandret et al. 2011).

Indications

- Adjuvant therapy in patients not cured by surgery that present mildly elevated serum GH and IGF- I levels [up to 2× the upper limit of normal (ULN)] (Giustina et al. 2014).
- In association with first-generation SA, especially in patients inadequately controlled with maximum SA dose that exhibit low levels of GH (<4.0–5.0 μg/L) and IGF-I (<2.2×ULN) (Mattar et al. 2010; Vilar et al. 2011).

Efficacy

In a meta-analysis of the literature, normalization of IGF-I was obtained in 34% of the patients and serum GH levels below 2.5 μg/L in 48% of patients with adjuvant cabergoline monotherapy (Sandret et al. 2011). The IGF-I reduction positively correlated with the dose of CAB, duration of treatment, and the presence of hyperprolactinemia and negatively correlated with baseline IGF-I levels. However, some studies included had preselection of patients (including patients who previously responded to bromocriptine), which may have overestimated the effectiveness of cabergoline (Kasuki et al. 2014). Studies evaluating the efficacy of CAB in adjuvant monotherapy in "real life" show much lower efficacy, as for example a recent study that showed control in 18% of the patients (Vandeva et al. 2015).

Cabergoline appears to be less effective than the SA; however, it can be considered in selected patients with mild to moderate disease (IGF-I up to 2×ULN), because it is an orally administered drug and costs less than the other two drug classes (Giustina et al. 2014).

In the same previously mentioned meta-analysis five studies (77 patients) that analyzed the combination therapy of CAB with first-generation SA were included. The normalization of IGF-I levels was obtained in 52% of the patients (Sandret et al. 2011). In two Brazilian prospective studies published later, the IGF-I normalization rate was approximately 40%, with better results in patients who had IGF-I levels up to 2.2×ULN and GH levels lower than 4.0–5.0 μg/L before combination treatment (Mattar et al. 2010, Vilar et al. 2011).

It is important to note that neither the presence of positive immunostaining for prolactin in the somatotropinoma nor elevated serum prolactin levels are predictors of response to combination therapy with CAB and should not be used as criteria to select patients (Sandret et al. 2011).

In terms of tumor size, decrease in tumor volume (>20%) was observed in 34% of patients in the adjuvant monotherapy and correlated with the presence of hyperprolactinemia and higher levels of IGF-I (Sandret et al. 2011).

Data regarding treatment with CAB are based on small studies, being the majority retrospective and in a meta-analysis, which makes impossible to accurately estimate the efficacy of this drug in the treatment of acromegaly (Kasuki et al. 2014).

Dose and Methods of Administration

The minimum recommended dose for the treatment of acromegaly is 1.5 mg/week that should be achieved with gradual increments of the dosage in the first weeks. The

drug should be orally administered after a meal, preferably after dinner. Monitoring of GH and IGF-I levels should be monthly, with gradual dose escalation in patients not controlled, to a maximum dose of 3.5 mg/week.

Side Effects

Cabergoline intolerance has been reported in only 3–4% of patients and the most common side effects include nausea, headache, dizziness, constipation, dry mouth, nasal congestion, and postural hypotension (Cook 2005). The major concern with the chronic use of this drug is the risk of valve regurgitation that has been described with the use of high doses for the treatment of Parkinson's disease (>3 mg/day) (Schade et al. 2007; Zanettini et al. 2007). The doses used in the treatment of acromegaly are therefore much smaller and no studies using the recommended dose for the treatment of acromegaly showed an increased risk of valve regurgitation, to date (Auriemma et al. 2015). Despite the lack of evidence of valve lesion in patients taking the CAB doses used for the treatment of acromegaly, it is recommended to carry out a baseline echocardiogram and annually during follow-up in patients using doses higher than 2 mg/week (Katznelson et al. 2014).

GH Receptor Antagonists

Pegvisomant (PEG) is the only medication of this drug class currently available (Giustina et al. 2017).

Indications

– As it acts peripherally in GH receptors and has no action in the tumor, PEG is not indicated as primary drug therapy being reserved for cases of failure of surgery and treatment with first-generation SA, either alone or in combination with other drug classes (Katznelson et al. 2014; Melmed et al. 2009).

Efficacy

Initial clinical trials with PEG alone showed greater than 90% efficacy in normalizing IGF-I in doses 10–40 mg/day (Trainer et al. 2000; van der Lely et al. 2001). However, a recent analysis of the Acrostudy data, which is an international observational database, showed normalization of IGF-I levels in only 63% of the cases (a total 1288 patients) (van der Lely et al. 2012). One possible explanation is the failure to properly scale the dose of medication, as evidenced by average daily dose of 20 mg in patients with high IGF-I levels. As PEG has a long half-life of 70 h some groups have proposed weekly administration of the drug in an attempt to improve adherence to treatment (Higham et al. 2009). In patients previously controlled with daily application, it was possible to reduce the dose of PEG in a portion of patients after trading the scheme for weekly administration, maintaining the biochemical control (Higham et al. 2009; Neggers et al. 2011).

In addition to monotherapy, PEG can be used in combination with first-generation SA with efficacy greater than 95% (Franck et al. 2015; Neggers et al. 2014). The combined treatment has the great advantage to act also in the tumor, reducing the risk

of tumor growth and is superior to the use of SA as monotherapy in relation to the carbohydrate metabolism (Neggers et al. 2014). The major concern with combination therapy is the incidence of hepatotoxicity, which occurs in about 15% of patients, but is mild in most cases (Neggers and van der Lely 2011). It is important to exclude cholestasis (because of the SA use) before correlating hepatotoxicity to PEG.

The PEG and CAB association has also been described in two studies in the literature with small numbers of patients (24 and 14 patients), being superior to the use of PEG isolated in the patients studied (Bernabeu et al. 2013; Higham et al. 2012).

Doses and Methods of Administration

The initial dose should be 10 mg/day subcutaneously with adjustment based only on IGF-I serum levels which should be measured 4–6 weeks after initiation of treatment. GH should not be used in monitoring, because there is no reduction in its release and because cross-reaction with PEG can occur in some GH assays, depending on the method used (Paisley et al. 2007). If normalization of IGF-I levels is not obtained, increments in the dose of 5 mg/day every 4–6 weeks (up to a maximum dose of 30 mg/day) should be performed, in order to reach IGF-I levels adjusted to age between 0 and +2 standard deviations. This way, it is possible to avoid the GH deficiency development that can occur if the IGF-I level is near the lower limit of normal. After stabilization of the dose, IGF-I levels can be measured every 3–6 months and sellar MRI should be requested after 6 months of starting PEG and annually thereafter.

Side Effects

One of the main concerns at the beginning of the clinical use of PEG was the increase in tumor volume, since there is blockage of peripheral GH action and no direct action on the tumor. However, this effect has not been observed in most studies (Buhk et al. 2010; van der Lely et al. 2012). Increase in tumor size possibly related to the PEG is found in only about 3% of patients, number similar to that found with other treatments, such as with first-generation SA (Tutuncu et al. 2012). The absence of radiation therapy and short-term therapy with SA prior to the use of PEG appear to be associated with increased risk of tumor growth. Therefore, considering the data available in the literature to date, treatment with PEG does not appear to be related to increased risk of tumor growth.

Hepatotoxicity is the major side effect of PEG, affecting about 2% of patients in monotherapy (van der Lely et al. 2001). However, elevation of liver enzymes is usually mild and transient, occurring in most cases in the first year of treatment (mainly in the first 3 months), and is independent of the dose (van der Lely et al. 2012). Usually, there is normalization of the transaminases even with the maintenance of treatment. Monitoring of transaminases should be performed in all patients and should be monthly for the first 6 months in patients with normal transaminases prior to treatment and then biannually (keep monthly monitoring for a year in patients with pretreatment transaminase elevations up to 3×ULN) (Vieira Neto

et al. 2011). In the case of transaminases elevation up to 3×ULN, the treatment can be maintained.

Another possible side effect is lipohypertrophy that occurs at the medication application site, as PEG can completely block the GH action at that location, allowing the lipogenic activity of insulin. This can be minimized by performing rotation of the application sites (van der Lely et al. 2012).

Temozolomide

Temozolomide is an oral alkylating agent that was used for the first time in 2006 to treat a pituitary carcinoma (Lim et al. 2006). After the first description it has been used in other pituitary carcinomas, but also in aggressive pituitary adenomas (Losa et al. 2016). Nine acromegaly patients treated with temozolomide have been described in the literature, with good response in three (33%) (Bengtsson et al. 2015; Losa et al. 2016; McCormack et al. 2011).

Temozolomide is well tolerated, but can lead to significant side effects, like severe neutropenia that can be observed in up to 14% of the patients (Villano et al. 2009). Therefore, it is reserved for aggressive tumors that are resistant to conventional acromegaly treatments (surgery, medical therapy with SA, CAB and PEG, and radiotherapy).

Radiotherapy

Radiation therapy is an effective therapeutic modality to control the disease, but its use is restricted for safety due to potential for serious side effects in the medium- and long-term follow-up, and especially due to the availability of other safer treatment modalities (Katznelson et al. 2014, Melmed et al. 2009).

Indication

– Third-line therapy: indicated for tumors not controlled with surgical and medical treatments (Katznelson et al. 2014).

Efficacy

Stereotactic radiation (directed) should be preferred unless only the conventional technique is available (Katznelson et al. 2014). Stereotactic radiotherapy may be applied in a few doses (radiosurgery – single dose) or fractionated (Minniti et al. 2011).

Radiotherapy with any technic allows control of tumor volume in more than 95% of the cases (Abu Dabrh et al. 2015; Lee et al. 2014). The biochemical control rates vary widely among studies, and are influenced by several factors, such as follow-up, since the effect of radiation therapy can take up to 20 years to occur (Abu Dabrh et al. 2015; Minniti et al. 2011). In a recent meta-analysis of the literature, the control rate with radiosurgery was 52% and with conventional fractionated radiotherapy it was 36% (Abu Dabrh et al. 2015).

Since the effect of radiation can take years, there is need for drug therapy until there is hormonal control. Periodic withdrawal of medication for evaluation of cure is recommended (Katznelson et al. 2014).

Side Effects

Side effects are the main limiting factors of radiation therapy, with hypopituitarism being the most common, affecting up to 80% of patients with the conventional technique after 15 years follow-up (Katznelson et al. 2011). The frequency appears to be lower with stereotactic techniques, but the follow-up with these techniques is smaller (Abu Dabrh et al. 2015). Annual assessment of pituitary function for long periods is required (Katznelson et al. 2011, 2014).

Besides hypopituitarism, damage of optic pathways or cranial nerves, cognitive impairment, cerebrovascular disease, and secondary malignancies may occur (Minniti et al. 2005, 2011). These adverse effects are best documented with the conventional technique, but it should be noted again that the follow-up with stereotactic techniques is shorter, thus it is not possible to date to assess the real risk of these complications with these more modern technics.

Future Perspectives

To date, the recommendations for the treatment of acromegaly are based on a "trial and error" approach, with generalized recommendations for all patients. Considering the particular characteristics of the different types of adenomas that can cause acromegaly or gigantism, which have treatment implications, and the range of choices now available for the treatment of acromegaly and gigantism, in the future, the treatment of these diseases will probably adopt a personalized approach based on biomarkers.

Several biomarkers of response to first-generation SA have been proposed, with better response observed in tumors with high expression of SSTR2 and AIP, densely granulated tumors, low expression of Ki-67, and hypointense signal on T2-weighted sequence MRI (Gadelha et al. 2013a; Giustina et al. 2017). However, to date, none of these markers alone have an accuracy that allows choosing other drugs as opposed to first-generation SA as the primary choice of treatment. In addition, there are no robust markers of response to CAB, PEG, or pasireotide LAR. With the ongoing advancement of translational medicine, it is expected that in the future, treatment of acromegaly will be individualized so that it will be possible to indicate the most appropriate treatment for a particular patient and at the ideal time (Gadelha 2015).

Summary

Acromegaly and gigantism are chronic diseases characterized by GH hypersecretion and an insidious course, what leads to a delay in its diagnosis. Earlier diagnosis is important to facilitate treatment, with surgery being the treatment of choice. However, in approximately half of the cases an adjuvant treatment will be necessary, with first-generation SA still being considered the mainstay of treatment for the majority of patients. Nevertheless, there are currently other treatment options (monotherapy or combination therapy) like CAB, pasireotide LAR, and PEG, and ideally the

development of biomarkers will allow choosing the right drug for the right patient at the right moment.

Cross-References

► Physiology of the Hypothalamus Pituitary Unit

References

Abu Dabrh AM, Mohammed K, Asi N, Farah WH, Wang Z, Farah MH, et al. Surgical interventions and medical treatments in treatment-naive patients with acromegaly: systematic review and meta-analysis. J Clin Endocrinol Metab. 2014;99(11):4003–14.

Abu Dabrh A, Asi N, Farah W, Mohammed K, Wang Z, Farah M, et al. Radiotherapy vs. radiosurgery in treating patients with acromegaly: systematic review and meta-analysis. Endocr Pract. 2015;18:1–33.

Annamalai AK, Webb A, Kandasamy N, Elkhawad M, Moir S, Khan F, et al. A comprehensive study of clinical, biochemical, radiological, vascular, cardiac, and sleep parameters in an unselected cohort of patients with acromegaly undergoing presurgical somatostatin receptor ligand therapy. J Clin Endocrinol Metab. 2013;98(3):1040–50.

Auriemma RS, Pivonello R, Ferreri L, Priscitelli P, Colao A. Cabergoline use for pituitary tumors and valvular disorders. Endocrinol Metab Clin N Am. 2015;44(1):89–97.

Baldelli R, Battista C, Leonetti F, Ghiggi MR, Ribaudo MC, Paoloni A, et al. Glucose homeostasis in acromegaly: effects of long-acting somatostatin analogues treatment. Clin Endocrinol. 2003;59(4):492–9.

Barkan A, Bronstein MD, Bruno OD, Cob A, Espinosa-de-los-Monteros AL, Gadelha MR, et al. Management of acromegaly in Latin America: expert panel recommendations. Pituitary. 2010;13(2):168–75.

Barker FG 2nd, Klibanski A, Swearingen B. Transsphenoidal surgery for pituitary tumors in the United States, 1996–2000: mortality, morbidity, and the effects of hospital and surgeon volume. J Clin Endocrinol Metab. 2003;88(10):4709–19.

Baumann G. Growth hormone heterogeneity: genes, isohormones, variants, and binding proteins. Endocr Rev. 1991;12(4):424–49.

Bengtsson BA, Eden S, Ernest I, Oden A, Sjogren B. Epidemiology and long-term survival in acromegaly. A study of 166 cases diagnosed between 1955 and 1984. Acta Med Scand. 1988;223(4):327–35.

Bengtsson D, Schroder HD, Andersen M, Maiter D, Berinder K, Feldt Rasmussen U, et al. Long-term outcome and MGMT as a predictive marker in 24 patients with atypical pituitary adenomas and pituitary carcinomas given treatment with temozolomide. J Clin Endocrinol Metab. 2015;100(4):1689–98.

Ben-Shlomo A, Melmed S. Acromegaly. Endocrinol Metab Clin N Am. 2008;37(1):101–22.

Bernabeu I, Alvarez-Escola C, Paniagua AE, Lucas T, Pavon I, Cabezas-Agricola JM, et al. Pegvisomant and cabergoline combination therapy in acromegaly. Pituitary. 2013;16(1):101–8.

Bevan JS, Newell-Price J, Wass JA, Atkin SL, Bouloux PM, Chapman J, et al. Home administration of lanreotide autogel by patients with acromegaly, or their partners, is safe and effective. Clin Endocrinol. 2008;68(3):343–9.

Buchfelder M, Schlaffer SM. Modern imaging of pituitary adenomas. Front Horm Res. 2010;38:109–20.

Buhk JH, Jung S, Psychogios MN, Goricke S, Hartz S, Schulz-Heise S, et al. Tumor volume of growth hormone-secreting pituitary adenomas during treatment with pegvisomant: a prospective multicenter study. J Clin Endocrinol Metab. 2010;95(2):552–8.

Carlsen SM, Lund-Johansen M, Schreiner T, Aanderud S, Johannesen O, Svartberg J, et al. Preoperative octreotide treatment in newly diagnosed acromegalic patients with macroadenomas increases cure short-term postoperative rates: a prospective, randomized trial. J Clin Endocrinol Metab. 2008;93(8):2984–90.

Carlsen SM, Svartberg J, Schreiner T, Aanderud S, Johannesen O, Skeie S, et al. Six-month preoperative octreotide treatment in unselected, de novo patients with acromegaly: effect on biochemistry, tumour volume, and postoperative cure. Clin Endocrinol. 2011;74(6):736–43.

Carmichael JD, Bonert VS, Mirocha JM, Melmed S. The utility of oral glucose tolerance testing for diagnosis and assessment of treatment outcomes in 166 patients with acromegaly. J Clin Endocrinol Metab. 2009;94(2):523–7.

Caron PJ, Bevan JS, Petersenn S, Flanagan D, Tabarin A, Prevost G, et al. Tumor shrinkage with lanreotide autogel 120 mg as primary therapy in acromegaly: results of a prospective multicenter clinical trial. J Clin Endocrinol Metab. 2014;99(4):1282–90.

Casagrande A, Bronstein MD, Jallad RS, Moraes AB, Elias PC, Castro M, et al. Long-term remission of acromegaly after octreotide withdrawal is an uncommon and frequently unsustainable event. Neuroendocrinology. 2017;104(3):273–9.

Casini AF, Araujo PB, Fontes R, Xavier SS, Gadelha MR. Cardiac morphology and performance alterations and analysis of determinant factors of left ventricular hypertrophy in 40 patients with acromegaly. Arq Bras Endocrinol Metabol. 2006;50(1):82–90.

Casini AF, Neto LV, Fontes R, Franca RF, Xavier SS, Gadelha MR. Aortic root ectasia in patients with acromegaly: experience at a single center. Clin Endocrinol. 2011;75(4):495–500.

Cazabat L, Bouligand J, Salenave S, Bernier M, Gaillard S, Parker F, et al. Germline AIP mutations in apparently sporadic pituitary adenomas: prevalence in a prospective single-center cohort of 443 patients. J Clin Endocrinol Metab. 2012;97(4):E663–70.

Chanson P, Salenave S, Kamenicky P, Cazabat L, Young J. Pituitary tumours: acromegaly. Best Pract Res Clin Endocrinol Metab. 2009;23(5):555–74.

Colao A, Spinelli L, Marzullo P, Pivonello R, Petretta M, Di Somma C, et al. High prevalence of cardiac valve disease in acromegaly: an observational, analytical, case-control study. J Clin Endocrinol Metab. 2003;88(7):3196–201.

Colao A, Ferone D, Marzullo P, Lombardi G. Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev. 2004;25(1):102–52.

Colao A, Pivonello R, Auriemma RS, Galdiero M, Savastano S, Lombardi G. Beneficial effect of dose escalation of octreotide-LAR as first-line therapy in patients with acromegaly. Eur J Endocrinol. 2007;157(5):579–87.

Colao A, Cappabianca P, Caron P, De Menis E, Farrall AJ, Gadelha MR, et al. Octreotide LAR vs. surgery in newly diagnosed patients with acromegaly: a randomized, open-label, multicentre study. Clin Endocrinol (Oxf). 2009a;70(5):757–68.

Colao A, Auriemma RS, Galdiero M, Lombardi G, Pivonello R. Effects of initial therapy for five years with somatostatin analogs for acromegaly on growth hormone and insulin-like growth factor-I levels, tumor shrinkage, and cardiovascular disease: a prospective study. J Clin Endocrinol Metab. 2009b;94(10):3746–56.

Colao A, Bronstein MD, Freda P, Gu F, Shen CC, Gadelha M, et al. Pasireotide versus octreotide in acromegaly: a head-to-head superiority study. J Clin Endocrinol Metab. 2014;99(3):791–9.

Colao A, Auriemma RS, Pivonello R, Kasuki L, Gadelha MR. Interpreting biochemical control response rates with first-generation somatostatin analogues in acromegaly. Pituitary. 2016;19(3):235–47.

Concolino P, Costella A, Capoluongo E. Multiple endocrine neoplasia type 1 (MEN1): an update of 208 new germline variants reported in the last nine years. Cancer Genet. 2016;209(1–2):36–41.

Cook DM. Long-term management of prolactinomas – use of long-acting dopamine agonists. Rev Endocr Metab Disord. 2005;6(1):15–21.

Correa LL, Taboada GF, Van Haute FR, Casini AF, Balarini GA, Vieira Neto L, et al. Evaluation of glucose metabolism in acromegalic patients before and after treatment with octreotide LAR. Arq Bras Endocrinol Metabol. 2008;52(1):55–64.

Correa R, Salpea P, Stratakis CA. Carney complex: an update. Eur J Endocrinol. 2015;173(4): M85–97.

Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A. High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege. Belgium J Clin Endocrinol Metab. 2006;91(12):4769–75.

Daly AF, Tichomirowa MA, Petrossians P, Heliovaara E, Jaffrain-Rea ML, Barlier A, et al. Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. J Clin Endocrinol Metab. 2010;95(11):E373–83.

Davi MV, Dalle Carbonare L, Giustina A, Ferrari M, Frigo A, Lo Cascio V, et al. Sleep apnoea syndrome is highly prevalent in acromegaly and only partially reversible after biochemical control of the disease. Eur J Endocrinol. 2008;159(5):533–40.

Dekkers OM, Biermasz NR, Pereira AM, Romijn JA, Vandenbroucke JP. Mortality in acromegaly: a metaanalysis. J Clin Endocrinol Metab. 2008;93(1):61–7.

Denes J, Swords F, Rattenberry E, Stals K, Owens M, Cranston T, et al. Heterogeneous genetic background of the association of pheochromocytoma/paraganglioma and pituitary adenoma: results from a large patient cohort. J Clin Endocrinol Metab. 2015;100(3):E531–41.

dos Santos Silva CM, Gottlieb I, Volschan ICM, Kasuki L, Warszawski L, Lima GA, et al. Low frequency of cardiomyopathy using cardiac magnetic resonance imaging in an acromegaly contemporary cohort. J Clin Endocrinol Metab. 2015a;100(12):4447–55.

Dos Santos Silva CM, Lima GA, Volschan IC, Gottlieb I, Kasuki L, Neto LV, et al. Low risk of coronary artery disease in patients with acromegaly. Endocrine. 2015b;50(3):749–55.

Fathalla H, Cusimano MD, Di Ieva A, Lee J, Alsharif O, Goguen J, et al. Endoscopic versus microscopic approach for surgical treatment of acromegaly. Neurosurg Rev. 2015;38(3):541–8.

Fatti LM, Scacchi M, Lavezzi E, Pecori Giraldi F, De Martin M, Toja P, et al. Effects of treatment with somatostatin analogues on QT interval duration in acromegalic patients. Clin Endocrinol. 2006;65(5):626–30.

Fernandez A, Karavitaki N, Wass JA. Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). Clin Endocrinol (Oxf). 2010;72(3):377–82.

Ferone D, Gatto F, Arvigo M, Resmini E, Boschetti M, Teti C, et al. The clinical-molecular interface of somatostatin, dopamine and their receptors in pituitary pathophysiology. J Mol Endocrinol. 2009;42(5):361–70.

Flitsch J, Ludecke DK, Stahnke N, Wiebel J, Saeger W. Transsphenoidal surgery for pituitary gigantism and galactorrhea in a 3.5 year old child. Pituitary. 2000;2(4):261–7.

Fougner SL, Casar-Borota O, Heck A, Berg JP, Bollerslev J. Adenoma granulation pattern correlates with clinical variables and effect of somatostatin analogue treatment in a large series of patients with acromegaly. Clin Endocrinol. 2012;76(1):96–102.

Fougner SL, Bollerslev J, Svartberg J, Oksnes M, Cooper J, Carlsen SM. Preoperative octreotide treatment of acromegaly: long-term results of a randomised controlled trial. Eur J Endocrinol. 2014;171(2):229–35.

Franck SE, Van der Lely AJ, Delhanty PJ, Jorgensen JO, Neggers SJ. Pegvisomant in combination with long-acting somatostatin analogues in acromegaly: the role of the growth hormone receptor deletion of exon 3. Eur J Endocrinol. 2015;173(5):553–61.

Freda PU, Katznelson L, van der Lely AJ, Reyes CM, Zhao S, Rabinowitz D. Long-acting somatostatin analog therapy of acromegaly: a meta-analysis. J Clin Endocrinol Metab. 2005;90(8):4465–73.

Gadelha MR. A paradigm shift in the medical treatment of acromegaly: from a 'trial and error' to a personalized therapeutic decision-making process. Clin Endocrinol. 2015;83(1):1–2.

Gadelha MR, Une KN, Rohde K, Vaisman M, Kineman RD, Frohman LA. Isolated familial somatotropinomas: establishment of linkage to chromosome 11q13.1-11q13.3 and evidence

for a potential second locus at chromosome 2p16-12. J Clin Endocrinol Metab. 2000;85(2):707–14.
Gadelha MR, Kasuki L, Korbonits M. Novel pathway for somatostatin analogs in patients with acromegaly. Trends Endocrinol Metab. 2013a;24(5):238–46.
Gadelha MR, Trivellin G, Hernandez Ramirez LC, Korbonits M. Genetics of pituitary adenomas. Front Horm Res. 2013b;41:111–40.
Gadelha MR, Bronstein MD, Brue T, Coculescu M, Fleseriu M, Guitelman M, et al. Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly (PAOLA): a randomised, phase 3 trial. Lancet Diabetes Endocrinol. 2014;2(11):875–84.
Gadelha MR, Kasuki L, Korbonits M. The genetic background of acromegaly. Pituitary. 2017a;20(1):10–21.
Gadelha MR, Wildemberg LE, Bronstein MD, Gatto F, Ferone D. Somatostatin receptor ligands in the treatment of acromegaly. Pituitary. 2017b;20(1):100–8.
Garby L, Caron P, Claustrat F, Chanson P, Tabarin A, Rohmer V, et al. Clinical characteristics and outcome of acromegaly induced by ectopic secretion of growth hormone-releasing hormone (GHRH): a French nationwide series of 21 cases. J Clin Endocrinol Metab. 2012; 97(6):2093–104.
Giustina A, Bonadonna S, Bugari G, Colao A, Cozzi R, Cannavo S, et al. High-dose intramuscular octreotide in patients with acromegaly inadequately controlled on conventional somatostatin analogue therapy: a randomised controlled trial. Eur J Endocrinol. 2009;161(2):331–8.
Giustina A, Chanson P, Bronstein MD, Klibanski A, Lamberts S, Casanueva FF, et al. A consensus on criteria for cure of acromegaly. J Clin Endocrinol Metab. 2010;95(7):3141–8.
Giustina A, Mazziotti G, Torri V, Spinello M, Floriani I, Melmed S. Meta-analysis on the effects of octreotide on tumor mass in acromegaly. PLoS One. 2012;7(5):e36411.
Giustina A, Chanson P, Kleinberg D, Bronstein MD, Clemmons DR, Klibanski A, et al. Expert consensus document: a consensus on the medical treatment of acromegaly. Nat Rev Endocrinol. 2014;10(4):243–8.
Giustina A, Arnaldi G, Bogazzi F, Cannavo S, Colao A, De Marinis L, et al. Pegvisomant in acromegaly: an update. J Endocrinol Invest. 2017;40(6):577–89.
Gunawardane K, Krarup Hansen T, Sandahl Christiansen J, Lunde Jorgensen JO. Normal physiology of growth hormone in adults. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, et al., editors. Endotext. South Dartmouth (MA); 2000.
Hatipoglu E, Bozcan S, Kadioglu P. Discontinuation of somatostatin analogs while acromegaly is in long-term remission. Pituitary. 2015;18(4):554–60.
Henry RR, Ciaraldi TP, Armstrong D, Burke P, Ligueros-Saylan M, Mudaliar S. Hyperglycemia associated with pasireotide: results from a mechanistic study in healthy volunteers. J Clin Endocrinol Metab. 2013;98(8):3446–53.
Herman V, Fagin J, Gonsky R, Kovacs K, Melmed S. Clonal origin of pituitary adenomas. J Clin Endocrinol Metab. 1990;71(6):1427–33.
Hernandez-Ramirez LC, Gabrovska P, Denes J, Stals K, Trivellin G, Tilley D, et al. Landscape of familial isolated and young-onset pituitary adenomas: prospective diagnosis in AIP mutation carriers. J Clin Endocrinol Metab. 2015;100(9):E1242–54.
Higham CE, Thomas JD, Bidlingmaier M, Drake WM, Trainer PJ. Successful use of weekly pegvisomant administration in patients with acromegaly. Eur J Endocrinol. 2009;161 (1):21–5.
Higham CE, Atkinson AB, Aylwin S, Bidlingmaier M, Drake WM, Lewis A, et al. Effective combination treatment with cabergoline and low-dose pegvisomant in active acromegaly: a prospective clinical trial. J Clin Endocrinol Metab. 2012;97(4):1187–93.
Hofland LJ, van der Hoek J, van Koetsveld PM, de Herder WW, Waaijers M, Sprij-Mooij D, et al. The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas in vitro. J Clin Endocrinol Metab. 2004; 89(4):1577–85.

Holdaway IM, Bolland MJ, Gamble GD. A meta-analysis of the effect of lowering serum levels of GH and IGF-I on mortality in acromegaly. Eur J Endocrinol. 2008;159(2):89–95.
Iacovazzo D, Caswell R, Bunce B, Jose S, Yuan B, Hernandez-Ramirez LC, et al. Germline or somatic GPR101 duplication leads to X-linked acrogigantism: a clinico-pathological and genetic study. Acta Neuropathol Commun. 2016;4(1):56.
Jahangiri A, Wagner J, Tran MT, Miller LM, Tom MW, Kunwar S, et al. Factors predicting postoperative hyponatremia and efficacy of hyponatremia management strategies after more than 1000 pituitary operations. J Neurosurg. 2013;119(6):1478–83.
Jallad RS, Musolino NR, Kodaira S, Cescato VA, Bronstein MD. Does partial surgical tumour removal influence the response to octreotide-LAR in acromegalic patients previously resistant to the somatostatin analogue? Clin Endocrinol. 2007;67(2):310–5.
Kasuki L, Vieira Neto L, Gadelha MR. Cabergoline treatment in acromegaly: cons. Endocrine. 2014;46(2):220–5.
Kato Y, Murakami Y, Sohmiya M, Nishiki M. Regulation of human growth hormone secretion and its disorders. Intern Med. 2002;41(1):7–13.
Katznelson L, Atkinson JL, Cook DM, Ezzat SZ, Hamrahian AH, Miller KK, et al. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the diagnosis and treatment of acromegaly – 2011 update: executive summary. Endocr Pract. 2011;17(4):636–46.
Katznelson L, Laws ER Jr, Melmed S, Molitch ME, Murad MH, Utz A, et al. Acromegaly: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2014;99(11):3933–51.
Kayano K, Takeo M, Morisue S, Yamamoto M, Mizuno Y, Meguro F. A case of human growth hormone (h-GH)-producing adenocarcinoma of the lung. Nippon Kyobu Geka Gakkai Zasshi. 1995;43(4):538–42.
Killinger Z, Payer J, Lazurova I, Imrich R, Homerova Z, Kuzma M, et al. Arthropathy in acromegaly. Rheum Dis Clin N Am. 2010;36(4):713–20.
Korbonits M, Storr H, Kumar AV. Familial pituitary adenomas – who should be tested for AIP mutations? Clin Endocrinol. 2012;77(3):351–6.
Kristof RA, Rother M, Neuloh G, Klingmuller D. Incidence, clinical manifestations, and course of water and electrolyte metabolism disturbances following transsphenoidal pituitary adenoma surgery: a prospective observational study. J Neurosurg. 2009;111(3):555–62.
Kropf LL, Madeira M, Vieira Neto L, Gadelha MR, de Farias ML. Functional evaluation of the joints in acromegalic patients and associated factors. Clin Rheumatol. 2013;32(7):991–8.
Laron Z. Insulin-like growth factor 1 (IGF-1): a growth hormone. Mol Pathol. 2001;54(5):311–6.
Lee CC, Vance ML, Xu Z, Yen CP, Schlesinger D, Dodson B, et al. Stereotactic radiosurgery for acromegaly. J Clin Endocrinol Metab. 2014;99(4):1273–81.
Lim S, Shahinian H, Maya MM, Yong W, Heaney AP. Temozolomide: a novel treatment for pituitary carcinoma. Lancet Oncol. 2006;7(6):518–20.
Lloyd RV, Kovacs K, Young WF Jr, Farrel WE, Asa SL, Trouillas J, et al. Pituitary tumours: introduction. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C, editors. WHO classification of tumours pathology and genetics tumours of endocrine organs. Lyon: IARC Press; 2004. p. 10–3.
Lorcy Y, Dejager S, Chanson P, French Octreotide LARG. Time course of GH and IGF-1 levels following withdrawal of long-acting octreotide in acromegaly. Pituitary. 2000;3(3):193–7.
Losa M, Bogazzi F, Cannavo S, Ceccato F, Curto L, De Marinis L, et al. Temozolomide therapy in patients with aggressive pituitary adenomas or carcinomas. J Neuro-Oncol. 2016;126(3):519–25.
Machado EO, Taboada GF, Neto LV, van Haute FR, Correa LL, Balarini GA, et al. Prevalence of discordant GH and IGF-I levels in acromegalics at diagnosis, after surgical treatment and during treatment with octreotide LAR. Growth Hormon IGF Res. 2008;18(5):389–93.
Madeira M, Neto LV, de Lima GA, Moreira RO, de Mendonca LM, Gadelha MR, et al. Effects of GH-IGF-I excess and gonadal status on bone mineral density and body composition in patients with acromegaly. Osteoporos Int. 2010;21(12):2019–25.
Madeira M, Neto LV, de Paula Paranhos Neto F, Barbosa Lima IC, Carvalho de Mendonca LM, Gadelha MR, et al. Acromegaly has a negative influence on trabecular bone, but not on cortical

bone, as assessed by high-resolution peripheral quantitative computed tomography. J Clin Endocrinol Metab. 2013a;98(4):1734–41.
Madeira M, Neto LV, Torres CH, de Mendonca LM, Gadelha MR, de Farias ML. Vertebral fracture assessment in acromegaly. J Clin Densitom. 2013b;16(2):238–43.
Manara R, Maffei P, Citton V, Rizzati S, Bommarito G, Ermani M, et al. Increased rate of intracranial saccular aneurysms in acromegaly: an MR angiography study and review of the literature. J Clin Endocrinol Metab. 2011;96(5):1292–300.
Marie P. Sur deux cas d'acromégalie: Hypertrophie singulière non congénitale des extremités supérieures, inférieures et céphaliques. Rev Med. 1886;6:297.
Mattar P, Alves Martins MR, Abucham J. Short- and long-term efficacy of combined cabergoline and octreotide treatment in controlling igf-I levels in acromegaly. Neuroendocrinology. 2010;92(2):120–7.
McCormack AI, Wass JA, Grossman AB. Aggressive pituitary tumours: the role of temozolomide and the assessment of MGMT status. Eur J Clin Investig. 2011;41(10):1133–48.
Melmed S. Acromegaly pathogenesis and treatment. J Clin Invest. 2009;119(11):3189–202.
Melmed S, Ezrin C, Kovacs K, Goodman RS, Frohman LA. Acromegaly due to secretion of growth hormone by an ectopic pancreatic islet-cell tumor. N Engl J Med. 1985;312(1):9–17.
Melmed S, Colao A, Barkan A, Molitch M, Grossman AB, Kleinberg D, et al. Guidelines for acromegaly management: an update. J Clin Endocrinol Metab. 2009;94(5):1509–17.
Melmed S, Casanueva FF, Klibanski A, Bronstein MD, Chanson P, Lamberts SW, et al. A consensus on the diagnosis and treatment of acromegaly complications. Pituitary. 2013;16(3):294–302.
Mercado M, Borges F, Bouterfa H, Chang TC, Chervin A, Farrall AJ, et al. A prospective, multicentre study to investigate the efficacy, safety and tolerability of octreotide LAR (long-acting repeatable octreotide) in the primary therapy of patients with acromegaly. Clin Endocrinol. 2007;66(6):859–68.
Mercado M, Gonzalez B, Vargas G, Ramirez C, de los Monteros AL, Sosa E, et al. Successful mortality reduction and control of comorbidities in patients with acromegaly followed at a highly specialized multidisciplinary clinic. J Clin Endocrinol Metab. 2014;99(12):4438–46.
Minniti G, Traish D, Ashley S, Gonsalves A, Brada M. Risk of second brain tumor after conservative surgery and radiotherapy for pituitary adenoma: update after an additional 10 years. J Clin Endocrinol Metab. 2005;90(2):800–4.
Minniti G, Scaringi C, Enrici RM. Radiation techniques for acromegaly. Radiat Oncol. 2011;6:167.
Muller TD, Nogueiras R, Andermann ML, Andrews ZB, Anker SD, Argente J, et al. Ghrelin. Mol Metab. 2015;4(6):437–60.
Murray PG, Higham CE, Clayton PE. 60 YEARS OF NEUROENDOCRINOLOGY: the hypothalamo-GH axis: the past 60 years. J Endocrinol. 2015;226(2):T123–40.
Neggers SJ, van der Lely AJ. Combination treatment with somatostatin analogues and pegvisomant in acromegaly. Growth Hormon IGF Res. 2011;21(3):129–33.
Neggers SJ, de Herder WW, Feelders RA, van der Lely AJ. Conversion of daily pegvisomant to weekly pegvisomant combined with long-acting somatostatin analogs, in controlled acromegaly patients. Pituitary. 2011;14(3):253–8.
Neggers SJ, Franck SE, de Rooij FW, Dallenga AH, Poublon RM, Feelders RA, et al. Long-term efficacy and safety of pegvisomant in combination with long-acting somatostatin analogs in acromegaly. J Clin Endocrinol Metab. 2014;99(10):3644–52.
Neggers SJ, Pronin V, Balcere I, Lee MK, Rozhinskaya L, Bronstein MD, et al. Lanreotide autogel 120 mg at extended dosing intervals in patients with acromegaly biochemically controlled with octreotide LAR: the LEAD study. Eur J Endocrinol. 2015;173(3):313–23.
Nomikos P, Buchfelder M, Fahlbusch R. The outcome of surgery in 668 patients with acromegaly using current criteria of biochemical 'cure'. Eur J Endocrinol. 2005;152(3):379–87.
Obari A, Sano T, Ohyama K, Kudo E, Qian ZR, Yoneda A, et al. Clinicopathological features of growth hormone-producing pituitary adenomas: difference among various types defined by cytokeratin distribution pattern including a transitional form. Endocr Pathol. 2008; 19(2):82–91.

Paisley AN, Hayden K, Ellis A, Anderson J, Wieringa G, Trainer PJ. Pegvisomant interference in GH assays results in underestimation of GH levels. Eur J Endocrinol. 2007;156(3):315–9.
Pendleton C, Adams H, Mathioudakis N, Quinones-Hinojosa A. Sellar door: Harvey Cushing's entry into the pituitary gland, the unabridged Johns Hopkins experience 1896–1912. World Neurosurg. 2013;79(2):394–403.
Pita-Gutierrez F, Pertega-Diaz S, Pita-Fernandez S, Pena L, Lugo G, Sangiao-Alvarellos S, et al. Place of preoperative treatment of acromegaly with somatostatin analog on surgical outcome: a systematic review and meta-analysis. PLoS One. 2013;8(4):e61523.
Ramirez C, Vargas G, Gonzalez B, Grossman A, Rabago J, Sosa E, et al. Discontinuation of octreotide LAR after long term, successful treatment of patients with acromegaly: is it worth trying? Eur J Endocrinol. 2012;166(1):21–6.
Reid TJ, Post KD, Bruce JN, Nabi Kanibir M, Reyes-Vidal CM, Freda PU. Features at diagnosis of 324 patients with acromegaly did not change from 1981 to 2006: acromegaly remains under-recognized and under-diagnosed. Clin Endocrinol. 2010;72(2):203–8.
Rokkas T, Pistiolas D, Sechopoulos P, Margantinis G, Koukoulis G. Risk of colorectal neoplasm in patients with acromegaly: a meta-analysis. World J Gastroenterol. 2008;14(22):3484–9.
Ronchi CL, Rizzo E, Lania AG, Pivonello R, Grottoli S, Colao A, et al. Preliminary data on biochemical remission of acromegaly after somatostatin analogs withdrawal. Eur J Endocrinol. 2008;158(1):19–25.
Rostomyan L, Daly AF, Petrossians P, Nachev E, Lila AR, Lecoq AL, et al. Clinical and genetic characterization of pituitary gigantism: an international collaborative study in 208 patients. Endocr Relat Cancer. 2015;22(5):745–57.
Salvatori R, Nachtigall LB, Cook DM, Bonert V, Molitch ME, Blethen S, et al. Effectiveness of self- or partner-administration of an extended-release aqueous-gel formulation of lanreotide in lanreotide-naive patients with acromegaly. Pituitary. 2010;13(2):115–22.
Sandret L, Maison P, Chanson P. Place of cabergoline in acromegaly: a meta-analysis. J Clin Endocrinol Metab. 2011;96(5):1327–35.
Schade R, Andersohn F, Suissa S, Haverkamp W, Garbe E. Dopamine agonists and the risk of cardiac-valve regurgitation. N Engl J Med. 2007 Jan 4;356(1):29–38.
Schilbach K, Bidlingmaier M. Growth hormone binding protein – physiological and analytical aspects. Best Pract Res Clin Endocrinol Metab. 2015;29(5):671–83.
Starke RM, Raper DM, Payne SC, Vance ML, Oldfield EH, Jane JA Jr. Endoscopic vs microsurgical transsphenoidal surgery for acromegaly: outcomes in a concurrent series of patients using modern criteria for remission. J Clin Endocrinol Metab. 2013;98(8):3190–8.
Taboada GF, Luque RM, Neto LV, Machado Ede O, Sbaffi BC, Domingues RC, et al. Quantitative analysis of somatostatin receptor subtypes (1-5) gene expression levels in somatotropinomas and correlation to in vivo hormonal and tumor volume responses to treatment with octreotide LAR. Eur J Endocrinol. 2008;158(3):295–303.
Thakker RV. Multiple endocrine neoplasia type 1 (MEN1) and type 4 (MEN4). Mol Cell Endocrinol. 2014;386(1–2):2–15.
Thakker RV, Newey PJ, Walls GV, Bilezikian J, Dralle H, Ebeling PR, et al. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). J Clin Endocrinol Metab. 2012;97(9):2990–3011.
Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, et al. Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. N Engl J Med. 2000;342(16):1171–7.
Trivellin G, Daly AF, Faucz FR, Yuan B, Rostomyan L, Larco DO, et al. Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. N Engl J Med. 2014;371(25):2363–74.
Tutuncu Y, Berker D, Isik S, Ozuguz U, Akbaba G, Kucukler FK, et al. Comparison of octreotide LAR and lanreotide autogel as post-operative medical treatment in acromegaly. Pituitary. 2012;15(3):398–404.

Uchoa HB, Lima GA, Correa LL, Vidal AP, Cavallieri SA, Vaisman M, et al. Prevalence of thyroid diseases in patients with acromegaly: experience of a Brazilian center. Arq Bras Endocrinol Metabol. 2013;57(9):685–90.

van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, et al. Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet. 2001;358(9295):1754–9.

van der Lely AJ, Biller BM, Brue T, Buchfelder M, Ghigo E, Gomez R, et al. Long-term safety of pegvisomant in patients with acromegaly: comprehensive review of 1288 subjects in ACROSTUDY. J Clin Endocrinol Metab. 2012;97(5):1589–97.

van Haute FR, Taboada GF, Correa LL, Lima GA, Fontes R, Riello AP, et al. Prevalence of sleep apnea and metabolic abnormalities in patients with acromegaly and analysis of cephalometric parameters by magnetic resonance imaging. Eur J Endocrinol. 2008;158(4):459–65.

Vandeva S, Elenkova A, Natchev E, Kirilov G, Tcharaktchiev D, Yaneva M, et al. Treatment outcome results from the Bulgarian Acromegaly Database: adjuvant dopamine agonist therapy is efficient in less than one fifth of non-irradiated patients. Exp Clin Endocrinol Diabetes. 2015;123(1):66–71.

Vieira Neto L, Taboada GF, Correa LL, Polo J, Nascimento AF, Chimelli L, et al. Acromegaly secondary to growth hormone-releasing hormone secreted by an incidentally discovered pheochromocytoma. Endocr Pathol. 2007;18(1):46–52.

Vieira Neto L, Abucham J, Araujo LA, Boguszewski CL, Bronstein MD, Czepielewski M, et al. Recommendations of Neuroendocrinology Department from Brazilian Society of Endocrinology and Metabolism for diagnosis and treatment of acromegaly in Brazil. Arq Bras Endocrinol Metabol. 2011;55(2):91–105.

Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, et al. Pituitary adenoma predisposition caused by germline mutations in the AIP gene. Science. 2006;312(5777):1228–30.

Vilar L, Azevedo MF, Naves LA, Casulari LA, Albuquerque JL, Montenegro RM, et al. Role of the addition of cabergoline to the management of acromegalic patients resistant to longterm treatment with octreotide LAR. Pituitary. 2011;14(2):148–56.

Vilar L, Fleseriu M, Naves LA, Albuquerque JL, Gadelha PS, dos Santos Faria M, et al. Can we predict long-term remission after somatostatin analog withdrawal in patients with acromegaly? Results from a multicenter prospective trial. Endocrine. 2014;46(3):577–84.

Villano JL, Seery TE, Bressler LR. Temozolomide in malignant gliomas: current use and future targets. Cancer Chemother Pharmacol. 2009;64(4):647–55.

Volschan I, Kasuki L, Silva CM, Alcantara ML, Saraiva RM, Xavier SS, et al. Two-dimensional speckle tracking echocardiography demonstrates no effect of active acromegaly on left ventricular strain. Pituitary. 2017;20:349–57.

Warszawski L, Kasuki L, Sa R, Dos Santos Silva CM, Volschan I, Gottlieb I, et al. Low frequency of cardiac arrhythmias and lack of structural heart disease in medically-naive acromegaly patients: a prospective study at baseline and after 1 year of somatostatin analogs treatment. Pituitary. 2016;19(6):582–9.

Wassenaar MJ, Cazemier M, Biermasz NR, Pereira AM, Roelfsema F, Smit JW, et al. Acromegaly is associated with an increased prevalence of colonic diverticula: a case-control study. J Clin Endocrinol Metab. 2010;95(5):2073–9.

Wildemberg LE, Gadelha MR. Pasireotide for the treatment of acromegaly. Expert Opin Pharmacother. 2016;17(4):579–88.

Wildemberg LE, Neto LV, Costa DF, Nasciuti LE, Takiya CM, Alves LM, et al. Low somatostatin receptor subtype 2, but not dopamine receptor subtype 2 expression predicts the lack of biochemical response of somatotropinomas to treatment with somatostatin analogs. J Endocrinol Investig. 2013;36(1):38–43.

Zanettini R, Antonini A, Gatto G, Gentile R, Tesei S, Pezzoli G. Valvular heart disease and the use of dopamine agonists for Parkinson's disease. N Engl J Med. 2007;356(1):39–46.

Physiopathology, Diagnosis, and Treatment of Nonfunctioning Pituitary Adenomas

4

Philippe Chanson, Anne-Lise Lecoq, Gerald Raverot, Frédéric Castinetti, Christine Cortet-Rudelli, Françoise Galland, and Sylvie Salenave

Contents

P. Chanson (✉) · A.-L. Lecoq
Service d'Endocrinologie et des Maladies de la Reproduction and Centre de Référence des Maladies Hypophysaires Rares, Hôpital de Bicêtre, Hôpitaux Universitaires Paris-Sud, Assistance Publique-Hôpitaux de Paris, Le Kremlin Bicêtre, France

Faculté de Médecine Paris-Sud, Univ Paris-Sud, UMR S1185, Le Kremlin-Bicêtre, France

INSERM U1185, Le Kremlin-Bicêtre, France
e-mail: philippe.chanson@bct.aphp.fr

G. Raverot
Fédération d'Endocrinologie, Groupement Hospitalier Est, Hospices Civils de Lyon, Lyon, France

INSERM U1028; CNRS UMR5292; Centre de Recherche Lyon Neuroscience, Université de Lyon, Lyon, France

F. Castinetti
Service d'Endocrinologie, Université Aix Marseille, Hôpital de la Conception, Marseille, France

C. Cortet-Rudelli
Service d'Endocrinologie-Métabolisme, Centre Hospitalier et Universitaire de Lille, Lille, France

F. Galland
Service d'Endocrinologie, Diabétologie, Nutrition, CHU Rennes, Hôpital Sud, Rennes, France

S. Salenave
Service d'Endocrinologie et des Maladies de la Reproduction and Centre de Référence des Maladies Hypophysaires Rares, Hôpital de Bicêtre, Hôpitaux Universitaires Paris-Sud, Assistance Publique-Hôpitaux de Paris, Le Kremlin Bicêtre, France

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_4

Abstract

Clinically nonfunctioning pituitary adenomas (NFPAs) include all pituitary adenomas that are not hormonally active and are therefore not associated with clinical syndromes such as amenorrhea-galactorrhea (prolactinomas), acromegaly, Cushing's disease, or hyperthyroidism (TSH-secreting adenomas). However, most such NFPAs in fact secrete gonadotropins or are actually gonadotroph pituitary adenomas. No univocal pathophysiological mechanism has been demonstrated. NFPA is usually diagnosed in a patient with signs and symptoms related to a mass effect (headache, visual impairment, sometimes pituitary apoplexy). More and more NFPAs are discovered incidentally. Biochemical workup often documents several pituitary insufficiencies. Unless contraindicated or in particular situations (e.g., incidentalomas at distance from optic pathways), surgery is the mainstay of treatment. Resection, generally via a transsphenoidal approach (with the help of an endoscope), should be performed by a neurosurgeon with extensive experience in pituitary surgery, in order to maximize the chances of complete resection and to minimize complications. If a tumor remnant persists (a frequent situation in patients with large and often invasive adenomas), watchful waiting is preferred to routine radiotherapy, as long as the tumor residue does not grow. NFPA can sometimes recur even after complete resection.

Postoperative irradiation is only considered in case of residual tumor growth or relapse.

Keywords
Nonfunctioning pituitary adenomas · Pituitary incidentalomas · Neurosurgery · Radiotherapy · Gonadotroph adenomas · Mass effect

"Clinically nonfunctioning pituitary adenomas" (NFPAs) include all pituitary adenomas that are not hormonally active and are therefore not associated with clinical syndromes such as amenorrhea-galactorrhea (prolactinomas), acromegaly, Cushing's disease, or hyperthyroidism (TSH-secreting adenomas). However, most such NFPAs, which are "chromophobic" on classical histology, in fact secrete gonadotropins or are actually gonadotroph pituitary adenomas. Immunolabeling is negative in 10% of cases ("null cell adenomas") but may occasionally be positive for GH, PRL, TSH, or ACTH despite absent or minimal secretion of these hormones in vivo; such cases are known as silent somatotroph, lactotroph, thyrotroph, or corticotroph adenomas. The French Endocrine Society recently published a series of reviews dealing extensively with NFPA (Abouaf et al. 2015; Castinetti et al. 2015; Chanson et al. 2015; Cortet-Rudelli et al. 2015; Galland et al. 2015; Raverot et al. 2015), and these serve as the basis for this chapter.

Pathogenesis

Nonfunctioning pituitary adenomas (NFPAs) mostly arise from gonadotroph cells but also include other subtypes such as null cell adenomas and oncocytomas. In common with other pituitary adenomas, NFPAs are of monoclonal origin, suggesting that somatic mutations precede clonal expansion of the cells (Herman et al. 1990). Another similarity between NFPAs and functioning adenomas is the existence of mechanisms restraining cell growth, protecting these tumors from malignant transformation. Senescence is one of them and appears to be mediated in NFPAs by clusterin, a protein which induces the cyclin-dependent kinase inhibitors p15, p16, and p27 (Chesnokova et al. 2007, 2011, 2012). Clusterin expression is upregulated in gonadotroph cells by FOXL2 and PTTG. Conversely, clusterin and FOXL2 suppress PTTG, thus protecting tumor cells from excessive proliferation and oncogenic transformation (Chesnokova et al. 2012).

The molecular mechanisms underlying NFPAs' tumorigenesis are still unclear. Some of the alterations and pathophysiologic mechanisms that have been reported are observed in several or all the pituitary lineages, and only few seem to be specific for NFPAs. The majority of the described molecular defects have been identified in human NFPAs samples, as only two transgenic mouse models developing NFPAs have been reported, one FSHβ-SV40 large T antigen transgenic mouse model developing null cell adenomas and one αGSU-PTTG1 transgenic mouse model with gonadotroph hyperplasia (Melmed 2015).

Familial NFPAs

Similarly to what is known in other types of adenomas, NFPAs rarely occur in a familial setting as a component of hereditary syndromes. Fifteen percent of pituitary adenomas encountered in multiple endocrine neoplasia type 1 (MEN1) kindreds are NFPAs, representing the second type of adenoma in terms of prevalence (de Laat et al. 2015; Verges et al. 2002). The clinical behavior of these *MEN1*-mutated NFPAs appears to be very similar to that of nonmutated NFPAs.

NFPAs have been described in 14.5% of familial isolated pituitary adenomas (FIPA), but germline *AIP* mutations were found in only one homogenous and three heterogeneous FIPA (Beckers et al. 2013). In our cohort of 766 sporadic pituitary adenomas, 3 out of 16 (18%) *AIP*-mutated patients were diagnosed with NFPA, but *AIP* mutations were encountered in less than 2% of all NFPAs (Lecoq et al. 2016).

Germline *CDKN1B* mutations have been reported in NFPAs in the context of MEN4 (Schernthaner-Reiter et al. 2016). *MEN1*, *AIP,* and *CDKN1B* are considered as tumor suppressor genes, but their precise role in pituitary tumorigenesis is still unknown (Lecoq et al. 2015). However, their tumor-suppressor effect could be mediated by cell cycle regulators.

One germline *SDHD* mutation has been found in a patient with a NFPA (Xekouki et al. 2015). However, this variant was not associated with pheochromocytoma nor paraganglioma and was also found in two endocrine-negative controls, questioning its pathogenicity.

Germline *GPR101* mutations have been reported in two patients with NFPAs (Lecoq et al. 2016). One was the p.E308D variant, previously associated with sporadic pituitary adenomas (Trivellin et al. 2014) but also present in unaffected persons, with an allelic frequency of 0.36% according to the ExAC database. The other was a rare *GPR101* variant p. (D358=), not reported in the ExAC database. Nevertheless, prevalence of *GPR101* germline variants is very low among sporadic pituitary adenomas (functioning and nonfunctioning) (Ferrau et al. 2016; Iacovazzo et al. 2016; Lecoq et al. 2016), and their pathogenicity has to be confirmed by in vitro functional analysis. Finally, no germline *PRKAR1A* mutations have been described so far in NFPAs.

Somatic Mutations

Whole-exome sequencing of DNA extracted from human NFPAs revealed no somatic mutations in genes previously associated with pituitary tumorigenesis such as *MEN1*, *AIP*, *CDKN1B*, *PRKAR1A*, *GNAS*, *HRAS*, *RB1*, *PTTG*, *PIK3CA*, and *ZAC1*. Twenty-four somatic variants have been found, representing a low mutation rate (Newey et al. 2013). These variants affected independent genes, suggesting that there is no common driver gene responsible for a high proportion of NFPAs.

The GnRH receptor gene, which was a candidate gene, is expressed in functioning rather than in nonfunctioning gonadotroph adenomas (Kottler et al. 1998), but no

mutations in its coding region have been found (Chanson et al. 1998; Kottler et al. 1998), indicating that activating mutations are not involved in their pathogenesis.

Altered Chromatin Remodeling

NFPAs demonstrate higher levels of methylation than other pituitary tumor type (Duong et al. 2012). Several of these epigenetic events have been described in other lineages and affect genes involved in cell cycle regulation. Silencing of the tumor suppressor gene *CDKN2A* by promoter hypermethylation is found in most human NFPAs (Simpson et al. 1999) and could be more common in null cell adenomas compared to other NFPAs such as gonadotroph tumors (Ruebel et al. 2001). Similarly, hypermethylation of CpG island in the *CDKN2B*, *CDKN2C*, and *RB1* promoters has been reported in NFPAs (Kirsch et al. 2009; Simpson et al. 2000; Yoshino et al. 2007). All these genes, coding the $p16^{Ink4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$, and Rb proteins, respectively, play a role in the G1 phase of the cell cycle that regulate exit from quiescence and progression into S phase. *GADD45γ*, a member of a family of genes that are induced by DNA damage and negatively regulate the cell growth, is downregulated in NFPAs as well as in hormone-secreting adenomas. This loss of expression is frequently associated with CpG island methylation (Bahar et al. 2004).

On the contrary, expression of MEG3 is selectively lost in NFPAs of gonadotroph origin (Gejman et al. 2008; Zhao et al. 2005). The *MEG3* gene belongs to the imprinted *DLK1-MEG3* locus located on 14q32. The protein is known to activate p53, stimulates expression of its target genes, and inhibits cell proliferation in vitro (Zhou et al. 2007). An increased methylation in *MEG3* promoter region and in the IG-DMR region regulating the imprinting of the *DLK1-MEG3* locus has been observed in NFPAs (Gejman et al. 2008; Zhao et al. 2005). Downregulation of other maternally and paternally imprinted genes within this locus has been reported in NFPAs but also in ACTH- and prolactin-secreting tumors (Cheunsuchon et al. 2011).

MiRNA

Altered miRNA expression has been reported in pituitary adenomas with most of them having *HMGA1*, *HMGA2*, and *E2F1* as target genes, which are involved in cell growth regulation (D'Angelo et al. 2012; Palmieri et al. 2012). Similarly, upregulation and downregulation of several miRNAs have been described in NFPAs (Mussnich et al. 2015). One of the most downregulated miRNAs, miR-410, belongs to one of the largest miRNA clusters in humans located on the *DLK1-MEG3* locus. In vitro, miR-410 inhibits the expression of *CCNB1* coding the cyclin B1 protein, thus decreasing cell proliferation (Mussnich et al. 2015). Among the upregulated miRNAs observed in NFPAs, miR-17 is able to decrease p21 levels, which enhances the G1-S transition and the cell growth (Mussnich et al. 2015). Other miRNAs within the *DLK1-MEG3* locus (miR-134, miR-323, miR-370, miR-432)

were also downregulated in NFPAs but not in functioning adenomas (Cheunsuchon et al. 2011). Some of these miRNAs are known to target the transcripts of cell cycle regulators, emphasizing the importance of cell cycle dysregulation in pituitary tumorigenesis.

Other Mechanisms

Growth Factors and Hormone Signaling

Several growth factors have been implicated in pituitary tumorigenesis (Melmed 2011). NFPAs are characterized by overexpression of *BRINP3*, a gene regulated by the growth factors bone morphogenetic protein (BMP) and retinoic acid (Shorts-Cary et al. 2007). BRINP3 is a mitochondrial protein which increases proliferation, migration, and invasion in vitro when overexpressed in pituitary gonadotroph cells.

Studies in *ERβ* knockout mice have shown a role for estrogens and their receptors ERα in gonadotroph adenomas development (Davis et al. 2006; Fan et al. 2010).

Other Altered Signaling Pathways

Raf/MEK/ERK and PI3K/Akt/mTOR pathways are upregulated in NFPAs as well as in secreting adenomas (Dworakowska et al. 2009). Wnt pathway inhibitors are downregulated in NFPAs and in functioning tumors (Elston et al. 2008). Interestingly, the *CCND1* gene (coding cyclin D1), which is a common target gene of Raf/MEK/ERK, PI3K/Akt/mTOR, and Wnt pathways, is upregulated specifically in NFPAs (Dworakowska et al. 2009; Elston et al. 2008).

Transcription Factors

Downstream targets of transcription factor SF1, such as *CYP11A1*, are upregulated in NFPAs and promote cell proliferation and survival (Lee et al. 2013).

Progenitor/Stem Cells

Progenitor/stem-like cells could play a role in pituitary tumorigenesis and especially in NFPAs. The presence of such cells has been shown in human NFPAs, particularly in those with invasive behavior (Peverelli et al. 2017). They were able in vitro to grow as spheres, expressed stem cell-specific markers and pituitary embryonic transcription factors involved in gonadotrophs differentiation, and were responsive to dopamine receptor DRD2 and somatostatin receptor SSTR2 agonists with a decrease in cell proliferation (Peverelli et al. 2017).

Markers of Invasiveness

Several studies have reported a specific gene expression profile of invasive NFPAs versus noninvasive. Thus, low levels of SMAD3 and phospho-SMAD3, which are involved in the TGFβ pathway and overexpression of IL-6R/JAK2/STAT3/MMP9

pathway and of MYO5A gene, have been shown in invasive NFPAs compared to noninvasive ones (Feng et al. 2016; Galland et al. 2015; Liu et al. 2016).

Pathology

NFPA are defined by default, i.e., by the absence of clinical picture related to hormone excess such as acromegaly, Cushing's syndrome, or amenorrhea-galactorrhea. However these tumors are exceptionally nonsecreting, i.e., with negative immunocytochemistry (the so-called null cell adenomas).

The Vast Majority of NFPAs Are in Fact Gonadotroph

The first patient with NFPA and increased serum levels of gonadotropins has been reported in 1976, introducing the concept of gonadotroph adenoma (Snyder and Sterling 1976). Two years later, Kovacs et al. described the histological and immunocytochemical features of FSH-secreting adenomas (Kovacs et al. 1978), and this was rapidly followed by publications of series of patients (Beckers et al. 1985; Trouillas et al. 1981). Progressively, with the use of specific monoclonal antibodies applied to the excised lesion, it has become clear that, in the vast majority of cases, these NFPAs (which are "chromophobic" with classical staining techniques), in fact, produce gonadotropins and/or their subunits (ßFSH, ßLH, αSU) and can thus be considered gonadotroph. This has been confirmed by gene expression studies (Jameson et al. 1987; Yamada et al. 1989).

Gonadotroph adenomas show a diffuse or, more often, cord arrangement, with extensive vascularization. In rare cases, they may be hemorrhagic or necrotic. The cells are well delineated, oval or polyhedral, generally without signs of secretion (small nuclei without nucleoli) (Asa 2011; Asa et al. 1992; Lloyd et al. 2004; Trouillas et al. 1981). All cells are strongly positive with anti-chromogranin A antibodies. The percentage of cells positive with anti-gonadotropin antibodies ranges from 100% to a few islands, but it is usually low (<20–30%). Some tumors contain cells positive for ßFSH, ßLH, and alpha subunit, while others contain cells that are only positive for ßFSH or, more rarely, ßLH or αSU (Black et al. 1988; Trouillas et al. 1981, 1986). When less than 5% of the adenomatous cells immunostain positively, the tumor is considered as "null cell adenoma." However, thanks to improved knowledge of pituitary cell differentiation and availability of cytogenetic markers, we now know that some null cell adenomas express steroidogenic factor 1 (SF-1), a transcription factor specific to the gonadotroph lineage, implying that they also belong to the gonadotroph family (Asa 2011; Asa et al. 1996; Lloyd et al. 2004; Mete and Asa 2012; Nishioka et al. 2015) or are able to secrete gonadotropins and/or subunits in vitro (Asa et al. 1992) and can thus be considered as gonadotroph adenomas. Plurihormonal gonadotroph adenomas are rare, except in MEN1 (Trouillas et al. 2008).

In the case of gonadotroph adenomas, it remains largely unknown why the gonadotropins are seldom secreted in the bloodstream and, when they are, why they are exceptionally responsible for clinical syndromes related to gonadotropins excess (Ntali et al. 2015) but, much more likely, are associated with hypogonadism. Chromatofocusing analysis showed that gonadotroph adenomas produce more basic FSH isoforms (Pigny et al. 1996) which, paradoxically, are considered as more biologically active (Borgato et al. 1996; Galway et al. 1990). As glycosylation of gonadotropins is essential for their biological activity, hypogonadism may be related to decreased biological activity of gonadotropins related to abnormal glycosylation of the isoforms produced by the adenoma.

Some NFPAs Are Silent Lactotroph, Somatotroph, or Corticotroph Adenomas

NFPAs may also be silent variants of lactotroph, somatotroph, or corticotroph adenomas. They can be suspected on histology as their aspect resembles that of their symptomatic clinical variant responsible for acromegaly or Cushing's disease. Immunocytochemistry allows to make the diagnosis. Silent corticotroph adenoma is also called "silent type 1 or 2 adenoma" (Raverot et al. 2010b). Positivity for Tpit, which determines corticotroph cell differentiation, is helpful for confirming the corticotroph nature of the adenoma (Nishioka et al. 2015). Silent somatotroph or mixed mammosomatotroph adenoma (GH-PRL $\pm$ TSH) is known as "silent subtype 3" (Erickson et al. 2009; Kovacs et al. 1989; Trouillas et al. 1991). Staining of silent adenomas for the transcription factor Pit-1, which determines somato-lacto-thyrotroph lineage, may help to classify these adenomas (Mete et al. 2016; Nishioka et al. 2015).

Markers of Proliferation

Three proliferation markers must be used to assess the risk of recurrence or progression, namely, the proliferation index (anti-Ki-67 antibody staining), mitotic activity, and p53 expression. Ki-67 expression in $>3\%$ of cells predicts recurrence/progression with high specificity but poor sensitivity (Filippella et al. 2006; Jaffrain-Rea et al. 2002; Righi et al. 2012).

Epidemiology

The prevalence of pituitary adenomas is 80–100 per 100,000 inhabitants, and 15–30% of these adenomas are "nonfunctioning" (Daly et al. 2006; Fernandez et al. 2009; Raappana et al. 2010). The annual incidence of NFPA is between 10 and 20 cases per million inhabitants (Olsson et al. 2015; Raappana et al. 2010).

NFPAs are generally diagnosed during the 5th or 6th decade and show a male predominance (Brochier et al. 2010; Ferrante et al. 2006; Kanner et al. 2009; Ntali et al. 2015; Olsson et al. 2015). NFPA is associated with increased mortality: recent studies show a standardized mortality ratio (SMR) ranging from 1.1 (95% CI, 1.00–1.20) in Sweden (Olsson et al. 2015) to 3.6 (2.9–4.5) in the UK (Ntali et al. 2015). Only older age was associated with mortality in the multivariate analysis of this latter study.

Clinical Presentation

Clinical Signs and Symptoms (Fig. 1)

Mass Effects

The vast majority of NFPAs, whether hormonally active (i.e., gonadotropin-secreting) or inactive (null cell adenomas), are revealed by mass effects on anatomic structures in the vicinity of the pituitary (headache, optic chiasm compression in 40–70% of cases, occasional cranial nerve III, IV, and VI compression) and/or on pituitary hormonal function, leading to hypopituitarism (Brochier et al. 2010; Ferrante et al. 2006; Greenman and Stern 2009b; Ntali et al. 2015; Olsson et al. 2015). Hypopituitarism can be caused by anterior pituitary compression, pituitary stalk interruption, or hypothalamic involvement. Sequential loss of hormone secretion usually begins with GH or gonadotropins; decrease in TSH and ACTH secretion may follow (Nomikos et al. 2004). Pituitary stalk compression can also produce hyperprolactinemia, by disinhibiting the dopaminergic tone that normally acts at the level of pituitary lactotrophs, causing amenorrhea and galactorrhea, but PRL serum levels are always below 150–200 ng/ml (Brochier et al. 2010; Karavitaki et al. 2006). This distinguishes them from macroprolactinomas, which are associated with much higher PRL levels, proportional to tumor size (Chanson and Maiter 2017; Klibanski 2010; Rogers et al. 2014).

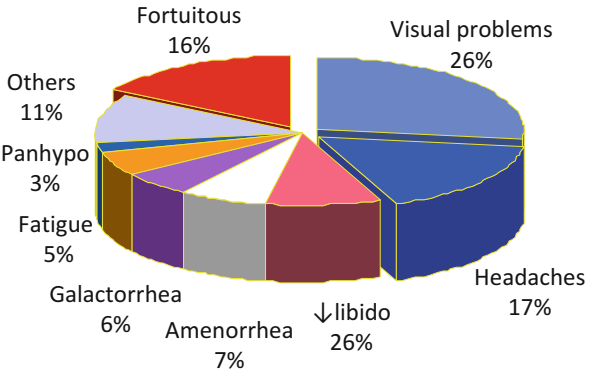


Fig. 1 NFPA presenting symptoms and signs (Adapted from Brochier et al. (2010))

Pituitary Apoplexy

Pituitary apoplexy may be the presenting feature of NFPA, with severe headaches of sudden onset, meningismus, a variably depressed sensorium, and visual disturbances (Briet et al. 2015). Between 2% and 12% of patients with all types of adenoma experience apoplexy, and the diagnosis of pituitary tumor was unknown at time of apoplexy in more than 3 out of 4 cases (Briet et al. 2015). If the NFPA (often incidentaloma) was already known and that a decision was made to manage them conservatively, the risk of pituitary apoplexy was calculated to be between 0.2 and 0.6 events per 100 person-years in two meta-analyses (Fernandez-Balsells et al. 2011; Sivakumar et al. 2011).

Hyperstimulation Syndromes

Very rarely, gonadotropin hypersecretion can stimulate the gonads: macroorchidism has been reported in males and an ovarian hyperstimulation syndrome in premenopausal women with FSH-secreting tumors (rev in Cooper et al. 2008; Ntali et al. 2014).

Incidentalomas

An increasing proportion of pituitary tumors (up to 15% of NFPAs) is detected by chance on computed tomography (CT) or magnetic resonance imaging (MRI) of the brain performed for unrelated reasons (Chanson and Young 2003; Freda et al. 2011; Galland et al. 2015; Molitch 2008). These tumors are called pituitary incidentalomas. In their great majority, they are small, less than 1 cm diameter and nonfunctioning, macroadenoma accounting for about 1% of incidentalomas (Ezzat et al. 2004). The main issues in these patients are to be sure that the lesion is a pituitary macroadenoma (Vasilev et al. 2016) and if it will grow or remain unchanged, in order to manage them adequately. Macroadenomas have a greater tendency to grow than microadenomas (12.5% of patients per year vs. 3.3%), and solid lesions show greater progression than cystic ones (5.7% of patients per year vs. 0.05%) (Fernandez-Balsells et al. 2011; Sanno et al. 2003). In a series of 115 NF incidentalomas, growth was reported in 20% of cases at 4 years (Fernandez-Balsells et al. 2011; Sanno et al. 2003). In a review of 353 macroincidentalomas (Molitch 2009), increase and decrease in size were observed in 24% and 12%, respectively; the rate of growth increased with the length of follow-up: 17% for follow-up <4 years (38/215) and 34% for follow-up 5–8 years (47/138).

Biochemical Evaluation

Gonadotropins and Subunit Secretion

When a functioning adenoma produces an excess hormone (e.g., GH or ACTH), a clinical syndrome (e.g., acromegaly or Cushing syndrome) is observed, and the diagnosis is readily confirmed by hormone assays (Molitch 2017). By definition, this important clinical marker is generally lacking in patients with NFPA. As already

mentioned, the vast majority of NFPAs are gonadotroph, i.e., able to produce gonadotropins or their subunits. However, baseline plasma dimeric FSH and/or LH levels are rarely elevated (see Chanson and Brochier 2005; Raverot et al. 2015 for review). Elevation of free subunit levels (mainly alpha LH, more rarely beta LH) is more common but is generally moderate.

When secretion is detected, it is usually moderate and rarely associated with specific clinical manifestations (see above) (Cooper et al. 2008; Ntali et al. 2014).

Careful interpretation of baseline plasma concentrations of dimeric FSH, dimeric LH, or the free alpha subunit shows that about half of male gonadotroph adenoma patients (as confirmed by immunocytochemistry or by in vitro tumor secretion studies) have excess secretion of FSH, LH, or their free subunits, in amount sufficient for measurement in serum (Chanson and Brochier 2005; Chanson et al. 1997; Daneshdoost et al. 1991, 1993). This situation is less common in premenopausal women (about 30% of cases). It is much harder to assess this secreting status after the menopause because the physiological elevation of gonadotropins and subunits levels. In fact, increased FSH levels in postmenopausal women, contrasting with low LH levels and generally low levels of all pituitary hormones, can help with the preoperative diagnosis of a gonadotroph adenoma (Chanson and Brochier 2005).

It was previously recommended to measure the response of gonadotropins and their free subunits to TRH and GnRH stimulation, but these tests are neither sensitive nor specific for indicating the gonadotroph nature of NFPAs (Raverot et al. 2015). Even if this is a rare event, they can also trigger pituitary apoplexy (Briet et al. 2015). Thus, such stimulation tests are no longer recommended (Raverot et al. 2015).

Other Pituitary Hormones

Patients with pituitary macroadenomas, whether discovered fortuitously or revealed by a mass effect, require assessment of secretion of the various pituitary hormone, for detecting any hypersecretion of a clinically silent corticotroph or somatotroph adenoma and diagnosing pituitary hormone deficiencies that may require preoperative replacement therapy.

The Search for "Silent" Hypersecretion of Other Pituitary Hormones

The abnormal secretion of pituitary hormones such as ACTH, PRL, or GH may be totally silent or subclinical. A silent corticotroph adenoma can be revealed preoperatively by an elevated ACTH level concomitant with a normal 8.00 am cortisol level or by a "paradoxically" normal cortisol level despite deficiency of all other pituitary hormones (Raverot et al. 2015). An overnight dexamethasone suppression test may be contributory, as may free urinary cortisol measurement.

By definition, IGF-I levels are normal in patients with a silent somatotroph adenoma; if they are not normal, then acromegaly should be diagnosed, even in the absence of clear clinical signs.

PRL is also routinely measured in patients with NFPA. If the pituitary adenoma is very large and PRL levels are moderately elevated, hyperprolactinemia is likely the

result of pituitary stalk lesion (see above). It might be kept in mind that a PRL assay artifact called "hook effect" can mask very high levels of PRL which normally would have been suggestive of a prolactinoma (Barkan and Chandler 1998; Frieze et al. 2002) and can falsely orientate the diagnosis to hyperprolactinemia related to stalk compression; fortunately, measurement of PRL in 1/10- to 1/100-diluted serum, by showing very high levels of PRL, allows to revise the diagnosis for that of macroprolactinoma.

Screening for Hypopituitarism

At the time of NFPA diagnosis, 60% to 85% of patients have at least one pituitary hormone deficiency. Gonadotropic deficiency is the most prevalent (>80% of cases), followed by somatotropic deficiency. Thyrotropic and corticotropic deficiencies are found in 20–50% of cases (Dekkers et al. 2008; Greenman and Stern 2009a, b; Greenman et al. 1995; Murad et al. 2010; Raverot et al. 2015).

Preoperative Assessment

Pituitary Imaging

Neuroradiological assessment of NFPA is based on MRI (Chanson et al. 2015; Raverot et al. 2015). On MRI, NFP macroadenomas appear as a mass centered on an enlarged sella turcica, with T1/T2 signal variations due to necrotic and/or hemorrhagic areas and possibly with a fluid level. Tumor enhancement is usually only weak (Fig. 2). The tumor may extend upward, toward the optic pathways, laterally into the cavernous sinuses (internal carotid artery encasement is a sign of cavernous sinus invasion, while visualization of the medial cavernous sinus veins indicates the absence of invasion), and/or downward into the sphenoid sinus, with lysis of the sellar floor. If discovered incidentally, it has to be differentiated from other pituitary lesions (Vasilev et al. 2016).

Ophthalmologic Assessment of NFPA Patients

NFPAs are a major source of visual disorders (Brochier et al. 2010; Ferrante et al. 2006; Kanner et al. 2009; Ntali et al. 2015; Olsson et al. 2015) and are diagnosed later than functioning adenomas, often with larger tumor volume. Patients may be unaware of their visual deficit, especially in case of bitemporal hemianopsia (Fig. 3), as the functioning visual field in one eye can long compensate for visual field loss in the other eye.

Neuro-ophthalmologic exploration is one of the main factors guiding the surgical decision. Ophthalmologic assessment includes sensory evaluation with measurement of visual acuity (VA) and visual field examination (VF), preferably using a central static and peripheral kinetic approach. Anterior segment and fundus examinations are also essential for interpreting the VA and VF data. Optic nerve optical coherence tomography (OCT) is contributory but not essential for estimating the visual prognosis (Abouaf et al. 2015).

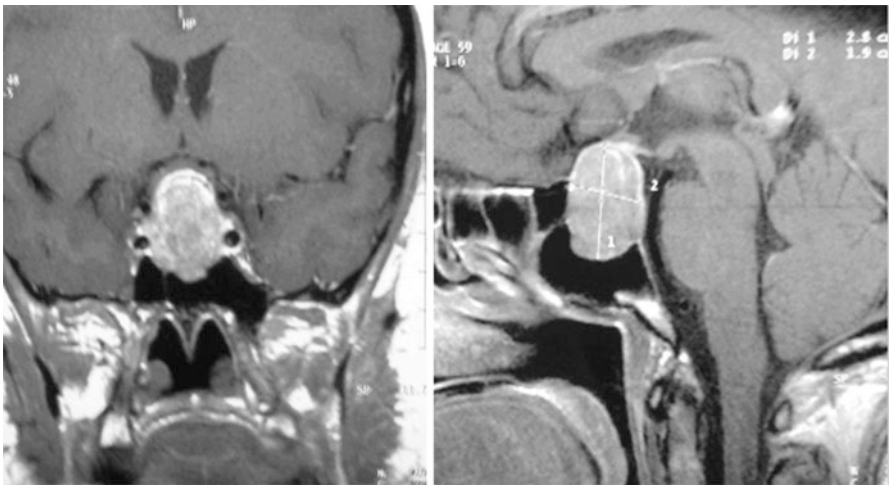


Fig. 2 Coronal and sagittal views of a NFPA with suprasellar extension

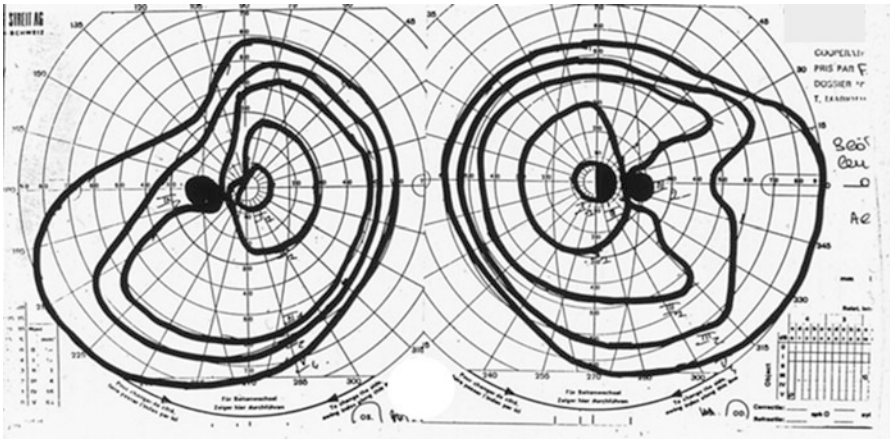


Fig. 3 Visual field (Goldmann perimetry) showing bitemporal hemianopsia in a patient with NFPA

General Principles of Treatment

The choice of treatment modality is determined by several factors:

- The need for immediate relief from a mass effect or hormonal abnormalities
- The chances of long-term control
- The type and frequency of possible morbidities

Surgery and radiotherapy are the two radical treatment options. A conservative approach may be appropriate for patients with an incidentally discovered pituitary adenoma ("incidentaloma"), provided the tumor is small, well defined, and has no suprasellar or lateral extension (risk of neurological or visual chiasm compression) and provided a meticulous hormonal workup has ruled out minimal hormonal hypersecretion insufficient to produce a clinical syndrome. Such watchful waiting may also be considered if surgery is contraindicated or declined.

Surgical Treatment of Nonfunctioning Pituitary Adenomas

Surgery is generally the first-line treatment for NFPAs, usually via a transsphenoidal approach (Castinetti et al. 2015).

The Results of Surgery

Remission Rate

Remission is defined as the disappearance of visible tumor on MRI after total surgical removal. The average remission rate after surgery is between 20% (95% CI: 12–57) according to one meta-analysis (Murad et al. 2010) and 47.3% (range, 3–92) at 1 year according to another (Roelfsema et al. 2012).

Results on Visual Defects

Vision improves (partially or fully) after surgery in some 80–90% of cases (Dehdashti et al. 2008; Tabaee et al. 2009a, b). Visual recovery may be very gradual, continuing up to 1 year after surgery. Some studies have shown a correlation between the degree of visual recovery and the duration and severity of visual disorders. Visual acuity <0.1 and optic atrophy both carry a poor visual prognosis (Dekkers et al. 2008; Gnanalingham et al. 2005). The urgency of surgery depends on the severity of visual disorders. There is no precise time limit after chiasm decompression beyond which visual recovery is no longer possible. Visual disorders are thus an indication for surgery, even though complete recovery cannot be guaranteed.

Results on Pituitary Deficiencies

The risk of onset of further pituitary deficiencies in patients with pituitary macroadenomas has been estimated at 12% per year (Castinetti et al. 2015). Arafah et al. found that preoperative deficiencies, headache, and hyperprolactinemia significantly influenced the chances of postoperative recovery: when all the criteria were met (which suggests an increased intrasellar pressure), deficiencies and headache were more likely to improve postoperatively (Arafah et al. 1994, 2000). Surgery normalized anterior pituitary function in about 20–30% of cases after an average follow-up of 1 year (Messerer et al. 2013; Murad et al. 2010; Nomikos et al. 2004), and the rate

is higher with earlier management. This explains why some authors recommend surgery even if the macroadenoma is asymptomatic (Messerer et al. 2013). The risk of a postoperative deterioration in pituitary function is 1–10% (Nomikos et al. 2004; Roelfsema et al. 2012; Tabaee et al. 2009a, b). Transcranial excision gives much poorer results (Murad et al. 2010; Nomikos et al. 2004). The risk of onset of diabetes insipidus is less than 5%.

Results on Headache

Headache is classically attributed to distension of the dural envelopes (Murad et al. 2010). Headache due to a mass effect is generally relieved by surgery (Comtois et al. 1991; Dekkers et al. 2008; Yu et al. 2016).

The Indications of Surgery

Taking into account its effects, as described above, surgery may not always be indicated and needs to be discussed on an individual basis (Castinetti et al. 2015).

Symptomatic Macroadenomas

Visual disorders are an undisputable indication for surgery, its urgency depending on the degree of visual impairment. As postoperative recovery from hypopituitarism is uncertain and as surgery is associated with a 5–10% risk of aggravating or inducing hypopituitarism, presence of a pituitary deficiency is probably not the main indication for surgery. Disabling headache likely due to the adenoma may be an indication for elective surgery, but the patient should be warned that relief cannot be guaranteed as causality is unproven.

In patients with large adenomas not associated with visual disorders, the surgical decision is made on an individual basis and depends on the rate of progression, as measured on two successive MRI scans.

Difficult Situations

Elderly

NFPAs account for 60–80% of pituitary adenomas occurring in the elderly, with an annual incidence of around 7/100,000 (Hong et al. 2008; Minniti et al. 2005a; Turner et al. 1999a). The main presenting symptom, as in younger patients, is visual impairment (50–70% of cases) (Minniti et al. 2005a). Most macroadenomas have a maximal diameter of 2–4 cm at diagnosis (Minniti et al. 2005a). Management decisions must take into account the visual impact, tumor proximity to the chiasm, and comorbidities (80% of elderly patients with NFPA have at least one comorbidity) (Minniti et al. 2005a). Mortality is not higher than in the general population (<1%) if the anesthetic risk is well controlled (Minniti et al. 2005a) and if the transsphenoidal route is preferred to the transcranial approach (Murad et al. 2010). The rates of recovery from visual disorders and pituitary deficiency are similar to those obtained in younger patients. The indications are the same in 65–75-year-old

patients as in younger patients, provided the comorbidities, the anesthetic risk, and the real impact to the patient are taken into account.

Patients Treated with Anticoagulants

The surgical decision is more difficult in patients taking antiplatelet drugs or anticoagulants. Indeed, patients on long-term anticoagulant or antiplatelet therapy have a higher risk of postoperative hemorrhagic complications. However, preoperative drug withdrawal (5 days ahead of surgery for aspirin and vitamin K antagonists and, ideally, 10 days for clopidogrel) is associated with increased morbidity and mortality, especially in patients with active stents (Castinetti et al. 2015).

Surgical Technique: Microsurgery or Endoscopy?

More than the technique itself, it is the neurosurgeon's experience that determines outcome. Endoscopy may be preferable to microscopy, as it offers better control of lateral and superior tumor extension, but in terms of surgical outcome, both techniques are comparable (Ammirati et al. 2013; Bastos et al. 2016). The complication rate seems similar, e.g., worsening of visual and pituitary functions in 2.4 and 13.7% cases, respectively, and persistent diabetes insipidus in 6.2% cases with an endoscopic approach, comparable to the microscopic approach in a recent study (Magro et al. 2016).

Postoperative Management of Patients with Nonfunctioning Pituitary Adenomas

Despite progress in neurosurgery, the size and frequent invasiveness of NFPAs often result in partial resection (see above), and the remnant (present in 50–80% of cases) may regrow (Cortet-Rudelli et al. 2015; Murad et al. 2010; Roelfsema et al. 2012). Recurrence is also possible, although rare, even when total resection has been achieved (Chen et al. 2012; Cortet-Rudelli et al. 2015; Murad et al. 2010; Roelfsema et al. 2012). Adjuvant therapy (surgical revision, radiation therapy, medications) may be indicated. Thanks to the quality of pituitary surveillance offered by MRI, it is now generally agreed that radiotherapy is not routinely required in the absence of postoperative remnant or if the remnant does not progress with time; watchful waiting is thus preferable (Fig. 4) (Chanson et al. 2015).

Factors Involved in NFPA Recurrence

Several recent meta-analyses (Chen et al. 2012; Murad et al. 2010; Roelfsema et al. 2012) have shown that NFPAs are more likely than secreting adenomas to recur, with little change over the past 30 years (Roelfsema et al. 2012).

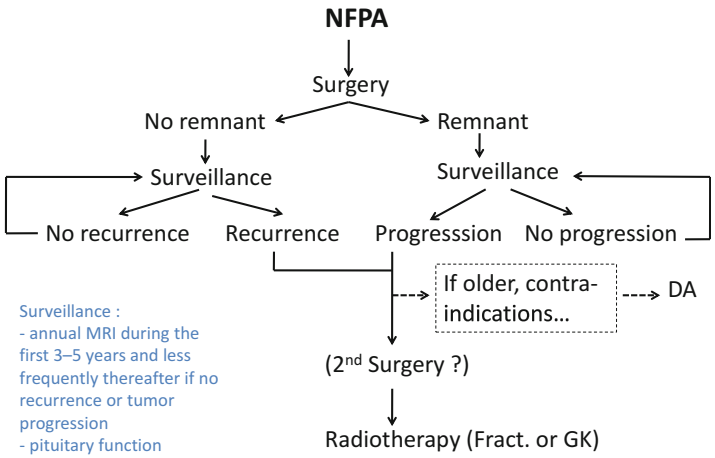


Fig. 4 Proposed NFPA patient management

Clinical Factors

The best-identified factor for recurrence is the presence of a postoperative adenoma remnant (Table 1). After apparently complete resection confirmed by MRI, the risk of recurrence is around 10–20% and 30%, 5 and 10 years after surgery, respectively (Brochier et al. 2010; Chen et al. 2012; Dekkers et al. 2006; Ferrante et al. 2006; Greenman et al. 2003; Karamouzis et al. 2015; Losa et al. 2008; Murad et al. 2010; O'Sullivan et al. 2009; Reddy et al. 2011; Roelfsema et al. 2012; Soto-Ares et al. 2002; Turner et al. 1999b; van den Bergh et al. 2007; Vargas et al. 2015; Woollons et al. 2000). In two meta-analyses, the mean recurrence rate in patients **without** a visible tumor residue was 11–12% (Chen et al. 2012; Roelfsema et al. 2012); the postoperative remission rate was 96% and 82% at 5 and 10 years, respectively (Chen et al. 2012); and most recurrences were diagnosed between 1 and 10 years postoperatively (Roelfsema et al. 2012). In patients **with** a postoperative tumor remnant, the recurrence rate was 25–40% and 50–60%, 5 and 10 years after surgery, respectively (Brochier et al. 2010; Chen et al. 2012; Dekkers et al. 2006; Ferrante et al. 2006; Greenman et al. 2003; Karamouzis et al. 2015; Losa et al. 2008; Murad et al. 2010; O'Sullivan et al. 2009; Reddy et al. 2011; Roelfsema et al. 2012; Soto-Ares et al. 2002; Turner et al. 1999b; van den Bergh et al. 2007; Vargas et al. 2015; Woollons et al. 2000). According to a meta-analysis, the mean recurrence rate was 46% in patients with a tumor residue; progression-free survival among these patients was 56% and 40% at 5 and 10 years, respectively; and the mean residual tumor volume doubling time was 3.4 years (Chen et al. 2012). Young age, gender, initial tumor size, and invasiveness had prognostic significance in some studies but not in others (Roelfsema et al. 2012).

Histologic Factors

The prognosis is classically poorer in case of a silent corticotroph adenoma (rev. in Cortet-Rudelli et al. 2015). As in other types of pituitary adenoma, expression of tumor

Table 1 Results of surgery and risk of recurrence according to postoperative remnant status. Reproduced from Cortet-Rudelli et al. 2015

First author (year)	Patient total/ patients without RT	Follow-up (years)	Type of study	Recurrence in the absence of remnant	Recurrence in the presence of remnant
Turner (1999)	65/65	6.3	Retrospective	9/31 (20%)	12/34 (35%)
Woolons (2000)	72/22	5.3	Retrospective	2/11 (18%)	8/11 (73%)
Soto-Ares (2002)	51/51	5.6	Prospective	0/17 (0%)	13/34 (38%)
Greenman (2003)	122/108	4.2	Prospective	6/30 (20%)	41/78 (53%)
Ferrante (2006)	226/150	8.1	Retrospective	14/73 (19%)	45/77 (58%)
Dekkers (2006)	97/91	6	Retrospective	1/27 (4%)	9/64 (14%)
Van den Bergh (2007)	122/46	8	Retrospective	1/18 (6%)	16/28 (57%)
Losa (2008)	436/355	4.5	Prospective	PFS 5 years: 87.1%	PFS 5 ans: 39.2%
O'Sullivan (2009)	126/126	5.7	Retrospective	0/26 (0%)	53/100 (53%)
Brochier (2010)	142/127	6.9	Retrospective	10/42 (24%)	46/85 (54%)
Reddy (2011)	144/144	6.1	Retrospective	2/29 (7%)	49/115 (43%)
Total	**1603/1285**			**45/304 (15%)**	**292/626 (47%)**

RT radiotherapy, *PFS* progression-free survival

biomarkers, particularly Ki-67 (see above), can help to assess the risk of recurrence (Mete and Asa 2012; Ramirez et al. 2012; Righi et al. 2012; Trouillas et al. 2013).

Postoperative Follow-Up

MRI and Ophthalmologic Monitoring

Usually, the first postoperative MRI is performed after 3 or 6 months. If no tumor remnant is present, the French Endocrine Society Consensus recommends yearly MRI follow-up for 5 years, then at 7, 10, and 15 years. If a remnant is present, MRI may be repeated annually for 5 years and then every 2 or 3 years in the absence of progression; the schedule is then redefined on a case-by-case basis, according to the size of the remnant and its distance from the optic pathways (Chanson et al. 2015; Cortet-Rudelli et al. 2015). If visual defects persist preoperatively, visual assessment should be performed regularly until the maximal improvement is achieved.

Hormonal Follow-Up

Biochemical hormonal assessment 3 months after surgery shows whether the preoperative pituitary deficiencies have improved and if any new deficiencies have occurred. Biochemical exploration is then repeated for adaptation of replacement therapies. If radiotherapy is decided, pituitary function needs to be assessed every year in order to detect possible late-onset deficiencies.

Options in Case of Tumor Recurrence or Remnant Progression

Repeat Surgery

Repeat surgery (Losa et al. 2008; O'Sullivan et al. 2009) may be indicated if the growing residue or recurrence is amenable to total resection, if optic pathway compression persists or recurs, or if surgery can help to achieve the anatomic conditions necessary for stereotactic radiotherapy (3–5 mm safety margin between the adenoma and optic pathways). However, repeat surgery is less effective in terms of tumor control (it often persists a remnant) and visual outcome, and complications (CSF leak) are more frequent than after initial surgery (Brada et al. 1993).

Radiation Therapy (RT)

Radiotherapy Techniques

- Fractionated conformal RT delivers high-energy X photons. The total dose is between 45 and 50 Gy, fractionated in 25 sessions of 1.8-2 Gy.
- Radiosurgery delivers radiation in a single session. It requires the use of an invasive stereotactic frame for precise positioning. The devices used are the Gamma Knife (201 ^{60}Co sources on a hemisphere) and linear accelerator (LINAC). This type of RT is only feasible if the target volume is clearly defined, small (<2–3 cm on the longer axis), and sufficiently remote from the optic pathways to ensure <8 Gy irradiation of the chiasm and optic nerves.
- Fractionated stereotactic RT combines the ballistic precision and multiple beam entries of radiosurgery with the principle of healthy tissue radioprotection by fractioning. The total dose is 45–50 Gy, administered in fractions of 1.8 to 2 Gy. The CyberKnife is a miniaturized accelerator with a robotic arm, allowing hypofractionated stereotactic RT (3–9 sessions) with a noninvasive contention system based on the technical and dosimetric principles of radiosurgery.
- Proton therapy is not widely available and is thus rarely used for NFPA.

Results of Radiotherapy

- Postoperative fractionated RT considerably reduces the risk of NFPA recurrence, with a 10-year progression-free survival rate higher than 90% in most series (Brada et al. 1993; Brochier et al. 2010; Chen et al. 2012; Gittoes et al. 1998; Jaffrain-Rea et al. 1993; Olsson et al. 2009; Park et al. 2004; Tsang et al. 1994; van den Bergh et al. 2007) (Tables 2 and 3). Almost all reports show a major

Table 2 Impact of fractionated conformational radiotherapy (FRT) on the risk of NFPA progression/recurrence in recent series. Reproduced from Cortet-Rudelli et al. 2015

First author (year)	N	Follow-up (years)	N patients with FRT/N patients without FRT	% free of recurrence at 5 years		% free of recurrence at 10 years	
				FRT (%)	No FRT (%)	FRT (%)	No FRT (%)
Jaffrain-Rea (1993)	57	7.1	24/33	100	70	96	55
Gittoes (1998)	126	9	81/355	93	68	93	47
Woollons (2000)	72	5.3	50/22	72	34	–	–
Park (2004)	176	4.3	44/132	98	85	98	50
Van den Bergh (2007)	122	8	76/46	95	49	95	22
Olsson (2009)	235	10	62/173	–	–	94	62
Brochier (2010)	142	6.9	15/127	100	70	91	52

benefit of RT in terms of the nonrecurrence rate at 5 years (66 ± 19% without RT, 94 ± 9% with RT) and at 10 years (52 ± 16% and 92 ± 6%, respectively). The overall relapse rate was about threefold lower with RT (55/468, 12% with RT; 321/986, 32% without RT). A recent meta-analysis (Murad et al. 2010) showed a relative risk of recurrence of 1.97 without RT compared with RT.

– Studies of fractionated stereotactic RT have involved fewer patients, and mean follow-up rarely exceeds 5 years (Astradsson et al. 2014; Bostrom et al. 2014; Colin et al. 2005; Milker-Zabel et al. 2001; Minniti et al. 2006, 2015; Paek et al. 2005; Schalin-Jantti et al. 2010). The results are generally similar to those of classical RT, with tumor control in more than 95% of patients at 5 years and in more than 90% of cases at 10 years.
– Most studies of radiosurgery also involved small numbers of patients, with less than 10 years of follow-up (Bir et al. 2015; Hoybye and Rahn 2009; Iwai et al. 2005; Lee et al. 2014; Liscak et al. 2007; Losa et al. 2011, 2016b; Mingione et al. 2006; Petrovich et al. 2003; Picozzi et al. 2005; Pollock et al. 2008; Sheehan et al. 2002, 2013; Wilson et al. 2012; Wowra and Stummer 2002). The results are generally similar to those of fractionated RT, with tumor control in 90–100% of patients at 5 years. Data for 512 patients managed in 9 North American centers showed tumor control rates of 95% and 85%, at 5 and 10 years, respectively (Sheehan et al. 2013). A meta-analysis showed better tumor control in patients with tumors smaller than 4 ml (Chen et al. 2013). There are no controlled studies comparing radiosurgery versus surgery alone.

Table 3 Efficacy and complications of pituitary irradiation modalities. Reproduced from Cortet-Rudelli et al. 2015

Type of radiotherapy	Conventional/conformational fractionated	Stereotactic multifractionated	CyberKnife	LINAC	Gamma Knife
Tumoral control	5 years: 72–100%	93–100%	93–98%	93–98%	89.9–100%
	10 years: 93–98%				
	20 years: 70–90%				
Hypopituitarism	↗with time 50–80% > 10 years	5–35%	0–20%	0–9.8%	7–40%
Visual complications	< 1%	< 3%	0–1%	< 2%	0–13.7%
Secondary intracranial neoplasms	1.3–2% at 10 years	?	?	?	Rare cases of malignant glioblastomas
	1.9–2.7% at 20 years				
Radionecrosis	↘↘↘	↘↘↘	?	Reported	?
Vascular	Conventional: RR 1.5–4 (risk factors: Dose, female gender, surgery)	Not reported	Not reported	Intracavernous carotid artery stenosis stroke	Intracavernous carotid artery stenosis
Others	?	?	?	?	Trigeminal neuralgia

Complications of Radiation Therapy (Table 3)

- Hypopituitarism is the most frequent complication of fractionated RT. It may occur after several years and progress with time. Its prevalence ranges from 50% to 80% in studies with follow-up exceeding 10 years (Brada et al. 1993): regular long-term follow-up is thus mandatory after RT. Severe ophthalmologic complications are rare (<1%) and may occur late (Erridge et al. 2009); they are more frequent when optic pathway was already impaired prior to RT and when systematic prospective ophthalmological follow-up is organized after fractionated stereotactic RT (Astradsson et al. 2014). Radiation-induced brain tumors (astrocytoma, glioma, glioblastoma sarcoma, or meningioma) are a rare but well-established risk of RT: in a series of 426 adenomas treated with RT between 1962 and 1994, the risk was 2% at 10 years, 2.4% at 20 years, and 8.5% at 30 years (Minniti et al. 2005b). Vascular inflammation secondary to RT may induce or aggravate atherosclerosis, which can also be aggravated by hypopituitarism and its treatment (e.g., overtreatment with glucocorticoids). An increased risk of stroke and cardio-/cerebrovascular death has been found in patients with hypopituitarism treated by radiotherapy (Brada et al. 1999, 2002; Plummer et al. 2011; Sherlock et al. 2010).
- Following Gamma Knife radiosurgery, the incidence of visual impairment ranges from 0% to 13.7%, depending on the series, and the incidence of RT-induced hypopituitarism ranges from 7% to 40% (Bir et al. 2015; Hoybye and Rahn 2009; Iwai et al. 2005; Lee et al. 2014; Liscak et al. 2007; Losa et al. 2011, 2016b; Mingione et al. 2006; Petrovich et al. 2003; Picozzi et al. 2005; Pollock et al. 2008; Sheehan et al. 2002, 2013; Wilson et al. 2012; Wowra and Stummer 2002).

Indications of Radiotherapy?

Most experts now agree that immediate postoperative RT is not indicated after complete tumor resection, as the risk of recurrence is low. Treatment can be postponed without loss of efficacy, but regular MRI surveillance should be maintained for many years. In case of recurrence, RT is generally proposed, preceded or not by a repeat surgery (see above).

In the presence of a significant tumor residue, especially if invasive, the indications for RT should take into account risk factors for tumor regrowth, the patient's age and history, and the presence of hypopituitarism. In most cases, regular monitoring is an acceptable option, treatment being postponed until the residue progresses and/or becomes threatening. RT is indicated if the tumor has a high growth potential, and the tumoral risk always takes precedence over hypopituitarism when considering management options.

The different RT modalities have similar efficacy in terms of tumor control, and the choice thus depends on the size, boundaries, and location of the tumor residue with respect to neighboring neural structures and also on the center's experience with and access to the different machines.

Medical Treatment

- Gn-RH agonists and antagonists have proved unable to reduce the volume of NFPAs (Colao et al. 2008).
- Dopamine agonists (DA) have been reported to reduce NFPA volume (Greenman et al. 2005). The available retrospective studies used variable drug doses and treatment durations (Colao et al. 2008). DA therapy sometimes improved visual disorders, despite no significant reduction in tumor volume (Colao et al. 2008). A recent study (Greenman et al. 2016) performed on 79 patients followed on average 8 years showed that DAs, either given upon residual tumor detection on postoperative MRI (preventive treatment (PT) group) or when tumor growth was subsequently detected during follow-up (remediable treatment (RT) group), are effective on tumor remnant growth, inducing stabilization or shrinkage of tumor volume more often than in a control group untreated. About 42% of patients in the control group required additional surgery or radiotherapy, compared with 38 and 13% subjects in the RT and PT groups, respectively ($P = 0.002$). Outcome measures were not related to NFPA D2 receptors abundance (Greenman et al. 2016).
- Somatostatin analogs were also reported to reduce tumor volume in a small percentage of cases (Colao et al. 2008).

The Particular Case of Nonfunctioning Pituitary Incidentalomas

Most pituitary incidentalomas are NFPAs. Patient management may differ somewhat from that of symptomatic pituitary adenomas (P Chanson and Young 2003; Freda et al. 2011; Galland et al. 2015; Molitch 2008). Macroadenomas increase in size more often than microadenomas (see above), which, given its initial size, may cause visual disorder. Attitude depends on macroadenoma size and also on proximity to the optic chiasm.

- If the adenoma is remote ($\geq$ 5 mm) from the optic chiasm, the French Endocrine Society Consensus suggests to perform MRI at 1 year with hormonal biochemical assessment looking for hypopituitarism (Chanson et al. 2015; Galland et al. 2015). In case of absence of progression, surveillance intervals may be increased to two yearly MRI. Annual hormonal biochemical assessment should be maintained in case of progression. Visual assessment (VA, VF) should be performed for lesions coming abutting the optic chiasm during follow-up. In case of progression, surgical resection may be considered.
- If the adenoma is close to the chiasm, the French Endocrine Society Consensus (Chanson et al. 2015; Galland et al. 2015) considers that surgery is not formally indicated but should be discussed with the patient, taking into account the natural history of NFPA, the low morbidity associated with surgery, and the need for the patient's compliance with surveillance, any plans for pregnancy, risk factors for

apoplexy, etc. If surgery is not decided on, control MRI should be performed at 6 months, completed by hormonal and visual assessment. MRI and hormonal assessment are thereafter continued annually, with visual evaluation every 6 months.

The guidelines of the Endocrine Society (Freda et al. 2011) recommend MRI at 6 months, then annually for 3 years, and then less frequently (whether the lesion is close to the optic chiasm or not).

In case of visual involvement, hypopituitarism, or tumoral progression, treatment strategy is similar to that proposed for symptomatic NFPA (see above).

The Particular Case of Nonfunctioning Pituitary Aggressive Tumors and Pituitary Carcinomas

Aggressive NFP tumors are invasive tumors with unusually rapid growth rate or clinically relevant tumor growth despite optimal standard therapies such as surgery, radiotherapy, and eventually DA (see above).

A large proportion of NF aggressive tumors or pituitary carcinomas are secreting but silent pituitary tumors (Chinezu et al. 2017; Raverot et al. 2010b). This is particularly true for silent corticotropic tumors. Indeed, if 15% of all corticotropic tumors are silent corticotropic tumors, meaning that approximately 2% of all pituitary tumors are silent corticotropic tumors, they represent about 30% of all pituitary carcinomas (Dudziak et al. 2011).

The analysis of proliferative markers (ki67) and p53 expression can help the prediction of tumor behavior and risk of recurrence or progression during the follow-up (Asa 2011; Lloyd et al. 2004; Trouillas et al. 2013). According to a new prognostic classification, grade 2b gonadotroph tumors are associated with a 7.5 higher risk of recurrence or progression compared to grade 1a (Trouillas et al. 2013).

Atypical nonfunctioning tumors represent 10 to 15% of all pituitary tumors (Chiloiro et al. 2015; Del Basso De Caro et al. 2017; Miermeister et al. 2015; Zada et al. 2011). This percentage may be overestimated in the group of gonadotroph adenoma but is more realistic in silent ACTH, GH, or PRL pituitary tumors.

Pituitary carcinomas are rare (0.2% of all pituitary tumors) and are rarely gonadotroph or non-immunoreactive in contrast with what is observed for benign adenomas. Indeed, pituitary carcinomas are mainly lactotroph (47%) or corticotroph (39%), less often non-immunoreactive (23%), and rarely somatotroph, thyrotroph, or gonadotroph (10% in total) (Dudziak et al. 2011).

Treatment options are limited. Temozolomide, an alkylating chemotherapy, can control tumor growth and secretion in about 40% of cases (Bengtsson et al. 2015; Bruno et al. 2015; Bush et al. 2010; Losa et al. 2016a; Raverot et al. 2010a). Only 19 gonadotroph tumors have been treated, and they appeared to be less sensitive to temozolomide compared to functioning tumors, with rare significant tumor shrinkage (Lasolle and Raverot 2016) and stable disease in the majority of cases.

Different alternative therapies should be evaluated including combination with capecitabine, anti-VEGF, tyrosine-kinase inhibitor, or peptide receptor radionuclide

therapy. Only case reports have been published with different success rate (Raverot et al. 2014).

Conclusion

NFPA is usually diagnosed in a patient with signs and symptoms related to a mass effect. Patient management is summarized in Fig. 4. Unless contraindicated, and in a few particular situations, surgery is the mainstay of treatment, after detailed hormonal, neuroradiologic, and ophthalmologic assessment. Resection, generally via a transsphenoidal approach (with the help of an endoscope), should be performed by a neurosurgeon with extensive experience in pituitary surgery, in order to maximize the chances of complete resection and to minimize complications. If a tumor remnant persists (a frequent situation in patients with large and often invasive adenomas), watchful waiting is preferred to routine radiotherapy, as long as the tumor residue does not grow. NFPA can sometimes recur even after complete resection. Postoperative irradiation is only considered in case of residual tumor growth or relapse. Nonfunctioning pituitary incidentalomas may, because of their fortuitous discovery, require a different approach, especially when they are small and/or remote from the optic pathways.

Acknowledgments French Endocrinology Society Nonfunctioning Pituitary Adenoma Working Group Members.

Philippe Chanson (Paris-Kremlin-Bicêtre), Gérald Raverot (Lyon), Christine Cortet-Rudelli (Lille), Françoise Galland (Rennes), Fréderic Castinetti (Marseille), Sylvie Salenave (Paris-Kremlin-Bicêtre), Laure Cazabat (Boulogne-Billancourt), Luc Foubert (Suresnes), Jean-François Bonneville (Besançon), Stephan Gaillard (Suresnes), Anne Boulin (Suresnes), Emmanuel Sonnet (Brest), Marie-Christine Vantyghem (Lille), Guillaume Assie (Paris), Vincent Rohmer (Angers), Brigitte Delemer (Rennes), Jean-Louis Sadoul (Nice), Isabelle Raingeard (Montpellier), Jacqueline Trouillas (Lyon), Chiara Villa (Paris), Jean-Marc Kuhn (Rouen), Henry Dufour (Marseille), Anne Barlier (Marseille), Lorraine Clavier (Créteil), Rachel Desailloud (Amiens), Emmanuel Jouanneau (Lyon), Dominique Maiter (Bruxelles, Belgique), Catherine Massart (Rennes), Françoise Borson-Chazot (Lyon), Michèle D'herbomez (Lille), Bruno Estour (Saint-Etienne), Gilles Brassier (Rennes) Muriel Cogne (Saint-Denis, La Réunion), François Cotton (Lyon), Maud Lebas (Rouen), Lucie Abouaf (Lyon), Alain Vighetto (Lyon), Bernard Coche Dequéant (Lille), Patrick Toussaint (Amiens), Gwenaëlle Vidal-Trécan (Paris), Alexandre Vasiljevic (Lyon), and Hervé Lefebvre (Rouen).

References

Abouaf L, Vighetto A, Lebas M. Neuro-ophthalmologic exploration in non-functioning pituitary adenoma. Ann Endocrinol (Paris). 2015;76(3):210–9. https://doi.org/10.1016/j.ando.2015.04.006.

Ammirati M, Wei L, Ciric I. Short-term outcome of endoscopic versus microscopic pituitary adenoma surgery: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2013;84(8):843–9. https://doi.org/10.1136/jnnp-2012-303194.

Arafah BM, Kailani SH, Nekl KE, Gold RS, Selman WR. Immediate recovery of pituitary function after transsphenoidal resection of pituitary macroadenomas. J Clin Endocrinol Metab. 1994;79(2):348–54. https://doi.org/10.1210/jcem.79.2.8045946.

Arafah BM, Prunty D, Ybarra J, Hlavin ML, Selman WR. The dominant role of increased intrasellar pressure in the pathogenesis of hypopituitarism, hyperprolactinemia, and headaches in patients with pituitary adenomas. J Clin Endocrinol Metab. 2000;85(5):1789–93.

Asa SL. Pituitary adenomas. In Asa SL, editors. Tumors of the pituitary gland. Vol. Fourth series fascicle 15. Washington, DC: Armed Forces Institute of Pathology; 2011. p. 55–172.

Asa SL, Cheng Z, Ramyar L, Singer W, Kovacs K, Smyth HS, Muller P. Human pituitary null cell adenomas and oncocytomas in vitro: effects of adenohypophysiotropic hormones and gonadal steroids on hormone secretion and tumor cell morphology. J Clin Endocrinol Metab. 1992;74(5):1128–34.

Asa SL, Bamberger AM, Cao B, Wong M, Parker KL, Ezzat S. The transcription activator steroidogenic factor-1 is preferentially expressed in the human pituitary gonadotroph. J Clin Endocrinol Metab. 1996;81(6):2165–70.

Astradsson A, Wiencke AK, Munck af Rosenschold P, Engelholm SA, Ohlhues L, Roed H, Juhler M. Visual outcome after fractionated stereotactic radiation therapy of benign anterior skull base tumors. J Neuro-Oncol. 2014;118(1):101–8. https://doi.org/10.1007/s11060-014-1399-0.

Bahar A, Bicknell JE, Simpson DJ, Clayton RN, Farrell WE. Loss of expression of the growth inhibitory gene GADD45gamma, in human pituitary adenomas, is associated with CpG island methylation. Oncogene. 2004;23(4):936–44.

Barkan AL, Chandler WF. Giant pituitary prolactinoma with falsely low serum prolactin: the pitfall of the "high-dose hook effect": case report. Neurosurgery. 1998;42(4):913–5; discussion 915-916.

Bastos RV, Silva CM, Tagliarini JV, Zanini MA, Romero FR, Boguszewski CL, Nunes VD. Endoscopic versus microscopic transsphenoidal surgery in the treatment of pituitary tumors: systematic review and meta-analysis of randomized and non-randomized controlled trials. Arch Endocrinol Metab. 2016;60(5):411–9. https://doi.org/10.1590/2359-3997000000204.

Beckers A, Stevenaert A, Mashiter K, Hennen G. Follicle-stimulating hormone-secreting pituitary adenomas. J Clin Endocrinol Metab. 1985;61:525–8.

Beckers A, Aaltonen LA, Daly AF, Karhu A. Familial isolated pituitary adenomas (FIPA) and the pituitary adenoma predisposition due to mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene. Endocr Rev. 2013;34(2):239–77. https://doi.org/10.1210/er.2012-1013.

Bengtsson D, Schroder HD, Andersen M, Maiter D, Berinder K, Feldt Rasmussen U, . . . Burman P. Long-term outcome and MGMT as a predictive marker in 24 patients with atypical pituitary adenomas and pituitary carcinomas given treatment with temozolomide. J Clin Endocrinol Metab. 2015;100(4):1689–98. https://doi.org/10.1210/jc.2014-4350.

Bir SC, Murray RD, Ambekar S, Bollam P, Nanda A. Clinical and radiologic outcome of gamma knife radiosurgery on nonfunctioning pituitary adenomas. J Neurol Surg B Skull Base. 2015;76(5):351–7. https://doi.org/10.1055/s-0035-1549309.

Black PM, Jameson JL, Hsu DN, Klibanski A, Hedley-Whyte ET, Ridgway EC, . . . Zervas NT. Hormone products of apparently non functioning pituitary adenomas. In Landolt AM, Heitz PU, Zapf J, Girard J, Del Pozo E, editors. Advances in pituitary adenoma research, vol 69. Oxford: Pergamon Press; 1988. p. 413.

Borgato S, Romoli R, Cortelazzi D, Asteria C, Persani L, Beck-Peccoz P. FSH bioactivity and serum inhibin a-a and a-ßa levels in patients with gonadotropinoma and non functioning pituitary adenomas. J Endocrinol Investig. 1996;19(Suppl to 5):34. (Abstract).

Bostrom JP, Meyer A, Pintea B, Gerlach R, Surber G, Lammering G, Hamm K. Risk-adapted single or fractionated stereotactic high-precision radiotherapy in a pooled series of nonfunctioning pituitary adenomas: high local control and low toxicity. Strahlenther Onkol. 2014;190(12):1095–103. https://doi.org/10.1007/s00066-014-0715-0.

Brada M, Rajan B, Traish D, Ashley S, Holmes-Sellors PJ, Nussey S, Uttley D. The long-term efficacy of conservative surgery and radiotherapy in the control of pituitary adenomas. Clin Endocrinol. 1993;38(6):571–8.

Brada M, Burchell L, Ashley S, Traish D. The incidence of cerebrovascular accidents in patients with pituitary adenoma. Int J Radiat Oncol Biol Phys. 1999;45(3):693–8.

Brada M, Ashley S, Ford D, Traish D, Burchell L, Rajan B. Cerebrovascular mortality in patients with pituitary adenoma. Clin Endocrinol (Oxf). 2002;57(6):713–7.

Briet C, Salenave S, Bonneville JF, Laws ER, Chanson P. Pituitary apoplexy. Endocr Rev. 2015;36(6):622–45. https://doi.org/10.1210/er.2015-1042.

Brochier S, Galland F, Kujas M, Parker F, Gaillard S, Raftopoulos C, ... Chanson P. Factors predicting relapse of nonfunctioning pituitary macroadenomas after neurosurgery: a study of 142 patients. Eur J Endocrinol. 2010;163(2):193–200. https://doi.org/10.1530/EJE-10-0255.

Bruno OD, Juarez-Allen L, Christiansen SB, Manavela M, Danilowicz K, Vigovich C, Gomez RM. Temozolomide therapy for aggressive pituitary tumors: results in a small series of patients from Argentina. Int J Endocrinol. 2015;2015:587893. https://doi.org/10.1155/2015/587893.

Bush ZM, Longtine JA, Cunningham T, Schiff D, Jane JA Jr, Vance ML, ... Lopes MB. Temozolomide treatment for aggressive pituitary tumors: correlation of clinical outcome with O(6)-methylguanine methyltransferase (MGMT) promoter methylation and expression. J Clin Endocrinol Metab. 2010;95(11):E280–90. https://doi.org/10.1210/jc.2010-0441.

Castinetti F, Dufour H, Gaillard S, Jouanneau E, Vasiljevic A, Villa C, Trouillas J. Non-functioning pituitary adenoma: when and how to operate? What pathologic criteria for typing? Ann Endocrinol (Paris). 2015;76(3):220–7. https://doi.org/10.1016/j.ando.2015.04.007.

Chanson P, Brochier S. Non-functioning pituitary adenomas. J Endocrinol Investig. 2005;28(Suppl 11):93–9.

Chanson P, Maiter D. Prolactinoma. In: Melmed S, editor. The pituitary. 4th ed. San Diego: Elsevier; 2017. p. 467–514.

Chanson P, Young J. Pituitary incidentalomas. Endocrinologist. 2003;13(2):124–35.

Chanson P, Pantel J, Young J, Couzinet B, Bidart JM, Schaison G. Free luteinizing-hormone beta-subunit in normal subjects and patients with pituitary adenomas. J Clin Endocrinol Metab. 1997;82(5):1397–402.

Chanson P, De Roux N, Young J, Bidart JM, Jaquet P, Misrahi M, ... Schaison G. Absence of activating mutations in the GnRH receptor gene in human pituitary gonadotroph adenomas. Eur J Med. 1998;139:157–60.

Chanson P, Raverot G, Castinetti F, Cortet-Rudelli C, Galland F, Salenave S, French Endocrinology Society non-functioning pituitary adenoma work group. Management of clinically non-functioning pituitary adenoma. Ann Endocrinol (Paris). 2015;76(3):239–47. https://doi.org/10.1016/j.ando.2015.04.002.

Chen Y, Wang CD, Su ZP, Chen YX, Cai L, Zhuge QC, Wu ZB. Natural history of postoperative nonfunctioning pituitary adenomas: a systematic review and meta-analysis. Neuroendocrinology. 2012;96(4):333–42. https://doi.org/10.1159/000339823.

Chen Y, Li ZF, Zhang FX, Li JX, Cai L, Zhuge QC, Wu ZB. Gamma knife surgery for patients with volumetric classification of nonfunctioning pituitary adenomas: a systematic review and meta-analysis. Eur J Endocrinol. 2013;169(4):487–95. https://doi.org/10.1530/EJE-13-0400.

Chesnokova V, Zonis S, Rubinek T, Yu R, Ben-Shlomo A, Kovacs K, ... Melmed S. Senescence mediates pituitary hypoplasia and restrains pituitary tumor growth. Cancer Res. 2007;67(21):10564–72.

Chesnokova V, Zonis S, Zhou C, Ben-Shlomo A, Wawrowsky K, Toledano Y, ... Melmed S. Lineage-specific restraint of pituitary gonadotroph cell adenoma growth. PLoS One. 2011;6(3):e17924. https://doi.org/10.1371/journal.pone.0017924.

Chesnokova V, Zonis S, Wawrowsky K, Tani Y, Ben-Shlomo A, Ljubimov V, ... Melmed S. Clusterin and FOXL2 act concordantly to regulate pituitary gonadotroph adenoma growth. Mol Endocrinol. 2012;26(12):2092–103. https://doi.org/10.1210/me.2012-1158.

Cheunsuchon P, Zhou Y, Zhang X, Lee H, Chen W, Nakayama Y, ... Klibanski A. Silencing of the imprinted DLK1-MEG3 locus in human clinically nonfunctioning pituitary adenomas. Am J Pathol. 2011;179(4):2120–30. https://doi.org/10.1016/j.ajpath.2011.07.002.

Chiloiro S, Doglietto F, Trapasso B, Iacovazzo D, Giampietro A, Di Nardo F, . . . Bianchi A. Typical and atypical pituitary adenomas: a single-center analysis of outcome and prognosis. Neuroendocrinology. 2015;101(2):143–50. https://doi.org/10.1159/000375448.

Chinezu L, Vasiljevic A, Trouillas J, Lapoirie M, Jouanneau E, Raverot G. Silent somatotroph tumour revisited from a study of 80 patients with and without acromegaly and a review of the literature. Eur J Endocrinol. 2017;176(2):195–201. https://doi.org/10.1530/EJE-16-0738.

Colao A, Di Somma C, Pivonello R, Faggiano A, Lombardi G, Savastano S. Medical therapy for clinically non-functioning pituitary adenomas. Endocr Relat Cancer. 2008;15(4):905–15.

Colin P, Jovenin N, Delemer B, Caron J, Grulet H, Hecart AC, . . . Rousseaux P. Treatment of pituitary adenomas by fractionated stereotactic radiotherapy: a prospective study of 110 patients. Int J Radiat Oncol Biol Phys. 2005;62(2):333–41. https://doi.org/10.1016/j.ijrobp.2004.09.058.

Comtois R, Beauregard H, Somma M, Serri O, Aris-Jilwan N, Hardy J. The clinical and endocrine outcome to trans-sphenoidal microsurgery of non secreting pituitary adenomas. Cancer. 1991;68:860–6.

Cooper O, Geller JL, Melmed S. Ovarian hyperstimulation syndrome caused by an FSH-secreting pituitary adenoma. Nat Clin Pract Endocrinol Metab. 2008;4(4):234–8.

Cortet-Rudelli C, Bonneville JF, Borson-Chazot F, Clavier L, Coche Dequeant B, Desailloud R, . . . Chanson P. Post-surgical management of non-functioning pituitary adenoma. Ann Endocrinol (Paris). 2015;76(3):228–38. https://doi.org/10.1016/j.ando.2015.04.003.

D'Angelo D, Palmieri D, Mussnich P, Roche M, Wierinckx A, Raverot G, . . . Fusco A. Altered microRNA expression profile in human pituitary GH adenomas: down-regulation of miRNA targeting HMGA1, HMGA2, and E2F1. J Clin Endocrinol Metab. 2012;97(7):E1128–38. https://doi.org/10.1210/jc.2011-3482.

Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A. High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. J Clin Endocrinol Metab. 2006;91(12):4769–75.

Daneshdoost L, Gennarelli TA, Bashey HM, Savino PJ, Sergott RC, Bosley TM, Snyder PJ. Recognition of gonadotroph adenomas in women. N Engl J Med. 1991;324:589–94.

Daneshdoost L, Gennarelli TA, Bashey HM, Savino PJ, Sergott RC, Bosley TM, Snyder PJ. Identification of gonadotroph adenomas in men with clinically non functioning adenomas by the luteinizing hormone ß subunit response to thyrotropin-releasing hormone. J Clin Endocrinol Metab. 1993;77:1352–5.

Davis JR, McNeilly JR, Norris AJ, Pope C, Wilding M, McDowell G, . . . McNeilly AS. Fetal gonadotrope cell origin of FSH-secreting pituitary adenoma – insight into human pituitary tumour pathogenesis. Clin Endocrinol (Oxf). 2006;65(5):648–54. https://doi.org/10.1111/j.1365-2265.2006.02644.x.

de Laat JM, Dekkers OM, Pieterman CR, Kluijfhout WP, Hermus AR, Pereira AM, . . . Valk GD. Long-term natural course of pituitary tumors in patients with MEN1: results from the Dutch-MEN1 Study Group (DMSG). J Clin Endocrinol Metab. 2015;100(9):3288–96. https://doi.org/10.1210/JC.2015-2015.

Dehdashti AR, Ganna A, Karabatsou K, Gentili F. Pure endoscopic endonasal approach for pituitary adenomas: early surgical results in 200 patients and comparison with previous microsurgical series. Neurosurgery. 2008;62(5):1006–15. https://doi.org/10.1227/01.neu.0000325862.83961.12; discussion 1015-1007.

Dekkers OM, Pereira AM, Roelfsema F, Voormolen JH, Neelis KJ, Schroijen MA, . . . Romijn JA. Observation alone after transsphenoidal surgery for nonfunctioning pituitary macroadenoma. J Clin Endocrinol Metab. 2006;91(5):1796–801.

Dekkers OM, Pereira AM, Romijn JA. Treatment and follow-up of clinically nonfunctioning pituitary macroadenomas. J Clin Endocrinol Metab. 2008;93(10):3717–26.

Del Basso De Caro M, Solari D, Pagliuca F, Villa A, Guadagno E, Cavallo LM, . . . Cappabianca P. Atypical pituitary adenomas: clinical characteristics and role of ki-67 and p53 in prognostic and therapeutic evaluation. A series of 50 patients. Neurosurg Rev. 2017;40(1):105–14. https://doi.org/10.1007/s10143-016-0740-9.

Dudziak K, Honegger J, Bornemann A, Horger M, Mussig K. Pituitary carcinoma with malignant growth from first presentation and fulminant clinical course–case report and review of the literature. J Clin Endocrinol Metab. 2011;96(9):2665–9. https://doi.org/10.1210/jc.2011-1166.

Duong CV, Emes RD, Wessely F, Yacqub-Usman K, Clayton RN, Farrell WE. Quantitative, genome-wide analysis of the DNA methylome in sporadic pituitary adenomas. Endocr Relat Cancer. 2012;19(6):805–16. https://doi.org/10.1530/ERC-12-0251.

Dworakowska D, Wlodek E, Leontiou CA, Igreja S, Cakir M, Teng M, ... Grossman AB. Activation of RAF/MEK/ERK and PI3K/AKT/mTOR pathways in pituitary adenomas and their effects on downstream effectors. Endocr Relat Cancer. 2009;16(4):1329–38. https://doi.org/10.1677/ERC-09-0101.

Elston MS, Gill AJ, Conaglen JV, Clarkson A, Shaw JM, Law AJ, ... McDonald KL. Wnt pathway inhibitors are strongly down-regulated in pituitary tumors. Endocrinology. 2008;149(3):1235–42. https://doi.org/10.1210/en.2007-0542.

Erickson D, Scheithauer B, Atkinson J, Horvath E, Kovacs K, Lloyd RV, Young WF Jr. Silent subtype 3 pituitary adenoma: a clinicopathologic analysis of the Mayo Clinic experience. Clin Endocrinol (Oxf). 2009;71(1):92–9.

Erridge SC, Conkey DS, Stockton D, Strachan MW, Statham PF, Whittle IR, ... Gregor A. Radiotherapy for pituitary adenomas: long-term efficacy and toxicity. Radiother Oncol. 2009;93(3):597–601. https://doi.org/10.1016/j.radonc.2009.09.011.

Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, McCutcheon IE. The prevalence of pituitary adenomas: a systematic review. Cancer. 2004;101(3):613–9.

Fan X, Gabbi C, Kim HJ, Cheng G, Andersson LC, Warner M, Gustafsson JA. Gonadotropin-positive pituitary tumors accompanied by ovarian tumors in aging female ERbeta$^{-/-}$ mice. Proc Natl Acad Sci USA. 2010;107(14):6453–8. https://doi.org/10.1073/pnas.1002029107.

Feng J, Yu SY, Li CZ, Li ZY, Zhang YZ. Integrative proteomics and transcriptomics revealed that activation of the IL-6R/JAK2/STAT3/MMP9 signaling pathway is correlated with invasion of pituitary null cell adenomas. Mol Cell Endocrinol. 2016;436:195–203. https://doi.org/10.1016/j.mce.2016.07.025.

Fernandez A, Karavitaki N, Wass JA. Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). Clin Endocrinol (Oxf). 2009;72:377–82.

Fernandez-Balsells MM, Murad MH, Barwise A, Gallegos-Orozco JF, Paul A, Lane MA, ... Montori VM. Natural history of nonfunctioning pituitary adenomas and incidentalomas: a systematic review and metaanalysis. J Clin Endocrinol Metab. 2011;96(4):905–12. https://doi.org/10.1210/jc.2010-1054.

Ferrante E, Ferraroni M, Castrignano T, Menicatti L, Anagni M, Reimondo G, ... Lania AG. Non-functioning pituitary adenoma database: a useful resource to improve the clinical management of pituitary tumors. Eur J Endocrinol. 2006;155(6):823–9.

Ferrau F, Romeo PD, Puglisi S, Ragonese M, Torre ML, Scaroni C, ... Cannavo S. Analysis of GPR101 and AIP genes mutations in acromegaly: a multicentric study. Endocrine. 2016;54(3):762–7. https://doi.org/10.1007/s12020-016-0862-4.

Filippella M, Galland F, Kujas M, Young J, Faggiano A, Lombardi G, ... Chanson P. Pituitary tumour transforming gene (PTTG) expression correlates with the proliferative activity and recurrence status of pituitary adenomas: a clinical and immunohistochemical study. Clin Endocrinol (Oxf). 2006;65(4):536–43. https://doi.org/10.1111/j.1365-2265.2006.02630.x.

Freda PU, Beckers AM, Katznelson L, Molitch ME, Montori VM, Post KD, ... Endocrine Society. Pituitary incidentaloma: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(4):894–904. https://doi.org/10.1210/jc.2010-1048.

Frieze TW, Mong DP, Koops MK. "Hook effect" in prolactinomas: case report and review of literature. Endocr Pract. 2002;8(4):296–303. https://doi.org/10.4158/EP.8.4.296.

Galland F, Vantyghem MC, Cazabat L, Boulin A, Cotton F, Bonneville JF, ... Chanson P. Management of nonfunctioning pituitary incidentaloma. Ann Endocrinol (Paris). 2015;76(3):191–200. https://doi.org/10.1016/j.ando.2015.04.004.

Galway AB, Hsue AJW, Daneshdoost L, Zhou MH, Pavlou SN, Snyder PJ. Gonadotroph adenomas in men produce biologically active follicle-stimulating hormone. J Clin Endocrinol Metab. 1990;71:907–12.

Gejman R, Batista DL, Zhong Y, Zhou Y, Zhang X, Swearingen B, ... Klibanski A. Selective loss of MEG3 expression and intergenic differentially methylated region hypermethylation in the MEG3/DLK1 locus in human clinically nonfunctioning pituitary adenomas. J Clin Endocrinol Metab. 2008;93(10):4119–25.

Gittoes NJL, Bates AS, Tse W, Bullivan B, Sheppard MC, Clayton RN, Stewart PM. Radiotherapy for non-functioning pituitary tumours. Clin Endocrinol. 1998;48:331–7.

Gnanalingham KK, Bhattacharjee S, Pennington R, Ng J, Mendoza N. The time course of visual field recovery following trans-sphenoidal surgery for pituitary adenomas: predictive factors for a good outcome. J Neurol Neurosurg Psychiatry. 2005;76(3):415–9. https://doi.org/10.1136/jnnp.2004.035576.

Greenman Y, Stern N. How should a nonfunctioning pituitary macroadenoma be monitored after debulking surgery? Clin Endocrinol (Oxf). 2009a;70(6):829–32. https://doi.org/10.1111/j.1365-2265.2009.03542.x.

Greenman Y, Stern N. Non-functioning pituitary adenomas. Best Pract Res Clin Endocrinol Metab. 2009b;23(5):625–38.

Greenman Y, Tordjman K, Kisch E, Razon N, Ouaknine G, Stern N. Relative sparing of anterior pituitary function in patients with growth hormone-secreting macroadenomas: comparison with nonfunctioning macroadenomas. J Clin Endocrinol Metab. 1995;80(5):1577–83.

Greenman Y, Ouaknine G, Veshchev I, Reider G II, Segev Y, Stern N. Postoperative surveillance of clinically nonfunctioning pituitary macroadenomas: markers of tumour quiescence and regrowth. Clin Endocrinol (Oxf). 2003;58(6):763–9.

Greenman Y, Tordjman K, Osher E, Veshchev I, Shenkerman G, Reider G II, ... Stern N. Postoperative treatment of clinically nonfunctioning pituitary adenomas with dopamine agonists decreases tumour remnant growth. Clin Endocrinol (Oxf). 2005;63(1):39–44.

Greenman Y, Cooper O, Yaish I, Robenshtok E, Sagiv N, Jonas-Kimchi T, ... Stern N. Treatment of clinically nonfunctioning pituitary adenomas with dopamine agonists. Eur J Endocrinol. 2016;175(1):63–72. https://doi.org/10.1530/EJE-16-0206.

Herman V, Fagin J, Gonski R, Kovacs K, Melmed S. Clonal origin of pituitary adenomas. J Clin Endocrinol Metab. 1990;71:1427–30.

Hong J, Ding X, Lu Y. Clinical analysis of 103 elderly patients with pituitary adenomas: trans-sphenoidal surgery and follow-up. J Clin Neurosci. 2008;15(10):1091–5. https://doi.org/10.1016/j.jocn.2007.11.003.

Hoybye C, Rahn T. Adjuvant Gamma Knife radiosurgery in non-functioning pituitary adenomas; low risk of long-term complications in selected patients. Pituitary. 2009;12(3):211–6. https://doi.org/10.1007/s11102-008-0163-x.

Iacovazzo D, Caswell R, Bunce B, Jose S, Yuan B, Hernandez-Ramirez LC, ... Korbonits M. Germline or somatic GPR101 duplication leads to X-linked acrogigantism: a clinico-pathological and genetic study. Acta Neuropathol Commun. 2016;4(1):56. https://doi.org/10.1186/s40478-016-0328-1.

Iwai Y, Yamanaka K, Yoshioka K, Yoshimura M, Honda Y, Matsusaka Y, ... Yasui T. [The usefulness of adjuvant therapy using gamma knife radiosurgery for the recurrent or residual nonfunctioning pituitary adenomas]. No Shinkei Geka. 2005;33(8):777–83.

Jaffrain-Rea ML, Derome P, Bataini JP, Thomopoulos P, Bertagna X, Luton JP. Influence of radiotherapy on long-term relapse in clinically non- secreting pituitary adenomas. A retrospective study (1970–1988). Eur J Med. 1993;2(7):398–403.

Jaffrain-Rea ML, Di Stefano D, Minniti G, Esposito V, Bultrini A, Ferretti E, ... Cantore G. A critical reappraisal of MIB-1 labelling index significance in a large series of pituitary tumours: secreting versus non-secreting adenomas. Endocr Relat Cancer. 2002;9(2):103–13.

Jameson JL, Klibanski A, Black PM, Zervas NT, Lindell CM, Hsu DW, ... Habener JF. Glycoprotein hormone genes are expressed in clinically nonfunctioning pituitary adenomas. J Clin Invest. 1987;80:1472–8.

Kanner AA, Corn BW, Greenman Y. Radiotherapy of nonfunctioning and gonadotroph adenomas. Pituitary. 2009;12(1):15–22.

Karamouzis I, Berardelli R, Prencipe N, Berton A, Bona C, Stura G, ... Grottoli S. Retrospective observational analysis of non-irradiated non-functioning pituitary adenomas. J Endocrinol Invest. 2015;38(11):1191–7. https://doi.org/10.1007/s40618-015-0361-0.

Karavitaki N, Thanabalasingham G, Shore HC, Trifanescu R, Ansorge O, Meston N, ... Wass JA. Do the limits of serum prolactin in disconnection hyperprolactinaemia need re-definition? A study of 226 patients with histologically verified non-functioning pituitary macroadenoma. Clin Endocrinol (Oxf). 2006;65(4):524–9.

Kirsch M, Morz M, Pinzer T, Schackert HK, Schackert G. Frequent loss of the CDKN2C (p18INK4c) gene product in pituitary adenomas. Genes Chromosomes Cancer. 2009;48(2): 143–54.

Klibanski A. Clinical practice. Prolactinomas. N Engl J Med. 2010;362(13):1219–26. https://doi.org/10.1056/NEJMcp0912025.

Kottler ML, Seret-Begue D, Lahlou N, Assayag M, Carre MC, Lagarde JP, ... Warnet A. The GnRH receptor gene is preferentially expressed in functioning gonadotroph adenomas and displays a Mae III polymorphism site [In process citation]. Clin Endocrinol (Oxf). 1998;49(1):115–23.

Kovacs K, Horvath E, Van Loon GR, Rewcastle NB, Ezrin C, Rosenbloom AA. Pituitary adenomas associated with elevated blood follicle-stimulating hormone levels: a histologic, immunocytologic, and electron microscopic study of two cases. Fertil Steril. 1978;29(6):622–8.

Kovacs K, Lloyd R, Horvath E, Asa SL, Stefaneanu L, Killinger DW, Smyth HS. Silent somatotroph adenomas of the human pituitary. A morphologic study of three cases including immunocytochemistry, electron microscopy, in vitro examination, and in situ hybridization. Am J Pathol. 1989;134(2):345–53.

Lasolle H, Raverot G. Therapeutic innovations in endocrine diseases – part 3: temozolomide and future therapeutics for aggressive pituitary tumors and carcinomas. Presse Med. 2016;45(6 Pt 2): e211–6. https://doi.org/10.1016/j.lpm.2016.05.008.

Lecoq AL, Kamenicky P, Guiochon-Mantel A, Chanson P. Genetic mutations in sporadic pituitary adenomas–what to screen for? Nat Rev Endocrinol. 2015;11(1):43–54. https://doi.org/10.1038/nrendo.2014.181.

Lecoq AL, Bouligand J, Hage M, Cazabat L, Salenave S, Linglart A, ... Kamenicky P. Very low frequency of germline GPR101 genetic variation and no biallelic defects with AIP in a large cohort of patients with sporadic pituitary adenomas. Eur J Endocrinol. 2016;174(4):523–30. https://doi.org/10.1530/EJE-15-1044.

Lee M, Marinoni I, Irmler M, Psaras T, Honegger JB, Beschorner R, ... Pellegata NS. Transcriptome analysis of MENX-associated rat pituitary adenomas identifies novel molecular mechanisms involved in the pathogenesis of human pituitary gonadotroph adenomas. Acta Neuropathol. 2013;126(1):137–50. https://doi.org/10.1007/s00401-013-1132-7.

Lee CC, Kano H, Yang HC, Xu Z, Yen CP, Chung WY, ... Sheehan JP. Initial Gamma Knife radiosurgery for nonfunctioning pituitary adenomas. J Neurosurg. 2014;120(3):647–54. https://doi.org/10.3171/2013.11.JNS131757.

Liscak R, Vladyka V, Marek J, Simonova G, Vymazal J. Gamma knife radiosurgery for endocrine-inactive pituitary adenomas. Acta Neurochir (Wien). 2007;149(10):999–1006. https://doi.org/10.1007/s00701-007-1253-7; discussion 1006.

Liu C, Li Z, Wu D, Li C, Zhang Y. Smad3 and phospho-Smad3 are potential markers of invasive nonfunctioning pituitary adenomas. Onco Targets Ther. 2016;9:2265–71. https://doi.org/10.2147/OTT.S99699.

Lloyd RV, Kovacs K, Young JWF, Farrel WE, Asa SL, Trouillas J, Kontogeorgos G, ... Horvath E. Tumours of the pituitary. In DeLellis RA, Lloyd RV, Heitz PU, Eng C, editors. Pathology and genetics of tumours of endocrine organs, vol. 8. 3rd ed. Lyon: IARC Press; 2004. p. 1–50.

Losa M, Mortini P, Barzaghi R, Ribotto P, Terreni MR, Marzoli SB, ... Giovanelli M. Early results of surgery in patients with nonfunctioning pituitary adenoma and analysis of the risk of tumor recurrence. J Neurosurg. 2008;108(3):525–32.

Losa M, Picozzi P, Motta M, Valle M, Franzin A, Mortini P. The role of radiation therapy in the management of non-functioning pituitary adenomas. J Endocrinol Investig. 2011;34(8):623–9. https://doi.org/10.3275/7618.

Losa M, Bogazzi F, Cannavo S, Ceccato F, Curto L, De Marinis L, . . . Scaroni C. Temozolomide therapy in patients with aggressive pituitary adenomas or carcinomas. J Neurooncol. 2016a;126(3):519–25. https://doi.org/10.1007/s11060-015-1991-y.

Losa M, Spatola G, Albano L, Gandolfi A, Del Vecchio A, Bolognesi A, Mortini P. Frequency, pattern, and outcome of recurrences after gamma knife radiosurgery for pituitary adenomas. Endocrine. 2016b. https://doi.org/10.1007/s12020-016-1081-8.

Magro E, Graillon T, Lassave J, Castinetti F, Boissonneau S, Tabouret E, . . . Dufour H. Complications related to the endoscopic endonasal transsphenoidal approach for nonfunctioning pituitary macroadenomas in 300 consecutive patients. World Neurosurg. 2016;89:442–53. https://doi.org/10.1016/j.wneu.2016.02.059.

Melmed S. Pathogenesis of pituitary tumors. Nat Rev Endocrinol. 2011;7(5):257–66. https://doi.org/10.1038/nrendo.2011.40.

Melmed S. Pituitary tumors. Endocrinol Metab Clin N Am. 2015;44(1):1–9. https://doi.org/10.1016/j.ecl.2014.11.004.

Messerer M, Dubourg J, Raverot G, Bervini D, Berhouma M, George I, . . . Jouanneau E. Non-functioning pituitary macro-incidentalomas benefit from early surgery before becoming symptomatic. Clin Neurol Neurosurg. 2013;115(12):2514–20. https://doi.org/10.1016/j.clineuro.2013.10.007.

Mete O, Asa SL. Clinicopathological correlations in pituitary adenomas. Brain Pathol. 2012;22(4):443–53. https://doi.org/10.1111/j.1750-3639.2012.00599.x.

Mete O, Gomez-Hernandez K, Kucharczyk W, Ridout R, Zadeh G, Gentili F, . . . Asa SL. Silent subtype 3 pituitary adenomas are not always silent and represent poorly differentiated monomorphous plurihormonal Pit-1 lineage adenomas. Mod Pathol. 2016;29(2):131–42. https://doi.org/10.1038/modpathol.2015.151.

Miermeister CP, Petersenn S, Buchfelder M, Fahlbusch R, Ludecke DK, Holsken A, . . . Buslei R. Histological criteria for atypical pituitary adenomas – data from the German pituitary adenoma registry suggests modifications. Acta Neuropathol Commun. 2015;3:50. https://doi.org/10.1186/s40478-015-0229-8.

Milker-Zabel S, Debus J, Thilmann C, Schlegel W, Wannenmacher M. Fractionated stereotactically guided radiotherapy and radiosurgery in the treatment of functional and nonfunctional adenomas of the pituitary gland. Int J Radiat Oncol Biol Phys. 2001;50(5):1279–86.

Mingione V, Yen CP, Vance ML, Steiner M, Sheehan J, Laws ER, Steiner L. Gamma surgery in the treatment of nonsecretory pituitary macroadenoma. J Neurosurg. 2006;104(6):876–83. https://doi.org/10.3171/jns.2006.104.6.876.

Minniti G, Esposito V, Piccirilli M, Fratticci A, Santoro A, Jaffrain-Rea ML. Diagnosis and management of pituitary tumours in the elderly: a review based on personal experience and evidence of literature. Eur J Endocrinol. 2005a;153(6):723–35.

Minniti G, Traish D, Ashley S, Gonsalves A, Brada M. Risk of second brain tumor after conservative surgery and radiotherapy for pituitary adenoma: update after an additional 10 years. J Clin Endocrinol Metab. 2005b;90(2):800–4.

Minniti G, Traish D, Ashley S, Gonsalves A, Brada M. Fractionated stereotactic conformal radiotherapy for secreting and nonsecreting pituitary adenomas. Clin Endocrinol (Oxf). 2006;64(5):542–8. https://doi.org/10.1111/j.1365-2265.2006.02506.x.

Minniti G, Scaringi C, Poggi M, Jaffrain Rea ML, Trillo G, Esposito V, . . . Enrici RM. Fractionated stereotactic radiotherapy for large and invasive non-functioning pituitary adenomas: long-term clinical outcomes and volumetric MRI assessment of tumor response. Eur J Endocrinol. 2015;172(4):433–41. https://doi.org/10.1530/EJE-14-0872.

Molitch ME. Nonfunctioning pituitary tumors and pituitary incidentalomas. Endocrinol Metab Clin N Am. 2008;37(1):151–71, xi.

Molitch ME. Pituitary tumours: pituitary incidentalomas. Best Pract Res Clin Endocrinol Metab. 2009;23(5):667–75. https://doi.org/10.1016/j.beem.2009.05.001.

Molitch ME. Diagnosis and treatment of pituitary adenomas: a review. JAMA. 2017;317(5):516–24. https://doi.org/10.1001/jama.2016.19699.

Murad MH, Fernandez-Balsells MM, Barwise A, Gallegos-Orozco JF, Paul A, Lane MA, ... Montori VM. Outcomes of surgical treatment for nonfunctioning pituitary adenomas: a systematic review and meta-analysis. Clin Endocrinol (Oxf). 2010;73(6):777–91. https://doi.org/10.1111/j.1365-2265.2010.03875.x.

Mussnich P, Raverot G, Jaffrain-Rea ML, Fraggetta F, Wierinckx A, Trouillas J, ... D'Angelo D. Downregulation of miR-410 targeting the cyclin B1 gene plays a role in pituitary gonadotroph tumors. Cell Cycle. 2015;14(16):2590–7. https://doi.org/10.1080/15384101.2015.1064207.

Newey PJ, Nesbit MA, Rimmer AJ, Head RA, Gorvin CM, Attar M, ... Thakker RV. Whole-exome sequencing studies of nonfunctioning pituitary adenomas. J Clin Endocrinol Metab. 2013;98(4):E796–800. https://doi.org/10.1210/jc.2012-4028.

Nishioka H, Inoshita N, Mete O, Asa SL, Hayashi K, Takeshita A, ... Yamada S. The complementary role of transcription factors in the accurate diagnosis of clinically nonfunctioning pituitary adenomas. Endocr Pathol. 2015;26(4):349–55. https://doi.org/10.1007/s12022-015-9398-z.

Nomikos P, Ladar C, Fahlbusch R, Buchfelder M. Impact of primary surgery on pituitary function in patients with non-functioning pituitary adenomas – a study on 721 patients. Acta Neurochir (Wien). 2004;146(1):27–35.

Ntali G, Capatina C, Grossman A, Karavitaki N. Clinical review: functioning gonadotroph adenomas. J Clin Endocrinol Metab. 2014;99(12):4423–33. https://doi.org/10.1210/jc.2014-2362.

Ntali G, Capatina C, Fazal-Sanderson V, Byrne JV, Cudlip S, Grossman AB, ... Karavitaki N. Mortality in patients with non-functioning pituitary adenoma is increased: systematic analysis of 546 cases with long follow-up. Eur J Endocrinol. 2015;174(2):137–45. https://doi.org/10.1530/EJE-15-0967.

O'Sullivan EP, Woods C, Glynn N, Behan LA, Crowley R, O'Kelly P, ... Agha A. The natural history of surgically treated but radiotherapy-naive nonfunctioning pituitary adenomas. Clin Endocrinol (Oxf). 2009;71(5):709–14.

Olsson DS, Buchfelder M, Schlaffer S, Bengtsson BA, Jakobsson KE, Johannsson G, Nilsson AG. Comparing progression of non-functioning pituitary adenomas in hypopituitarism patients with and without long-term GH replacement therapy. Eur J Endocrinol. 2009;161(5):663–9. https://doi.org/10.1530/EJE-09-0572.

Olsson DS, Nilsson AG, Bryngelsson IL, Trimpou P, Johannsson G, Andersson E. Excess mortality in women and young adults with nonfunctioning pituitary adenoma: a Swedish nationwide study. J Clin Endocrinol Metab. 2015;100(7):2651–8. https://doi.org/10.1210/jc.2015-1475.

Paek SH, Downes MB, Bednarz G, Keane WM, Werner-Wasik M, Curran WJ Jr, Andrews DW. Integration of surgery with fractionated stereotactic radiotherapy for treatment of nonfunctioning pituitary macroadenomas. Int J Radiat Oncol Biol Phys. 2005;61(3):795–808. https://doi.org/10.1016/j.ijrobp.2004.07.688.

Palmieri D, D'Angelo D, Valentino T, De Martino I, Ferraro A, Wierinckx A, ... Fusco A. Downregulation of HMGA-targeting microRNAs has a critical role in human pituitary tumorigenesis. Oncogene. 2012;31(34):3857–65. https://doi.org/10.1038/onc.2011.557.

Park P, Chandler WF, Barkan AL, Orrego JJ, Cowan JA, Griffith KA, Tsien C. The role of radiation therapy after surgical resection of nonfunctional pituitary macroadenomas. Neurosurgery. 2004;55(1):100–6; discussion 106-107.

Petrovich Z, Yu C, Giannotta SL, Zee CS, Apuzzo ML. Gamma knife radiosurgery for pituitary adenoma: early results. Neurosurgery. 2003;53(1):51–9; discussion 59-61.

Peverelli E, Giardino E, Treppiedi D, Meregalli M, Belicchi M, Vaira V, ... Mantovani G. Dopamine receptor type 2 (DRD2) and somatostatin receptor type 2 (SSTR2) agonists are effective in inhibiting proliferation of progenitor/stem-like cells isolated from nonfunctioning pituitary tumors. Int J Cancer. 2017;140(8):1870–80. https://doi.org/10.1002/ijc.30613.

Picozzi P, Losa M, Mortini P, Valle MA, Franzin A, Attuati L, . . . Giovanelli M. Radiosurgery and the prevention of regrowth of incompletely removed nonfunctioning pituitary adenomas. J Neurosurg. 2005;102(Suppl):71–74.

Pigny P, Henric B, Lahlou N, Christin S, Mazzuca M, Dewailly D, Boersma A. A gonadotroph adenoma with a high proportion of basic FSH isohormones by chromatofocusing. J Clin Endocrinol Metab. 1996;81(6):2407–8.

Plummer C, Henderson RD, O'Sullivan JD, Read SJ. Ischemic stroke and transient ischemic attack after head and neck radiotherapy: a review. Stroke. 2011;42(9):2410–8. https://doi.org/10.1161/STROKEAHA.111.615203.

Pollock BE, Cochran J, Natt N, Brown PD, Erickson D, Link MJ, . . . Schomberg PJ. Gamma knife radiosurgery for patients with nonfunctioning pituitary adenomas: results from a 15-year experience. Int J Radiat Oncol Biol Phys. 2008;70(5):1325–9. https://doi.org/10.1016/j.ijrobp.2007.08.018.

Raappana A, Koivukangas J, Ebeling T, Pirila T. Incidence of pituitary adenomas in Northern Finland in 1992–2007. J Clin Endocrinol Metab. 2010;95(9):4268–75. https://doi.org/10.1210/jc.2010-0537.

Ramirez C, Cheng S, Vargas G, Asa SL, Ezzat S Gonzalez B, . . . Mercado M. Expression of Ki-67, PTTG1, FGFR4, and SSTR 2, 3, and 5 in nonfunctioning pituitary adenomas: a high throughput TMA, immunohistochemical study. J Clin Endocrinol Metab. 2012;97(5):1745–51. https://doi.org/10.1210/jc.2011-3163.

Raverot G, Sturm N, de Fraipont F, Muller M, Salenave S, Caron P, . . . Brue T. Temozolomide treatment in aggressive pituitary tumors and pituitary carcinomas: a French multicenter experience. J Clin Endocrinol Metab. 2010a;95(10):4592–9. https://doi.org/10.1210/jc.2010-0644.

Raverot G, Wierinckx A, Jouanneau E, Auger C, Borson-Chazot F, Lachuer J, . . . Trouillas J. Clinical, hormonal and molecular characterization of pituitary ACTH adenomas without (silent corticotroph adenomas) and with Cushing's disease. Eur J Endocrinol. 2010b;163(1):35–43. https://doi.org/10.1530/EJE-10-0076.

Raverot G, Jouanneau E, Trouillas J. Management of endocrine disease: clinicopathological classification and molecular markers of pituitary tumours for personalized therapeutic strategies. Eur J Endocrinol. 2014;170(4):R121–32. https://doi.org/10.1530/EJE-13-1031.

Raverot G, Assie G, Cotton F, Cogne M, Boulin A, Dherbomez M, . . . Massart C. Biological and radiological exploration and management of non-functioning pituitary adenoma. Ann Endocrinol (Paris). 2015;76(3):201–9. https://doi.org/10.1016/j.ando.2015.04.005.

Reddy R, Cudlip S, Byrne JV, Karavitaki N, Wass JA. Can we ever stop imaging in surgically treated and radiotherapy-naive patients with non-functioning pituitary adenoma? Eur J Endocrinol. 2011;165(5):739–44. https://doi.org/10.1530/EJE-11-0566.

Righi A, Agati P, Sisto A, Frank G, Faustini-Fustini M, Agati R, . . . Foschini MP. A classification tree approach for pituitary adenomas. Hum Pathol. 2012;43(10):1627–37. https://doi.org/10.1016/j.humpath.2011.12.003.

Roelfsema F, Biermasz NR, Pereira AM. Clinical factors involved in the recurrence of pituitary adenomas after surgical remission: a structured review and meta-analysis. Pituitary. 2012;15(1):71–83. https://doi.org/10.1007/s11102-011-0347-7.

Rogers A, Karavitaki N, Wass JA. Diagnosis and management of prolactinomas and non-functioning pituitary adenomas. BMJ. 2014;349:g5390.

Ruebel KH, Jin L, Zhang S, Scheithauer BW, Lloyd RV. Inactivation of the p16 gene in human pituitary nonfunctioning tumors by hypermethylation is more common in null cell adenomas. Endocr Pathol. 2001;12(3):281–9.

Sanno N, Oyama K, Tahara S, Teramoto A, Kato Y. A survey of pituitary incidentaloma in Japan. Eur J Endocrinol. 2003;149(2):123–7.

Schalin-Jantti C, Valanne L, Tenhunen M, Setala K, Paetau A, Sane T, Kouri M. Outcome of fractionated stereotactic radiotherapy in patients with pituitary adenomas resistant to conventional treatments: a 5.25-year follow-up study. Clin Endocrinol (Oxf). 2010;73(1):72–7. https://doi.org/10.1111/j.1365-2265.2009.03755.x.

Schernthaner-Reiter MH, Trivellin G, Stratakis CA. MEN1, MEN4, and carney complex: pathology and molecular genetics. Neuroendocrinology. 2016;103(1):18–31. https://doi.org/10.1159/000371819.

Sheehan JP, Kondziolka D, Flickinger J, Lunsford LD. Radiosurgery for residual or recurrent nonfunctioning pituitary adenoma. J Neurosurg. 2002;97(Suppl 5):408–14.

Sheehan JP, Starke RM, Mathieu D, Young B, Sneed PK, Chiang VL, ... Lunsford LD. Gamma Knife radiosurgery for the management of nonfunctioning pituitary adenomas: a multicenter study. J Neurosurg. 2013;119(2):446–56. https://doi.org/10.3171/2013.3.JNS12766.

Sherlock M, Ayuk J, Tomlinson JW, Toogood AA, Aragon-Alonso A, Sheppard MC, ... Stewart PM. Mortality in patients with pituitary disease. Endocr Rev. 2010;31(3):301–42. https://doi.org/10.1210/er.2009-0033.

Shorts-Cary L, Xu M, Ertel J, Kleinschmidt-Demasters BK, Lillehei K, Matsuoka I, ... Wierman ME. Bone morphogenetic protein and retinoic acid-inducible neural specific protein-3 is expressed in gonadotrope cell pituitary adenomas and induces proliferation, migration, and invasion. Endocrinology. 2007;148(3):967–75. https://doi.org/10.1210/en.2006-0905.

Simpson DJ, Bicknell JE, McNicol AM, Clayton RN, Farrell WE. Hypermethylation of the p16/CDKN2A/MTSI gene and loss of protein expression is associated with nonfunctional pituitary adenomas but not somatotrophinomas. Genes Chromosomes Cancer. 1999;24(4):328–36.

Simpson DJ, Hibberts NA, McNicol AM, Clayton RN, Farrell WE. Loss of pRb expression in pituitary adenomas is associated with methylation of the RB1 CpG island. Cancer Res. 2000;60(5):1211–6.

Sivakumar W, Chamoun R, Nguyen V, Couldwell WT. Incidental pituitary adenomas. Neurosurg Focus. 2011;31(6):E18. https://doi.org/10.3171/2011.9.FOCUS11217.

Snyder PJ, Sterling FH. Hypersecretion of LH and FSH by a pituitary adenoma. J Clin Endocrinol Metab. 1976;42:544–50.

Soto-Ares G, Cortet-Rudelli C, Assaker R, Boulinguez A, Dubest C, Dewailly D, Pruvo JP. MRI protocol technique in the optimal therapeutic strategy of non-functioning pituitary adenomas. Eur J Endocrinol. 2002;146(2):179–86.

Tabaee A, Anand VK, Barron Y, Hiltzik DH, Brown SM, Kacker A, ... Schwartz TH. Endoscopic pituitary surgery: a systematic review and meta-analysis. J Neurosurg. 2009a;111(3):545–54. https://doi.org/10.3171/2007.12.17635.

Tabaee A, Anand VK, Barron Y, Hiltzik DH, Brown SM, Kacker A, ... Schwartz TH. Predictors of short-term outcomes following endoscopic pituitary surgery. Clin Neurol Neurosurg. 2009b;111(2):119–22. https://doi.org/10.1016/j.clineuro.2008.09.010.

Trivellin G, Daly AF, Faucz FR, Yuan B, Rostomyan L, Larco DO, ... Stratakis CA. Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. N Engl J Med. 2014;371(25):2363–74. https://doi.org/10.1056/NEJMoa1408028.

Trouillas J, Girod C, Sassolas G, Claustrat B, Lheritier M, Dubois MP, Goutelle A. Human pituitary gonadotropic adenoma; histological, immunocytochemical, and ultrastructural and hormonal studies in eight cases. J Pathol. 1981;135(4):315–36.

Trouillas J, Girod C, Sassolas G, Claustrat B. The human gonadotropic adenoma: pathologic diagnosis and hormonal correlations in 26 tumors. Semin Diagn Pathol. 1986;3(1):42–57.

Trouillas J, Sassolas G, Loras B, Velkeniers B, Raccurt M, Chotard L, ... Girod C. Somatotropic adenomas without acromegaly. Pathol Res Pract. 1991;187(8):943–9.

Trouillas J, Labat-Moleur F, Sturm N, Kujas M, Heymann MF, Figarella-Branger D, ... Calender A. Pituitary tumors and hyperplasia in multiple endocrine neoplasia type 1 syndrome (MEN1): a case-control study in a series of 77 patients versus 2509 non-MEN1 patients. Am J Surg Pathol. 2008;32(4):534–43.

Trouillas J, Roy P, Sturm N, Dantony E, Cortet-Rudelli C, Viennet G, ... Tabarin A. A new prognostic clinicopathological classification of pituitary adenomas: a multicentric case-control study of 410 patients with 8 years post-operative follow-up. Acta Neuropathol. 2013;126(1):123–35. https://doi.org/10.1007/s00401-013-1084-y.

Tsang RW, Brierley JD, Panzarella T, Gospodarowicz MK, Sutcliffe SB, Simpson WJ. Radiation therapy for pituitary adenoma: treatment outcome and prognostic factors. Int J Radiat Oncol Biol Phys. 1994;30(3):557–65.

Turner HE, Adams CB, Wass JA. Pituitary tumours in the elderly: a 20 year experience. Eur J Endocrinol. 1999a;140(5):383–9.

Turner HE, Stratton IM, Byrne JV, Adams CB, Wass JA. Audit of selected patients with non-functioning pituitary adenomas treated without irradiation – a follow-up study. Clin Endocrinol (Oxf). 1999b;51(3):281–4.

van den Bergh AC, van den Berg G, Schoorl MA, Sluiter WJ, van der Vliet AM, Hoving EW, . . . Dullaart RP. Immediate postoperative radiotherapy in residual nonfunctioning pituitary adenoma: beneficial effect on local control without additional negative impact on pituitary function and life expectancy. Int J Radiat Oncol Biol Phys. 2007;67(3):863–9.

Vargas G, Gonzalez B, Ramirez C, Ferreira A, Espinosa E, Mendoza V, . . . Mercado M. Clinical characteristics and treatment outcome of 485 patients with nonfunctioning pituitary macroadenomas. Int J Endocrinol. 2015;2015:756069. https://doi.org/10.1155/2015/756069.

Vasilev V, Rostomyan L, Daly AF, Potorac I, Zacharieva S, Bonneville JF, Beckers A. Management of endocrine disease: pituitary 'incidentaloma': neuroradiological assessment and differential diagnosis. Eur J Endocrinol. 2016;175(4):R171–84. https://doi.org/10.1530/EJE-15-1272.

Verges B, Boureille F, Goudet P, Murat A, Beckers A, Sassolas G, . . . Calender A. Pituitary disease in MEN type 1 (MEN1): data from the France-Belgium MEN1 multicenter study. J Clin Endocrinol Metab. 2002;87(2):457–65. https://doi.org/10.1210/jcem.87.2.8145.

Wilson PJ, De-Loyde KJ, Williams JR, Smee RI. A single centre's experience of stereotactic radiosurgery and radiotherapy for non-functioning pituitary adenomas with the linear accelerator (Linac). J Clin Neurosci. 2012;19(3):370–4. https://doi.org/10.1016/j.jocn.2011.07.025.

Woollons AC, Hunn MK, Rajapakse YR, Toomath R, Hamilton DA, Conaglen JV, Balakrishnan V. Non-functioning pituitary adenomas: indications for postoperative radiotherapy. Clin Endocrinol (Oxf). 2000;53(6):713–7.

Wowra B, Stummer W. Efficacy of gamma knife radiosurgery for nonfunctioning pituitary adenomas: a quantitative follow up with magnetic resonance imaging-based volumetric analysis. J Neurosurg. 2002;97(Suppl 5):429–32. https://doi.org/10.3171/jns.2002.97.supplement.

Xekouki P, Szarek E, Bullova P, Giubellino A, Quezado M, Mastroyannis SA, . . . Stratakis CA. Pituitary adenoma with paraganglioma/pheochromocytoma (3PAs) and succinate dehydrogenase defects in humans and mice. J Clin Endocrinol Metab. 2015;100(5):E710–9. https://doi.org/10.1210/jc.2014-4297.

Yamada S, Asa SL, Kovacs K, Muller P, Smyth HS. Analysis of hormone secretion by clinically nonfunctioning human pituitary adenomas using the reverse hemolytic plaque assay. J Clin Endocrinol Metab. 1989;68(1):73–80.

Yoshino A, Katayama Y, Ogino A, Watanabe T, Yachi K, Ohta T, . . . Fukushima T. Promoter hypermethylation profile of cell cycle regulator genes in pituitary adenomas. J Neurooncol. 2007;83(2):153–62.

Yu B, Ji N, Ma Y, Yang B, Kang P, Luo F. Clinical characteristics and risk factors for headache associated with non-functioning pituitary adenomas. Cephalalgia. 2016. https://doi.org/10.1177/0333102416648347.

Zada G, Woodmansee WW, Ramkissoon S, Amadio J, Nose V, Laws ER Jr. Atypical pituitary adenomas: incidence, clinical characteristics, and implications. J Neurosurg. 2011;114(2):336–44. https://doi.org/10.3171/2010.8.JNS10290.

Zhao J, Dahle D, Zhou Y, Zhang X, Klibanski A. Hypermethylation of the promoter region is associated with the loss of MEG3 gene expression in human pituitary tumors. J Clin Endocrinol Metab. 2005;90(4):2179–86.

Zhou Y, Zhong Y, Wang Y, Zhang X, Batista DL, Gejman R, . . . Klibanski A. Activation of p53 by MEG3 non-coding RNA. J Biol Chem. 2007;282(34):24731–42. https://doi.org/10.1074/jbc.M702029200.

Craniopharyngioma and Posttreatment Pituitary Dysfunction in Brain Tumors

5

Francesco Felicetti, Nunzia Prencipe, Enrico Brignardello, and Emanuela Arvat

Contents

Francesco Felicetti and Nunzia Prencipe contributed equally to this work.

F. Felicetti (✉)
Transition Unit for Childhood Cancer Survivors, Department of Oncology, Città della Salute e della Scienza di Torino and Department of Medical Science, University of Torino, Torino, Italy
e-mail: ffelicetti@cittadellasalute.to.it

N. Prencipe
Department of Medical Science, Division of Endocrinology, Diabetology and Metabolism, Città della Salute e della Scienza di Torino, University of Torino, Torino, Italy
e-mail: nunzia.prencipe@gmail.com

E. Brignardello
Transition Unit for Childhood Cancer Survivors, Department of Oncology, Città della Salute e della Scienza di Torino, Torino, Italy
e-mail: ebrignardello@cittadellasalute.to.it

E. Arvat
Division of Endocrinological Oncology, Città della Salute e della Scienza di Torino and Department of Medical Science, University of Torino, Torino, Italy
e-mail: emanuela.arvat@unito.it

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_5

Abstract

Central nervous system tumors are a heterogeneous group of malignancies. They are rare diseases but represent a significant cause of mortality, especially in children and young adult patients. Craniopharyngioma, an embryogenic lesion of the sellar area with low-grade histological malignancy, represents approximately 3% of all intracranial tumors. Despite a relatively high rate of tumor recurrence, survival is generally good. Presenting symptoms are related to endocrine problems, mass effect, and cognitive dysfunction. Non-aggressive surgery followed by radiotherapy is currently the most widely used treatment, achieving the best long-term outcome. Other available treatments are intracystic irradiation, intracystic instillation of antineoplasmatic agents, and stereotactic radiotherapy. Complete tumor resection and potential cure should be balanced with a more conservative approach, aiming to avoid treatment-associated long-term morbidity as hypopituitarism and hypothalamic damage (obesity, adipsic or polidipsic diabetes insipidus, sleep disorders, neurocognitive impairment).

In the last decades, thanks to the improvement of anticancer therapies, the rate of patients that can be effectively cured is dramatically improved. The majority of patients with childhood and adolescent non-pituitary central nervous system tumors have a high survival expectancy. In these as in all cancer patients, the treatment choice should take into account the potential adverse effects of anticancer therapies. Any organ system can be damaged by oncological treatments, and endocrine disorders are the most common long-term effects in cancer survivors, with a cumulative incidence of about 50% after a 15-year follow-up from cancer recovery. Endocrine dysfunctions can play a role in the long-term health of these patients but also affect the short-term health, particularly in children. The main responsible for pituitary damage in survivors of central nervous system tumors is radiotherapy that, in combination with neurosurgery and/or chemotherapy, represents the mainstay for treatment of these tumors. Hypothalamic-pituitary dysfunction in survivors of central nervous system tumors is characterized by peculiar features that should be carefully kept in mind by endocrinologists who have to cope with these peculiar patients. The diagnosis, treatment, and clinical management of endocrinopathies in this context show some differences from those applied to patients in the routine endocrine practice. Therefore, the endocrinologist should play a prominent role in the multidisciplinary team that takes care of cancer survivors.

Keywords

Craniopharyngioma · Brain tumors · Cancer survivors · Pituitary dysfunction

Craniopharyngioma

Epidemiology and Pathophysiology of Craniopharyngioma

Craniopharyngiomas (CP) are rare embryogenic lesions of the sellar area with low-grade histological malignancy. They are tumors that arise from squamous epithelial remnants of Rathke's pouch. These cells can extend anywhere from the nasopharynx

to the tuber cinereum and may arise within the sphenoid bone, sella, or suprasellar region. Macroscopically CP are predominantly cystic or mixed lesions, although solid tumors might also occur. The size varies from small solid and circumscribed tumor to large multilocular cysts invading the sella turcica and adjacent structures. Most tumors are located in the sellar/parasellar region with suprasellar extension. A smaller number is confined to the sella or arises in the third ventricle or within the optic system. Purely intrasellar CP occur approximately in 4% of cases. Intra- and suprasellar CP comprise about 21% of these lesions, and suprasellar lesions, which occasionally extend into the third ventricle, occur in about 75% of cases. Calcifications are common in CP and may occur in about 60% of cases.

CP are divided in two main histological subtypes, the adamantinomatous and the papillary types, but transitional and mixed variants have also been reported. The adamantinomatous tumor is typically seen in the pediatric age. It often contains calcifications and usually has both cystic and solid components. The second major variant, the papillary CP, is often a solid tumor, and it is more common in adults than in children. The papillary type rarely presents with calcifications, it is generally well-circumscribed, and tumor infiltration of surrounding tissue is less frequent than the adamantinomatous type. The molecular mechanisms owing to CP are widely unknown. In patients with the adamantinomatous variant, mutations in the beta-catenin gene CTNNB1, leading to degradation-resistant mutant forms of beta-catenin, have been described (Campanini et al. 2010). In rodents, permanent activation of the Wnt signaling pathway results in high expression beta-catenin levels causing growth of pituitary tumors, similarly to human adamantinomatous CP. Cyst formation and their size seem to be associated with the carbonic anhydrase IX expression, an enzyme causing fluid production, by mechanisms not so far clarified (Proescholdt et al. 2011). In most cases, the origin of the tumor is in the infundibulum, and the tumors frequently cause visual loss because of their proximity to the optic chiasm.

CP represents approximately 3% of all intracranial tumors, and they are much more common in children than in adults. They have a bimodal age distribution with peaks at ages 5–14 years and, later in life, at ages 50–74 years. The actual incidence is 0.13 cases per 100,000 population per year in the United States with approximately 350 new cases diagnosed annually. There is no significant gender preference. Craniopharyngioma is the most common suprasellar tumor and non-glial primary intracranial tumor in children (10% of pediatric brain tumors with 100 new cases annually in the United States).

Despite a relatively high rate of tumor recurrence, survival in children treated for CP is generally good, although disease-related mortality can occur many years after treatment. Data on survival includes primarily surgically treated patients. The reported postsurgical 5-year overall survival is 88–94% (Müller et al. 2006), and the reported 10-year overall survival is 70–92%, with a 20-year survival of 76% (Poretti et al. 2004). Causes of late mortality include those directly related to the tumor or treatment such as progressive disease with multiple recurrences, hormonal deficiencies, chronic hypothalamic insufficiency, cerebrovascular disease, and seizure-related events. Despite these high survival rates (87–95% in recent series),

quality of life (QOL) is frequently impaired in long-term survivors due to sequelae caused by the anatomical proximity of CP to the optic nerve, pituitary gland, and hypothalamus.

Symptoms, Signs, and Diagnosis

In CP, presenting symptoms can be categorized as those related to endocrine problems (different in pediatric and adult population), those related to mass effect, and finally those related to cognitive dysfunction. As CP are in general slowly growing, symptoms may develop gradually, and this may contribute to the reported delay of 1–2 years between symptom onset and diagnosis (Garnett et al. 2007).

In Children

The history of childhood CP patients shows that the initial symptoms often occur long before the diagnosis is made (Müller et al. 2006). In children, neurological disturbances such as headache and visual field defects, along with manifestations of endocrine deficiencies such as growth retardation and delayed puberty, are the most common presenting symptoms. At diagnosis, 40–87% of patients have at least one hormone deficiency, and endocrine deficiencies are also common after specific treatment for CP. Reduced GH secretion is the most frequent endocrinopathy and can be present in up to 75% of patients, followed by FSH/LH deficiency, which can be seen in 40% of patients, and ACTH and TSH deficiency in 25% (Müller 2013); diabetes insipidus (DI) is present in 17–27% of pediatric patients at diagnosis (Müller 2008). Despite the fact that CP are frequently large at presentation, the pituitary stalk is usually not disrupted, and hyperprolactinemia secondary to pituitary stalk compression is found in only 20% of patients. Some pediatric patients may be unaware of visual field loss as it often progresses quite slowly. Hydrocephalus, when it occurs, often produces dramatic symptoms, including severe headache and projectile vomiting. Obstruction of the cerebral aqueduct and the foramen of Monro may also occur, making a shunt necessary.

In Adults

The most common presenting clinical symptoms in adults are those related to hypopituitarism and visual field deficits. Overall, GH deficiency is most common, followed by gonadotropin, ACTH, and TSH deficiency, which are present in 86, 74, 58, and 42% of adult cases, respectively. In a series of 78 adult patients, 57% of the female patients complained about menstrual irregularities or amenorrhea, and 28% reported impaired sexual function. Nausea and vomiting (26%), fatigue (32%), and lethargy (26%) are also frequent in these patients due to anterior pituitary dysfunction. Compression of the pituitary stalk causes DI with polydipsia and polyuria in 17–38.5% of cases. Significant weight gain is a presenting symptom in 13–15.4% of adult patients, and it is due to involvement of the hypothalamus by the tumor (Karavitaki et al. 2005; Mortini et al. 2011). Severe headache is also frequent (56%) and may be caused either by high intracranial pressure due to the tumor mass itself or

to meningeal irritation by spread cyst content or to obstructive hydrocephalus resulting from compression of the third ventricle (Karavitaki et al. 2006). Parasellar tumor extension with infiltration of the cavernous sinus may cause nerve palsies with diplopia and paresis of ocular muscles. Involvement of the temporal lobe might trigger seizure, and, especially in the elderly, wide tumors may cause impairment of cognitive abilities and personality changes (Karavitaki et al. 2006), most probably related to a combination of endocrinopathy and direct effect on the structures of the hypothalamic region. Eventually, in about 40–80% of the patients, suprasellar tumor extension pressurizes the optic chiasm causing loss of visual acuity and visual field abnormalities (mostly asymmetric bitemporal hemianopsia).

Physical examination is crucial to reach a diagnosis. Symptoms suggestive of CP in adults are headache, loss of libido, amenorrhea, fatigue and tiredness, polyuria, and polydipsia and, primarily, for visual field deficit or loss of visual acuity (Zoicas and Schöfl 2012). In children, CP may present with growth failure and abnormal sexual development. Laboratory diagnosis is based on the measurement of serum electrolytes and water balance and the evaluation of anterior pituitary hormonal function including IGF-1, TSH, free thyroxin, ACTH, cortisol, FSH, LH, testosterone/estradiol, and prolactin blood concentrations. An assessment of the visual fields is necessary in all patients in whom CP is suspected, looking specifically for the presence of bitemporal hemianopsia, which is characteristic of chiasma involvement. Visual acuity can also be affected when there is direct pressure on the optic nerves. The optic fundi need to be examined to detect the presence of optic atrophy and papilledema in children. Both in adults and children, the most accurate way to obtain the anatomic diagnosis of these lesions is neuroimaging. The availability of high-resolution MR imaging has greatly improved the visualization and radiological diagnosis of CP. Diagnosis is mainly based on the presence of cystic, solid, and calcified components in the tumor that is characteristic of CPs. The cystic component usually constitutes the major part of the tumor and shows different signals depending on the chemical-physical properties of its content. A fluid content will appear hypointense in T1-weighted and hyperintense in T2-weighted series, whereas a lipid (due to cholesterol), methemoglobin, or protein content will appear as hyperintense in T1 and T2 sequences. The solid portion has an isointense signal in T1- and a hyperintense signal in T2-weighted images with an enhancement after gadolinium (Karavitaki 2014). Calcifications can appear as areas of low signal in all sequences but are generally visualized better with computerized tomography scans (TC). The differential diagnosis should be made with the different types of lesions that occur in this region and can mimic a CP, including Rathke's cleft cyst, pituitary adenoma, astrocytoma of the hypothalamus or optic chiasm, and suprasellar arachnoid cyst, among others (Karavitaki et al. 2006).

Craniopharyngioma Treatment

The choice of treatment depends on the age at presentation, the size of the tumor and its localization or extension, the presence of intracranial pressure, compression

symptoms, and, eventually, pituitary dysfunction. There are no evidence-based guidelines or a clear consensus on which is the best treatment of primary or recurrent CP in adults. In children, the best approach to avoid substantial treatment-associated long-term morbidity must be sought.

Surgery is the first-line treatment of CP, and complete surgical resection is the goal of initial therapy. According to the literature, radical surgery can lead to a complete tumor removal in 18–84% of selected childhood and adult cases (Karavitaki et al. 2006). Aggressive surgery (gross total resection), however, may result in significant and devastating postoperative morbidity, especially after resection of tumors invading the hypothalamus. If complete tumor removal is unlikely to be achieved, subtotal or partial resections are alternative approaches effective in reducing pressure on adjacent structures and/or reestablishing normal cerebrospinal fluid circulation. In any case, the benefits of surgery must be balanced against the risks of treatment-related morbidity. For pediatric patients, a preoperative grading system has been proposed according to the degree of hypothalamic involvement (Puget et al. 2007). With this grading system, a significant relationship between postsurgical morbidity and the preoperative tumor grade can be obtained. According to it, gross tumor resection should only be attempted in patients without hypothalamic involvement (grade 0) or with a distorted or elevated but still visible hypothalamus (grade 1). In grade 2 CP (hypothalamic structures not discernible), a subtotal resection leaving in situ the hypothalamic part is recommended (Puget et al. 2007). Since no similar studies have been performed in adults, it seems reasonable to follow the recommendations used in pediatric patients. For CP with major cystic portions, stereotactic cyst decompression is a treatment option for acute symptomatic therapy, prior to CP resection, or whenever a total cyst excision is not indicated (Honegger and Tatagiba 2008). The pituitary stalk is important for pituitary function preservation, and many studies reported that the pituitary stalk preservation/maintenance may reduce postoperative anterior pituitary dysfunction. The correlation between stalk preservation and recurrence risk is also important, although in a recent meta-analysis, no significant correlations were detected (Li et al. 2015). CP treatment may worsen neuroendocrine function. In a study by Mortini et al. (2011), 82.3%, 75.9%, 72.7%, and 66.7% of patients with normal baseline values for GH, ACTH, TSH, and gonadotropins developed a newly onset deficiency of the respective pituitary axis after surgery. Postsurgical onset of DI was observed in 69.6% of their patients. Transient DI has been described in almost all patients after surgery in most series; permanent DI after surgery was reported in 40–93% of cases (Poretti et al. 2004). The risk for treatment-related hormone deficiencies appears to be lower after transsphenoidal intervention, while, in contrast to pituitary adenomas, recovery of pre-existing pituitary dysfunction after surgery is rare.

Radiotherapy is suggested in patients for whom surgery is contraindicated, like those with residual tumor after subtotal or partial resection or with recurrent disease. Modern stereotactic radiotherapy and radiosurgery techniques allow the radiation dose to be tightly conformed to the target tumor, allowing structural sparing of adjacent critical structures such as the optic apparatus, hypothalamus, temporal lobes, and pituitary gland. Techniques used for the treatment of CP include

fractionated stereotactic radiotherapy (FSRT), radiosurgery, intensity-modulated radiation therapy (IMRT), and proton beam therapy (PBT). In cases of non-radical surgery, adjuvant radiotherapy reduces significantly the local recurrence rates (10–63% at 10-year follow-up), while RT alone provides 10 years recurrence rates between 0 and 23%. These results are based on conventional, fractionated external beam RT, while 5-year progression-free survival with newer, higher precision techniques, such as FSRT, is greater than 90% (Minniti et al. 2007). For FSRT schedules, the probability of local tumor control is best with radiation doses above 54–55 Gray (Gy) and less than 61 Gy to lower the risk of radiation-associated side effects such as visual impairment, pituitary deficiency, impaired cognitive function, and development of secondary malignancies (Merchant 2006). Radiosurgery with delivery of a single high-dose radiation to the tumor is an attractive technique but applicable to small tumors only and requiring adequate safety margins of adjacent critical structures. For patients with residual pituitary function, the risk of endocrine deficits increases with doses delivered to the hypothalamic-pituitary structures higher than 45 Gy and a fraction size higher than 1.8 Gy, and, when the pituitary is already dysfunctional, with doses lower than 40 Gy. Gross tumor resection was significantly correlated with a higher risk of endocrinopathy than subtotal resection and radiotherapy. More recent series treated with FSRT after conservative surgery, in fact, showed no significant deterioration in the endocrine morbidity over and above that shown postoperatively. Intracavitary irradiations with stereotactically guided instillation of radioisotopes or intracystic bleomycin are other proposed approaches for treatment of mono- or multicystic tumors, but long-term studies are still necessary to establish efficacy and safety of these techniques (Barriger et al. 2011).

Hypopituitarism and Hypothalamic Damage after CP Treatment

Hypopituitarism

It was already said that the long-term morbidity in patients with CP is substantial and mainly based on the tumor location and size, its treatment, and recurrence rate. The morbidity is represented by hypopituitarism, hypothalamic involvement (obesity, thirst disorders, thermoregulatory disorders, somnolence and sleep apnea, and cardiac arrhythmia), increased cardiovascular risk factors, visual and neurological problems, as well as reduced quality of life and cognitive function. Most of the patients chronically suffer from partial or complete hypopituitarism as well as DI, with approximately 80% requiring substitution of more than two hormones. Complete hypopituitarism is found in the majority of CP patients. At least three pituitary hormone deficiencies have been reported in 54–100% of patients with CP (Karavitaki et al. 2006). The long-term prevalence rate of total anterior pituitary insufficiency is near 89%, and for GH, gonadotropin, ACTH, and TSH deficiency, it is 91%, 93.5%, 92%, and 86%, respectively. The prevalence of DI is 81% (Crowley et al. 2010). Pituitary deficiency per se and its treatment, through various metabolic effects, might contribute to the enhanced cardiovascular morbidity and mortality detected in epidemiological studies. Previous studies reported that patients

with CP not only have three- to fivefold higher morbidity and mortality rates compared with the general population but also have higher morbidity and mortality rates compared with patients with other causes of hypopituitarism, namely, those with nonfunctioning pituitary adenomas (NFPA) (Tomlinson et al. 2001).

GH deficiency (GHD) is probably the most frequent pituitary hormone deficiency, and it is associated with increased mortality rate. Few studies compared the clinical characteristics and patient-reported outcomes of GHD adults with childhood-onset CP (COCP) with those of patients with other causes of childhood-onset hypothalamic-pituitary dysfunction (Tomlinson et al. 2001) showing that CP patients had an increased standardized mortality rate (SMR 5.55–9.28), which was significantly higher than in other groups of hypopituitary patients, such as the NFPA group (SMR 1.70). The main causes of the increased SMR were mainly cerebro- and cardiovascular accidents. This finding cannot easily be explained but suggests important differences in tumoral behavior and/or therapeutic approach between CP and other causes of hypopituitarism. Furthermore, in adult COCP patients, the impact of hypothalamic damage on clinical features and quality of life is difficult to differentiate from that of GHD per se. In addition, the availability of recombinant GH encouraged the use of GH replacement therapy in all causes of GHD, including CP (Jorgensen et al. 1989). In most of the studies on GHD and GH replacement therapy, 10–20% of adult patients had CP, but these latter were not analyzed separately, making impossible to establish whether CP patients were distinct from non-tumor-related GH-deficient patients (Simpson et al. 2002). In the observational KIMS study (Abs et al. 2005), hypopituitary patients with adult-onset CP and NFPA were compared in terms of body weight, body composition, metabolic and endocrine dysfunctions, quality of life, employment, and marital status. The effects of GH therapy in CP patients were also separately analyzed. CP patients showed worst metabolic parameters, endocrine function, and body composition compared with NFPA patients. GH replacement therapy prompted a significant improvement in serum IGF-I, fat-free mass, LDL cholesterol, and quality of life in both groups of patients. However, no decrease in fat mass was observed in CP patients after 2 years of GH therapy, in contrast to observations in NFPA patients and other GHD patients.

In a recent published guideline about hormonal replacement in adults with hypopituitarism, the authors recommend offering GH replacement to those patients with proven GHD and no contraindications (Fleseriu et al. 2016). Because of the known mitogenic effect of GH, it has been hypothesized that GH replacement therapy (GHRT) might increase the tumor recurrence rate or enlargement in patients with a history of pituitary tumor. Some findings indicate that this is not the case in patients with NFPA (Olsson et al. 2009). Craniopharyngioma patients have a high tumor progression rate, and after 10 years of follow-up in 50% of those who do not receive RT, there is tumor progression (Karavitaki et al. 2006). There are only a few studies examining the tumor progression rate in CP patients with and without GHRT. Karavitaki et al. (2006) reported no influence of 6-year GHRT on tumor progression rate in 32 GH-treated patients. In a study by Müller (2010), there was no difference in tumor progression between 54 CP patients treated with GHRT and 60 CP patients without GHRT after a mean follow-up period of 2.8 years. In a more recent study

(Olsson et al. 2012), the 10-year recurrence-free survival ratio after adjustment for initial RT, residual tumor after primary treatment, and gender was 85% for the GHRT-treated patients and 65% for patients not treated with GHRT with no difference in IGF-I levels, which are closely correlated with the biological effect of GH. In these studies (Müller 2010; Olsson et al. 2012), the two most important negative predictive factors for tumor progression/recurrence were initial RT and the absence of a residual tumor after primary tumor treatment. Finally in HypoCCS study (Child et al. 2015), no significant differences in the recurrence rates for CP or pituitary adenoma between GH-treated and untreated hypopituitary patients were observed.

Replacement therapy for hypothyroidism, hypogonadism, and hypocortisolism in CP patients is not different from that used in patients with hypopituitarism form other causes (for each condition, see dedicated chapter of this book). However, CP patients are often on antiepileptic drugs, and it is therefore mandatory to titrate steroid and L-thyroxine therapy because of their higher requirement due the accelerated clearance owing to these drugs (Fleseriu et al. 2016). Stress doses of glucocorticoid for intercurrent illnesses and minor stress are similar to those used in Addison disease (Bornstein et al. 2016).

Hypothalamic Damage

Hypothalamic damage is one of the most feared complications in patients with CP, resulting in a severe impairment in quality of life and frequently life-threatening morbidity. For this reason, avoiding or minimizing hypothalamic damage is the most important concern when defining a treatment strategy for a newly diagnosed patient with CP. Severe hypothalamic morbidity has been closely correlated with the extent of surgical resection and preoperative hypothalamic involvement, as seen on MRI, while it is not unequivocally attributable to RT when the hypothalamic function is originally intact. Tumor- or treatment-related damage of the ventromedial hypothalamus may lead to the impairment of mechanisms controlling satiety, hunger, and energy expenditure resulting in severe obesity.

Hypothalamic obesity is the most common manifestation of hypothalamic complications. At presentation, approximately 15% of adult patients complain of weight gain or are obese (Karavitaki et al. 2005; Mortini et al. 2011). During follow-up, excessive weight gain has been reported in up to 67% of patients after surgery with and without adjuvant RT (Karavitaki et al. 2005). Obesity develops early after treatment, with body weight rapidly increasing in the first 6–12 months after surgery or RT. Later on, in spite of body mass index (BMI) stabilization, obesity remains a problem. Hypothalamic obesity is often associated with disastrous metabolic and psychological consequences leading to severe morbidity, impaired quality of life, and reduced life expectancy. Features of the metabolic syndrome like abdominal obesity, insulin resistance with hyperinsulinemia, dyslipidemia, and elevated blood pressure are commonly seen in patients with CP (Kendall-Taylor et al. 2005). In children, as in adults, hypothalamic dysfunction is common, and it is present at diagnosis in 35% of the former. It dramatically increases after treatment and, in some series, is reported to occur in up to 65–80% of patients. This complication can significantly compromise the quality of life and be extremely resilient to treatment.

In a retrospective analysis of the KIMS database patients (Kendall-Taylor et al. 2005), obese patients with adult-onset disease had a greater waist circumference and higher cholesterol and triglycerides levels compared to patients with childhood-onset disease. These metabolic alterations contribute to hypertension, diabetes, and atherosclerosis and, consequently, to an increase in the cardiovascular disease risk. Treatment of hypothalamic obesity is difficult, and patients need to comply with dietary and behavioral modifications such as undertaking regular physical activities and psychological counseling and the use of anti-obesity drugs or bariatric surgery. Rapid weight gain in children treated for CP occurs despite adequate replacement of pituitary hormone deficiencies. Possible contributing mechanisms include lack of sensitivity to endogenous leptin, vagally mediated hyperinsulinemia, and autonomic imbalance, as well as reduced physical activity, rather than increased energy intake, which is partly due to the neurological defects, visual failure, and somnolence (Harz et al. 2003). In fact, the hypothalamic disturbance in energy management contributes to obesity and is further worsened by factors limiting activity. The results of the evaluation of body composition and fat distribution in children with CP are conflicting. Some studies showed increased fat-free and muscle mass in CP patients compared with controls (Holmer et al. 2010), while others found no significant differences between patients and controls. Adults with childhood-onset CP and obesity also reported a higher tendency to restrict food intake for controlling body weight compared with controls. Excess weight has a great negative impact on self-esteem and quality of life, and it is associated with difficulties dealing with daily activities.

Diabetes *insipidus* with absent sense of thirst is another hypothalamic complication resulting in serious water and electrolyte imbalance and was reported in almost 20% of adult patients after surgery with and without adjuvant RT (Smith et al. 2004). Diabetes *insipidus* and thirst abnormalities in CP patients can be the result of marked tumor size, surgical trauma to the hypothalamus, and tumor adhesiveness to the pituitary stalk. Early studies (Devile et al. 1996) documented that hypodipsia was more common in patients with tumor size greater than 3.5 cm^2 than in those with a tumor size smaller than 3.5 cm^2. The presence of hydrocephalus owing to third ventricular and hypothalamic involvement is associated with increased morbidity, including adipsia, and mortality in these patients. Also radical surgical excision of the tumor was suggested to contribute to the pathogenesis of impaired thirst sensation, even though preservation of the pituitary stalk does not exclude the development of DI (Lehrnbecher et al. 1998).

Treatment of adipsic DI represents a very difficult management problem. Regular desmopressin therapy is required to treat DI, and patients must be trained to drink approximately 2 l of fluid daily. They also need regular medical follow-up and daily control of body weight at home to detect changes in fluid balance status (Thompson et al. 1986), though poor patient compliance occasionally limits the effectiveness of this strategy. Some authors reported that chlorpropamide as well as clofibrate can improve thirst sensation in patients with adipsic DI, while others showed no benefits from the use of these agents. Behavior modification techniques have also been used with modest success (Johnston et al. 1991). The clinical management of these patients is therefore based on a regular schedule of water

intake according to daily weights, urine output, and frequent monitoring of plasma sodium concentrations and plasma osmolality. Polydipsia can be another problem in CP patients with DI. Polydipsic patients drink more water than healthy controls in response to a given rise in plasma osmolality; they do not suppress thirst during drinking and continue to feel thirsty even when imbibing large volumes of water (Thompson et al. 1991). Management of primary polydipsia is particularly difficult in the presence of DI. Without desmopressin therapy, polyuria can be severe and compromise significantly daily activities. On the other hand, desmopressin treatment can be dangerous for the significant risk of water intoxication and consequent hyponatremia, because lowering of plasma osmolality does not abolish thirst and inhibit drinking. The thirst disturbances described in published studies are unique to CP and not a clinical characteristic of all sellar tumors. This, probably, reflects the proximity of CP to the hypothalamic osmoreceptors for thirst, which causes changes in osmolality and generates the thirst sensation (Ramsay 1989). The abnormalities of thirst in these patients can lead to life-threatening metabolic consequences and increased mortality and underline the importance of routinely assessing the thirst response in postoperative CP patients.

Sleep disorders and increased daytime somnolence, caused by disruption of the circadian rhythm, occur in up to one-third of adult CP patients. Daytime sleepiness and obesity in these patients were both correlated with low nocturnal and early morning melatonin levels. Initial experiences with melatonin substitution in patients with childhood CP were encouraging, as normalized melatonin levels reduced daytime sleepiness and improved physical activity. Polysomnographic studies in patients with childhood CP and severe daytime sleepiness revealed sleeping patterns typical of hypersomnia and secondary narcolepsy (O'Gorman et al. 2010). Treatment with central stimulating agents such as dextroamphetamine had a significantly beneficial effect on daytime sleepiness in these patients. This drug, in fact, by inducing weight reduction or stabilizing patients' BMI, produced substantial intensification in physical activity and, therefore, in alertness (Müller et al. 2006).

Impaired thermoregulation with hyper- or hypothermia is another common feature of CP patients. Defective thermoregulation is usually ascribed to lesions in the region of the third ventricle. In most cases, the exact mechanism is not clear. Direct hypothalamic lesions, segregation of endogenous pyrogens, or deficits in perceiving temperature elevations are the proposed pathogenic mechanisms. The "human thermostat" for temperature regulation is located in the anterior preoptic area, and other centers modulating body temperature have been identified in the posterior hypothalamus. Although suprasellar lesions often compress the hypothalamus, deregulation of body temperature is an extremely rare presenting feature probably because of the bilateral localization of hypothalamic nuclei and tracts and the adaptability of central connections. In contrast, in the postoperative period, dysthermia may be noted either temporarily or permanently (Clar 1985). A number of cases have been reported in which direct hypothalamic lesions are implicated in the pathogenesis of central fever. The extent of the resection is one of the major factors implicated in the abnormal thermoregulation. In a comparative study by

Bucci et al. (1987), patients undergoing a 95% or greater resection of CP had statistically significant fluctuations in sodium, temperature, and glucose. The authors postulated that slow preoperative tumor growth allows compensatory processes to take place, while abrupt postoperative changes in local perfusion eliminate these compensatory mechanisms leading to overt abnormalities.

Neurocognitive impairment linked to hypothalamic damage is another important concern in CP patients, especially in children. Whether the neurocognitive deficit is due to tumor growth itself or to the treatment is still debated. In some studies, no impairment of neuropsychological functioning after surgical removal was reported (Karavitaki et al. 2006), while in others, a more conservative approach to surgical treatment was advised to minimize the occurrence of cognitive deficits, especially in patients with tumors in the hypothalamic area (Merchant et al. 2002a). However, prospective studies with modern surgical and radiotherapy techniques are lacking. Radiation-induced cognitive decline correlates significantly with the irradiation volume and tumor location, suggesting that high precision radiotherapy techniques, such as FSRT and proton beam therapy, especially if delivered as IMPT, may reduce this risk. The youngest children (<5 years) represent the most vulnerable subgroup for treatment-related neural injury and consequent decline of functional/quality of life outcomes regardless of the cause. Behavioral problems and deteriorated cognitive functions are relatively common, contributing to decreased academic and occupational performance and difficulties in maintaining family and social relationships and resulting in a loss in quality of life (Karavitaki et al. 2006).

Non-pituitary Central Nervous System Tumors

Pituitary dysfunction in cancer patients should be considered as part of the complex scenario of the anticancer treatment late effects. Cancer survivors (CS) are a growing population. In the last decades, thanks to the improvement of anticancer therapies, the rate of patients diagnosed with cancer that can be effectively cured is dramatically improved, and patients with childhood- and adolescence-onset tumors, in many cases, have a high survival expectancy. It has been estimated that more than 420,000 people in the US population are survivors of a cancer diagnosed before the age of 21 years, meaning that one in every 750 individuals who live in the United States has been diagnosed with cancer before the age of 21 years (Robison and Hudson 2014; Howlader et al. 2015).

Although any organ system can be damaged by oncological treatments, endocrine disorders are the most common late effects diagnosed in CS, with a cumulative incidence of about 50% after a 15-year follow-up from cancer recovery (Brignardello et al. 2013). Endocrine dysfunctions can play a role in the long-term health of these patients but also diminish the short-term health, particularly in children (Chemaitilly et al. 2015). Hence, it follows that the endocrinologist is an essential component of the multidisciplinary team that should monitor CSs for many years after the completion of anticancer treatments (Brignardello et al. 2013).

Epidemiology and Pathophysiology of Non-pituitary Central Nervous System Tumors

Central nervous system (CNS) tumors are a heterogeneous group of malignancies (McNeill 2016). As a whole, they are a rare disease but represent a significant cause of mortality, especially in children and young adult patients (Ostrom et al. 2014).

In the last four decades, a significant increase in the incidence of CNS tumors has been reported (McNeill 2016) owing to the introduction, in the routine practice, of new imaging techniques like the magnetic resonance imaging (MRI). Changing in classification of the different tumors, increased access of patients to health care, and the amelioration of cancer registries might have also contributed to the increased incidence of these tumors (Ostrom et al. 2014).

Meningioma is the most common CNS tumors in the adult population, and its cumulative incidence is probably underestimated, as suggested by the high rate of asymptomatic benign (grade 1 according to WHO) meningiomas incidentally diagnosed or found in autoptical series. Atypical and malignant meningiomas are instead characterized by a significant morbidity and mortality, due to the local invasiveness and the high rate of recurrences (Morris et al. 2009; Vernooij et al. 2007).

The second most common CNS tumors in adults are the gliomas that account for about 25% of all CNS tumors (5). As meningiomas, they also are categorized into four groups, according to their malignant potential. Pilocytic astrocytoma, or grade 1 glioma, shows excellent survival rates, whereas only the 5% of patients diagnosed with glioblastoma (grade 4 glioma) survive 5 or more years after the diagnosis. If only malignant CNS tumors are considered, the 75% of them is represented by malignant gliomas. Oligodendrogliomas, ependymal tumors, and other CNS tumors are extremely rare in adults (McNeill 2016; Ostrom et al. 2014).

In the pediatric population, CNS tumors are the most common solid tumors and the most common cause of cancer-related mortality. Pilocytic astrocytoma and other low-grade gliomas represent the majority of CNS tumors in children, followed by embryonal tumors and other rare histologic subtypes (McNeill 2016; Ostrom et al. 2015; Rickert and Paulus 2001). Among embryonal tumors, medulloblastoma and primitive neuroectodermal tumors (PNETs) show similar histologic characteristics but different locations (the posterior fossa and supratentorial region, respectively). Both medulloblastoma and PNETs usually occur before the age of 10 years, affecting more boys than girls (Packer et al. 2012).

As summarized in Table 1, thanks to the improvement in treatment protocols, the survival rates of pediatric patients affected by CNS tumors are today markedly increased and higher than those of adult patients, reaching 80% for most histologic subtypes (Ostrom et al. 2015).

Pituitary Damage in CNS Tumor Survivors

In patients with CNS tumors, pituitary dysfunction is one of the most relevant complications of anticancer treatments. The main responsibility for pituitary damage

Table 1 Survival rates of pediatric CNS tumors, according to histology

Histology	5-year survival (%)	10-year survival (%)
Gliomas	77	73
Pilocytic astrocytoma	98	96
Other low-grade gliomas	90	85
High-grade glioma	30	26
Ependymal tumors	80	64
Other glioma	93	91
Embryonal tumors	62	56
Medulloblastoma	70	63
PNET	56	49
Atypical Teratoid/Rhabdoid tumors	28	26
Other embryonal tumors	65	63
Tumors of the pineal region	89	89
Germ cell tumors	87	80
Tumors of meninges	56	48
Choroid plexus tumors	61	58
Neuronal and mixed neuronal-glial tumors	55	43
Total	73	69

Modified from McNeill (2016)

is radiotherapy (RT) that, in combination with neurosurgery and/or chemotherapy (CT), represents the mainstay of CNS tumors treatment (Massimino et al. 2016; Vitanza and Cho 2016; Darzy and Shalet 2005c).

The Impact of Tumor Mass and Neurosurgery

When the tumor involves the hypothalamic-pituitary region, the endocrine disorder may be the presenting feature of the tumor itself. More than half of the children affected by CNS tumors were reported to have some evidence of neuroendocrinopathy before irradiation (Merchant et al. 2002b). More recently, in a cohort of 192 pediatric patients with localized primary brain tumors, a pre-irradiation GH deficiency (GHD) was diagnosed by provocative testing of growth hormone (GH) secretion in 22.9% of them (Merchant et al. 2011).

Data on adult patients are limited. The incidence of postsurgery pituitary dysfunction was reported to vary between 21.6 and 64.7% in patients with non-pituitary CNS neoplasm, independent of tumor location and volume and neurosurgical approach (Taku et al. 2016). Schneider et al. reported that 7 out of 17 patients developed a pituitary dysfunction after neurosurgery alone, with an elapsed time after the intervention of 0.8–9 years (Schneider et al. 2006). An interesting study performed in 37 patients with a mean age of 54.6 years, who underwent neurosurgery for benign CNS tumors not involving the hypothalamic-pituitary region, showed some degree of hypopituitarism 3 months after surgery in 43.2% of them. Specifically, hypogonadism was present in four, adrenal insufficiency in four, and hypothyroidism in two patients. Two patients showed mild hyperprolactinemia, and

seven (18.9%) had GHD, whereas none had diabetes insipidus. Twelve months after surgery, some degree of hypopituitarism persisted in eight patients, and precisely hypogonadism was present in two patients and hypothyroidism in one. GHD was present in five patients, while none showed adrenal insufficiency (De Marinis et al. 2006). In contrast with the results of this study, Wachter and colleagues showed that surgery had no impact on the pituitary function evaluated before and after neurosurgery (on days 1 and 7) in 54 patients with supratentorial non-sellar tumors. Before surgery, 24 of the 54 patients (44.4%) already exhibited pituitary dysfunction, and hormone testing performed after surgery revealed no changes (Wachter et al. 2011) in comparison with the initial assessment.

The Impact of Chemotherapy

Controversial data on hypothalamic-pituitary function have been reported in survivors of childhood-onset non-CNS tumors treated with chemotherapy (CT) alone (Rose et al. 2004). In CNS tumors, CT is almost always employed in combination with RT or neurosurgery and is therefore difficult to distinguish the role played by CT in the development of hypothalamic-pituitary damage. Some studies showed that in patients treated for medulloblastoma, CT may increase the sensitivity of pituitary gland to the detrimental effect of radiation (Gleeson et al. 2004; Olshan et al. 1992; Ogilvy-Stuart and Shalet 1995). Moreover, the development of transient posterior pituitary dysfunction (diabetes insipidus) was reported in a patient following treatment with temozolomide (Faje et al. 2013). Finally, adrenal insufficiency secondary to the use of corticosteroids frequently employed in cancer patients to minimize the side effects of chemotherapy or to prevent the cerebral edema due to the tumor itself or cranial RT should be mentioned. Adrenal insufficiency in these patients is usually transient, but the recovery of a normal adrenal function requires sometimes a long time and a very slow and careful tapering of drug dose (Crowne et al. 2015).

The Impact of Radiotherapy: Pathophysiology

The radiation-induced pituitary dysfunction has been widely investigated in the last years, in parallel with the evolution of cancer treatment protocols and the increased survival of CNS tumor patients (Crowne et al. 2015; Clement et al. 2014; Darzy and Shalet 2005c, 2009). Due to the higher rates of recovery reached in pediatric oncology and to the greater health impact of pituitary dysfunction in younger patients (Darzy 2013), the majority of available data refer to childhood cancer survivors, and specifically to those treated with conventional RT, whereas the impact of new RT techniques (IMRT, stereotactic RT, etc.) still results poorly investigated (Clement et al. 2014; Darzy 2013).

The potential damage induced by radiation therapy is always limited to the anterior pituitary. To date, there is no evidence for a role of RT in the development of diabetes insipidus which, instead, can be the presenting feature of a para-sellar neoplasia as well as a severe complication of brain surgery.

The sensitivity to radiation is quite different for each hypothalamic-pituitary axis (Fig. 1). The GH-secreting cells are the most sensitive, followed by the FSH- and LH-secreting cells, while those secreting ACTH and TSH are the most resistant

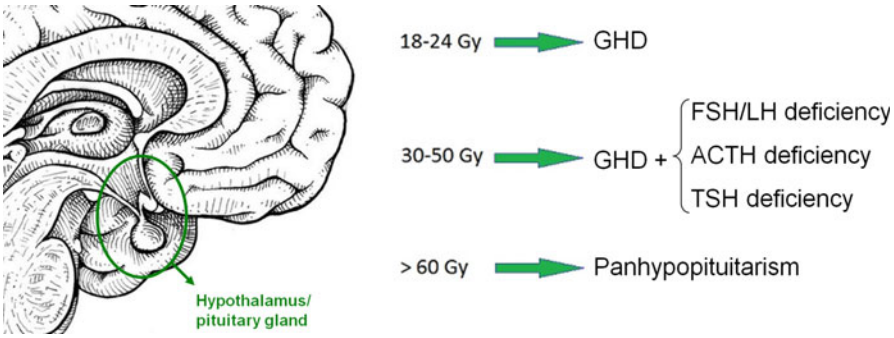


Fig. 1 Association between the dose of cranial irradiation and anterior pituitary hormone deficits (Modified from Rutter and Rose 2007)

(Darzy and Shalet 2006; Rutter and Rose 2007). In agreement with these observations, a single dose of 3 Gy has been reported to impair the in vitro secretion of GH (and prolactin) by pituitary cells, whereas TSH-secreting cells were resistant to doses higher than 10 Gy (Hochberg et al. 1983).

The pathophysiology of radiation-induced pituitary damage is not fully understood. Previous studies indicated that the hypothalamus is the main site of radiation-induced pituitary damage (Darzy et al. 2003; Lustig et al. 1985; Schmiegelow et al. 2000), but the current evidence suggests that cranial irradiation at doses <40 Gy causes direct pituitary injury (Darzy and Shalet 2009; Darzy et al. 2005, 2007, 2009). With radiation doses >40 Gy, both the pituitary and hypothalamus are damaged (Darzy 2013). Darzy et al., through a dynamic evaluation of GH secretion, reported that in patients with radiation-induced GHD acquired during childhood, both pulsatile GH secretion and diurnal variation are maintained. Thus, radiation seems to cause mostly a quantitative damage, leading to amplitude-dependent dampening of GH secretion, with relative preservation of tonic GH secretion. However, GH secretion was reported to be also more disorganized in these patients, suggesting a coexisting qualitative alteration in the hypothalamic control (Darzy et al. 2005). The occurrence of hyperprolactinemia in patients treated with RT at doses higher than 40 Gy (for nasopharyngeal or skull-base tumors), probably due to a reduction in hypothalamic release of dopamine, seems to confirm the presence of a hypothalamic impairment (Lam et al. 1991; Pai et al. 2001; Samaan et al. 1982; Chen et al. 1989; Lam et al. 1986). In contrast, hyperprolactinemia was not reported in patients treated with lower RT doses (Darzy 2013; Constine et al. 1993; Rose et al. 1999).

The pituitary damage is directly related to the total radiation dose (Clayton and Shalet 1991) and to the lapse of time after the completion of radiotherapy (Darzy 2013). More debated is the role of other factors such as age at irradiation, fraction size, sex, and concomitant CT (Mulder et al. 2009).

Younger age at RT seems to be a negative factor for the development of GHD, especially when low doses are employed, as shown in patients treated with total body

irradiation (TBI) as conditioning regimen for hematopoietic stem cell transplantation (Ogilvy-Stuart et al. 1992; Littley et al. 1991).

The Impact of Radiotherapy: Clinical Features

GHD

The somatotrophic axis is the most sensitive to the effect of radiation. The prevalence of GHD in CS varies significantly in the available studies, mainly due to the different assessment methods and cutoff values employed. In a pooled analysis of three studies, Mulder et al. reported a GHD prevalence of 35.6% in childhood CS previously treated with cranial RT at doses ranging between 32 and 55 Gy (Mulder et al. 2009). Merchant and colleagues evaluated patients with localized primary CNS tumors using provocative testing of GH secretion by the secretagogues arginine and L-dopa, before 6, 12, 36, and 60 months after conformal RT. The GH peak was modeled as an exponential function of the time after RT and the mean radiation dose to the hypothalamus. The authors predicted that the average patient might develop GHD with three different combinations of time after RT and mean dose to the hypothalamus: 12 months and more than 60 Gy, 36 months and 25 to 30 Gy, or 60 months and 15 to 20 Gy. The mean radiation dose to the hypothalamus required to achieve a 50% risk of GHD at 5 years was found to be 16.1 Gy (Merchant et al. 2011).

A lower prevalence of GHD was reported in adult patients (Agha et al. 2005; Madaschi et al. 2011), if compared with pediatric CNS tumors survivors. However, a recent study performed on a quite large cohort of adult patients affected by CNS tumors (Kyriakakis et al. 2016) showed that partial or severe GHD was present in more than 80% of patients treated with cranial RT.

The clinical presentation of GHD in pediatric versus adult CNS tumor survivors is significantly different. Clearly, the impaired growth is the most important effect of GH deficiency in children (Brennan et al. 1998; Clarson and Del Maestro 1999; Darendeliler et al. 1990; Lannering et al. 1998; Ilveskoski et al. 1997; Clayton et al. 1988). In older studies on childhood CS, it was recognized that a growth deceleration occurs in pediatric patients affected by brain tumors, independent of GHD and mainly attributable to chemotherapy and the disease itself (Schriock et al. 1991; Hokken-Koelega et al. 1993; Moëll et al. 1988; Shalet et al. 1978; Brauner et al. 1989; Brown et al. 1983). The same observations were done in children affected by acute lymphoblastic leukemia (ALL) in whom a catch-up growth was reported after the completion of anticancer treatments. In CNS cancer patients, however, GHD is more severe and occurs earlier than in patients with other malignancies (Albertsson-Wikland et al. 1987). The concomitant spinal RT, which is frequently employed to treat medulloblastoma patients, might play a role in worsening the growth impairment. During the first years after radiation, subjects treated with combined cranial and spinal RT showed a significantly lower growth in comparison with those who underwent cranial RT only (Shalet et al. 1978; Olshan et al. 1992). The effects of GHD becomes evident 2 or more years after RT, and in children who received cranium-spinal RT, the early growth retardation observed during the first 2 years

post-RT seems to be mainly due to a reduced spinal growth. Moreover, it has been demonstrated that the additive detrimental effect of spinal RT still persists in the following years (Olshan et al. 1992). Besides the effects of spinal RT, children who receive cranial irradiation can develop a premature activation of the pituitary-gonadal axis resulting in precocious puberty. Therefore, the poor outcome in stature frequently observed in children treated for CNS tumors might also be caused by the acceleration of bone maturation and the precocious epiphyseal closure, in combination with the reduced pubertal growth spurt due to GHD.

Finally, other conditions such as reduced nutritional intake, steroid administration, psychological dysfunction, hypothyroidism, and hypogonadism can play an additional role on the impaired growth induced by GHD (Darzy and Shalet 2006).

In pediatric CS, the persistence of GHD in adulthood has been related with an increased risk of metabolic syndrome, obesity, and reduced bone mineral density (BMD) (Felicetti et al. 2015; van Waas et al. 2010; Steffens et al. 2008), as it happens in patients with adult-onset GHD. In GHD adult patients, a polymorphic syndrome characterized by weight gain, increased fat mass with central adiposity, and a negative lipid profile is likely to be responsible for their increased cardiovascular mortality. In addition, GHD adult patients show a reduction in BMD, lean body mass, muscle strength, exercise capacity, cognitive function, and quality of life (De Boer et al. 1995; Rosen and Bengtsson 1990).

Precocius Puberty and Central Hypogonadism

The hypothalamic-pituitary gonadal axis is more resistant than somatotropic axis to the injury of RT. However, both gonadotropin deficiency and precocious puberty can be observed in patients who received RT involving the hypothalamus and/or pituitary gland.

Precocious puberty is defined as the development of pubertal changes at an age younger than the accepted lower limits for the onset of puberty, namely, before age 8 years in girls and 9 years in boys. Early progression of secondary sexual characteristics, rapid bone maturation, reduced adult height, inappropriate perception of body image, and behavioral abnormalities represent the most important clinical manifestation of this clinical condition, though in children with malignancy, growth acceleration may not be clearly evident if GHD coexists (Berberoğlu 2009). An accelerated progression of pubertal development can result from the release of hypothalamic gonadotropin-releasing hormone (GnRH) triggered by irradiation, the effects of CNS tumor itself, and the raised intracranial pressure (Oberfield et al. 1996). Cranial RT seems to cause a damage to γ-aminobutyric-acid secreting neurons, resulting in premature activation of GnRH neurons. Female sex and younger age at cancer treatment increase the risk of precocious puberty. Low-dose RT (18 Gy), usually employed in ALL patients, was reported to cause precocious puberty in females, but not in males. With radiation doses increased to 25–50 Gy, the incidence of precocious puberty becomes similar in girls and boys. In females, age $\leq$4 years at time of diagnosis increases the risk of early menarche fourfold. Moreover, after cranial irradiation for CNS tumors, puberty might start on time but advance rapidly (Rose et al. 2016).

Radiation doses higher than 30 Gy are able to induce central hypogonadism, both in patients treated for CNS neoplasm in childhood as well as in adulthood. A reduced GnRH hypothalamic release and/or a direct damage of pituitary FSH/LH producing cells, due to radiation, has been hypothesized as pathophysiologic mechanisms (Darzy 2013).

In children treated with cranial RT for CNS tumors, the incidence of gonadotrophin deficiency was estimated to be 20–50% after a prolonged follow-up period (Darzy and Shalet 2009; Lam et al. 1991; Constine et al. 1993; Rappaport et al. 1982). This wide range of incidence is probably due to the different definitions of "gonadotrophin deficiency," besides the classic one characterized by impaired responses of gonadotrophins to dynamic evaluation by GnRH test, severe clinical manifestation, and reduced sex hormone levels (Darzy and Shalet 2009). Central hypogonadism is also frequently seen in adult survivors of CNS tumor. After a median follow-up of 8 years since RT, Kyriakakis et al. recently reported a 34.6% rate of gonadotrophin deficiency in a cohort of 107 adult patients treated with cranial RT (Kyriakakis et al. 2016).

In survivors of childhood CNS tumors, hypogonadism causes not only the absence of normal pubertal development and the impairment of sexual function but also severe metabolic alterations that can affect the long-term health of these patients. Brain tumor survivors, in fact, show an increased risk of metabolic syndrome, osteoporosis, and cardiovascular disease if compared with the general population (Felicetti et al. 2015; van Waas et al. 2010), and a misdiagnosed hypogonadism can significantly worsen this conditions.

Finally, it is important to consider the potential interaction between the impairment in gonadotrophin production and the quite common damage induced by chemotherapy on testicular and ovarian function. Indeed, commonly employed antineoplastic drugs such as alkylating agents and platinum-derived compounds can induce transient or permanent damage to the germinal cells. Moreover, RT directly involving the ovaries or the testes can also induce hypogonadism (Brignardello et al. 2013; Brignardello et al. 2016). Hence it follows that mixed clinical situations, with both central and primary dysfunctions of the gonadal axis, can sometimes be observed in this specific context.

ACTH Deficiency

The hypothalamic-pituitary-adrenal axis is quite resistant to the detrimental effect of radiation; hence, adrenal insufficiency is rarely observed in CNS tumor survivors. ACTH production seems to be unaffected by low radiation doses (<20 Gy; 50,51), whereas a low rate of adrenal insufficiency (about 3%) has been reported in patients receiving doses higher than 20 Gy but lower than 50 Gy of cranial RT (Darzy 2013). In patients receiving more than 50 Gy, a significant increase in ACTH deficiency was observed, with a cumulative incidence of 27–35% after a 15-year observation period (Darzy and Shalet 2009; Lam et al. 1991; Samaan et al. 1982). In survivors of childhood-onset CNS tumors, older age at RT seems to be a risk factor for ACTH deficiency (Agha et al. 2005). Recently, in a study on long-term survivors of pediatric ALL previously treated with moderate cranial radiation doses (18–30 Gy),

Follin et al. reported an increased risk of central adrenal insufficiency 20 years after anticancer treatments (Follin et al. 2014).

Adrenal insufficiency is a potential life-threatening condition, and adrenal crisis is one of the most feared medical emergencies in endocrinology. Nevertheless, in patients treated with cranial RT for non-pituitary brain tumors, ACTH deficiency is usually partial, and few patients show clinical signs or symptoms of adrenal insufficiency requiring daily hydrocortisone replacement therapy. This was also observed by Kyriakakis et al. who reported a 23.4% incidence of ACTH deficiency in adult non-pituitary brain tumors survivors, but only 11 of 25 affected patients required replacement therapy (Kyriakakis et al. 2016) in baseline conditions.

In addition to a deficiency in ACTH secretion, Darzy et al. reported a surprisingly increased activity of the CRH-ACTH-adrenal axis in CS. The authors evaluated the stimulated and 24-h spontaneous release of cortisol in 34 subjects who had received conventional cranial RT for CNS tumors or leukemia (all with normal peak cortisol responses to the ITT) and in 33 age-, gender-, and body mass index-matched controls. The patients showed a significant increase in integrated 24-h circulating cortisol levels, with a significant 20% increase in cortisol production rates determined by deconvolution analysis, compared with controls (Darzy and Shalet 2005a). The authors hypothesized that the chronic stress that many CS may have, due to poor quality of life and long-term disabilities, may account for this increase in cortisol levels (Darzy 2013).

TSH Deficiency

The hypothalamic-pituitary-thyroid axis is the more resistant to radiation-induced damage. No significant impairment of TSH production was reported after moderate-low-dose cranial RT (Darzy 2013; Darzy and Shalet 2009), whereas the incidence of central hypothyroidism significantly increases with higher doses (>40 Gy). A 3–6% of cumulative incidence was reported in survivors of childhood-onset CNS tumors, while a slightly higher incidence was shown in patients receiving cranial RT in adulthood (Kyriakakis et al. 2016; Agha et al. 2005; Madaschi et al. 2011). In older studies, the presence of a so-called "hidden" central hypothyroidism in survivors of childhood cancer was hypothesized, based on abnormal baseline and stimulated TSH secretion (as in central hypothyroidism) observed in a relevant proportion of euthyroid irradiated children (Lam et al. 1991; Rose et al. 1999; Schmiegelow et al. 2003; Darzy and Shalet 2005b). These abnormalities in dynamic TSH secretion, however, should not be considered a real pathology as more recent studies did not show a subsequent development of "overt" central hypothyroidism in these patients (Darzy 2013). Therefore, only in the presence of subnormal fT4 levels with clinical signs or symptoms of hypothyroidism even if associated to normal TSH levels, the diagnosis of central hypothyroidism should be made and replacement therapy started.

As previously discussed for gonadotrophin deficiency, a final consideration should be made about patients who received both cranial and spinal/neck irradiation. The thyroid gland is highly sensitive to the effect of RT, and primary hypothyroidism is frequently observed after neck irradiation at doses >20 Gy (Brignardello et al. 2013; Jereczek-Fossa et al. 2004). Being the radiation doses able to induce central

hypothyroidism higher than those able to induce primary hypothyroidism, it is not rare to observe patients with combined central and primary hypothyroidism.

Prolactin

After high-dose cranial RT (usually >40 Gy), hyperprolactinemia occurs in a high percentage of patients (20–50%), whereas it is rare in patients who receive lower doses (Lam et al. 1991; Constine et al. 1993; Clayton et al. 2011; Ogilvy-Stuart et al. 1992; Agha et al. 2005; Littley et al. 1989). Age at irradiation and sex do not seem to affect the development of hyperprolactinemia that results from the impaired hypothalamic production of dopamine with a consequent reduction of its inhibitory effect on the pituitary gland. In the great majority of patients, a mild hyperprolactinemia is reported not sufficient to impair gonadotrophin secretion that in most cases is already affected by the RT itself. A progressive reduction in prolactin levels with time was described, most probably related to the progression of the radiation-induced injury to the pituitary (Littley et al. 1989; Darzy 2013).

Management of Pituitary Dysfunction in CNS Tumor Survivors

Hypothalamic-pituitary dysfunction in survivors of CNS tumors is characterized by distinct features that should be carefully considered by the endocrinologists who deal with these patients (Brignardello et al. 2013). The diagnosis, treatment, and clinical management of endocrinopathy in this context show some differences if compared with the routine clinical practice.

GHD

In patients with cancer, a prompt diagnosis of GHD during childhood is crucial. The Children's Oncology Group (COG) guidelines (2008) recommend to monitor height, weight, BMI, and pubertal development every 6 months until adult height, in all children with an increased risk of GHD (i.e., treated with cranial irradiation ≥18 Gy). Coexisting hypothyroidism and other conditions that could affect growth rate, like chronic unresolved illness, undernutrition, and depression, should be ruled out. The evaluation of pubertal status is crucial, considering that a coexisting precocious puberty can augment the growth rate and mask the GHD (Rose et al. 2016; Children's Oncology Group Guidelines 2008; Chemaitilly and Sklar 2010). Measurements should be done using appropriate instruments and values plotted on growth chart specific for the ethnic group of the patient and taking into account parental height. In children treated with spinal radiotherapy for medulloblastoma, it is preferable to measure leg length (Crowne et al. 2015). In the presence of a reduced growth, GH status should be assessed by dynamic tests as IGF-1 and IGFBP3 levels might be normal even in the presence of GHD (Rose et al. 1988, 2016; Sklar et al. 1993). Multiple tests have been proposed to asses GH production in children with a history of malignancy. If not contraindicated (seizures or cardiac diseases), insulin tolerance test (ITT) is the preferable test (Rose et al. 2016; Darzy 2013). GHRH + arginine or glucagon stimulating tests can be considered as alternative tests.

In children, according to some authors (Darzy 2013), an abnormal GH response to ITT, even in the presence of normal response to other stimulation tests, may indicate that the GH axis cannot meet the increased demand of GH production and thus requires hormone replacement.

In adult patients, signs and symptoms of GHD are more subtle, and the assessment of GH axis function using proper provocative tests is usually performed only in patients who are candidate to receive GH replacement therapy (Crowne et al. 2015), as recently recommended by the Endocrine Society Guidelines (Fleseriu et al. 2016).

Despite the well-proven beneficial effect of GH administration on the growth pattern of children, the potential side effects of GH administration have been one of the most debated topics on cancer survivors. In children with cancer, one concern is the increased risk of disease recurrence, although available studies seem to rule out an increased incidence of cancer recurrence in patients treated with GH replacement during childhood (Felicetti et al. 2016). Another concern is the supposed role of GH administration on the development of second neoplasms (SNs), which is one of the most feared late complications of anticancer treatments (Reulen et al. 2011; Oeffinger and Bhatia 2009; Travis et al. 2012). Many confounding factors might play a role in the development of second SNs, and contrasting data were reported. The incidence of SNs should be evaluated many years after cancer treatment and GH exposure and can be influenced by several factors unrelated to the disease and its treatment, such as lifestyle, family history, etc. The most important risk factor for second neoplasm in patients treated for CNS tumors is RT, making the oncological risk due to GH administration hardly distinguishable from the risk due to radiation itself. In a recent publication of the Childhood Cancer Survivor Study, after a long observation period (median 15 years), no increased rate of meningioma, glioma, or any CNS neoplasm was associated with GH therapy, even after adjusting for the radiation doses and duration of follow-up (Patterson et al. 2014). No difference in the risk of SNs between GH-treated and untreated patients was also reported by Mackenzie et al. in 2011 (Mackenzie et al. 2011) and by Brignardello et al. in 2015 (Brignardello et al. 2015). However, some studies reported an early occurrence (Brignardello et al. 2015) and an increased risk of SN after a short follow-up period (Sklar et al. 2002; Ergun-Longmire et al. 2006) probably related to the promoting rather than initiating effect on carcinogenesis of GH and IGF-1. Hence, GH administration may be not responsible for cancer initiation, but it might accelerate the growth of a second neoplasm and cause its earlier clinical appearance (Felicetti et al. 2016; Ahmid et al. 2016). If there is no evidence of tumor recurrence, recommendations based on expert opinion advise to wait at least 1 year after completion of cancer therapy before initiating GH therapy during childhood (Rose et al. 2016).

When survivors of childhood cancer become adults, or in patients treated for adult-onset malignancies, the question about GH treatment is even more debated. As summarized in a recently published review (Ahmid et al. 2016), short-term studies showed improved lipid profile and bone mineralization in these patients after GH replacement. A definite conclusion as to whether GH therapy should be

recommended in adult patients cannot be drawn, as large studies with a long follow-up are lacking making impossible to predict if GH replacement therapy carried on until late adulthood would significantly affect long-term health of cancer survivors. Moreover, the majority of adult-onset non-pituitary CNS tumors shows a high malignant potential (McNeill 2016; Ostrom et al. 2014), and GH therapy is not recommended in patients who had multiple cancer recurrences, metastases, highly malignant tumors, or genetic cancer predisposition (Rose et al. 2016; Molitch et al. 2011). Thus, if GH administration in childhood CS during childhood is advisable to obtain a normal growth, after the attainment of adult height, the decision to treat should be taken by a multidisciplinary team composed by endocrinologists, oncologists, and/or radiation oncologists, after careful evaluation of the potential advantages and disadvantages and in agreement with the patient's preferences (Allen et al. 2016).

Precocious Puberty and Central Hypogonadism

The diagnosis of precocious puberty in children treated for CNS tumors is substantially based on the clinic, and therefore, pubertal development should be carefully evaluated in these patients at every follow-up visit. When clinically indicated, LH, FSH, and sex steroids should be measured at baseline and after GnRH stimulation test that represents the gold standard for the diagnosis of precocious puberty (Rose et al. 2016; Walvoord 2010).

Pharmacologic treatment of precocious puberty with GnRH analogue (GnRHa) can be started during anticancer treatments or even before. The decision to initiate treatment with GnRHa must be based on a variety of factors such as progression of secondary sexual characteristics, skeletal maturation, predicted adult height, probability of menarche before age 9 years, and also neuropsychological development and maturity sometimes impaired in pediatric cancer patients (Carel and Leger 2008).

Available guidelines (Children's Oncology Group Guidelines 2008) recommend annual evaluation of pubertal status until the patient is sexually mature. After pubertal development, subsequent evaluation of gonadal function should be done on the basis of clinical sign or symptoms suggestive for hypogonadism (Children's Oncology Group Guidelines 2008). Replacement therapy should be administered using standard protocols for patients with hypogonadism (Fleseriu et al. 2016). In patients with delayed puberty, the treatment with estrogens/progesterone or testosterone should be started not too early and carefully monitoring growth, due to the risk of prematurely fusion of growth plates (DiVasta and Gordon 2008). A gradual increase in the hormone replacement therapy doses is recommended to mimic normal pubertal progression (Rose et al. 2016; DiVasta and Gordon 2008; Han and Bouloux 2010). In patients with central hypogonadism, infertility can be treated with gonadotropin replacement therapy (Milsom et al. 2012; Dwyer et al. 2015). However, in the specific context of CS, concomitant primary gonadal damage due to gonadotoxic CT and/or RT (Brignardello et al. 2013; Brignardello et al. 2016), as well as uterine injury following abdominal radiotherapy, might reduce the possibility of successful fertility restoration (Waimey et al. 2015; Critchley et al. 1992; Ben-Nagi and Penay 2014).

Central Hypothyroidism and Hypoadrenalism

In patients at risk for hypothyroidism, annual evaluation of TSH and free tetraiodothyroinine (fT4) is recommended (Children's Oncology Group Guidelines 2008). The diagnosis of central hypothyroidism may be sometimes difficult, especially in the presence of low or progressively reducing levels of fT4 with normal TSH levels. Hence, some studies suggested the use of a morning/afternoon ratio (with normal values >1.3) as a marker of preserved circadian variation or, in case of alteration, of impaired hypothalamic-pituitary-thyroid axis. During childhood, early treatment of mild hypothyroidism was associated with better growth and quality of life (Rose et al. 2016; Rose 1995). Before starting treatment, in patients at risk, ACTH deficiency should be carefully ruled out to avoid adrenal crisis due to the increased cortisol clearance induced by thyroid hormones. Daily doses and subsequent monitoring of replacement therapy in CS patients are not different from those used in the general population (Fleseriu et al. 2016).

Adrenal insufficiency is a rare occurrence in CS, but it should always be kept in mind as a misdiagnosis can lead to an adrenal crisis with life-threatening consequences. The most common symptoms of adrenal insufficiency (asthenia, weight loss, abdominal pain, etc.) are often subtle and found frequently in cancer patients with normal cortisol production. In all patients at risk (cranial RT at doses >30 Gy, surgery involving the sellar or suprasellar region), an annual evaluation of cortisol blood concentration should be performed. For replacement therapy, oral immediate-release hydrocortisone was used so far. The recently available modified-release hydrocortisone, which is administered once daily, may represent a valuable therapeutic option especially in younger patients. In patients at risk for adrenal insufficiency and in whom GH or thyroxine replacement therapy has to be started, hypothalamic-pituitary-adrenal axis should be carefully evaluated due to the risk of adrenal crisis. GH in fact, by inhibiting 11β-hydroxysteroid dehydrogenase type 1 that transforms cortisone to cortisol, and thyroxine by increasing cortisol clearance may lead to reduced cortisol blood concentrations (Rose et al. 2016).

Summary

In summary, thanks to the improvement of anticancer therapies, the rate of patients diagnosed with cancer that can be effectively cured is improved. Patients with childhood and adolescence central nervous system tumors, in many cases, have a high survival expectancy and it is necessary to take under consideration the risk or the presence of adverse effects of anticancer-therapies. Pituitary dysfunction is commonly diagnosed in survivors of central nervous system tumors and can play a significant role in the long-term health of these patients, but also diminish their short term health, particularly in children.

Craniopharyngioma represents approximately 3% of all intracranial tumours. Non-aggressive surgery followed by radiotherapy is currently the most widely treatment. Even in this case, hypopituitarism but also hypothalamic damage (obesity,

adipsic or polidipsic diabetes insipidus, sleep disorders, neurocognitive impairment) are the most common side effects of treatment.

The diagnosis, therapy and clinical management of endocrine dysfunctions in these contexts show some differences if compared with the routine clinical practice. Therefore, the endocrinologist should play an essential role in the multidisciplinary team that take care of this kind of patients.

References

Abs R, Mattsson AF, Bengtsson BA, Feldt-Rasmussen U, Góth MI, Koltowska-Häggström M, Monson JP, Verhelst J, Wilton P, KIMS Study Group. Isolated growth hormone (GH) deficiency in adult patients: baseline clinical characteristics and responses to GH replacement in comparison with hypopituitary patients. A sub-analysis of the KIMS database. Growth Horm IGF Res. 2005;15(5):349–59.

Agha A, Sherlock M, Brennan S, O'Connor SA, O'Sullivan E, Rogers B, Faul C, Rawluk D, Tormey W, Thompson CJ. Hypothalamic-pituitary dysfunction after irradiation of nonpituitary brain tumours in adults. J Clin Endocrinol Metab. 2005;90:6355–60.

Ahmid M, Perry CG, Ahmed SF, Shaikh MG. Growth hormone deficiency during adulthood and the benefits of growth hormone replacement. Endocrine Connect. 2016;5:R1–R11.

Albertsson-Wikland K, Lannering B, Marky I. A longitudinal study on growth and spontaneous growth hormone (GH) secretion in children with irradiated brain tumors. Acta Paediatr Scand. 1987;76:966–73.

Allen DB, Backeljauw P, Bidlingmaier M, Biller BM, Boguszewski M, Burman P, Butler G, Chihara K, Christiansen J, Cianfarani S, Clayton P, Clemmons D, Cohen P, Darendeliler F, Deal C, Dunger D, Erfurth EM, Fuqua JS, Grimberg A, Haymond M, Higham C, Ho K, Hoffman AR, Hokken-Koelega A, Johannsson G, Juul A, Kopchick J, Lee P, Pollak M, Radovick S, Robison L, Rosenfeld R, Ross RJ, Savendahl L, Saenger P, Toft Sorensen H, Stochholm K, Strasburger C, Swerdlow A, Thorner M. GH safety workshop position paper: a critical appraisal of recombinant human GH therapy in children and adults. Eur J Endocrinol. 2016;174:P1–9.

Barriger RB, Chang A, Lo SS, Timmerman RD, DesRosiers C, Boaz JC, Fakiris AJ. Phosphorus-32 therapy for cystic craniopharyngiomas. Radiother Oncol. 2011;98(2):207–12.

Ben-Nagi J, Penay N. Premature ovarian insufficiency: how to improve reproductive outcome? Climacteric. 2014;17:22–6.

Berberoğlu M. Precocious puberty and normal variant puberty: definition, etiology, diagnosis and current management. J Clin Res Pediatr Endocrinol. 2009;1(4):164–74.

Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, Husebye ES, Merke DP, Murad MH, Stratakis CA, Torpy DJ. Diagnosis and treatment of primary adrenal insufficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2016;101(2):364–89.

Brauner R, Rappaport R, Prevot C, Czernichow P, Zucker JM, Bataini P, Lemerle J, Sarrazin D, Guyda HJ. A prospective study of the development of growth hormone deficiency in children given cranial irradiation, and its relation to statural growth. J Clin Endocrinol Metab. 1989;68:346–51.

Brennan BM, Rahim A, Mackie EM, Eden OB, Shalet SM. Growth hormone status in adults treated for acute lymphoblastic leukaemia in childhood. Clin Endocrinol (Oxf). 1998;48:777–83.

Brignardello E, Felicetti F, Castiglione A, Chiabotto P, Corrias A, Fagioli F, Ciccone G, Boccuzzi G. Endocrine health conditions in adult survivors of childhood cancer: the need for specialized adult-focused follow-up clinics. Eur J Endocrinol. 2013;168(3):465–72.

Brignardello E, Felicetti F, Castiglione A, Fortunati N, Matarazzo P, Biasin E, Sacerdote C, Ricardi U, Fagioli F, Corrias A, Arvat E. GH replacement therapy and second neoplasms in adult

survivors of childhood cancer: a retrospective study from a single institution. J Endocrinol Invest. 2015;38:171–6.
Brignardello E, Felicetti F, Castiglione A, Nervo A, Biasin E, Ciccone G, Fagioli F, Corrias A. Gonadal status in long-term male survivors of childhood cancer. J Cancer Res Clin Oncol. 2016;142(5):1127–32.
Brown IH, Lee TJ, Eden OB, Bullimore JA, Savage DC. Growth and endocrine function after treatment for medulloblastoma. Arch Dis Child. 1983;58:722–7.
Bucci MN, Chin LS, Hoff JT. Perioperative morbidity associated with operative resection of craniopharyngioma: a review of ten years experience. Neurochirurgia. 1987;30:135–8.
Campanini ML, Colli LM, Paixao BM, Cabral TP, Amaral FC, Machado HR, Neder LS, Saggioro F, Moreira AC, Antonini SR, De Castro M. CTNNB1 gene mutations, pituitary transcription factors, and MicroRNA expression involvement in the pathogenesis of adamantinomatous craniopharyngiomas. Horm Cancer. 2010;1(4):187–96.
Carel JC, Leger J. Precocious puberty. N Engl J Med. 2008;358:2366–77.
Chemaitilly W, Sklar CA. Endocrine complications in long-term survivors of childhood cancers. Endocr Relat Cancer. 2010;17(3):R141–59.
Chemaitilly W, Li Z, Huang S, Ness KK, Clark KL, Green DM, Barnes N, Armstrong GT, Krasin MJ, Srivastava DK, Pui CH, Merchant TE, Kun LE, Gajjar A, Hudson MM, Robison LL, Sklar CA. Anterior hypopituitarism in adult survivors of childhood cancers treated with cranial radiotherapy: a report from the St Jude lifetime cohort study. J Clin Oncol. 2015;33(5):492–500.
Chen MS, Lin FJ, Huang MJ, Wang PW, Tang S, Leung WM, Leung W. Prospective hormone study of hypo- thalamic-pituitary function in patients with nasopharyngeal carcinoma after high dose irradiation. Jpn J Clin Oncol. 1989;19:265–70.
Child CJ, Conroy D, Zimmermann AG, Woodmansee WW, Erfurth EM, Robison LL. Incidence of primary cancers and intracranial tumour recurrences in GH-treated and untreated adult hypopituitary patients: analyses from the hypopituitary control and complications study. Eur J Endocrinol. 2015;172(6):779–90.
Children's Oncology Group. Long-term follow-up guidelines for survivors of childhood, adolescent, and young adult cancers. Version 3.0. 2008. http://www.survivorshipguidelines.org/.
Clar HE. Disturbances of hypothalamic thermoregulation. Acta Neurochir. 1985;75:106–12.
Clarson CL, Del Maestro RF. Growth failure after treatment of pediatric brain tumors. Pediatrics. 1999;103:E37.
Clayton PE, Shalet SM. Dose dependency of time of onset of radiation-induced growth hormone deficiency. J Pediatr. 1991;118:226–8.
Clayton PE, Shalet SM, Price DA. Growth response to growth hormone therapy following craniospinal irradiation. Eur J Pediatr. 1988;147:597–601.
Clayton PE, Banerjee I, Murray PG, Renehan AG. Growth hormone, the insulin-like growth factor axis, insulin and cancer risk. Nat Rev Endocrinol. 2011;7:11–24.
Clement SC, Meeteren AY, Kremer LC, van Trotsenburg AS, Caron HN, van Santen HM. High prevalence of early hypothalamic-pituitary damage in childhood brain tumor survivors: need for standardized follow-up programs. Pediatr Blood Cancer. 2014;61(12):2285–9.
Constine LS, Woolf PD, Cann D, Mick G, McCormick K, Raubertas RF, Rubin P. Hypothalamic-pituitary dysfunction after radiation for brain tumors. N Engl J Med. 1993;328:87–94.
Critchley HO, Wallace WH, Shalet SM, Mamtora H, Higginson J, Anderson DC. Abdominal irradiation in childhood: the potential for pregnancy. Br J Obstet Gynaecol. 1992;99:392–4.
Crowley RK, Hamnvik OP, O'Sullivan EP, Behan LA, Smith D, Agha A, Thompson CJ. Morbidity and mortality in patients with craniopharyngioma after surgery. Clin Endocrinol (Oxf). 2010;73(4):516–21.
Crowne E, Gleeson H, Benghiat H, Sanghera P, Toogood A. Effect of cancer treatment on hypothalamic–pituitary function. Lancet Diabetes Endocrinol. 2015;3:568–76.
Darendeliler F, Livesey EA, Hindmarsh PC, Brook CG. Growth and growth hormone secretion in children following treatment of brain tumours with radiotherapy. Acta Paediatr Scand. 1990;79:950–6.

Darzy KH. Radiation-induced hypopituitarism. Curr Opin Endocrinol Diabetes Obes. 2013;20(4): 342–53.

Darzy KH, Shalet SM. Absence of adrenocorticotropin (ACTH) neurosecretory dysfunction but increased cortisol concentrations and production rates in ACTH-replete adult cancer survivors after cranial irradiation for non-pituitary brain tumors. J Clin Endocrinol Metab. 2005a;90(9): 5217–25.

Darzy KH, Shalet SM. Circadian and stimulated thyrotropin secretion in cranially irradiated adult cancer survivors. J Clin Endocrinol Metab. 2005b;90:6490–7.

Darzy KH, Shalet SM. Hypopituitarism as a consequence of brain tumours and radiotherapy. Pituitary. 2005c;8(3–4):203–11.

Darzy KH, Shalet SM. Pathophysiology of radiation-induced growth hormone deficiency: efficacy and safety of GH replacement. Growth Horm IGF Res. 2006;16(Suppl A):S30–40.

Darzy KH, Shalet SM. Hypopituitarism following radiotherapy. Pituitary. 2009;12(1):40–50.

Darzy KH, Aimaretti G, Wieringa G, Gattamaneni HR, Ghigo E, Shalet SM. The usefulness of the growth hormone (GH)-releasing hormone and arginine stimulation test in the diagnosis of radiation-induced GH deficiency is dependent on the post-irradiation time interval. J Clin Endocrinol Metab. 2003;88:95–102.

Darzy KH, Pezzoli S, Thorner M, Shalet S. The dynamics of GH secretion in adult cancer survivors with severe GH deficiency acquired after brain irradiation in childhood for non pituitary brain tumors: evidence for preserved pulsatility and diurnal variation with increase secretory disorderliness. J Clin Endocrinol Metab. 2005;90:2794–803.

Darzy KH, Pezzoli S, Thorner M, Shalet S. Cranial irradiation and growth hormone neurosecretory dysfunction: a critical appraisal. J Clin Endocrinol Metab. 2007;92:1666–72.

Darzy KH, Thorner M, Shalet S. Cranially irradiated adult cancer survivors may have normal spontaneous GH secretion in the presence of discordant peak GH response to stimulation tests (compensated GH deficiency). Clin Endocrinol (Oxf). 2009;70:287–93.

De Boer H, Blok GJ, Van der Veen EA. Clinical aspects of growth hormone deficiency in adults. Endocr Rev. 1995;16:63–86.

De Marinis L, Fusco A, Bianchi A, Aimaretti G, Ambrosio MR, Scaroni C, Cannavo S, Di Somma C, Mantero F, degli Uberti EC, Giordano G, Ghigo E. Hypopituitarism findings in patients with primary brain tumors 1 year after neurosurgical treatment: preliminary report. J Endocrinol Invest. 2006;29(6):516–22.

Devile CJ, Grant DB, Hayward RD, Stanhope R. Growth and endocrine sequelae of craniopharyngioma. Arch Dis Child. 1996;75(2):108–14.

DiVasta AD, Gordon CM. Hormone replacement therapy for the adolescent patient. Ann N Y Acad Sci. 2008;1135:204–11.

Dwyer AA, Raivio T, Pitteloud N. Gonadotrophin replacement for induction of fertility in hypogonadal men. Best Pract Res Clin Endocrinol Metab. 2015;29:91–103.

Ergun-Longmire B, Mertens AC, Mitby P, Qin J, Heller G, Shi W, Yasui Y, Robison LL, Sklar CA. Growth hormone treatment and risk of second neoplasms in the childhood cancer survivor. J Clin Endocrinol Metab. 2006;91:3494–8.

Faje AT, Nachtigall L, Wexler D, Miller KK, Klibanski A, Makimura H. Central diabetes insipidus: a previously unreported side effect of temozolomide. J Clin Endocrinol Metab. 2013;98: 3926–31.

Felicetti F, D'Ascenzo F, Moretti C, Corrias A, Omedè P, Marra WG, Arvat E, Fagioli F, Brignardello E, Gaita F. Prevalence of cardiovascular risk factors in long-term survivors of childhood cancer: 16 years follow up from a perspective registry. Eur J Prev Cardiol. 2015;22:762–70.

Felicetti F, Fortunati N, Arvat E, Brignardello E. GH deficiency in adult survivors of childhood cancer. Best Pract Res Clin Endocrinol Metab. 2016;30(6):795–804.

Fleseriu M, Hashim IA, Karavitaki N, Melmed S, Murad MH, Salvatori R, Samuels MH. Hormonal replacement in hypopituitarism in adults: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2016;101(11):3888–921.

Follin C, Wiebe T, Moell C, Erfurth EM. Moderate dose cranial radiotherapy causes central adrenal insufficiency in long-term survivors of childhood leukaemia. Pituitary. 2014;17:7–12.

Garnett MR, Puget S, Grill J, Sainte-Rose C. Craniopharyngioma. Orphanet J Rare Dis. 2007;2:18.
Gleeson HK, Gattamaneni HR, Smethurst L, Brennan BM, Shalet SM. Reassessment of growth hormone status is required at final height in children treated with growth hormone replacement after radiation therapy. J Clin Endocrinol Metab. 2004;89:662–6.
Han TS, Bouloux PM. What is the optimal therapy for young males with hypogonadotrophic hypogonadism? Clin Endocrinol (Oxf). 2010;72:731–7.
Harz KJ, Müller HL, Waldeck E, Pudel V, Roth C. Obesity in patients with craniopharyngioma: assessment of food intake and movement counts indicating physical activity. J Clin Endocrinol Metab. 2003;88(11):5227–31.
Hochberg Z, Kuten A, Hertz P, Tatcher M, Kedar A, Benderly A. The effect of single-dose radiation on cell survival and growth hormone secretion by rat anterior pituitary cells. Radiat Res. 1983;94:508–12.
Hokken-Koelega AC, van Doorn JW, Hählen K, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL. Long-term effects of treatment for acute lymphoblastic leukemia with and without cranial irradiation on growth and puberty: a comparative study. Pediatr Res. 1993;33:577–82.
Holmer H, Pozarek G, Wirfält E, Popovic V, Ekman B, Björk J, Erfurth EM. Reduced energy expenditure and impaired feeding-related signals but not high energy intake reinforces hypothalamic obesity in adults with childhood onset craniopharyngioma. J Clin Endocrinol Metab. 2010;95(12):5395–402.
Honegger J, Tatagiba M. Craniopharyngioma surgery. Pituitary. 2008;11(4):361–73.
Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, et al., editors. SEER cancer statistics review, 1975–2012. Bethesda: National Cancer Institute; 2015. http://seer.cancer.gov/csr/1975_2012/. (2015).
Ilveskoski I, Saarinen UM, Wiklund T, Sipilä I, Mäkipernaa A, Perkkiö M, Lanning M, Salmi TT, Pihko H. Growth impairment and growth hormone therapy in children treated for malignant brain tumours. Eur J Pediatr. 1997;156:764–9.
Jereczek-Fossa BA, Alterio D, Jassem J, Gibelli B, Tradati N, Orecchia R. Radiotherapy-induced thyroid disorders. Cancer Treat Rev. 2004;30(4):369–84.
Johnston S, Burgess J, McMillan T, Greenwood R. Management of adipsia by a behavioural modification technique. J Neurol Neurosurg Psychiatry. 1991;54:272–4.
Jorgensen JO, Pedersen SA, Thuesen L, Jorgensen J, Ingemann-Hansen T, Skakkebaek NE, Christiansen JS. Beneficial effects of growth hormone treatment in GH-deficient adults. Lancet. 1989;1:1221–5.
Karavitaki N. Management of craniopharyngiomas. J Endocrinol Invest. 2014;37(3):219–28.
Karavitaki N, Brufani C, Warner JT, Adams CB, Richards P, Ansorge O, Shine B, Turner HE, Wass JA. Craniopharyngiomas in children and adults: systematic analysis of 121 cases with long-term follow-up. Clin Endocrinol (Oxf). 2005;62(4):397–409.
Karavitaki N, Cudlip S, Adams CB, Wass JA. Craniopharyngiomas. Endocr Rev. 2006;27(4):371–97.
Kendall-Taylor P, Jönsson PJ, Abs R, Erfurth EM, Koltowska-Häggström M, Price DA, Verhelst J. The clinical, metabolic and endocrine features and the quality of life in adults with childhood-onset craniopharyngioma compared with adult-onset craniopharyngioma. Eur J Endocrinol. 2005;152(4):557–67.
Kyriakakis N, Lynch J, Orme SM, Gerrard G, Hatfield P, Loughrey C, Short SC, Murray RD. Pituitary dysfunction following cranial radiotherapy for adult-onset nonpituitary brain tumours. Clin Endocrinol (Oxf). 2016;84(3):372–9.
Lam KS, Wang C, Yeung RT, Ma JT, Ho JH, Tse VK, Ling N. Hypothalamic hypopituitarism following cranial irradiation for nasopharyngeal carcinoma. Clin Endocrinol (Oxf). 1986;24:643–65.
Lam KS, Tse VK, Wang C, Yeung RT, Ho JH. Effects of cranial irradiation on hypothalamic-pituitary function: a 5-year longitudinal study in patients with nasopharyngeal carcinoma. Q J Med. 1991;78:165–76.

Lannering B, Marky I, Mellander L, Albertsson-Wikland K. Growth hormone secretion and response to growth hormone therapy after treatment for brain tumour. Acta Paediatr Scand. 1998;343:146–51.

Lehrnbecher T, Müller-Scholden J, Danhauser-Leistner I, Sörensen N, von Stockhausen HB. Perioperative fluid and electrolyte management in children undergoing surgery for craniopharyngioma. A 10-year experience in a single institution. Childs Nerv Syst. 1998;14(6): 276–9.

Li K, Lu X, Yang N, Zheng J, Huang B, Li L. Association of pituitary stalk management with endocrine outcomes and recurrence in microsurgery of craniopharyngiomas: a meta-analysis. Clin Neurol Neurosurg. 2015;136:20–4.

Littley MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML. Hypopituitarism following external radiotherapy for pituitary tumours in adults. Q J Med. 1989;70:145–60.

Littley MD, Shalet SM, Morgenstern GR, Deakin DP. Endocrine and reproductive dysfunction following fractionated total body irradiation in adults. Q J Med. 1991;78:265–74.

Lustig RH, Schriock EA, Kaplan SL, Grumbach MM. Effect of growth hormone-releasing factor on growth hormone release in children with radiation induced growth hormone deficiency. Pediatrics. 1985;76:274–9.

Mackenzie S, Craven T, Gattamaneni HR, Swindell R, Shalet SM, Brabant G. Long-term safety of growth hormone replacement after CNS irradiation. J Clin Endocrinol Metab. 2011;96:2756–276.

Madaschi S, Fiorino C, Losa M, Lanzi R, Mazza E, Motta M, Perna L, Brioschi E, Scavini M, Reni M. Time course of hypothalamic-pituitary deficiency in adults receiving cranial radiotherapy for primary extrasellar brain tumors. Radiother Oncol. 2011;99:23–8.

Massimino M, Biassoni V, Gandola L, Garrè ML, Gatta G, Giangaspero F, Poggi G, Rutkowski S. Childhood medulloblastoma. Crit Rev Oncol Hematol. 2016;105:35–51.

McNeill KA. Epidemiology of brain tumors. Neurol Clin. 2016;34:981–98.

Merchant TE. Craniopharyngioma radiotherapy: endocrine and cognitive effects. J Pediatr Endocrinol Metab. 2006;19(Suppl 1):439–46.

Merchant TE, Kiehna EN, Sanford RA, Mulhern RK, Thompson SJ, Wilson MW, Lustig RH, Kun LE. Craniopharyngioma: the St. Jude Children's Research Hospital experience 1984–2001. Int J Radiat Oncol Biol Phys. 2002a;53(3):533–42.

Merchant TE, Williams T, Smith JM, Rose SR, Danish RK, Burghen GA, Kun LE, Lustig RH. Preirradiation endocrinopathies in pediatric brain tumor patients determined by dynamic tests of endocrine function. Int J Radiat Oncol Biol Phys. 2002b;54(1):45–50.

Merchant TE, Rose SR, Bosley C, Wu S, Xiong X, Lustig RH. Growth hormone secretion after conformal radiation therapy in pediatric patients with localized brain tumors. J Clin Oncol. 2011;29(36):4776–80.

Milsom S, Duggan K, O'sullivan S, Ogilvie M, Gunn AJ. Treatment of infertility with hypogonadotropic hypogonadism: 10-year experience in Auckland, New Zealand. Aust N Z J Obstet Gynaecol. 2012;52:293–8.

Minniti G, Saran F, Traish D, Soomal R, Sardell S, Gonsalves A, Ashley S, Warrington J, Burke K, Mosleh-Shirazi A, Brada M. Fractionated stereotactic conformal radiotherapy following conservative surgery in the control of craniopharyngiomas. Radiother Oncol. 2007;82(1):90–5.

Moëll C, Garwicz S, Marky I, Mellander L, Karlberg J. Growth in children treated for acute lymphoblastic leukemia with and without prophylactic cranial irradiation. Acta Paediatr Scand. 1988;77:688–92.

Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Vance ML. Endocrine Society. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1587–609.

Morris Z, Whiteley WN, Longstreth WT Jr, et al. Incidental findings on brain mag- netic resonance imaging: systematic review and meta-analysis. BMJ. 2009;339:b3016.

Mortini P, Losa M, Pozzobon G, Barzaghi R, Riva M, Acerno S, Angius D, Weber G, Chiumello G, Giovanelli M. Neurosurgical treatment of craniopharyngioma in adults and children: early and long-term results in a large case series. J Neurosurg. 2011;114(5):1350–9.

Mulder RL, Kremer LC, van Santen HM, Ket JL, van Trotsenburg AS, Koning CC, Schouten-van Meeteren AY, Caron HN, Neggers SJ, van Dalen EC. Prevalence and risk factors of radiation-induced growth hormone deficiency in childhood cancer survivors: a systematic review. Cancer Treat Rev. 2009;35:616–32.

Müller HL. Childhood craniopharyngioma. Recent advances in diagnosis, treatment and follow-up. Horm Res. 2008;69(4):193–202.

Müller HL. Childhood craniopharyngioma. Current concepts in diagnosis, therapy and follow-up. Nat Rev Endocrinol. 2010;6(11):609–18.

Müller HL. Childhood craniopharyngioma. Pituitary. 2013;16(1):56–67.

Müller HL, Gebhardt U, Pohl F, Flentje M, Emser A, Warmuth-Metz M, Kolb R, Calaminus G, Sörensen N. Relapse pattern after complete resection and early progression after incomplete resection of childhood craniopharyngioma. Klin Padiatr. 2006;218(6):315–20.

O'Gorman CS, Simoneau-Roy J, Pencharz P, MacFarlane J, MacLusky I, Narang I, Adeli K, Daneman D, Hamilton J. Sleep-disordered breathing is increased in obese adolescents with craniopharyngioma compared with obese controls. J Clin Endocrinol Metab. 2010;95(5): 2211–8.

Oberfield SE, Soranno D, Nirenberg A, Heller G, Allen JC, David R, Levine LS, Sklar CA. Age at onset of puberty following high-dose central nervous system radiation therapy. Arch Pediatr Adolesc Med. 1996;150:589–92.

Oeffinger KC, Bhatia S. Second primary cancers in survivors of childhood cancer. Lancet. 2009;374:1484–5.

Ogilvy-Stuart AL, Shalet SM. Effect of chemotherapy on growth. Acta Paediatr Suppl. 1995;411:52–6.

Ogilvy-Stuart AL, Clark DJ, Wallace WH, Gibson BE, Stevens RF, Shalet SM, Donaldson MD. Endocrine deficit after fractionated total body irradiation. Arch Dis Child. 1992;67:1107–10.

Olshan JS, Gubernick J, Packer RJ, D'Angio GJ, Goldwein JW, Willi SM, Moshang T Jr. The effects of adjuvant chemotherapy on growth in children with medulloblastoma. Cancer. 1992;70:2013–7.

Olsson DS, Buchfelder M, Schlaffer S, Bengtsson BA, Jakobsson KE, Johannsson G, Nilsson AG. Comparing progression of non-functioning pituitary adenomas in hypopituitarism patients with and without long-term GH replacement therapy. Eur J Endocrinol. 2009;161(5):663–9.

Olsson DS, Buchfelder M, Wiendieck K, Kremenevskaja N, Bengtsson BÅ, Jakobsson KE, Jarfelt M, Johannsson G, Nilsson AG. Tumour recurrence and enlargement in patients with craniopharyngioma with and without GH replacement therapy during more than 10 years of follow-up. Eur J Endocrinol. 2012;166(6):1061–8.

Ostrom QT, Gittleman H, Liao P, Rouse C, Chen Y, Dowling J, Wolinsky Y, Kruchko C, Barnholtz-Sloan J. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007–2011. Neuro Oncol. 2014;16(Suppl 4):iv1–iv63.

Ostrom QT, de Blank PM, Kruchko C, Petersen CM, Liao P, Finlay JL, Stearns DS, Wolff JE, Wolinsky Y, Letterio JJ, Barnholtz-Sloan JS. Alex's Lemonade Stand Foundation Infant and childhood primary brain and central nervous system tumors diagnosed in the United States in 2007–2011. Neuro Oncol. 2015;16(Suppl 10):x1–x36.

Packer RJ, Macdonald T, Vezina G, Keating R, Santi M. Medulloblastoma and primitive neuro-ectodermal tumors. Handb Clin Neurol. 2012;105:529–48.

Pai HH, Thornton A, Katznelson L, Finkelstein DM, Adams JA, Fullerton BC, Loeffler JS, Leibsch NJ, Klibanski A, Munzenrider JE. Hypothalamic/pituitary function following high-dose conformal radiotherapy to the base of skull: demonstration of a dose-effect relationship using dose-volume histogram analysis. J Radiat Oncol Biol Phys. 2001;49:1079–92.

Patterson BC, Chen Y, Sklar CA, Neglia J, Yasui Y, Mertens A, Armstrong GT, Meadows A, Stovall M, Robison LL, Meacham LR. Growth hormone exposure as a risk factor for the development of subsequent neoplasms of the central nervous system: a report from the childhood cancer survivor study. J Clin Endocrinol Metab. 2014;99:2030–7.

Poretti A, Grotzer MA, Ribi K, Schönle E, Boltshauser E. Outcome of craniopharyngioma in children: long-term complications and quality of life. Dev Med Child Neurol. 2004;46(4):220–9.

Proescholdt M, Merrill M, Stoerr EM, Lohmeier A, Dietmaier W, Brawanski A. Expression of carbonic anhydrase IX in craniopharyngiomas. J Neurosurg. 2011;115(4):796–801.

Puget S, Garnett M, Wray A, Grill J, Habrand JL, Bodaert N, Zerah M, Bezerra M, Renier D, Pierre-Kahn A, Sainte-Rose C. Pediatric craniopharyngiomas: classification and treatment according to the degree of hypothalamic involvement. J Neurosurg. 2007;106(1 Suppl):3–12.

Ramsay DJ. The importance of thirst in maintenance of fluid balance. Baillieres Clin Endocrinol Metab. 1989;3(2):371–91.

Rappaport R, Brauner R, Czernichow P, Thibaud E, Renier D, Zucker JM, Lemerle J. Effect of hypothalamic and pituitary irradiation on pubertal development in children with cranial tumors. J Clin Endocrinol Metab. 1982;54:1164–8.

Reulen RC, Frobisher C, Winter DL, Kelly J, Lancashire ER, Stiller CA, Pritchard-Jones K, Jenkinson HC, Hawkins MM. British Childhood Cancer Survivor Study Steering Group. Long-term risks of subsequent primary neoplasms among survivors of childhood cancer. J Am Med Assoc. 2011;305:2311–9.

Rickert CH, Paulus W. Epidemiology of central nervous system tumors in child- hood and adolescence based on the new WHO classification. Childs Nerv Syst. 2001;17(9):503–11.

Robison LL, Hudson MM. Survivors of childhood and adolescent cancer: life-long risks and responsibilities. Nat Rev Cancer. 2014;14:61–70.

Rose SR. Isolated central hypothyroidism in short stature. Pediatr Res. 1995;38:967–73.

Rose SR, Ross JL, Uriarte M, Barnes KM, Cassorla FG, Cutler GB Jr. The advantage of measuring stimulated as compared with spontaneous growth hormone levels in the diagnosis of growth hormone deficiency. N Engl J Med. 1988;319:201–7.

Rose SR, Lustig RH, Pitukcheewanont P, Broome DC, Burghen GA, Li H, Hudson MM, Kun LE, Heideman RL. Diagnosis of hidden central hypothyroidism in survivors of childhood cancer. J Clin Endocrinol Metab. 1999;84:4472–9.

Rose SR, Schreiber RE, Kearney NS, Lustig RH, Danish RK, Burghen GA, Hudson MM. Hypothalamic dysfunction after chemotherapy. J Pediatr Endocrinol Metab. 2004;17(1):55–66.

Rose SR, Horne VE, Howell J, Lawson SA, Rutter MM, Trotman GE, Corathers SD. Late endocrine effects of childhood cancer. Nat Rev Endocrinol. 2016;12(6):319–36.

Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. Lancet. 1990;336:285–8.

Rutter MM, Rose SR. Long-term endocrine sequelae of childhood cancer. Curr Opin Pediatr. 2007;19:480–7.

Samaan NA, Vieto R, Schultz PN, Maor M, Meoz RT, Sampiere VA, Cangir A, Ried HL, Jesse RH Jr. Hypothalamic, pituitary and thyroid dysfunction after radiotherapy to the head and neck. Int J Radiat Oncol Biol Phys. 1982;8:1857–67.

Schmiegelow M, Lassen S, Poulsen HS, Feldt-Rasmussen U, Schmiegelow K, Hertz H, Müller J. Growth hormone response to a growth hormone-releasing hormone stimulation test in a population-based study following cranial irradiation of childhood brain tumors. Horm Res. 2000;54:53–9.

Schmiegelow M, Feldt-Rasmussen U, Rasmussen AK, Poulsen HS, Muller J. A population-based study of thyroid function after radiotherapy and chemotherapy for a childhood brain tumor. J Clin Endocrinol Metab. 2003;88:136–40.

Schneider HJ, Rovere S, Corneli G, Croce CG, Gasco V, Rudà R, Grottoli S, Stalla GK, Soffietti R, Ghigo E, Aimaretti G. Endocrine dysfunction in patients operated on for non-pituitary intracranial tumors. Eur J Endocrinol. 2006;155(4):559–66.

Schriock EA, Schell MJ, Carter M, Hustu O, Ochs JJ. Abnormal growth patterns and adult short stature in 115 long term survivors of childhood leukemia. J Clin Oncol. 1991;9:400–5.

Shalet SM, Beardwell CG, Aarons BM, Pearson D, Jones PH. Growth impairment in children treated for brain tumours. Arch Dis Child. 1978;53:491–4.

Simpson H, Savine R, Sönksen P, Bengtsson BA, Carlsson L, Christiansen JS, Clemmons D, Cohen P, Hintz R, Ho K, Mullis P, Robinson I, Strasburger C, Tanaka T, Thorner M, GRS Council. Growth hormone replacement therapy for adults: into the new millennium. Growth Horm IGF Res. 2002;12:1–133.

Sklar C, Sarafoglou K, Whittam E. Efficacy of insulin-like growth factor binding protein 3 in predicting the growth hormone response to provocative testing in children treated with cranial irradiation. Acta Endocrinol. 1993;129:511–5.

Sklar CA, Mertens AC, Mitby P, Occhiogrosso G, Qin J, Heller G, Yasui Y, Robison LL. Risk of disease recurrence and second neoplasms in survivors of childhood cancer treated with growth hormone: a report from the Childhood Cancer Survivors Study. J Clin Endocrinol Metab. 2002;87:3136–41.

Smith D, Finucane F, Phillips J, Baylis PH, Finucane J, Tormey W, Thompson CK. Abnormal regulation of thirst and vasopressin secretion following surgery for craniopharyngioma. Clin Endocrinol (Oxf). 2004;61(2):273–9.

Steffens M, Beauloye V, Brichard B, Robert A, Alexopoulou O, Vermylen C, Maiter D. Endocrine and metabolic disorders in young adult survivors of childhood acute lymphoblastic leukaemia (ALL) or non-Hodgkin lymphoma (NHL). Clin Endocrinol (Oxf). 2008;69:819–27.

Taku N, Gurnell M, Burnet N, Jena R. Time dependence of radiation-induced hypothalamic-pituitary axis dysfunction in adults treated for non-pituitary, intracranial neoplasms. Clin Oncol (R Coll Radiol). 2016. pii: S0936-6555(16)30306-5. [Epub ahead of print]. https://doi.org/10.1016/j.clon.2016.09.012.

Thompson CJ, Bland J, Burd J, Baylis PH. The osmotic thresholds for thirst and vasopressin release are similar in healthy man. Clin Sci. 1986;71:651–6.

Thompson CJ, Edwards CR, Baylis PH. Osmotic and non-osmotic regulation of thirst and vasopressin secretion in patients with compulsive water drinking. Clin Endocrinol (Oxf). 1991;35(3):221–8.

Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, Sheppard MC, Stewart PM. Association between premature mortality and hypopituitarismWest Midlands Prospective Hypopituitary Study Group. Lancet. 2001;357:425–31.

Travis LB, Ng AK, Allan JM, Pui CH, Kennedy AR, Xu XG, Purdy JA, Applegate K, Yahalom J, Constine LS, Gilbert ES, Boice JD Jr. Second malignant neoplasms and cardiovascular disease following radiotherapy. J Natl Cancer Inst. 2012;104:357–70.

van Waas M, Neggers SJ, Pieters R, van den Heuvel-Eibrink MM. Components of metabolic syndrome in 500 adult long-term survivors of childhood cancer. Ann Oncol. 2010;21:1121–6.

Vernooij MW, Ikram MA, Tanghe HL, Vincent AJ, Hofman A, Krestin GP, Niessen WJ, Breteler MM, van der Lugt A. Incidental findings on brain MRI in the general population. N Engl J Med. 2007;357(18):1821–8.

Vitanza NA, Cho YJ. Advances in the biology and treatment of pediatric central nervous system tumors. Curr Opin Pediatr. 2016;28(1):34–9.

Wachter D, Gondermann N, Oertel MF, Nestler U, Rohde V, Böker DK. Pituitary insufficiency after operation of supratentorial intra- and extraaxial tumors outside of the sellar-parasellar region? Neurosurg Rev. 2011;34(4):509–16.

Waimey KE, Smith BM, Confino R, Jeruss JS, Pavone ME. Understanding fertility in young female cancer patients. J Womens Health. 2015;24:812–8.

Walvoord E. The timing of puberty: is it changing? Does it matter? J Adolesc Health. 2010;47:433–9.

Zoicas F, Schöfl C. Craniopharyngioma in adults. Front Endocrinol (Lausanne). 2012;3:46.

Physiopathology, Diagnosis, and Treatment of Functional Pituitary Dysfunction

6

Dragana Miljic, Sandra Pekic, Marko Stojanovic, and Vera Popovic

Contents

D. Miljic · S. Pekic · M. Stojanovic
Clinic for Endocrinology, Diabetes and Metabolic Diseases, Neuroendocrine Unit, Clinical Centre of Serbia, Belgrade, Serbia

University of Belgrade, School of Medicine, Belgrade, Serbia

V. Popovic (✉)
University of Belgrade, School of Medicine, Belgrade, Serbia
e-mail: popver@gmail.com

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_6

Abstract

Neuroendocrine signals are integrated by the hypothalamus. Both hypothalamic and peripheral hormone signals are executed in the pituitary. Functional hypothalamic-pituitary changes may develop as a result of chronic stress, energy imbalance (both positive-obesity and negative-anorexia nervosa), and critical illness. Abnormalities of the endocrine system include increased activity of hypothalamic-pituitary-adrenal axis (HPA), impaired growth hormone axis, gonadal and thyroid dysfunction, and alteration in prolactin secretion. These hormonal alterations may also contribute to the development of underlying condition. A skillful use of dynamic tests together with careful clinical evaluation and follow-up are necessary to establish the correct diagnosis. Some of these changes are viewed as adaptive, and improvement in pituitary dysfunctions is expected with reversal of causation.

Keywords

Obesity · Amenorrhea · Eating disorders · Thyroid · Pseudo-Cushing's

List of Abbreviations

ACTH	Adrenocorticotropic hormone
AgRP	Agouti-related-peptide
aMSH	Alpha-melanocyte-stimulating hormone
AN	Anorexia nervosa
ATP	Adenosine tri-phosphate
AVP	Arginine vasopressin
BMD	Bone mineral density
BMI	Body mass index
CFS	Chronic fatigue syndrome
CRH	Corticotropin-releasing hormone
CS	Cushing's syndrome
DM	Diabetes mellitus
E2	Estradiol
FFA	Free fatty acids

FGF-21	Fibroblast growth factor-21
FHA	Functional hypothalamic amenorrhea
FM	Fibromyalgia
FSH	Follicle-stimulating hormone
fT3	Free tri-iodothyronine
fT4	Free thyroxine
GC	Glucocorticoid
GH	Growth hormone
GHD	Growth hormone deficiency
GHRH	Growth hormone releasing hormone
GnRH	Gonadotropin-releasing hormone
GR	Glucocorticoid receptors
HPA	Hypothalamo-pituitary-adrenal axis
HPG	Hypothalamo-pituitary-gonadal axis
HPO	Hypothalamo-pituitary-ovary
HPT	Hypothalamo-pituitary-thyroid
ICU	Intensive care unit
IGF-1	Insulin-like growth factor 1
IGFBP	Insulin-like growth hormone binding protein
IHH	Idiopathic hypogonadotropic hypogonadism
IL	Interleukin
ITT	Insulin tolerance test
LH	Luteinizing hormone
MCH	Melanine-concentrating hormone
MR	Mineralocorticoid receptors
MRI	Magnetic resonance imaging
NEFA	Non-esterified fatty acids
NPY	Neuropeptide Y
NTIS	Non-thyroidal illness syndrome
OSAS	Obstructive sleep apnea syndrome
PCOS	Polycystic ovary syndrome
PCS	Pseudo- Cushing`s syndrome
PRL	Prolactin
PTSD	Posttraumatic stress disorder
RAI	Relative adrenal insufficiency
rT3	Reverse tri-iodothyronine
SHBG	Sex hormone binding globulin
T_3	Tri-iodothyronine
T4	Thyroxine
TNFa	Tumor necrosis factor alpha
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
UFC	Urinary free cortisol

Functional Pituitary Disorders in Obesity

Introduction

Obesity is associated with various derangements of pituitary hormonal axes. Several of these may be causing obesity, but likewise functional pituitary disorders often arise as a consequence of obesity (Seetho and Wilding 2013). Increased activity of hypothalamo-pituitary-adrenal (HPA) axis, impaired growth hormone – Insulin-like growth factor 1 (GH-IGF-1) axis, gonadal and thyroid dysfunction, and hyperprolactinemia, may be consequences of severe obesity but possibly also contribute to its development, perpetuation, or aggravation (Seetho and Wilding 2013). Some changes represent adaptation to obesity rather than true hormonal axis impairments. Elevated serum leptin and insulin in obesity appear implicated in several of these dysfunctions.

The rising prevalence of obesity imposes concerns about endocrine testing and interpretation in the obese (Seetho and Wilding 2013). A thorough assessment of the severely obese enables the identification of possible causative neuroendocrine dysfunctions. Endocrine investigations should always be guided by expected benefit from treatment and not performed routinely without substantial clinical rationale. General reference values for many hormonal analyses might not be applicable to obese populations. Performing pituitary imaging in severely obese poses another challenge due to body size (Seetho and Wilding 2013). Reversibility of pituitary dysfunctions with normalization of body weight is often expected.

Hypothalamo-Pituitary-Adrenal (HPA) Axis in Obesity

HPA axis is frequently dysregulated in obesity, particularly in abdominal or visceral phenotype often leading to a state of "functional hypercortisolism" (Pasquali and Vicennati 2000). HPA axis alterations in abdominal obesity include an increased cortisol metabolic clearance rate; increased cortisol production in adipose tissue; increased or normal daily urinary free cortisol (UFC); increased HPA sensitivity to noradrenergic tone; increased cortisol response to meals; increased adrenocorticotropic hormone (ACTH) response to acute stress, corticotropin-releasing hormone (CRH), and arginine vasopressin (AVP); exaggerated cortisol response to ACTH; altered ACTH pulsatility; and reduced cortisol suppressibility to dexamethasone. (Pasquali and Vicennati 2000)

Baseline cortisol and ACTH and even the daily rhythm are usually normal in obesity. (Kokkoris and Pi-Sunyer 2003). Urinary free cortisol (UFC) is elevated in abdominal obesity, correlating with visceral fat distribution. Night-to-day ratio of UFC is increased (Pasquali et al. 2006). Long-term cortisol output measured from hair is more consistently elevated (Incollingo Rodriguez et al. 2015). ACTH pulse frequency increases and pulse amplitude decreases, most prominently in the peak of the daily cycle.

Responses to stimulatory and suppressive tests provide the most convincing evidence of HPA dysregulation in abdominal obesity (Pasquali et al. 2006). HPA

axis is hypersensitized resulting in slightly but inappropriately elevated net cortisol production, continuous or episodic. Cortisol reactivity upon physiological and psychological stress and meals was consistently elevated in obesity. Reduced glucocorticoid feedback sensitivity (suppressibility by dexamethasone) was also associated with obesity. Animal and human studies consistently report on alterations of adipocyte cortisol metabolism. Local 11beta-hydroxysteriod dehydrogenase-1 (11b-HSD-1) overactivity in adipose tissue is increased, escalating cortisone to cortisol conversion, while hepatic cortisol production is downregulated (Incollingo Rodriguez et al. 2015). This local cortisol overproduction is labeled as "local Cushing`s syndrome of adipose tissue." Meanwhile impaired liver 11b-HSD-1 might cause compensatory HPA axis overactivation (Incollingo Rodriguez et al. 2015).

Why Is HPA Axis Affected in Obesity?

Limbic-hypothalamic-pituitary-adrenal (LHPA) axis activity in the face of stress exposure is a factor implicated in the excess food intake and increased fat accumulation. This amplifies ACTH secretion, but increased cortisol production in obesity is balanced by enhanced cortisol clearance (Pasquali et al. 2006).

The fat-derived hormone leptin causes upregulation of corticotropin-releasing hormone (CRH) expression. Leptin has little effect on basal cortisol but when the HPA axis is activated, leptin attenuates the neuroendocrine response by enhancing glucocorticoid negative feedback. Cateholamines are important modulators of CRH and ACTH secretion during acute or chronic stress. Altered HPA axis sensitivity to central noradrenergic tone promotes its inappropriate excitation following neuropeptide stimulation. Reduced sensitivity to inhibition by dexamethasone is attributed to downregulation of central glucocorticoid receptors. Diminished cortisol suppression is more pronounced in women, progressing with increased abdominal fat (Pasquali et al. 2006).

Increased cortisol concentrations may perpetuate (abdominal) obesity, if not cause it. Obesity could lead to prolonged stress-induced increases in cortisol concentrations and overall output, promoting further adipose tissue accumulation and weight gain, leading to cardiometabolic morbidity.

How to Approach HPA Axis in Obesity?

Obesity in itself affects many of diagnostic tests for hypercortisolism. It is often challenging to differentiate patients with true Cushing`s syndrome (CS) from the obese population (Pasquali et al. 2006). Screening for occult CS in severely obese patients is not routinely recommended but should be considered according to clinical judgment (Seetho and Wilding 2013). No single marker is sufficient to discriminate subtle HPA alterations in abdominal obesity. Night-time UFC and salivary-free cortisol may be promising (Pasquali et al. 2006). HPA axis overactivation is potentially reversible with significant weight loss. Abnormalities of 11b-HSD enzymes do not appear to be correctable by weight loss. They may open focus for possible new therapeutic approaches (Incollingo Rodriguez et al. 2015).

Hypothalamo-Pituitary-Thyroid (HPT) Axis in Obesity

Elevated TSH with normal fT4 and normal or slightly elevated fT3 is frequently observed in obesity, even after excluding patients with symptomatic hypothyroidism or with positive antithyroid antibodies (Moulin de Moraes et al. 2005). Mean daily TSH directly correlates to BMI (Camastra et al. 2009). Obesity is characterized by alterations in circadian TSH secretion, with higher nocturnal than diurnal values (Camastra et al. 2009).

Why Is HPT Axis Affected in Obesity?

Leptin appears crucial in linking obesity to HPT axis derangement. A direct correlation of integrated 24 h TSH and 24 h leptin was observed in obesity (Camastra et al. 2009). Leptin stimulates TSH production both directly and through the neurotransmitters targeting TRH neurons – neuropeptide Y (NPY), agouti-related-peptide (AgRP), and alpha-melanocyte-stimulating hormone (aMSH). Reduction of hypothalamic T3 receptors and decrease in D2 deiodinase in the pituitary can disturb negative feedback and promote pituitary resistance to thyroid hormones. Obesity is associated with low-grade inflammation. Proinflammatory cytokines (namely TNFa, IL-1, and IL-6) inhibit sodium-iodide symporter and may lead to compensatory TSH rise (Rumińska et al. 2016).

HPT axis changes in obesity may be viewed as adaptive, counteracting fat accumulation by increasing energy expenditure or compensating for decreased tissue responsiveness to circulating thyroid hormones (Sanyal and Raychaudhuri 2016). Change of HPT axis set point could represent an adaptation by increasing thermogenesis required by the increased fat mass (Dall'Asta et al. 2010; Michalaki et al. 2006).

How to Approach HPT Axis in Obesity?

The increasing prevalence of obesity confounds definition of normal TSH range in population studies (Sanyal and Raychaudhuri 2016). Elevated TSH in obesity is found to revert after weight loss. Improvement in body composition, even with unchanged BMI, can lead to TSH decrease implying that these HPT axis alterations are largely functional (Sanyal and Raychaudhuri 2016).

Raised TSH may just be a functional consequence of obesity, but true subclinical hypothyroidism should not go undiagnosed in obese patients. It could contribute to further weight increase and lipid profile derangement. Subclinical hypothyroidism in the obese should not be diagnosed based on elevated TSH alone, unsupported by thyroid autoantibodies. No data supports thyroid hormone supplementation in obese patients to control body weight, except in those with established hypothyroidism (Sanyal and Raychaudhuri 2016).

Reproductive Axis in Obesity

Obesity and reproductive axis derangement are bidirectionaly related in both sexes.

Male Reproductive Axis in Obesity

Large epidemiological studies associate an elevated risk of infertility to male obesity. Obese men exhibit a decrease in testosterone and sex hormone binding globulin (SHBG) and elevated estradiol (E2) (Seetho and Wilding 2013). Moderate obesity predominantly decreases total testosterone due to effects of insulin resistance on SHBG. Free testosterone initially remains normal, particularly in younger men (Kokkoris and Pi-Sunyer 2003). More severe obesity causes reduction in free testosterone due to hypothalamo-pituitary-gonadal (HPG) axis suppression (Ng Tang Fui et al. 2014). Testosterone decrease correlates with severity of obesity. Men with BMI above 35–40 kg/m^2 have on average 50% reduction in total and free testosterone. Decline in testosterone by moving from nonobese to obese state is comparable to that of advancing 10 years in age (Ng Tang Fui et al. 2014). Unlike age-dependent decline in testosterone levels which is partly compensated by increased LH secretion, in obesity LH rise is blunted (Ng Tang Fui et al. 2014).

Why Is Male Reproductive Axis Affected in Obesity?

Association of male fertility impairment and obesity is likely multifactorial. SHBG decrease mediated by hyperinsulinemia emphasizes negative feedback effect of elevated total E2. Adipocyte aromatase activity is particularly increased in inflamed, insulin-resistant state. Increased testosterone to E2 conversion leads to inhibition of gonadotrophin-releasing hormone (GnRH) secretion and to a "hypogonadal-obesity cycle" (Kokkoris and Pi-Sunyer 2003). Increased endorphin levels in obesity may also lead to decrease in LH pulse amplitude and GnRH production. Energy-sensing hypothalamic pathways of kisspeptin/neurokin B, which drive GnRH pulsatile secretion, participate in alterations of LH pulse amplitude. Persistent state of overweight can suppress hypothalamic kisspeptin expression (Roa and Tena-Sempere 2014). Proinflamatory cytokines released by visceral fat can also inhibit HPG axis at multiple levels (Ng Tang Fui et al. 2014). Decreased total and free testosterone and increased E2 result in impaired spermatogenesis.

Additional confounders of male infertility in obesity are erectile dysfunction and reduced coital frequency (Chambers and Richard 2015). Obesity may directly alter spermatogenesis. Inhibin B decrease appears greater then FSH decrease. Increased scrotal temperature caused by hip and abdominal or scrotal fat also causes concern. Increased accumulation of endocrine disruptors in enlarged fat tissue is another possible confounder (Sermondade et al. 2013).

How to Approach Male Reproductive Axis in Obesity?

Obesity-associated hypotestosteronemia is functional, but its reversal requires substantial weight loss. Low testosterone can in itself aggravate obesity creating self-perpetuating cycle of metabolic complications. Progressive accumulation of total and visceral fat is importantly complicated by sarcopenia which further diminishes basal metabolism. Reduced testosterone in men also creates lack of motivation to exercise (Ng Tang Fui et al. 2014).

Weight loss is beneficial for male reproductive function (Seetho and Wilding 2013). Rise of testosterone with BMI reduction was greater in younger and more obese men (Chambers and Richard 2015). Minor weight loss is associated with modest increase in total testosterone mostly awing to increased SHBG. Only more substantial weight loss results in marked increase in total testosterone and free testosterone elevation. This HPG axis reactivation is evidenced by significant LH rise (Ng Tang Fui et al. 2014). Diet and exercise were reported to improve sperm parameters, but this appears to take as long as 2 years (Chambers and Richard 2015).

In obese men with low testosterone, a need for evaluation for underlying intrinsic HPG axis pathology must be balanced with the likelihood of nonspecific obesity-related testosterone demise. Thorough clinical evaluation of symptoms and signs of androgen deficiency (loss of libido, poor concentration, erectile dysfunction) or end-organ deficits (mild, unexplained anemia, trabecular-predominant osteoporosis, reduced muscle mass) is mandated. Analysis of PRL and other pituitary axes hormones might be indicated. Pituitary imaging is advised in men with testosterone repeatedly low and non-raised gonadotrophins (Ng Tang Fui et al. 2014).

Due to insufficient evidence regarding risk-benefit ratio, testosterone treatment is not advised for the sole aim of weight loss. Testosterone replacement may be considered in cases of confirmed and clinically symptomatic hypogonadism, after exclusion of contraindications for testosterone treatment. Potential concerns include obstructive sleep apnea syndrome and adverse effect on coronary heart disease or prostate events in older men. In younger men, impairing spermatogenesis is a risk. Alternative approaches include aromatase inhibitors, selective estrogen receptor modulators, and gonadotrophins (Ng Tang Fui et al. 2014).

Reference ranges for free testosterone are not well established, particularly in older men as SHBG increases with age (Ng Tang Fui et al. 2014). Measurement of increased circulating E2 is sometimes elusive and misrepresentive of local tissue-specific increase (Ng Tang Fui et al. 2014).

Female Reproductive Axis in Obesity

Many aspects of obesity may at different levels interfere with reproductive outcomes in women. Anovulation or subfertility may be first manifestations of obesity-related morbidity in younger women (Jungheim et al. 2012). Anovulatory obese women, without criteria for PCOS, may prove that obesity itself affects hypothalamo-pituitary-ovary (HPO) axis leading to oligomenorrhoea and infertility (Seetho and Wilding 2013).

Why Is Female Reproductive Axis Affected in Obesity?

Adipokines (leptin, TNFa, IL-6, free fatty acids (FFA) and adiponectin) may be an important link in obesity-related impairment of female fertility. (Jungheim et al. 2012) At hypothalamic level leptin signals the initiation of reproductive maturation. Hyperleptinemia in obesity with central leptin resistance may lead to hypogonadism, recognized by altered pulsatile LH amplitude in obese women. (Jungheim et al. 2012) Decreased LH pulse amplitude leads to decreased excretion of progesterone metabolites. Abnormal LH pulsatility results in anovulation, impaired ovarian

follicular steroidogenesis, abnormal oocyte recruitment, poor oocyte quality, or altered endometrial development (Jungheim et al. 2012). Adiponectin impairs pre-embryonic development and implantation while FFA impairs oocyte maturation (Jungheim et al. 2012).

How to Approach Female Reproductive Axis in Obesity?

Subfertility in ovulatory obese women is attributable to poorer oocyte and embryo quality or impairment in embryo implantation. Weight loss in obese women improves ovulatory function and pregnancy outcomes. Obesity may provide an important opportunity for preconceptional intervention (Jungheim et al. 2012). BMI analysis may not be sufficient. Better predictors could be the quantity of visceral adipose tissue or intrahepatic triglyceride content.

GH-IGF-1 Axis in Obesity

Obesity is a condition of functional GH insufficiency. Besides diminished spontaneous GH secretion and enhanced clearance, somatotroph response to all known stimuli is clearly reduced in both adults and children. Serum GH is uniformly lower in the obese during 24 h cycle, while nocturnal increase is absent (Camastra et al. 2009). Degree of obesity negatively correlates with GH peek (Lee et al. 2013). Adiposity, visceral in particular, is a major independent determinant of pulsatile GH secretion (Steyn et al. 2013). An inverse correlation of waist-to-hip ratio to GH is observed across different degrees of obesity. A linear reduction of peak GH response to stimulatory test (GHRH+arginine) was established for each 1 cm increase in waist circumference. Mean total IGF-1 is only slightly reduced in spite of marked GH insufficiency. Free IGF-1 levels are found to be increased in obesity.

Why Is GH-IGF-1 Axis Affected in Obesity?

Alterations in GH-IGF-1 axis in obesity could represent an adaptation or a true impairment of the axis activity. Suppressed GH predominantly results from systemic signals acting directly on somatotrophs. Crucial effect on GH-IGF-1 axis in obesity comes from insulin, leptin and free fatty acids (FFA), along with enhanced negative IGF-1 feedback (Maccario et al. 2000). Hyperinsulinemia associated with visceral obesity might provide negative feedback for GH secretion (Camastra et al. 2009). Insulin is able to directly inhibit GH synthesis and release from somatotrophs (Maccario et al. 2000; Steyn et al. 2013). Hyperinsulinism could enhance peripheral sensitivity to GH and reduce IGFBP-1 levels, thus increasing free IGF-1 levels which could exercise negative feedback on GH in obesity. On hypothalamic level insulin may promote catecholamine-mediated somatostatin release thus inhibiting GH secretion (Steyn et al. 2013). Pituitary insulin receptors remain functional despite systemic insulin resistance. Central leptin resistance characteristic for obesity contributes to reduced GH secretion (Steyn et al. 2013). FFA inhibit somatotroph secretion, by interfering with cell membrane depolarization. Antilypolitic treatment ameliorates the effect of obesity on GH. (Lee et al. 2013)

Impaired pulsatile GH secretion in mouse and human models occurs alongside progressive dietary-induced weight gain even preceding the development of obesity. GH may be protective in modulating insulin-induced lipogenesis throughout positive energy balance. GH suppression with increased food consumption improves meal tolerance by ameliorating insulin resistance and preventing hyperlipidemia. Discordance between GH and IGF-1 levels is thought to result from increased hepatic sensitivity to GH. Hyperinsulinemia induces upregulation of GH receptors and GH sensitivity, possibly explaining preserved IGF-1 levels. IGF-1 response to a single bolus of GH in obese subjects is increased (Popovic 2013).

How to Approach GH-IGF-1 Axis in Obesity?

General assumption is that functional hyposomatotropism in obesity is reversible after weight loss. Even short-term fasting increases GH response to stimuli but full normalization of BMI is required to fully restore somatotroph secretion (Maccario et al. 2000). Serum GH increased threefold after gastric bypass with restoration in the GH secretion dynamics (Camastra et al. 2009). No clear evidence supports the use of GH treatment for achieving weight loss in obese subjects without GH deficiency (GHD) (Seetho and Wilding 2013). Testing obese patients for GHD without appropriate underlying clinical setting can cause false-positive results. GH stimulation tests should be avoided in obese subjects with very low pretest probability. BMI-related cutoff of GH responses to stimulation tests is needed. Insulin resistance poses an obstacle for the most widely used test – insulin tolerance test (ITT). Achieving hypoglycemia may be difficult and adjustment of insulin dose is advised (Popovic 2013).

PRL in Obesity

Across species PRL participates in determining deposition and mobilization of fat (Kopelman 2000). During lactation PRL acts in diverting fuel substrates from adipose tissue to mammary gland. In obesity, it may have a different action (Mingrone et al. 2008). Integrated 24 h PRL secretion is elevated in the obese (Camastra et al. 2009). PRL release is enhanced proportionally to BMI and more directly to visceral fat area. PRL response to different stimuli such as ITT or TRH is blunted in obesity (Kokkoris and Pi-Sunyer 2003). Alteration in circadian PRL secretion in obesity is characterized by delay in the nocturnal rise (Kopelman 2000; Mingrone et al. 2008).

Why Is PRL Affected in Obesity?

Hyperinsulinemia may link obesity and impaired PRL response. In pituitary cell culture, insulin significantly stimulated basal PRL secretion (Mingrone et al. 2008). PRL response to hypoglycemia inversely correlated to increasing waist-to-hip ratio. Patients with impaired PRL response to ITT may, in fact, be initially prone to obesity by an intrinsic disorder of hypothalamic function, as demonstrated by inadequate rise of noradrenaline to hypoglycemia. Altered PRL secretion in obesity could be

explained by alterations in central dopaminergic and serotoninergic tone (Kopelman 2000). An association between fasting plasma leptin and PRL is found in obese men.

How to Address PRL in Obesity?

PRL alterations in obesity appear to be reversible. After bariatric surgery PRL levels decreased to control values (Camastra et al. 2009). Weight reduction normalizes PRL responses to stimuli (Kopelman 2000). Daily PRL rhythm could be restored after 12 days of fasting. PRL secretion rate was significantly reduced, but not normalized, after 50% reduction of excess body weight (Mingrone et al. 2008). A consistent decrease of PRL was observed throughout daily cycle, after bariatric surgery in severely obese women. Peak PRL shifted from late evening to early morning, characteristic of healthy lean subjects. Decrease in PRL significantly correlated with the decrease in insulin and lipoprotein lipase activity (Mingrone et al. 2008).

Functional Hypothalamic Amenorrhea

Introduction

Functional hypothalamic amenorrhea (FHA) is a relatively common and potentially reversible form of central hypogonadism and is defined as the absence of menstrual cycles for more than 6 months without evidence of anatomic or organic abnormalities (Liu 1990; Gordon 2010; Santoro 2011; Fourman and Fazelli 2015; Liu et al. 2016). It is characterized by reduced gonadotropin-releasing hormone (GnRH) drive, concomitant decreased LH pulse frequency (hypogonadotropic state) and hypoestrogenic state, without organic abnormalities (Reame et al. 1985; Berga et al. 1989; Silveira and Latronico 2013). FHA develops in women exposed to nutritional (undernutrition) or physical stress (excessive exercise, emotional stress, chronic disease), as an adaptive response of suppression of reproductive function during unfavorable period for pregnancy to conserve energy for the most essential function. Various menstrual abnormalities are reported in women in all types of recreational and competitive athletic activities, with excessive energy expenditure and decreased energy intake (Misra 2014).

FHA is a diagnosis of exclusion (Fig. 1), meaning that the diagnosis of FHA should be made after exclusion of other causes of central hypogonadism, congenital or acquired (Rosen and Cedars 2007).

Epidemiology

FHA can affect women of any reproductive age and is one of the most common causes of secondary amenorrhea. FHA can also present as delayed puberty and delayed menarche in girls. FHA is responsible for approximately 3% of primary amenorrhea (Meczakalski et al. 2014).

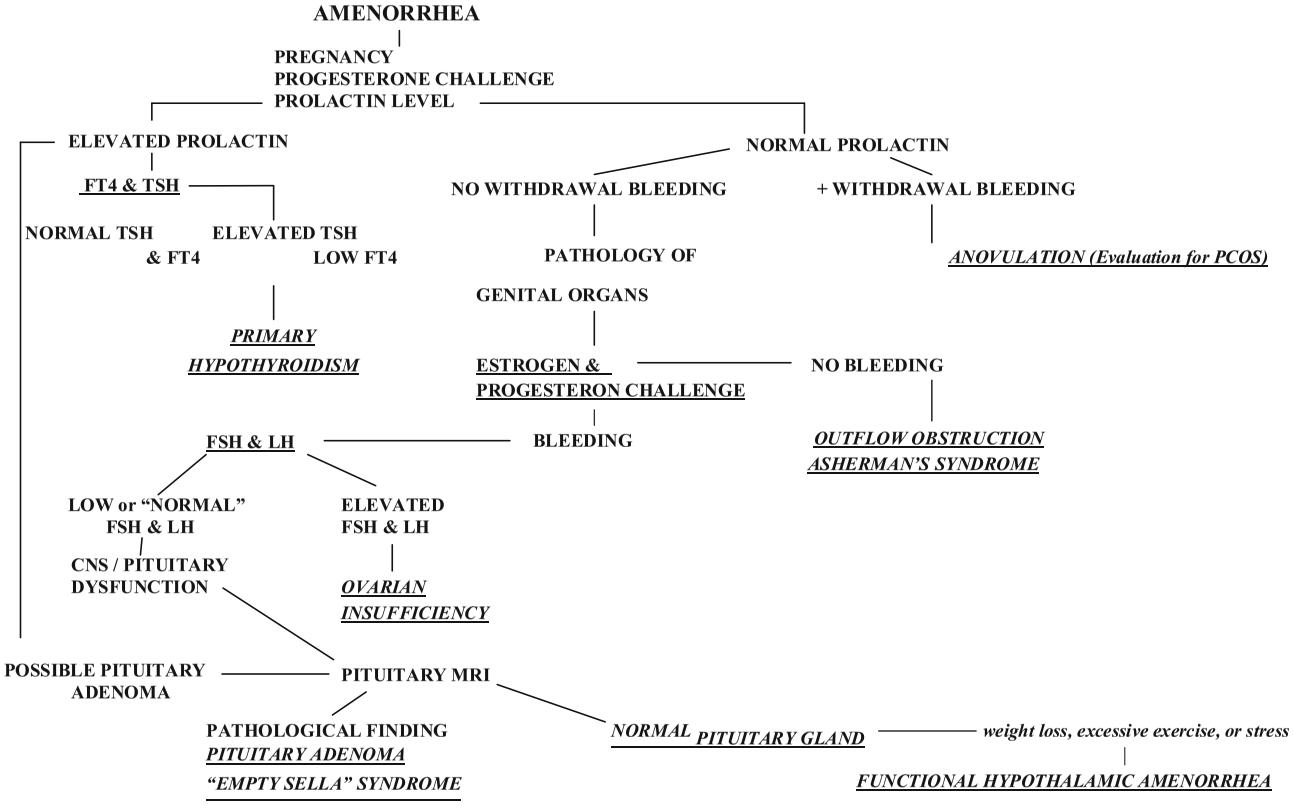


Fig. 1 **A suggested diagnostic algorithm for evaluating patients with secondary amenorrhea** (*fT4* free thyroxine, *TSH* thyroid-stimulating hormone, *PCOS* polycystic ovary syndrome, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *MRI* magnetic resonance imaging)

The incidence of menstrual abnormalities in females varies from 5% to 25% in recreational and competitive athletic sports. The incidence of disordered eating and amenorrhea is higher (6–43%) in "lean sports" (sports with low-body weight physique, like ballet, gymnastics, middle and long distance running) (Liu et al. 2016). It is less frequent in bicyclers and swimmers (12%) (Warren 1999).

Etiology

FHA and chronically reduced GnRH drive have been attributed to the metabolic and psychogenic stresses. It is frequently linked with stress, weight loss, or excessive and persistent exercise (Meczekalski et al. 2008, 2014).

Various subjective and objective stressors may elicit the FHA. Subjective stressors which reflect attitudes of self or society may not be easy to recognize and quantitate, while objective stressors (food deprivation, violence, stressful life event – divorce, death of relative/friend, job stresses, pressure during schooling) are more obvious.

The patients with FHA are typically intelligent, thin or with normal body weight. They are usually single, with obsessive-compulsive habits and abnormal eating patterns. FHA patients have a lower intake of carbohydrate-rich foods and higher intake of fiber, with lower energy density and fat content (Melin et al. 2016). Some patients often use sedatives or hypnotic drugs and have history of sexual abuse or disrupted sexual function and depression.

It is well known that women vary in their susceptibility to inhibition of the reproductive axis by stressors. There are some data showing that this variability may reflect a genetic predisposition to hypothalamic amenorrhea. Mutations in genes involved in idiopathic hypogonadotropic hypogonadism (IHH), a congenital form of GnRH deficiency were identified in 13% of patients with FHA (Caronia et al. 2011). It is speculated that these mutated genes may cause smaller-than-normal number of GnRH-producing cells that have complete migration to the hypothalamus during embrional life, or a suboptimal maturation of the GnRH network during puberty, or a defective regulation of GnRH secretion during adulthood (Caronia et al. 2011).

Recently, variants in several IHH genes (*IL17RD* and *TAC3*) and rare mutations in a gene called Immunoglobulin Superfamily Member 10 (*IGSF10*) were identified as a cause of some cases of delayed puberty and hypothalamic amenorrhea (Zhu et al. 2015; Howard et al. 2016). *IGSF10* has a likely role in the early migration of GnRH neurons (Howard et al. 2016).

Hormonal Mediators of FHA

Hypothalamo-Pituitary-Ovarian Axis

FHA is characterized by **hypogonadotropic hypogonadism** (low gonadotropin levels, hypoestrogenemia) (Table 1). The basic neuroendocrine abnormality in patients with FHA is the failure of the hypothalamus to increase GnRH secretion

Table 1 An overview of neuroendocrine changes in patients with functional hypothalamic amenorrhea (FHA)

Axis	Hormone	Changes
HPA axis	CRH	↑
	ACTH	↑
	Cortisol	↑
HPG axis	GnRH	↓
	FSH/LH	↓,=
	Estradiol	↓,=
HPT axis	T_3	↓
	TSH	↓,=
	GH	↑
	IGF-1	↓
	Ghrelin	↑
	Insulin	↓
	Adiponectin	↑
	Leptin	↓
	Kisspeptin	↓

in the state of severe hypoestrogenism, with slowing of the GnRH pulse generator, decreasing in pulsatile LH secretion, and reducing estradiol production (Loucks et al. 1998; Liu et al. 2016). The aberration of the pulsatile secretion of GnRH ranges from undetectable pulses (regression to a prepubertal pattern) to variations in amplitude or frequency (Perkins et al. 1999). Response to exogenous GnRH in FHA patients may be absent, normal or supranormal, depending on prior endogenous GnRH priming.

Limbic-Hypothalamic-Pituitary-Adrenal Axis

The activity of **limbic-hypothalamic-pituitary-adrenal axis (LHPA)** is increased, with elevated levels of CRH, ACTH, and cortisol (Berga et al. 1989; Loucks et al. 1989; Biller et al. 1990; Fourman and Fazelli 2015). Elevated circulating levels of cortisol are not present in women with other causes of anovulation, only in patients with FHA (Berga et al. 1997). Interestingly, increased circulating and central cortisol levels (in the cerebrospinal fluid) of women with FHA did not suppress central CRH level (Berga et al. 2000). These data indicate resistance to cortisol feedback inhibition, possibly due to altered hippocampal corticosteroid reception and neuromodulation (Brundu et al. 2006). It has been shown that CRH directly inhibits GnRH secretion in experimental animals and humans at the hypothalamic level. In addition, increased secretion of ACTH and cortisol suppress pituitary response to GnRH at the pituitary level. Cortisol is a gluconeogenic hormone, and elevated cortisol level is an adaptive response to maintain euglycemia (Misra 2014).

Hypothalamo-Pituitary-Thyroidal Axis

The **hypothalamic-pituitary-thyroidal axis** is suppressed, with a decrease of TSH and tri-iodothyronine T_3 (Berga et al. 1989; Gordon 2010).

Leptin

Patients with FHA have decreased **leptin** level with a loss of the normal diurnal rhythm (Laughlin and Yen 1997; Miller et al. 1998; Santoro 2011). Leptin is an anorexigenic peptide, primarily produced by the adipocytes, which regulates energy homeostasis. Leptin is a signal to the hypothalamus that energy reserves are sufficient to sustain a pregnancy. In energy-deficient conditions, lower leptin level is associated with a decrease of the LH pulse frequency and compensatory down-regulation of reproductive function (Ackerman et al. 2012). The effect is indirect and mediated by **kisspeptin**, through leptin receptor expressed by Kiss1 neurons of the arcuate nucleus (Smith et al. 2006). The hypothalamic expression of Kiss1 mRNA is reduced after food deprivation (Castellano et al. 2005).

Ghrelin

Another energy-balance peptide, **ghrelin,** an orexigenic peptide and growth hormone (GH) secretagogue, is inversely associated with fat mass. FHA patients have increased ghrelin concentrations, likely an adaptive response (Ackerman et al. 2012).

Insulin

The patient with significant weight loss and FHA also had nutritionally acquired GH resistance, reduced IGF-1 levels, low serum insulin, increased adiponectin level, and increased insulin sensitivity (Laughlin et al. 1998; Misra 2014). Alteration in all these axes has the potential to impact the hypothalamo-pituitary-gonadal axis causing central hypogonadism (Misra 2014).

Androgens

A subgroup of athletic females with exercise-induced HA (sports emphasizing strength over leanness) may have also androgen excess, with higher LH to FSH ratio, in some cases meeting criteria for PCOS (Warren and Perlroth 2001; Javed et al. 2015). It is associated with adverse metabolic health markers (higher blood pressure and fasting serum glucose), but with improved skeletal health (less stress fractures and higher Z scores; Javed et al. 2015).

Fibroblast Growth Factor-21

Recent data showed that FGF-21, a fasting-induced hepatokine, is upregulated during starvation. Data from animal model suggested that FGF-21 may act through hypothalamic Kiss-1 neurons causing central hypogonadism and anovulation (Owen et al. 2013).

Skeletal Problems in Patients with FHA

Neuroendocrine alterations and undernutrition in FHA exert a negative influence on the skeletal system, causing diminished bone mineral density, failure to achieve peak bone mass, osteopenia, and osteoporosis (Kaufman et al. 2002; Lambrinoudaki et al. 2010;

Meczakalski et al. 2014, Misra 2014). FHA patients have low bone turnover with depressed bone markers (Kaufman et al. 2002). Bone density is affected at the lumbar spine, a site of predominantly trabecular bone and at the total hip and these patients are at increased risk for stress fractures and skeletal fragility. Several factors are responsible for osteopenia in FHA patients: estrogen deficiency, chronic dieting, hypometabolic state, low leptin, insulin and IGF-1 levels, and high serum cortisol (Kaufman et al. 2002; Misra 2014).

Therapy

Since FHA is associated with abnormal psychosocial and dietary behavior, the management includes nonpharmacological treatment, dietary intervention, and medications.

Individual psychological therapy, accommodation to stressors, or modification of life style should be applied to treat affected women. Cognitive behavior therapy may restore ovarian activity and ovulation, without weight changes (Berga et al. 2003). The possible mechanism is the reduction of LHPA activation and restoration of GnRH pulsatility (Berga et al. 1997, 2003; Michopoulos et al. 2013).

Dietary interventions (increased caloric intake) can improve reproductive function, resulting in follicular growth, ovulation, and menstrual resumption (Kyriakidis et al. 2016).

In females with exercise-induced amenorrhea, the neuroendocrine abnormalities may be reversible with a decrease in exercise intensity. These patients should decrease the level or intensity of exercise and improve the diet.

Some patients recover their menstrual function, while others fail to resume normal cycles and ovulation. A trial with clomiphene citrate may be advised (25–50 mg for 5 days) to increase pulsatile GnRH secretion. If this approach failed, alternative treatment with human menopausal gonadotropins or exogenous GnRH portable pump may be appropriate (Martin et al. 1993; Christin-Maitre et al. 2007; Dumont et al. 2016).

FHA is characterized by low leptin levels, and previous studies have suggested that leptin administration may improve reproductive status, hormonal and metabolic parameters, and bone markers in FHA patients (Licinio et al. 2004; Welt et al. 2004; Chou et al. 2011; Sienkiewicz et al. 2011). Kisspeptin administration potently stimulates LH release in women with FHA (Jayasena et al. 2014).

If amenorrhea persists for a long period of time and pregnancy is not immediately desired, estrogen replacement therapy or oral contraceptive pills may be indicated. This approach will not correct hypercortisolism, hypothalamic hypothyroidism, and associated metabolic disturbances (osteopenia, cardiovascular disease). Exogenous sex steroid replacement may not fully prevent or reverse osteopenia and cardiovascular disease associated with chronic stress and FHA (Michopoulos et al. 2013).

Conclusion

Functional hypothalamic amenorrhea is not an isolated reproductive abnormality, but a mixture of neuroendocrine abnormalities due to chronic stress and energy imbalance. Even seemingly minor psychosocial and metabolic stressors, potentially in combination with genetic predisposition, can compromise reproductive function. Long-term consequences of amenorrhea, such as premature bone demineralization or inadequate bone formation, may significantly affect the quality of life of these patients.

Neuroendocrine Abnormalities in Anorexia Nervosa

Introduction

Anorexia nervosa (AN) is severe eating disorder associated with neuroendocrine and reproductive abnormalities (Gorwood et al. 2016; Misra and Klibanski 2016). It is characterized by extreme food restriction and weight loss (greater than 25% of body weight, with very low body fat mass), distortion of body self-image, intense fear of weight gaining and becoming obese, and hyperactivity (Vigersky et al. 1976; Gorwood et al. 2016; Misra and Klibanski 2016). According to the revised Diagnostic and Statistical Manual-V (DSM-V) amenorrhea is no longer required for the diagnosis of AN (APA 2013; Misra and Klibanski 2014). The AN patients have obsessive-compulsive personality (preoccupied with food and calorie counting) and increased incidence of sexual abuse. They have secondary amenorrhea, low bone mass, and hypothermia. The mortality rate is high (9%) due to cardiac arrhythmia (precipitated by reduced heart muscle mass and electrolyte abnormalities-hypokalemia due to diuretic or laxative abuse, vomiting) and suicide.

The estimated prevalence of AN in adolescents and young adults is 0.2–4% (Misra and Klibanski 2016). Anorexia nervosa is primarily a disease of females, but it is now increasingly recognized in males. Approximately 5–15% of all patients with AN are males.

Etiology

The etiology of AN is complex and still not completely understood. Recently, seven models of AN were proposed, based on three different approaches – (1) neuroimmunoendocrine (2) imaging (functional MRI), and (3) psychological and clinical approach (Gorwood et al. 2016). AN can be considered as a starvation addiction, driven by abnormalities of the food reward pathway, with dopamine dysfunction and elevated opioid activity (Kaye et al. 1989; Gorwood et al. 2016). Several groups have proposed another model of AN as a state of ghrelin-specific resistance. These patients have elevated ghrelin levels, but with no ability to induce appetite in AN patients (Miljic et al. 2006). Third model of AN proposed that AN might be a

pathology of chronic stimulation of the reward system for starvation or purging behaviors by orexigenic neuropeptides of the lateral hypothalamic area (orexin A and B, melanine-concentrating hormone MCH, 26RFa) (Gorwood et al. 2016). Modification of the gut microbiota was proposed also as an important model of AN etiology. Other groups suggested the concept of neuropeptide signaling dysfunction in AN (Inui 2001). These neuropeptides are important for regulation of food intake, satiety, pleasure, sleep, anxiety, digestive motility, endocrine functions, and bone metabolism (Berthoud 2011). Premorbid vulnerability factors determining childhood feeding and eating behavior (food preferences, inherited taste factors, early inadequate food intakes) were proposed as a base for AN (Gorwood et al. 2016). Finally, AN can be considered as an attempt to preserve mental homeostasis in patients with genetic susceptibility to the disorder after the precipitating stressful life event or negative emotional states (Gorwood et al. 2016).

Neuroendocrine Changes in Anorexia Nervosa

Hypothalamo-Pituitary-Adrenal Axis

Anorectic patients have hyperactivation of HPA axis, with elevated cortisol levels, increased 24 h free cortisol excretion, incomplete suppression of the HPA axis by dexamethasone, and blunted pituitary responses to CRH stimulation (Boyar et al. 1997; Liu et al. 2016). CRH levels in cerebrospinal fluid are elevated (Kaye et al. 1987). Despite biochemical hypercortisolism, these patients do not express signs of hypercortisolism due to the reduction of glucocorticoid receptors (Kontula et al. 1982).

Hypothalamo-Pituitary-Ovarian Axis

Like patients with FHA, AN patients have low (prepubertal) levels of LH due to diminished GnRH pulsatile frequency and amplitude. Several neuroendocrine abnormalities cause impaired GnRH and gonadotropin secretion, as an adaptation to low energy availability and low-fat mass, such as elevated cortisol and ghrelin and decreased IGF-1 and leptin levels (Misra and Klibanski 2016). After weight gain and recovery of normal body weight and composition, half of patients resume normal GnRH function and LH secretion. Half of patients remain anovulatory despite normalization of body weight and body composition.

Hypothalamo-Pituitary-Thyroid Axis

AN patients have low TSH levels, decreased conversion of thyroxine to T_3, and increased conversion to reverse T_3, with lower basal metabolism rate and defective thermoregulation with hypothermia.

Leptin

Leptin, an anorexigenic adipokine, decreases with energy deprivation and in conditions associated with low-fat mass, like AN (Casanueva et al. 1997; Popovic and Casanueva 2002; Chan and Mantzoros 2005; Miljic et al. 2006). This decrease of leptin is an adaptive response to prevent further suppression of food intake in these patients. Leptin correlates positively with fat mass, and increase in BMI (and partial recovery in weight)

led to a normalization of IGF-1 levels and a significant rise in leptin levels (Casanueva et al. 1997). Nutritional factors other than percent body fat may control leptin secretion, such as disordered eating behavior, low-fat consumption, and low IGF-1.

Growth Hormone

AN patients are also GH resistant, with an increase of basal and pulsatile GH secretion, associated with low IGF-1 levels (suggesting nutritionally acquired hepatic resistance to GH) (Fazeli and Klibanski 2014). GH receptors are downregulated and GH-binding protein is low (Misra 2014). This state of GH resistance and hypercortisolism are likely adaptive to severe undernutrition because GH and cortisol are potent stimulators of gluconeogenesis and are involved in the maintenance of euglycemia.

Ghrelin

Ghrelin is an appetite-stimulating peptide and GH secretagogue secreted by the oxyntic cells of the stomach. There are some studies on association of polymorphism of the ghrelin gene with eating disorders (Misra and Klibanski 2014). Restrictive AN patients have elevated ghrelin levels, inversely associated with BMI, fat mass, and insulin levels. Elevated ghrelin level is also an adaptation to starvation to increase food intake. Furthermore, ghrelin stimulates GH and ACTH secretion, consistent with an activation of contraregulatory mechanisms to maintain euglycemia. Ghrelin inhibits gonadotropin secretion and may contribute to the hypogonadal state in AN. Ghrelin infusion in patients with AN led to a reduction of gastrointestinal symptoms (decrease postprandial bloating) and increased hunger and caloric intake (Hotta et al. 2009). Other studies showed that GH response to ghrelin was blunted in patients with AN and there was no effect on appetite (Miljic et al. 2006).

Insulin

Patients with AN have lower glucose levels and lower fasting insulin levels (an adaptive phenomenon to help preserve euglycemia; Misra and Klibanski 2014).

Fibroblast Growth Factor 21

Fibroblast growth factor 21 (FGF-21) is a hepatokine induced by fasting and in starvation. Although we might expect that levels of FGF-21 are elevated in AN, these patients may have lower or normal levels of FGF-21 (Dostálová et al. 2008; Fazeli et al. 2015).

Other Neuroendocrine Abnormalities

AN patients have increased central opioid activity with increased levels of beta-endorphins in cerebrospinal fluid (Kaye et al. 1987).

Skeletal Abnormalities in AN Patients

Patients with AN have lower bone mineral density (BMD), with decrease in bone formation and an increase in bone resorption markers (Faje et al. 2013; Singhal et al. 2014). The cortical and trabecular thickness are reduced causing a decrease in

strength and increase risk for fractures (Faje et al. 2013). Lower estradiol levels in females and testosterone levels in males are an important causative factor of low BMD, together with low IGF-1 and leptin levels and elevated cortisol levels (Misra and Klibanski 2016).

Therapy

Therapeutic approaches to AN patients are complex and include group and individual psychotherapy, behavior modification, hypercaloric diet, and estrogen replacement therapy for patients who fail to resume menstrual function after restoration of body weight.

Increase in fat mass in patients with AN is the strongest predictor of resumption of menses. In these patients, inhibin B level (marker for ovarian follicular development) correlates with leptin and it may serve as an early marker of gonadal activity and with weight gain, for awakening of the reproductive function (Popovic et al. 2004).

Despite multidisciplinary team approach, the success rates for treatment of these patients remain low. Half of the patients recover, but 30% demonstrate only partial recovery and 20% have chronic AN or remissions and relapses (Misra and Klibanski 2014).

Conclusion

Anorexia nervosa is a severe eating disorder with complex etiology, in which an abnormally low body weight is associated with an intense fear of gaining weight. It is associated with severe neuroendocrine abnormalities and impaired skeletal health. The treatment is multidisciplinary and the success rate of therapy is still low.

Functional Disorders of Hypothylamo-Pituitary-Thyroid (HPT) Axis

Nonthyroidal Illness Syndrome

A major challenge in interpretation of the thyroid function tests and a common cause of nonstructural hypothalamo-pituitary-thyroid (HPT) axis dysfunction is the nonthyroidal illness syndrome (NTIS) also referred to as the “sick euthyroid syndrome” or “low T3 syndrome”. NTIS is most often defined by laboratory profile of decreased serum triiodothyronine (T3) and thyroxine (T4), increased serum reverse T3 (rT3), and unaltered or inappropriately normal or low thyroid-stimulating hormone (TSH) in the setting of acute or chronic illness with no previous or current intrinsic thyroid disease (De Vries et al. 2015).

The Scope of the Problem

NTIS has been reported in practically every severe illness, acute or chronic, physical and mental (Farwell 2013). It is very frequent among hospitalized patients even those not critically ill, and it is associated with increased mortality (Economidou et al. 2011; Pappa et al. 2011). In the ICU setting, the prevalence of abnormal thyroid function tests is so high that more than 70% of patients exhibit low T3 and 50% have low T4 (Ray et al. 2002). NTIS is often viewed as a part of the generalized systemic endocrine response to illness, in association with an increase in adrenocorticotrope hormone and free cortisol and reduction in serum gonadotropins and sex hormone concentration (Economidou et al. 2011). Many of the effects of NTIS could be viewed as adaptive, namely as a mean for energy preservation, but could possibly also evolve to be maladaptive. The changes observed during an acute phase of illness may be beneficial but become harmful during prolonged critical illness. The stage and severity of illness are major determinants of NTIS character (De Vries et al. 2015). In many acute and chronic illnesses, such as cardiovascular, cerebrovascular diseases, and respiratory failure, NTIS has also been considered as a prognostic factor (Pappa et al. 2011). Currently available evidence does not support treatment of NTIS with thyroid hormone supplementation although there are data suggesting possible benefit in small subset of patients (Farwell 2013; Lee and Farwell 2016).

Mechanisms and Levels of Impairment

Multiple mechanisms causing NTIS involve all levels of HPT axis and beyond and are influenced by both physiologic and pharmacologic factors including concomitant malnutrition and medications (Table 2).

Hypothalamic level: Thyroid function tests pattern in NTIS implies a central down-regulation of HPT axis. A decrease in hypothalamic TRH expression has been observed early in acute illness possibly preceding the decrease in circulating

Table 2 Mechanisms behind nonthyroidal illness syndrome and levels of impairment

Level	Impairment	Mechanism
Hypothalamus	Reduced TRH	Increased hypothalamic deiodinase activity
		Inflammatory cytokines
		Malnutrition via reduced leptin
Pituitary	Suppressed TSH	Inflammatory cytokines
		Glucocorticoids, dopamine
Thyroid	Reduced T4 and T3 production	Cytokines
Circulation	Impaired binding to TBG	Acute phase protein alterations
		Nonesterified fatty acids
		Heparin
Peripheral tissues	Impaired transport	ATP depletion
	Impaired T4 to T3 conversion	Altered deiodinase activity

thyroid hormone levels (De Vries et al. 2015). TRH reduction is influenced by an increase of local T3 bioavailability at hypothalamic level awing to D2 deiodinase activation (De Vries et al. 2015). Inflammatory cytokines (IL1b) and corticosterone can also contribute to TRH decrease in inflammation (De Vries et al. 2015). Malnutrition also may play a part in NTIS at hypothalamic level via decreased leptin (Farwell 2013). Animal data (Boelen et al. 2006) indicates that NTIS alterations are separate and only partly overlapping with effects of starvation.

Pituitary level: Locally produced or systemic proinflammatory cytokines (particularly IL-6) may exert a negative feedback on TSH release. Medications administered to acutely ill patients may also suppress TSH, particularly high dose systemic glucocorticoids, opiates or adrenergic agonists (Lee and Farwell 2016). Alteration of TSH pulsatility with decrease or absence of nocturnal surge has been reported in NTIS (Farwell 2013).

Thyroid level: Cytokines alone or synergistically are able to downregulate various stages of thyroid hormone synthesis pathway, leading to decreased T4 and T3 secretion (De Vries et al. 2015). IL1a and IL1b inhibit TSH-induced thyroglobulin expression and diminish iodine incorporation in T4 and T3 (Warner and Beckett 2010) Il1b impairs basal and TSH stimulated iodide uptake by the sodium-iodide symporter (De Vries et al. 2015).

Circulation: Thyroid hormone transport in circulation is altered in NTIS. Decrease of thyroxine-binding globulin (TBG) in the acute phase response and activity of substances interfering with TBG binding capacity such as nonesterified fatty acids (NEFA) lead to total T4 and total T3 decrease. The fall in TBG is due to impaired synthesis, rapid breakdown, and movement out of plasma space and can be as high as 60% in 12 h (Warner and Beckett 2010). Drugs known to interfere with thyroid hormone binding include heparin, furosemide, salicylates, and anticonvulsants (Lee and Farwell 2016). NEFA are also believed to inhibit T4 to T3 conversion and block T4 transport.

Tissue level: Data regarding thyroid hormone membrane transporters and nuclear receptors is conflicting (Lee and Farwell 2016). As patients with NTIS are usually in a negative energy balance, depleted ATP may impair energy-dependent process of cellular uptake of thyroid hormones (Pappa et al. 2011). Traditionally considered the most important factor in NTIS, deiodinases might have a lesser role than initially believed. Animal models of D1/D2 knock out demonstrate essentially the same pattern of NTIS (Warner and Beckett 2010). Animal model of NTIS decrease in plasma T3 and T4 precedes the changes in D1 and D2 activity. Deiodinases derangements in NTIS might thus represent a consequence rather than a cause. Altered thyroid hormone receptor activity is observed as mostly increased in the chronic illness and decreased in acute illness (Farwell 2013). Changes in coactivators and corepressors of thyroid hormone receptor may also play a role in NTIS. SRC-1 is a shared coactivator for thyroid hormone receptor and inflammatory signaling pathways. Competition for limiting amounts of coactivator is possible in acute illness (De Vries et al. 2015; Warner and Beckett 2010). Alternative pathways of thyroid hormone degradation and clearance such as sulfation and glucuronidation might play a part in the NTIS (De Vries et al. 2015).

The Course of NTIS

NTIS presents with a continuum of changes that depend on severity of illness and follow a sequential progression of several distinct stages (Lee and Farwell 2016). Mechanisms of NTIS also depend on the stage of illness. The rapid fall of T3 in the acute illness is more likely to represent the impaired thyroid production due to central hypothyroidism or the change in serum binding proteins due to the acute phase response pattern (fall in TBG and accumulation of substances that lower thyroid hormone binding capacity). Serum TSH usually remains normal during early phases of acute illness. With progression of illness, TSH steadily decreases (Lee and Farwell 2016). Low T3 stage can occur as early as 24 h after the onset of illness. Over half of patients admitted to medical services will have reduced T3. Low T3 is predictor of both all-cause and cardiac mortality in critical care patients (Lee and Farwell 2016). Low T4 stage is a sign of further progression and a poor prognostic factor in ICU setting. Initially, there is a decrease of total T4 while fT4 remains normal, but if the illness is severe or protracted, low fT4 is found. It is a sign of further severity and poor outcome (Economidou et al. 2011). Finally, the recovery phase of NTIS may be characterized with a modest increase in serum TSH rising transiently even above the reference range.

Diagnosis and Differential Diagnosis of NTIS

Analysis of the thyroid function tests (TSH and fT4) relies on the assumption of a steady-state condition which can be importantly challenged by an associated illness or concomitant medications. In the setting of inappropriately normal or low TSH, thyroid status should be reassessed in 4–6 weeks before making a diagnosis, whenever possible (Pantalone and Nasr 2010). The goal of thyroid testing in the critically ill should only be to identify those thyroid disorders (often pre-existing) that could be positively influenced by therapy. Since thyroid hormone supplementation is not found beneficial in NTIS, the utility of routine thyroid function testing in hospitalized patients can be questioned since it rarely leads to beneficial therapeutic intervention (Bao et al. 2012). It is advisable to test thyroid function tests in seriously ill patient only if there is a strong suspicion of thyroid dysfunction (Economidou et al. 2011) (Fig. 2). A possible alternative motive for thyroid function testing in critically ill could come from its prognostic value (Economidou et al. 2011). In myocardial infarction patients, rT3 levels were found to be independently associated with 1-year mortality. In acute stroke patients, low T3 was an independent predictor of short-term and long-term survival. NTIS was a predictor of higher mortality in hospitalized patients with respiratory failure. Elevated serum rT3 in the elderly was speculated to reflect declining health (Pappa et al. 2011).

Judgment on thyroid hormone status in acute illness is possibly further complicated by preanalytical and analytical pitfalls. Serum TSH assays that have a detection limit of 0.01 mIU/l should be used when assessing thyroid function in critically ill patients (Economidou et al. 2011). Due to the inaccuracy of fT4 assays, particularly in the setting of critical illness, repeating fT4 by another method is advised before establishing diagnosis. Many routine fT4 assays are prone to artifacts leading to underestimation of fT4 (Warner and Beckett 2010).

Thyroid function test values depend on the severity and course of illness. A low fT3, normal fT4, and low-normal TSH are the most common abnormalities; however, a

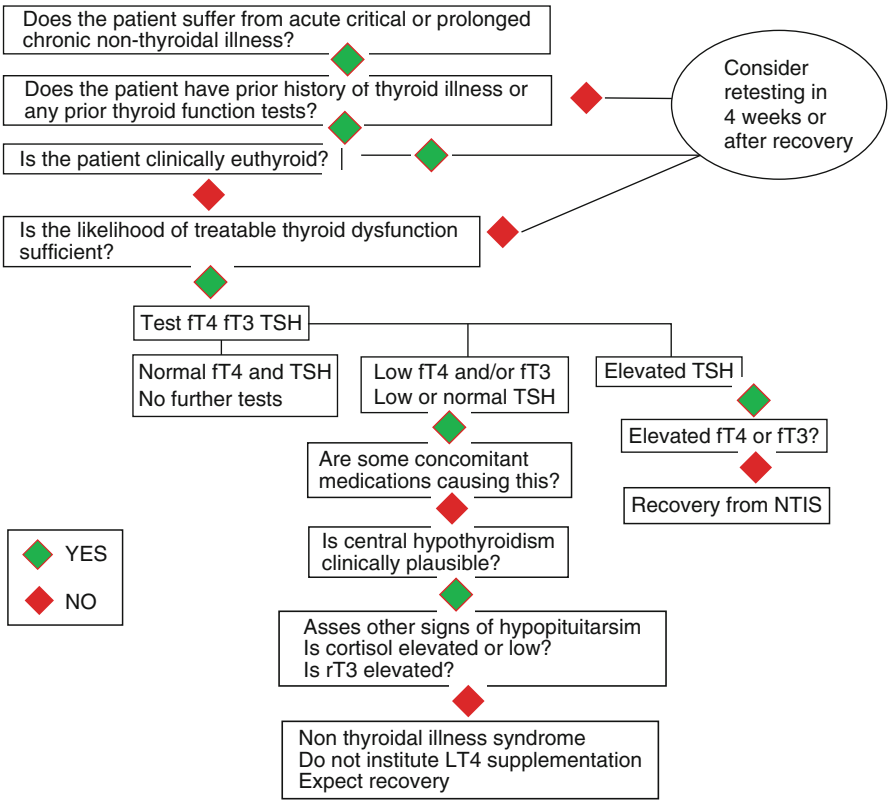


Fig. 2 A suggested diagnostic approach in nonthyroidal illness syndrome (NTIS) (*LT4* levothyroxine, *TSH* thyroid-stimulating hormone, *fT4* free thyroxine, *fT3* free tri-iodothyronine)

spectrum of abnormal thyroid function tests could be found. Serum TSH has been reported as normal in about 50%, low in 30%, but a marked suppression of TSH (< 0.1 mcIU/ml) is seen in only about 7% of NTIS patients and is mainly confounded with the independent effect of TSH lowering medications (dopamine and corticosteroids).

Low fT4 and fT3 with low or normal TSH should also invite central hypothyroidism as a differential diagnosis, or possibly the disequilibrium phase of the resolving stage of subacute thyroiditis in which TSH is still suppressed but thyroid hormones start to decrease (Pantalone and Nasr 2010). Patients with NTIS are most often clinically euthyroid, so signs of overt hypothyroidism (e.g., bradycardia, hypothermia, respiratory acidosis, pleural effusion) should raise suspicion (Economidou et al. 2011). Along with clinical judgment, the history of present illness (especially if it is prolonged or critical), and concomitant medications, a review of previous thyroid function tests (before the onset of critical illness) can all be crucial in assessing the nature of thyroid tests impairment in critical illness (Pantalone and Nasr 2010). Previous results are often unavailable and critically ill patients often unable to provide a history or to report the symptoms. When hypopituitarism is suspected, an assessment is advisable for signs

and symptoms of headache, nausea, vomiting, visual field defects, head trauma, infiltrative diseases, known cancer, abnormal water balance, or stigmata of gonadal insufficiency. Sometimes a complete evaluation of the remaining hypothalamic-pituitary axes is necessary to correctly interpret this pattern of thyroid function tests. Distinguishing between NTIS and central hypothyroidism is greatly important due to markedly different prognosis and treatment options. In central hypothyroidism, a low or an inappropriately normal (for critical illness setting) cortisol level could also be found due to hypopituitarism while in the NTIS elevated cortisol is expected. Undetectable TSH is more consistent with central hypothyroidism but not incompatible with NTIS (Bao et al. 2012). Elevated serum PRL and low gonadotropins levels can be found in both NTIS and central hypothyroidism. Without known pre-existing underlying hypothalamo-pituitary disease, it would be very rare to newly diagnose central hypothyroidism by routine testing in a critically ill patient, while on the other hand, the probability of NTIS is quite high (Bao et al. 2012). Although not routinely done, measurement of rT3 may be useful in differentiating NTIS (in which fT3 is high) from central hypothyroidism (in which rT3 is low) (Economidou et al. 2011).

The rise of TSH in the recovery phase of NTIS may precede the rise of low fT4 thus resembling a pattern of primary hypothyroidism (Economidou et al. 2011).

A normal variant of low TSH should also be considered. The normal reference range always covers plus or minus 2 standard deviations from the mean, leaving 2.5% of euthyroid individuals below and 2.5% above the reference range. Discriminating a normal variant low TSH person from one with asymptomatic subclinical hyperthyroidism is difficult and follow up is warranted (Pantalone and Nasr 2010).

Level of TSH in NTIS could be prognostic of the thyroid status in the aftermath of acute illness. Patients with TSH > 0.05 and < 3 are most often found to be euthyroid after reassessment upon the recovery from acute illness. Patients with elevated TSH but < 20 in the recovery phase of NITS are at risk of transient hypothyroidism while TSH > 20 could be announcing permanent hypothyroidism. Rare patients with TSH < 0.01 often evolve to hyperthyroidism (Economidou et al. 2011).

Treatment of NTIS

Routine supplementation with thyroid hormones in NTIS is not recommended but advantages are advocated in certain groups of patients. Current evidence has not found significant positive effect but no clear deleterious effect was found either (Lee and Farwell 2016). The debate is still ongoing but the rationale for supplementation mostly rests on the presumption of maladaptive nature of the prolonged NTIS. If however NTIS is viewed as part of adaptive mechanism, attempts to restore thyroid hormone levels might even be harmful (Lee and Farwell 2016). Most NTIS patients appear eumetabolic despite changes in thyroid hormone levels (Lee and Farwell 2016). Patients with a mild NTIS of short duration may be mostly euthyroid, but on the contrary those with severe or prolonged NTIS might actually be tissue hypothyroid and could represent a group that might benefit from supplementation treatment (Pappa et al. 2011). LT4 alone treatment is expected to have little effect due to pronounced inhibition of T4 to T3 conversion in NTIS (Economidou et al. 2011). No benefit of T4/T3 supplementation was demonstrated in trials involving ICU patients

with acute renal failure or renal transplant, burn patients, and premature infants (with even deleterious effect in some age groups) (Farwell 2013). Some trials have suggested promising effects in cardiac surgery patients (mostly in children) and congestive heart failure. Consensus statements, unsupported by meta-analysis have recommended the use of LT3 in improving donor heart function prior to transplantation (Farwell 2013). TRH therapy has also been considered and positive effects have been reported on cardiometabolic function on a small number of patients (Lee and Farwell 2016). Treatment other than thyroid hormone supplementation was inspired by a view that NTIS is a compensatory response to oxidative stress of acute illness (Lee and Farwell 2016). The use of a potent antioxidant (N-acetyl cystein) in preventing NTIS in myocardial infarction patients has restored thyroid function abnormalities but with no effect on mortality or length of hospital stay (Lee and Farwell 2016). Some studies aimed at early nutritional support in acute illness claiming it could prevent NTIS or at least the malnutrition component (Lee and Farwell 2016). Supplementation with selenium may lead to faster T4 and rT3 normalization (Economidou et al. 2011).

Other Nonstructural HPT Axis Dysfunctions

Other than NTIS, a functional HPT axis impairment worth recognizing is HPT axis activation in acute psychiatric patients. In patients with acute psychosis or mood disorders raised T4 with normal or elevated TSH is frequently found (Bunevicius et al. 2014). This indicates a pattern of central HPT axis activation, sometimes referred to as transient euthyroid hyperthyroxinemia. Serum T3 is usually not elevated. Elevated serum SHBG as a marker of tissue effects of thyroid hormones was also observed. These changes were also found after excluding patients with autoimmune thyroid disease or prior thyroid dysfunction and untreated with antipsychotics (Steiblienė et al. 2012). In most instances, the thyroid status normalized within 2 weeks, and no treatment directed at thyroid gland was indicated. Possible explanations for this phenomenon included disturbed T4 uptake or metabolism in the central nervous system or centrally mediated TSH hypersecretion (Steiblienė et al. 2012). In some instances, a high T4 stage is viewed as an initial stage of NTIS due to acute inhibition of D1 deiodinase or increase in TBG levels often in elderly or psychiatric patients, but progressing further to low T4 stage of NTIS (Lee and Farwell 2016).

Pseudo-Cushing's Syndrome and Functional Disorders of Hypothalamus-Pituitary-Adrenal (HPA) Axis

Introduction

Functional hypercortisolism is caused by conditions able to chronically activate HPA axis. Although usually reversible and mild, in some patients, functional hypercortisolism can mediate systemic complications similar to those observed in

Cushing's syndrome (Tirabassi et al. 2014). Some of these states, like alcoholism and depression, belong to pseudo-Cushing states, which are difficult to distinguish from Cushing's syndrome, on the basis of biochemical and clinical findings attributed to hypercortisolism, but tend to disappear upon resolution of the underlying condition (Newell-Price et al. 1998).

Cushing's syndrome refers to a state of chronic hypercortisolism regardless of its etiology. Deleterious effects of chronic overexposure to glucorticoid excess comprise a phenotype characterized by typical body composition changes (centripetal adiposity, sarcopenia with proximal miopathy, decreased bone mineral content and quality, skin changes with plethora, acne, striae, hirsutism, and bruises). Associated metabolic, cardiovascular, and psychiatric comorbidities (including hypertension, obesity, osteoporosis with fractures, impaired immune function, hyperlipidemia, glucose intolerance or diabetes, depression, and anxiety) account for high multisystem morbidity and mortality rates in patients with Cushing's syndrome. The need for causative treatment of hypercortisolism makes it imperative to distinguish between patients with true Cushing's syndrome and those with functional hypercortisolism or pseudo-Cushing's syndrome due to hypothalamo-pituitary-adrenal axis over-activation from other causes (Newell-Price et al. 1998). A skillful use of dynamic tests together with careful clinical evaluation and follow-up are necessary to establish the correct diagnosis. Biochemical evidence of hypercortisolemia, resistance to dexamethasone, and blunting of the circadian rhythm are common in patients with major depression, critical illness, and those with alcohol-induced pseudo-Cushing's syndrome. In depressed patients, there is usually a cortisol response to adequate insulin-induced hypoglycemia, while such response is unusual for patients with Cushing's syndrome. The most diagnostically reliable tests to distinguish between patients with Cushing's syndrome and those with pseudo-Cushing's are represented by combined dexamethasone/CRH test, midnight cortisol (serum or salivary), and desmopressin test (Pecori Giraldi et al. 2007; Tirabassi et al. 2010; Tirabassi et al. 2011). Causes of functional hypercortisolism are listed in Table 3.

Organization of the Hypothalamus-Pituitary-Adrenal HPA Axis and Stress Response

Adrenocorticotropic hormone (ACTH) is the key regulator of glucocorticoid (cortisol) secretion by the adrenal cortex. In healthy individuals, ACTH secretion from the pituitary is stimulated at the hypothalamic level by CRH and AVP signals integrating the pulsatile diurnal rhythm with stress and feeding responses. Cortisol then interacts with the glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) in multiple tissues, including those regulating the HPA axis in the hypothalamus, pituitary, and other brain regions responsible for the feedback inhibition. In peripheral tissues, availability of cortisol is controlled by 11β-hydroxysteroid dehydrogenases (11β-HSD1 and 11β-HSD2). 11β-HSD2 is highly expressed in mineralocorticoid target tissues including the salivary gland, kidney, and colon to protect mineralocorticoid receptor from unwanted activation by cortisol (Ricketts et

Table 3 Causes of pseudo-Cushing's syndrome and functional hypercortisolism

Alcoholism, drug addiction, and substance abuse
Depression (melancholic type)
Obsessive-compulsive disorder
Panic disorders
Generalized anxiety disorder
Anorexia and bulimia nervosa (AN, BN)
Shift work
Diabetes mellitus
Obesity and obstructive sleep apnea syndrome (OSAS)
Polycystic ovarian syndrome (PCOS)
Critical illness
End-stage renal disease

al. 1998). 11β-HSD1 is more widely distributed, especially in key metabolic tissues including adipose tissue, liver, and skeletal muscle. Tissue-specific 11β-HSD1 activity is the major determinant of the metabolic manifestations of GC excess by local regeneration of cortisol (Morgan et al. 2014).

The hypothalamus-pituitary-adrenal axis (HPA axis) with its end product cortisol mediates response to stress. The stress response is an innate, stereotypic, adaptive response to stressors that has evolved in purpose to restore the nonstressed homeostatic set point. It is encoded in specific neuroanatomical sites that activate a repertoire of cognitive, behavioral, and physiologic phenomena (Tsigos and Chrousos 2002). When the organism is challenged, the HPA-axis contributes to the adaptation by giving higher priority to the mobilization of energy and lower priority to digestion, growth, and reproduction. Insulin resistance, inflammation, and a prothrombotic state emerge in anticipation of threatening injury. Function of the prefrontal cortex is attenuated to disinhibit amygdala, HPA axis, and sympathic nervous system to promote anxiety and diminish sleep and appetite. Adaptive responses, though essential for survival, can become dysregulated and result in disease. In fact, defective stress responses with hyper- or hyporesponsiveness of HPA axis characterise many disorders. In general, HPA axis reactivity, to various stressors, is determined by genetic predisposition and epigenetic changes usually induced by early life adversities.

States of Functional Hypercortisolism

Pseudo-Cushing's States: Alcoholism and Depression

Alcoholism

PCS was initially described by Smalls et al. in 1976 (Smalls et al. 1976) in three patients with chronic alcohol abuse featuring typical Cushingoid phenotype and biochemical abnormalities of CS, which disappeared after few weeks of abstinence

from alcohol. Clinical and biochemical features of alcohol-induced PCS vary widely and no criteria are available to distinguish between CS and alcohol-induced PCS (Besemer et al. 2011). Signs and symptoms tend to normalize after at least a month of abstinence from alcohol. In a study by Somer et al., rats given 15% alcohol for 3 months showed a greater number of corticotropin producing cells with increased secretory activity (Somer et al. 1996). Central HPA axis activation due to heavy alcohol consumption impairs negative feedback toward CRH hypothalamic secretion due to alcohol-induced damage of hippocampus, structure that highly expresses receptors for glucocorticoids (Beresford et al. 2006). Impaired cortisol metabolism by the liver has also been suggested as the underlying mechanisms of alcohol-induced PCS.

Depression

In patients with major depression, hypercortisolism is evident in approximately 50% and is particularly characteristic of the melancholic subtype (Pariante and Lightman 2008). Melancholic and atypical depression have opposite effects on HPA axis activation. Melancholic depression is characterized by activation and persistence of the stress response, whereas atypical depression resembles a stress response that has been excessively inhibited. In melancholic depression, symptoms are worst in the morning when HPA axis activity is peaking, while in atypical depression symptoms are worst in the evening when cortisol levels fall. Melancholic patients experience anorexia and insomnia, whereas atypical patients experience hyperphagia and hypersomnia (Gold 2015). In patients with major depression reversible adrenal gland enlargement and increased cortisol secretion are caused by feed-forward drive from an activated limbic-hypothalamic system and defective GC feedback (Pariante and Lightman 2008). Hyperactivation of the HPA axis in patients with major depression has been associated with increased risk of dementia, diabetes, hypertension, and osteoporosis (Gold 2015). A hyperactivation of the HPA axis has also been reported for obsessive-compulsive disorder, panic disorder, and anorexia nervosa, while a decreased functioning of the HPA axis has been associated with atypical depression, chronic fatigue syndrome (CFS), posttraumatic stress disorder (PTSD), and fibromyalgia.

Other Causes of Functional Hypercortisolism: Anorexia Nervosa, Obesity, Diabetes Mellitus, Shift Work, Polycystic Ovarian Syndrome, End Stage Renal Disease, and Critical Illness

Anorexia Nervosa (AN)

Anorexia nervosa (AN) is an eating disorder mostly affecting young females, characterized by chronically decreased caloric intake and self-induced starvation. Prolonged starvation in these patients causes adaptive metabolic and endocrine changes in reproductive, thyroid, adrenal, and somatotrope axes. Baseline cortisol levels are significantly elevated in patients with AN due to CRH-driven hyperactivation of the limbic-hypothalamic-pituitary-adrenal system in response to

chronic stressors (Peters et al. 2004). Hypoactivation of numerous regions of the brain involved in food motivation and appetite regulation have been found in AN compared to healthy controls on functional magnetic resonance imaging (Holsen et al. 2012). Influence of CRH and cortisol in these regions with high expression of CRH and GC receptors has been suggested to influence hedonic and homeostatic perception of appetite in AN (Lawson et al. 2013).

Diabetes Mellitus (DM)

Prevalence of CS is higher in patients with type two diabetes than in the general population (Catargi et al. 2003; Gungunes et al. 2014). Diabetes exerts metabolic stress on cells and provokes chronic increase in HPA activity. Hyperortisolism correlates with metabolic disturbances and complications in a type 2 diabetic patients (Chiodini et al. 2007; Oltmanns KM et al. 2006; Prpić-Križevac et al. 2012). HPA axis dysregulation in patients with diabetes appears to involve complex interactions between impaired sensitivity to negative GC feedback that may increase the central drive of HPA axis, as well as peripheral alterations characterized by increased conversion of cortisone to cortisol due to increased expression and activity of subcutaneous and visceral fat 11 beta-HSD1 (Stimson et al. 2011). Patients with type two diabetes have elevated ACTH levels and urinary cortisol levels both basal and after dexamethasone suppression (Chiodini et al. 2007; Prpić-Križevac et al. 2012). Diurnal pattern of cortisol hypersecretion is altered with lower cortisol awakening response, slower cortisol decline in the afternoon, higher bedtime cortisol values and increased response to stressors in patients with type two diabetes (Siddiqui et al. 2015).

Shift Work

Shift work, although not a disease, predisposes to type two diabetes and increased cardio-vascular risk (Pan et al. 2011). Hypercortisolism resulting from impaired pattern and rhythm of cortisol secretion induces adverse metabolic actions in these subjects, favoring the onset of obesity, diabetes, arterial hypertension, and cardio-vascular diseases (Manenschijn et al. 2011).

Obesity

Postulated mechanisms of hyperactivation of HPA axis in obesity include hyper-responsiveness to different neuropeptides, stress events, dietary factors, and augmented peripheral activation of cortisol due to increased 11β-HSD1 activity in subcutaneous and visceral adipose tissue promoting the conversion of inactive cortisone to active cortisol (Desbriere et al. 2006; Pasquali and Vicennati 2000; Walker and Andrew 2006; Valsamakis et al. 2004). Hypercortisolism caused by visceral adiposity further promotes features of the metabolic syndrome and adipocyte differentiation. In obese patients with obstructive sleep apnea syndrome (OSAS), sleep fragmentation and hypoxia are thought to be additional factors which interfere with the normal rhythm of cortisol secretion.

Polycystic Ovarian Syndrome (PCOS)

In PCOS, increased spontaneous pulsatile ACTH and cortisol production is balanced by increased metabolic clearance of cortisol (Gambineri et al. 2009; Inviti et al. 1998). Pituitary sensitivity to GC suppression is increased in PCOS (Milutinovic et al. 2011) as well as ACTH and cortisol responses to social stress (Bensson et al. 2009). Obesity, insulin resistance, and diabetes can modulate HPA axis responses at both central level and periphery (role of hyperinsulinism on ACTH-driven adrenal androgen production) (Romuladi et al. 2007). Hypercortisolism is more severe in women with diabetes adding to impaired glucose-insulin feedback and components of the metabolic syndrome.

End-Stage Renal Disease

Cortisol clearance and metabolism by 11 beta-hydroxysteroid dehydrogenase type 2 in kidneys are reduced and responsible for hypercortisolism in patients with end-stage renal failure (Raff and Trivedi 2012). Hypercortisolemia in these patients is associated with higher blood pressure and markers of adverse cardiovascular outcomes (Sarnak et al. 2003).

Critical Illness

Critical illness is considered to be a condition of severe stress-induced HPA-axis activation with several-fold increased cortisol production. New insights revealed that high circulating levels of cortisol during critical illness are explained more by reduced cortisol breakdown than by elevated cortisol production. This was explained by suppressed expression and activity of A-ring reductases in the liver and 11 beta-hydroxysteroid dehydrogenase type 2 in kidneys (Boonen et al. 2013).

States of Functional Hypocortisolism

States of functional hypocortisolism are characterized by inadequate basal cortisol production and inappropriate ACTH and cortisol responses to stressful stimuli. The term “adrenal fatigue” has evolved to describe maladaptive state in which cortisol production is significantly diminished in response to chronic and prolonged physical or emotional stress transforming initial “alarm stress response” into resistance and exhaustion. An increase in the HPA axis sensitivity during periods of excessive cortisol production can induce negative feedback control of cortisol release resulting in hypocortisolism. Primary and secondary hypocorticism, if unrecognized and untreated, can lead to potentially life-threatening adrenal crisis and therefore should be considered and ruled out in the differential diagnosis of functional hypocortisolism. Causes of functional hypocortisolism are listed in Table 4.

Posttraumatic Stress Disorder (PTSD)

Exposure to trauma (threat of death, serious injury, or sexual violation) is related to the development of several psychiatric disorders including posttraumatic stress

Table 4 Causes of functional hypocortisolism

Atypical depression
Chronic fatigue syndrome (CFS)
Fibromyalgia
Posttraumatic stress disorder (PTSD)
Relative adrenal insufficiency (RAI) in critically ill patients

disorder (PTSD) in approximately 10% of individuals. PTSD is characterized by symptoms of re-experiencing, avoidance, negative mood, and cognition. The neurocircuitry model of PTSD suggests hyperresponsivity of amygdala, and hyporesponsivity of prefrontal cortex and hippocampus unable to inhibit the amygdala. During chronic stress, HPA activity and cortisol secretion decrease with lower morning and afternoon cortisol levels as well as lower daily cortisol output and enhanced cortisol suppression (Morris et al. 2012). An increase in hypothalamic CRH release with downregulation of CRH-R1 expression in pituitary has been described in the rat model of PTSD (Li et al. 2015).

Fibromyalgia (FM) and Chronic Fatigue Syndrome (CFS)

Fibromyalgia is a chronic disease with unknown etiology, characterized by widespread pain, fatigue and altered functioning of the HPA axis. Patients with the syndrome of chronic fatigue also have a hypoactive stress response and reduced basal ACTH and cortisol secretion with reduced ACTH and cortisol responses to desmopressin and CRH and reduced adrenal responsiveness to ACTH. Reduced bioavailability of cortisol due to increased frequency of the mutation in the cortisol binding globulin gene has also been reported. Immune activation induced by hypocortisolism characterized by increased levels of pro-inflammatory cytokines and increased sympathic nervous activity add to impaired glucocorticoid receptor functioning, stress sensitivity, pain and fatigue in these patients (Heim et al. 2000).

Relative Adrenal Insufficiency (RAI)

Dissociation between ACTH and cortisol levels was found in intensive care unit (ICU) patients. High plasma cortisol secretion and reduced breakdown can reduce plasma ACTH concentrations via feedback inhibition leading to dysfunction of the adrenal cortex and development of relative adrenal insufficiency (RAI). In ICU patients treated for shock and septicemia, RAI should be suspected if hypotension refractory to fluids and vasopressors occurs, especially when other symptoms of adrenal failure are absent. Random cortisol level of less than 500 nmol/L in a patient with septic shock and clinical suspicion of adrenal failure is an indication for initiation of steroid therapy (Dellinger et al. 2013).

Acknowledgments This work was supported by the Serbian Ministry of Science (project number 17503).

References

Ackerman KE, Slusarz K, Guereca G, Pierce L, Slattery M, Mendes N, Herzog DB, Misra M. Higher ghrelin and lower leptin secretion are associated with lower LH secretion in young amenorrheic athletes compared with eumenorrheic athletes and controls. Am J Phys Endocrinol Metab. 2012;302:E800–6.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5. DSM-5. Washington, DC: APA; 2013.

Bao S, Oiknine R, Fisher SJ. Differentiating nonthyroidal illness syndrome from central hypothyroidism in the acutely ill hospitalized patient. Endocrine. 2012;42(3):758–60.

Bensson S, Arck PC, Tan S, Hahn S, Mann K, Rifaie N, Janssen OE, Schedlowski M, Elsenbruch S. Disturbed stress responses in women with polycystic ovary syndrome. Psychoneuroendocrinology. 2009;34(5):727–35.

Beresford TP, Arciniegas DB, Alfers J, Clapp L, Martin B, Beresford HF, Du Y, Liu D, Shen D, Davatzikos C, Laudenslager ML. Hypercortisolism in alcohol dependence and its relation to hippocampal volume loss. J Stud Alcohol. 2006;67:861–7.

Berga SL, Daniels TL, Giles DE. Women with functional hypothalamic amenorrhea but not other forms of anovulation display amplified cortisol concentrations. Fertil Steril. 1997;67:1024–30.

Berga SL, Loucks-Daniels TL, Adler LJ, Chrousos GP, Cameron KL, Methews KA, Marcus MD. Cerebrospinal fluid levels of corticotropin-releasing-hormone in women with functional hypothalamic amenorrhea. Am J Obstet Gynecol. 2000;182:776–84.

Berga SL, Marcus MD, Loucks TL, Hlastala S, Ringham R, Krohn MA. Recovery of ovarian activity in women with functional hypothalamic amenorrhea (FHA) treated with cognitive behavior therapy (CBT). Fertil Steril. 2003;80:976–81.

Berga SL, Mortola JF, Girton L, Suh B, Laughlin G, Pham P, Yen SSC. Neuroendocrine aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab. 1989;68:301–8.

Berthoud HR. Metabolic and hedonic drives in the neural control of appetite: who is the boss? Curr Opin Neurobiol. 2011;21:888–96.

Besemer F, Pereira AM, Smit JWA. Alcohol-induced Cushing syndrome: Hypercortisolism caused by alcohol abuse. Neth J Med. 2011;69:318–23.

Biller BMK, Federoff HJ, Koenig JI, Klibanski A. Abnormal cortisol secretion and responses to corticotropin-releasing hormone in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab. 1990;70:311–7.

Boelen A, Kwakkel J, Wiersinga WM, Fliers E. Chronic local inflammation in mice results in decreased TRH and type 3 deiodinase mRNA expression in the hypothalamic paraventricular nucleus independently of diminished food intake. J Endocrinol. 2006;191(3):707–14.

Boonen E, Vervenne H, Meersseman P, Andrew R, Mortier L, Declercq PE, Vanwijngaerden Y-M, Spriet I, Wouters PJ, Vander Perre S, Langouche L, Vanhorebeek I, Walker BR, Van Den Berghe G. Reduced cortisol metabolism during critical illness. N Engl J Med. 2013;368:1477–88.

Boyar RM, Hellman LD, Roffwarg H, Katz J, Zumoff B, O'Connor J, Bradlow HL, Fukushima DK. Cortisol secretion and metabolism in anorexia nervosa. N Engl J Med. 1997;296:190–3.

Brundu B, Loucks TL, Adler LJ, Cameron JL, Berga SL. Increased cortisol in the cerebrospinal fluid of women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab. 2006;91:1561–5.

Bunevicius R, Steibliene V, Prange AJ Jr. Thyroid axis function after in-patient treatment of acute psychosis with antipsychotics: a naturalistic study. BMC Psychiatry. 2014;14:279.

Camastra S, Manco M, Frascerra S, Iaconelli A, Mingrone G, Ferrannini E. Daylong pituitary hormones in morbid obesity: effects of bariatric surgery. Int J Obes. 2009;33(1):166–72.

Caronia LM, Martin C, Welt CK, Sykiotis GP, Quinton R, Thambundit A, Avbelj M, Dhruvakumar S, Plummer L, Hughes V, Seminara SB, Boepple PA, Sidis Y, Crowley WF Jr, Martin KA, Hall JE, Pitteloud N. (2011). A genetic basis for functional hypothalamic amenorrhea. N Engl J Med, 364, 215-225.

Casanueva FF, Dieguez C, Popovic V, Peino R, Considine R, Caro J. Serum immunoreactive leptin concentrations in patients with anorexia nervosa before and after partial weight recovery. Biochem Mol Med. 1997;60:116–20.

Castellano JM, Navarro VM, Fernández-Fernández R, Nogueiras R, Tovar S, Roa J, Vazquez MJ, Vigo E, Casanueva FF, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M. Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. Endocrinology. 2005;146:3917–25.

Catargi B, Rigalleau V, Poussin A, Ronci-Chaix N, Bex V, Vergnot V, Gin H, Roger P, Tabarin A. Occult Cushing's syndrome in type-2 diabetes. J Clin Endocrinol Metab. 2003;88(12):5808–13.

Chambers TJ, Richard RA. The impact of obesity on male fertility. Hormones (Athens). 2015;14(4):563–8.

Chan JL, Mantzoros CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhea and anorexia nervosa. Lancet. 2005;366:74–85.

Chiodini I, Adda G, Scillitani A, Coletti F, Morelli V, Di Lembo S, Epaminonda P, Masserini B, Beck-Peccoz P, Orsi E, Ambrosi B, Arosio M. Cortisol secretion in patients with type 2 diabetes: relationship with chronic complications. Diabetes Care. 2007;30:83–8.

Chou SH, Chamberland JP, Liu X, Matarese G, Gao C, Stefanakis R, Brinkoetter MT, Gong H, Arampatzi K, Mantzoros CS. Leptin is an effective treatment for hypothalamic amenorrhea. Proc Natl Acad Sci U S A. 2011;108:6585–90.

Christin-Maitre S, de Crecy M, Groupe Francais des popes. GnRH pregnancy outcomes following pulsatile GnRH treatment: results of a large multicenter retrospective study. J Gynecol Obstet Biol Reprod. 2007;36:8–12.

Dall'Asta C, Paganelli M, Morabito A, Vedani P, Barbieri M, Paolisso G, Folli F, Pontiroli AE. Weight loss through gastric banding: effects on TSH and thyroid hormones in obese subjects with normal thyroid function. Obesity (Silver Spring). 2010;18(4):854–7.

De Vries EM, Fliers E, Boelen A. The molecular basis of the non-thyroidal illness syndrome. J Endocrinol. 2015;225(3):R67–81.

Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock. Crit Care Med. 2013;41(2):580–637.

Desbriere R, Vuaroqueaux V, Achard V, Boullu-Ciocca S, Labuhn M, Dutour A, Grino M. 11beta-hydroxysteroid dehydrogenase type 1 mRNA is increased in both visceral and subcutaneous adipose tissue of obese patients. Obesity (Silver Spring). 2006;14:794–8.

Dostálová I, Kaválková P, Haluzíková D, Lacinová Z, Mráz M, Papezová H, Haluzík M. Plasma concentrations of fibroblast growth factors 19 and 21 in patients with anorexia nervosa. *J Clin Endocrinol Metab*. 2008;*93*:3627–32.

Dumont A, Dewailly D, Plouvier P, Catteau-Jonard S, Robin G. Does polycystic ovarian morphology influence the response to treatment with pulsatile GnRH in functional hypothalamic amenorrhea? Reprod Biol Endocrinol. 2016;14:24–9.

Economidou F, Douka E, Tzanela M, Nanas S, Kotanidou A. Thyroid function during critical illness. Hormones (Athens). 2011;10(2):117–24.

Faje AT, Karim L, Taylor A, Lee H, Miller KK, Mendes N, Meenaghan E, Goldstein MA, Bouxsein ML, Misra M, Klibanski A. Adolescent girls with anorexia nervosa have impaired cortical and trabecular microarchitecture and lower estimated bone strength at the distal radius. J Clin Endocrinol Metab. 2013;98:1923–9.

Farwell AP. Nonthyroidal illness syndrome. Curr Opin Endocrinol Diabetes Obes. 2013;20(5):478–84.

Fazeli PK, Faje AT, Cross EJ, Lee H, Rosen CJ, Bouxsein ML, Klibanski A. Serum FGF-21 levels are associated with worsened radial trabecular bone microarchitecture and decreased radial bone strength in women with anorexia nervosa. Bone. 2015;77:6–11.

Fazeli PK, Klibanski A. Determinants of GH resistance in malnutrition. J Endocrinol. 2014;220:R57–65.

Fourman LT, Fazelli PK. Neuroendocrine causes of amenorrhea – an update. J Clin Endocrinol Metab. 2015;100:812–24.

Fui MN, Dupuis P, Grossmann M. Lowered testosterone in male obesity: mechanisms, morbidity and management. Asian J Androl. 2014;16(2):223–31.

Gambineri A, Forlani G, Munarini A, Tomassoni F, Cognigni GE, Ciampaglia W, Pagotto U, Walker BR, Pasquali R. Increased clearance of cortisol by 5 beta-reductase in a subgroup of women with adrenal hyperandrogenism in polycystic ovary syndrome. J Endocrinol Investig. 2009;32(3):210–8.

Gold PW. The organization of the stress system and its dysregulation in depressive illness. Mol Psychiatry. 2015;20:32–47.

Gordon CM. Clinical practice.Functional hypothalamic amenorrhea. N Engl J Med. 2010;363:365–71.

Gorwood P, Blanchet-Collet C, Chartrel N, Duclos J, Dechelotte P, Hanachi M, Fetissov S, Godart N, Melchior J, Ramoz N, Rovere-Jovene C, Tolle V, Viltart O, Epelbaum J, on behalf of the GIR-AFDAS-TCA Group. (2016). New insights in anorexia nervosa. Front Neurosci, 10, 256.

Gungunes A, Sahin M, Demirci T, Ucan B, Cakir E, Arslan MS, Unsal IO, Karbek B, Caliskan M, Ozbek M, Cakal E, Delibasi T. Cushing's syndrome in type 2 diabetes patients with poor glycemic control. Endocrine. 2014;47(3):895–900.

Heim C, Ehlert U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology. 2000;25(1):1–35.

Holsen LM, Lawson EA, Blum J, Ko E, Makris N, Fazeli PK, Klibanski A, Goldstein JM. Food motivation circuitry hypo activation related to hedonic and non-hedonic aspects of hunger and in women with active and weight-restored anorexia nervosa. J Psychiatry Neurosci. 2012;37 (5):322–32.

Hotta M, Ohwada R, Akamizu T, Shibasaki T, Takano K, Kangawa K. Ghrelin increases hunger and food intake in patients with restricting-type anorexia nervosa: a pilot study. Endocr J. 2009;56:1119–28.

Howard SR, Guasti L, Ruiz-Babot G, Mancini A, David A, Storr HL, Metherell LA, Sternberg MJE, Cabrera CP, Warren HR, Barnes MR, Quinton R, de Roux N, Young J, Guiochon-Mantel A, Wehkalampi K, Andre V, Gothilf Y, Cariboni A, Dunkel L. IGSF10 mutations dysregulate gonadotropin-releasing hormone neuronal migration resulting in delayed puberty. EMBO Mol Med. 2016;8:626–42.

Incollingo Rodriguez AC, Epel ES, White ML, Standen EC, Seckl JR, Tomiyama AJ. Hypothalamic-pituitary-adrenal axis dysregulation and cortisol activity in obesity: a systematic review. Psychoneuroendocrinology. 2015;62:301–18.

Inui A. Eating behavior in anorexia nervosa–an excess of both orexigenic and anorexigenic signalling? Mol Psychiatry. 2001;6:620–4.

Inviti C, De Martin M, Delitala G, Veldhuis JD, Cavagnini F. Altered morning and night time pulsatile corticotropin and cortisol release in polycystic ovary syndrome. Metabolism. 1998;47 (2):143–8.

Javed A, Kashyap R, Lteif AN. Hyperandrogenism in female athletes with functional hypothalamic amenorrhea: a distinct phenotype. Int J Wom Health. 2015;7:103–11.

Jayasena CN, Abbara A, Veldhuis JD, Comninos AN, Ratnasabapathy R, De Silva A, Nijher GM, Ganiyu-Dada Z, Mehta A, Todd C, Ghatei MA, Bloom SR, Dhillo WS. Increasing LH pulsatility in women with hypothalamic amenorrhoea using intravenous infusion of Kisspeptin-54. J Clin Endocrinol Metab. 2014;99:E953–61.

Jungheim ES, Travieso JL, Carson KR, Moley KH. Obesity and reproductive function. Obstet Gynecol Clin N Am. 2012;39(4):479–93.

Kaufman BA, Warren MP, Dominguez JE, Wang J, Heymsfield SB, Pierson RN. Bone density and amenorrhea in ballet dancers are related to a decreased resting metabolic rate and lower leptin levels. J Clin Endocrinol Metab. 2002;87:2777–83.

Kaye WH, Gwirtsman HE, George DT, Ebert MH, Jimerson DC, Tomai TP, Chrousos GP, Gold PW. Elevated cerebrospinal fluid levels of immunoreactive corticotropin-releasing

hormone in anorexia nervosa: relation to state of nutrition, adrenal function, and intensity of depression. J Clin Endocrinol Metab. 1987;64:203–8.
Kaye WH, Berrettini WH, Gwirtsman HE, Gold PW, George DT, Jimerson DC, Ebert MH. Contribution of CNS neuropeptide (NPY, CRH, and beta-endorphin) alterations to psychophysiological abnormalities in anorexia nervosa. 1989;25:433–8.
Kokkoris P, Pi-Sunyer FX. Obesity and endocrine disease. Endocrinol Metab Clin N Am. 2003;32(4):895–914.
Kontula K, Anderson LC, Huttumen M, Pelkonen R. Reduced level of cellular glucocorticoid receptors in patients with anorexia nervosa. Horm Metab Res. 1982;14:619–20.
Kopelman PG. Physiopathology of prolactin secretion in obesity. Int J Obes Relat Metab Disord. 2000;24(Suppl 2):S104–8.
Kyriakidis M, Caetano L, Anastasiadou N, Karasu T, Lashen H. Functional hypothalamic amenorrhea: leptin treatment, dietary intervention and counseling as alternatives to traditional practice – systematic review. Eur J Obstet Gynecol Reprod Biol. 2016;198:131–7.
Lambrinoudaki I, Papadimitriou D. Pathophysiology of bone loss in the female athlete. Ann N Y Acad Sci. 2010;1205:45–50.
Laughlin GA, Yen SSC. Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. J Clin Endocrinol Metab. 1997;82:318–21.
Laughlin GA, Dominguez CE, Yen SS. Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea. J ClinEndocrinolMetab. 1998;83:25–32.
Lawson EA, Holsen LM, DeSanti R, McKale S, Meenaghan E, Herzog DB, Goldstein JM, Klibanski A. Increased hypothalamic-pituitary-adrenal drive is associated with decreased appetite and hypo activation of food motivation neurocircuitry in anorexia nervosa. Eur J Endocrinol. 2013;169(5):639–47.
Lee J, Yoon J, Kang MJ, Lee YA, Lee SY, Shin CH, Yang SW. Influence of body mass index on the growth hormone response to provocative testing in short children without growth hormone deficiency. J Korean Med Sci. 2013;28(9):1351–5.
Lee S, Farwell AP. Euthyroid sick syndrome. Compr Physiol. 2016;6(2):1071–80.
Li C, Liu Y, Lu C, Liu D, Jianq H, Pan F. Long-term effects of early adolescent stress: dysregulation of hypothalamic-pituitary-adrenal axis and central corticotropin releasing factor receptor 1 expression in adult male rats. Behav Brain Res. 2015;288:39–49.
Licinio J, Caglayan S, Ozata M, Yildiz BO, Miranda PB, O'Kirwan F, Whitby R, Liang L, Cohen P, Bhasin S, Krauss RM, Veldhuis JD, Wagner AJ, AM DP, SM MC, Wong ML. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc Natl Acad Sci U S A. 2004;101:4531–6.
Liu JH, Patel B, Collins G. Central causes of amenorrhea. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, Mc Lachlan R, New M, Rebar R, Singer F, Vinik A, Weickert MO, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc; 2016. 2000-2016 Mar 1.
Liu JH. Hypothalamic amenorrhea: Clinical perspectives, pathophysiology, and management. Am J Obstet Gynecol. 1990;163:1732–6.
Loucks AB, Mortola JF, Girton L, Yen SSC. Alterations in the hypothalamic-pituitary-ovarian and hypothalamic-pituitary-adrenal axes in athletic women. J Clin Endocrinol Metab. 1989;68:402–11.
Loucks AB, Verdun M, Heath EM. Low energy availability, not stress or exercise, alters LH pulsatility in exercising women. J Appl Physiol. 1998;84:37–46.
Maccario M, Grottoli S, Procopio M, Oleandri SE, Rossetto R, Gauna C, Arvat E, Ghigo E. The GH/IGF-I axis in obesity: influence of neuro-endocrine and metabolic factors. Int J Obes Relat Metab Disord. 2000;24(Suppl 2):S96–9.
Manenschijn L, van Kruysbergen RG, de Jong FH, Koper JW, van Rossum EF. Shift work at young age is associated with elevated long-term cortisol levels and body mass index. J Clin Endocrinol Metab. 2011;96:1862–5.

Martin KA, Hall JE, Adams JM, Crowley Jr WF. (1993). Comparison of exogenous gonadotropins and pulsatile gonadotropin-releasing hormone for induction of ovulation in hypogonadotropic amenorrhea. J Clin Endocrinol Metab, 77, 125-129.

Meczakalski B, Katulski K, Czyzyk A, Podfigurna-Stopa A, Maciejewska-Jeske M. Functional hypothalamic amenorrhea and its influence on women′s health. J Endocrinol Investig. 2014;37:1049–56.

Meczekalski B, Podfigurna-Stopa A, Warenk-Szymankiewicz A, Genazzani AR. Functional hypothalamic amenorrhea: current view on neuroendocrine aberrations. Gynecol Endocrinol. 2008;24:4–11.

Melin A, Tornberg AB, Skouby S, Moller SS, Faber J, Sundgot-Borgen J, Sjodin A. Low-energy density and high fiber intake are dietary concerns in female endurance athletes. Scand J Med Sci Sports. 2016;26:1060–71.

Michalaki MA, Vagenakis AG, Leonardou AS, Argentou MN, Habeos IG, Makri MG, Psyrogiannis AI, Kalfarentzos FE, Kyriazopoulou VE. Thyroid function in humans with morbid obesity. Thyroid. 2006;16(1):73–8.

Michopoulos V, Mancini F, Loucks TL, Berga SL. Neuroendocrine recovery initiated by cognitive behavioral therapy in women with functional hypothalamic amenorrhea: a randomized controlled trial. Fertil Steril. 2013;99:2084–91.

Miller KK, Parulekar MS, Schoenfeld E, Anderson E, Hubbard J, Klibanski A, Grinspoon SK. Decreased leptin levels in normal weight women with hypothalamic amenorrhea: the effects of body composition and nutritional intake. J Clin Endocrino lMetab. 1998;83:2309–12.

Miljic D, Pekic S, Djurovic M, Doknic M, Milic N, Casanueva FF, Ghatei M, Popovic V. Ghrelin has partial or no effect on appetite, growth hormone, prolactin, and cortisol release in patients with anorexia nervosa. J Clin Endocrinol Metab. 2006;91:1491–5.

Milutinovic DV, Macut D, Bozic I, Nestorov J, Damjanovic S, Matic G. Hypothalamic-pituitary-adrenocortical axis hypersensitivity and glucocorticoid receptor expression and function in women with polycystic ovarian syndrome. Exp Clin Endocrinol Diabetes. 2011;119:636–43.

Mingrone G, Manco M, Iaconelli A, Gniuli D, Bracaglia R, Leccesi L, Calvani M, Nolfe G, Basu S, Berria R. Prolactin and insulin ultradian secretion and adipose tissue lipoprotein lipase expression in severely obese women after bariatric surgery. Obesity (Silver Spring). 2008;16(8):1831–7.

Misra M, Klibanski A. Anorexia nervosa and its associated endocrinopathy in young people. Horm Res Paediatr. 2016;85:147–57.

Misra M, Klibanski A. Endocrine consequences of anorexia nervosa. Lancet Diabetes Endocrinol. 2014;*2*:581–92.

Misra M. Neuroendocrine mechanisms in athletes. Handb Clin Neurol. 2014;124:373–86.

Morgan SA, McCabe EL, Gathercole LL, Hassan-Smith ZK, Larner DP, Bujalska IJ, Stewart PM, Tomlinson JW, Lavery GG. 11beta-HSD1 is the major regulator of the tissue-specific effects of circulating glucocorticoid excess. PNAS. 2014;111:E2482–91.

Morris MC, Compas BE, Garber J. Relations among posttraumatic stress disorder, co morbid major depression, and HPA function: a systematic review and meta-analysis. Clin Psychol Rev. 2012;32(4):301–15.

Moulin de Moraes CM, Mancini MC, de Melo ME, Figueiredo DA, Villares SM, Rascovski A, Zilberstein B, Halpern A. Prevalence of subclinical hypothyroidism in a morbidly obese population and improvement after weight loss induced by Roux-en-Y gastric bypass. Obes Surg. 2005;15(9):1287–91.

Newell-Price J, Trainer P, Besser M, Grossman A. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. Endocr Rev. 1998;19(5):647–72.

Oltmanns KM, Dodt B, Schultes B, Raspe HH, Schweiger U, Born J, Fehm HL, Peters A. Cortisol correlates with metabolic disturbances in a population study of type 2 diabetic patients. Eur J Endocrinol. 2006;154:325–31.

Owen BM, Bookout AL, Ding X, Lin VY, Atkin SD, Gautron L, Kliewer SA, Mangelsdorf DJ. FGF21 contributes to neuroendocrine control of female reproduction. Nat Med. 2013;19:1153–6.

Pan A, Schernhammer ES, Sun Q, Hu FB. Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. PLoS Med. 2011;8(12):e1001141.

Pantalone KM, Nasr C. Approach to a low TSH level: patience is a virtue. Cleve Clin J Med. 2010;77(11):803–11.

Pappa TA, Vagenakis AG, Alevizaki M. The nonthyroidal illness syndrome in the non-critically ill patient. Eur J Clin Investig. 2011;41(2):212–20.

Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 2008;31:464–8.

Pasquali R, Vicennati V, Cacciari M, Pagotto U. The hypothalamic-pituitary-adrenal axis activity in obesity and the metabolic syndrome. Ann N Y Acad Sci. 2006;1083:111–28.

Pasquali R, Vicennati V. Activity of the hypothalamic-pituitary-adrenal axis in different obesity phenotypes. Int J Obes Relat Metab Disord. 2000;24(Suppl 2):S47–9.

Pecori Giraldi F, Pivonello R, Ambrogio AG, De Martin MC, Scacchi M, Colao A, Toja PM, Lombardi G, Cavagnini F. The dexamethasone-suppressed corticotropin-releasing hormone stimulation test and the desmopressin test to distinguish Cushing's syndrome from pseudo-Cushing's states. Clin Endocrinol. 2007;66:251–7.

Perkins RB, Hall JE, Martin KA. Neuroendocrine abnormalities in hypothalamic amenorrhea: spectrum, stability, and response to neurotransmitter modulation. J Clin Endocrinol Metab. 1999;84:1905–11.

Peters A, Schweiger U, Pellerin L, Hubold C, Oltmanns KM, Conrad M, Schultes B, Born J, Fehm HL. The selfish brain: competition for energy resources. Neurosci Biobehav Rev. 2004;28:143–80.

Popovic V, Casanueva FF. Leptin, nutrition and reproduction: new insight. Hormones. 2002;1:204–17.

Popovic V, Djurovic M, Cetkovic A, Vojvodic D, Pekic S, Spremovic S, Petakov M, Damjanovic S, Milic N, Dieguez C, Casanueva FF. Inhibin B: a potential marker of gonadal activity in patients with anorexia nervosa during weight recovery. J Clin Endocrinol Metab. 2004;89:1838–43.

Popovic V. Approach to testing growth hormone (GH) secretion in obese subjects. J Clin Endocrinol Metab. 2013;98(5):1789–96.

Prpić-Križevac I, Canecki-Varžić S, Bilić-Ćurčić I. Hyperactivity of the hypothalamic-pituitary-adrenal axis in patients with type 2 diabetes and relations with insulin resistance and chronic complications. Wien Klin Wochenschr. 2012;124(11-12):403–11.

Raff H, Trivedi H. Circadian rhythm of salivary cortisol, plasma cortisol, and plasma ACTH in end-stage renal disease. Endocr Connect. 2012;2(1):23–31.

Ray DC, Macduff A, Drummond GB, Wilkinson E, Adams B, Beckett GJ. Endocrine measurements in survivors and non-survivors from critical illness. Intensive Care Med. 2002;28(9):1301–8.

Reame NE, Sauder SE, Case GD, Kelch RP, Marshall JC. Pulsatile gonadotropin secretion in women with hypothalamic amenorrhea: evidence that reduced frequency of gonadotropin-releasing hormone secretion is the mechanism of persistent anovulation. J Clin Endocrinol Metab. 1985;61:851–8.

Ricketts ML, Verhaeg JM, Bujalska I, Howie AJ, Rainey WE, Stewart PM. Immunohistochemical localization of type 1 11betahydroxysteroid dehydrogenase in human tissues. J Clin Endocrinol Metab. 1998;83:1325–35.

Roa J, Tena-Sempere M. Connecting metabolism and reproduction: roles of central energy sensors and key molecular mediators. Mol Cell Endocrinol. 2014;397(1-2):4–14.

Romuladi D, Giuliani M, Draisci G, Costantini B, Cristello F, Lanzone A, Guido M. Pioglitezone reduces the adrenal androgen response to corticotropin-releasing factor without changes in ACTH release in hyperinsulinemic women with polycystic ovary syndrome. Fertil Steril. 2007;88(1):131–8.

Rosen MP, Cedars ML. Female reproductive endocrinology and infertility. In: Gardner D, Shoback D, editors. Greenspan's basic & clinical endocrinology. 8th ed. USA: McGraw-Hill Comp; 2007. p. 502–61.

Rumińska M, Witkowska-Sędek E, Majcher A, Pyrżak B. Thyroid function in obese children and adolescents and its association with anthropometric and metabolic parameters. Adv Exp Med Biol. 2016;912:33–41.

Santoro N. Update in hyper- and hypogonadotropic amenorrhea. J Clin Endocrinol Metab. 2011;96:3281–8.

Sanyal D, Raychaudhuri M. Hypothyroidism and obesity: an intriguing link. Indian J Endocrinol Metab. 2016;20(4):554–7.

Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW. American Heart Association Councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention: kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. Hypertension. 2003;42(5):1050–65.

Seetho IW, Wilding JP. How to approach endocrine assessment in severe obesity? Clin Endocrinol. 2013;79(2):163–7.

Sermondade N, Faure C, Fezeu L, Shayeb AG, Bonde JP, Jensen TK, Van Wely M, Cao J, Martini AC, Eskandar M, Chavarro JE, Koloszar S, Twigt JM, Ramlau-Hansen CH, Borges E Jr, Lotti F, Steegers-Theunissen RP, Zorn B, Polotsky AJ, La Vignera S, Eskenazi B, Tremellen K, Magnusdottir EV, Fejes I, Hercberg S, Lévy R, Czernichow S. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. Hum Reprod Update. 2013;19(3):221–31.

Siddiqui A, Madhu SV, Sharma SB, Desai NG. Endocrine stress responses and risk of type 2 diabetes mellitus. Stress. 2015;18(5):498–506.

Sienkiewicz E, Magkos F, Aronis KN, Brinkoetter M, Chamberland JP, Chou S, Arampatzi KM, Gao C, Koniaris A, Mantzoros CS. Long-term metreleptin treatment increases bone mineral density and content at the lumbar spine of lean hypoleptinemic women. Metabolism. 2011;60:1211–21.

Silveira LFG, Latronico AC. Approach to the patient with hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2013;98:1781–8.

Singhal V, Misra M, Klibanski A. Endocrinology of anorexia nervosa in young people: recent insights. Curr Opin Endocrinol Diabetes Obes. 2014;21:64–70.

Smalls AG, Kloppenborg PW, Njo KT, Knoben JM, Ruland CM. Alcohol-induced Cushingoid syndrome. Br Med J. 1976;2:1298.

Smith JT, Acohido BV, Clifton DK, Steiner RA. Kiss-1 neurons are direct targets for leptin in the ob/ob mouse. J Neuroendocrinol. 2006;18:298–303.

Somer L, Matavulj M, Hadzic B. The hypophyseal-adrenal axis in chronic alcoholism. Med Pregl. 1996;49:349–55.

Steiblienė V, Mickuvienė N, Prange AJ Jr, Bunevičius R. (2012). Concentrations of thyroid axis hormones in psychotic patients on hospital admission: the effects of prior drug use Medicina (Kaunas) 48(5):229-234.

Steyn FJ, Xie TY, Huang L, Ngo ST, Veldhuis JD, Waters MJ, Chen C. Increased adiposity and insulin correlates with the progressive suppression of pulsatile GH secretion during weight gain. J Endocrinol. 2013;218(2):233–44.

Stimson IRH, Andrew R, McAvoy NC, Tripathi D, Hayes PC, Walker BR. Increased whole-body and sustained liver cortisol regeneration by 11 beta-hydroxysteroid dehydrogenase type 1 in obese men with type 2 diabetes provides a target for enzyme inhibition. Diabetes. 2011;60:720–5.

Tirabassi G, Boscaro M, Arnaldi G. Harmful effects of functional hypercortisolism: a working hypothesis. Endocrine. 2014;46(3):370–86.

Tirabassi G, Faloia F, Papa R, Furlani G, Boscaro M, Arnaldi G. Use of the desmopressin test in the differential diagnosis of pseudo-Cushing state from Cushing's disease. J Clin Endocrinol Metab. 2010;95:1115–22.

Tirabassi G, Papa R, Faloia E, Boscaro M, Arnaldi G. Corticotrophin-releasing hormone and desmopressin tests in the differential diagnosis between Cushing's disease and pseudo-Cushing state: a comparative study. Clin Endocrinol. 2011;75:666–72.

Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res. 2002;53:865–71.

Valsamakis G, Anwar A, Tomlinson JW, Shackleton CH, McTernan PG, Chetty R, Wood PJ, Banerjee AK, Holder G, Barnett AH, Stewart PM, Kumar S. 11beta-hydroxysteroid dehydrogenase type 1 activity in lean and obese males with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2004;89:4755–61.

Vigersky RA, Loriaux D, Andersen AE, Lipsett MR. Anorexia nervosa: behavioral and hypothalamic aspects. Clin Endocrinol Metab. 1976;5:515–35.

Walker BR, Andrew R. Tissue production of cortisol by 11beta-hydroxysteroid dehydrogenase type 1 and metabolic disease. Ann N Y Acad Sci. 2006;1083:165–84.

Warner MH, Beckett GJ. Mechanisms behind the non-thyroidal illness syndrome: an update. J Endocrinol. 2010;205(1):1–13.

Warren MP, Perlroth NE. The effects of intense exercise on the female reproductive system. J Endocrinol. 2001;170:3–11.

Warren MP. Health issues for women athletes: exercise-induced amenorrhea. J Clin Endocrinol Metab. 1999;84:1892–6.

Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, Karalis A, Mantzoros CS. Recombinant human leptin in women with hypothalamic amenorrhea. N Engl J Med. 2004;351:987–97.

Zhu J, Choa RE, Guo MH, Plummer L, Buck C, Palmert MR, Hirschhorn JN, Seminara SB, Chan YM. A shared genetic basis for self-limited delayed puberty and idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2015;100:E646–54.

Physiopathology, Diagnosis, and Treatment of Secondary Hypothyroidism

7

Andrzej Lewiński and Magdalena Stasiak

Contents

Abstract

Secondary (or central) hypothyroidism (CH) is characterized by decreased thyroid hormone synthesis due to absent or insufficient thyroid stimulation by pituitary thyrotropin (TSH). The disease results from anatomical and/or functional disorder of the pituitary and/or the hypothalamus. The thyroid morphology and potential of hormone synthesis are usually normal, and the thyroid

A. Lewiński (✉)
Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, Polish Mother's Memorial Hospital Research Institute, Lodz, Poland
e-mail: alewin@csk.umed.lodz.pl

M. Stasiak
Department of Endocrinology and Metabolic Diseases, Polish Mother's Memorial Hospital Research Institute, Lodz, Poland
e-mail: magdi.g@poczta.fm

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_7

insufficiency is caused by dysfunction of the upper parts of the hypothalamus-pituitary-thyroid axis.

Rarely is CH an isolated insufficiency of thyrotropic cells and, in most cases, it is a part of combined (or multiple) pituitary hormone deficiency (CPHD).

The typical biochemical test results in CH include low free thyroxine (FT4) with decreased, low normal, or simply normal serum TSH concentration. Congenital CH (CoCH) is an important diagnostic challenge since routine screening for thyroid function, usually including TSH level only, can give misleading conclusion of normal thyroid function or even of hyperthyroidism. In most cases of CoCH, the genetic basis of hypothalamic and/or pituitary pathology is undefined. Among the already known molecular causes of CoCH, rarely occurring isolated TSH deficiency is a result of mutations in genes which control TSH synthesis (*TSHβ, TRHR, TBL1X*). Much more often CoCH is a part of CPHD resulting from mutations in one of several transcription factors important for the entire pituitary development, such as *HESX1, LHX3, LHX4, SOX2, SOX3, OTX2, GATA2, ISL1,* PITX1/2, *PROP1*, or *POU1F1*.

Acquired CH or CPHD of various extent and severity can be caused by intrasellar or extrasellar tumors, with pituitary adenomas and craniopharyngiomas being the most common ones. Surgical treatment of such tumors, as well as head irradiation, may lead to CH – either isolated or coexisting with additional pituitary deficiencies. Other important potential causes of CH include brain injuries, pituitary apoplexy, empty sella syndrome, parasellar aneurysm, subarachnoid hemorrhage, lymphocytic hypophysitis, and infiltrative or infectious diseases.

In every case of CH, detailed investigation of secretion of other pituitary hormones is obligatory, as additional anterior pituitary hormone deficiencies may be present in the majority of patients. Magnetic resonance imaging of the pituitary and the hypothalamus is mandatory in every case when CH is confirmed or suspected.

Treatment of CH is challenging as TSH cannot guide levothyroxine (L-T4) dose adjustment. Similarly to primary hypothyroidism, oral daily dose of L-T4 is a treatment of choice in CH. Before starting L-T4 administration, concomitant secondary adrenal insufficiency in the course of CPHD should always be excluded. If present, secondary adrenal insufficiency should be substituted before L-T4 treatment to avoid exacerbation of cortisol deficiency, which may even precipitate adrenal crisis.

The target level of FT4 during treatment should be maintained within the upper half of the normal range. Majority of CH patients require on average 1.5–1.6 μg L-T4/kg body weight daily. Older patients need lower doses of L-T4 than the young (approximately 1.1 μg L-T4/kg body weight daily). In infants and young children with CH, higher doses of L-T4 are required comparing to adults. It is recommended to start treatment in neonates with 10–15 μg/kg body weight daily and the dose should be decreased as the child gets older.

Concomitant treatment of CPHD significantly influences L-T4 requirement, and the dose should be re-established when estrogens, androgens, or recombinant

human growth hormone (rhGH) are introduced or in the patients in whom hydrocortisone therapy is introduced or modified during L-T4 treatment.

Keywords
Central hypothyroidism · Central congenital hypothyroidism · Pituitary · Hypothalamus · Thyroid · L-T4 · TSH · FT4 · *TSHβ* gene · *PROP1* · *POU1F1*

General Information

Secondary (or central) hypothyroidism (CH) is characterized by decreased thyroid hormone synthesis due to absent or insufficient thyroid stimulation by pituitary thyrotropin (TSH). The disease results from anatomical and/or functional disorder of the pituitary itself or the superior level of regulation – the hypothalamus. The thyroid morphology and potential of hormone synthesis are usually normal, and the thyroid insufficiency is caused by dysfunction of upper parts of the hypothalamus-pituitary-thyroid axis.

TSH secretion is physiologically stimulated by hypothalamic thyrotropin-releasing hormone (TRH) and regulated by thyroid hormone levels in the mechanism of negative feedback. Despite the long half-life of TSH molecule (about 6 weeks), its secretion is characterized by circadian pattern, with a nocturnal surge at early night hours. Many factors can influence TSH secretion with the most important being glucocorticoids, dopamine, and its analogs or somatostatin, as well as leptin or other hormones associated with fat tissue or sleep.

Rarely is the CH an isolated insufficiency of thyrotropic cells, and in most cases, the failure of TSH secretion is a part of combined (or multiple) pituitary hormone deficiency (CPHD). The diagnosis is based on biochemical test findings of low free thyroxine (FT4) with decreased, low normal, or simply normal serum TSH concentration. Therefore, CH (either congenital or acquired) is an important diagnostic challenge, since routine screening for thyroid function usually includes TSH level only and can give misleading conclusion of normal thyroid function or even of hyperthyroidism.

Epidemiology of CH

Most cases are sporadic forms of CH which can affect people of all ages with no gender predominance. Familial cases are rare.

CH prevalence in general population is estimated as ranging between 1:20,000 and 1:80,000 and as 1:1000 in all hypothyroid patients. Congenital CH (CoCH) seems to be much more common than it was previously considered. Although its prevalence is estimated as 1:160,000 newborns in Japan, it reaches up to 1:16,000 in the Netherlands. Many cases of CoCH may be missed because in most countries neonatal thyroid screening comprises only TSH level, and FT4 concentration is

assessed later on in children with elevated TSH, as the next step of hypothyroidism diagnostics. Thus, some countries have already introduced neonatal thyroid screening based on evaluation of both TSH and T4 levels or sometimes initially T4 level only. Unfortunately, taking into account the rarity of CoCH, T4 evaluation in neonatal screening is considered as not cost-effective and in most countries has not been introduced.

Pathogenesis

CH may be a result of either hypothalamic or pituitary insufficiency, or both. In many cases, the cause of CH remains unknown.

The failure of TSH secretion is usually quantitative – with low TSH concentration which is typical for most genetic cases of CH, such as – for example, *TSHβ* gene mutation, in which dimerization with αGSU to produce normal TSH molecule is impossible. In acquired CH, quantitative impairment frequently coexists with qualitative defect of the secreted TSH with preserved immunoreactivity but insufficient bioactivity for the receptor stimulation. TSH concentration in such cases may be normal or even slightly elevated.

Congenital CH

In most cases of CoCH, the genetic (or maybe even epigenetic) basis of hypothalamic and/or pituitary pathology is still undefined. Among the already known molecular causes of CoCH, rarely occurring isolated TSH deficiency is a result of mutations in genes which control TSH synthesis, including TSH β subunit gene (*TSHβ*), TRH receptor gene (*TRHR*), and transducin β-like protein 1 gene *(TBL1X*). Immunoglobulin superfamily member 1 gene (*IGSF1*) mutation used to be described as a cause of isolated CoCH, but currently concomitant growth hormone (GH) and prolactin (PRL) deficiencies are often reported (Asakura et al. 2015; Schoenmakers et al. 2015).

The most frequently CoCH is a part of CPHD resulting from mutations in one of several transcription factor genes important for entire pituitary development. Differentiation of certain pituitary cell types is dependent on complex interactions between many signaling molecules and transcription factors, such as *HESX1, LHX3, LHX4, SOX2, SOX3, OTX2, GATA2, ISL1,* PITX1/2, *PROP1*, and *POU1F1*. Among those, the most important for development of thyrotropic cells are *PROP1* and *POU1F1*.

Isolated CoCH

TSHβ Mutations

TSH molecule is a heterodimer comprised of common α subunit (αGSU) which is the same in other glycoprotein hormones (follicle-stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG)) and specific β

subunit (TSHβ). Mutations in *TSHβ* gene (located in the short arm of chromosome 1) lead to its truncation or to structural changes which impair heterodimeric integrity. So far described naturally occurring *TSHβ* mutations include missense, nonsense, frameshift, and splice site mutations, as well as – recently reported – homozygous *TSHβ* deletion (Hermanns et al. 2014). The most commonly reported *TSHβ* mutation is a single-nucleotide deletion (c373delT), leading to cysteine 125 to valine change (C125V) and braking of the Cys-Cys 39–125 disulfide bridge. This mutation causes frameshift and stop codon formation at position 134. In the cases of mutations leading to disruption of heterodimer formation, serum TSH levels are generally undetectable. Some mutations lead to formation of abnormal, biologically inactive TSH heterodimer, which is, however, recognized by monoclonal anti-TSH antibodies used in immunoassays. In such situation, TSH may be detectable despite the lack of its activity.

TSH mutations with biallelic loss of function lead to severe CH in neonates. Except for the low levels of thyroid hormones and TSH, increased concentration of pituitary glycoprotein α subunit (αGSU) and impaired TSH response to TRH administration with concurrent normal increase in serum PRL concentration are hallmarks of this condition.

TRHR Mutations

Thyrotropin-releasing hormone receptor gene (*TRHR*) (located in chromosome 8) mutations are very rare causes of CoCH. Four (4) cases in three (3) unrelated families have already been described (Bonomi et al. 2009; Collu et al. 1997; Koulouri et al. 2016). In male probants of two of these families, serum T4 concentrations were decreased with inappropriately normal TSH levels, and clinical manifestation included growth retardation with delayed bone age. Although in the youngest male patient, the diagnosis was established at the age of 9, no neurodevelopmental retardation was found, which indicated sufficient thyroid hormone supply in infancy, when brain development strictly depends on the thyroid status. Synthesis and rhythmic secretion of TSH were preserved in those cases and no abnormality in pituitary morphology was found. Interestingly, a woman with homozygous, nonsense *TRHR* mutation was diagnosed as having CH at the age of 33 after two normal pregnancies and lactations (Bonomi et al. 2009; Collu et al. 1997). Very recently a female infant with novel *TRHR* deleterious missense mutation was reported (Koulouri et al. 2016). In this infant, thyroid function was assessed due to prolonged neonatal jaundice and – similarly to the previously described cases – the severity of CoCH was mild. In patients (males and females) with biallelic *TRHR* mutations, there is no TSH or PRL response to exogenous TRH. The numbers of lactotropes and thyrotropes are not reduced and pregnancy and lactation are normal despite decreased serum PRL level. No extrapituitary manifestation of *TRHR* mutation was observed.

So far, no defect of *TRH* gene itself has been discovered, but – as it was mentioned above – impaired TRH action has been described with complete TRH resistance. In the reported cases, no specific symptoms were present throughout early childhood and even a normal physiologic pregnancy and lactation was achieved.

It therefore seems that TSH secretion, circadian rhythm, and bioactivity are not completely dependent on TRH action.

TBL1X Mutation

TBL1X (Transducin β-like protein 1, X-linked) located in chromosome X (Xp22.31) encodes a protein which is – among others – a part of thyroid hormone receptor-corepressor complex. TBL1X mRNA and protein are expressed in many organs, including human hypothalamus and pituitary. TBL1X protein potential role in the hypothalamus and the pituitary development or thyrotropic cell differentiation is not described. Very recently, a missense mutation in *TBL1X* gene was identified in three relatives with isolated CoCH (Heinen et al. 2016). Not only males were affected. Mutations in *TBL1X* were demonstrated to be associated with a novel syndrome of familial isolated CoCH with concomitant hearing loss, presumably resulting from impaired function of the nuclear NCoR/SMRT corepressor complex. The severity of CoCH is usually mild and the pituitary MRI is normal (Heinen et al. 2016).

Combined Pituitary Hormone Deficiencies

IGSF1 (Immunoglobulin Superfamily, Member 1) Gene Mutations

The incidence of CoCH caused by *IGSF1* mutations is estimated at up to 1:100,000. *IGSF1* gene is located in the X chromosome; thus, overt CoCH occurs in all affected males. Precise role of *IGSF1* in human pituitary and hypothalamic physiology is still undefined, but in murine pituitary the presence of IGSF1 protein was proven in thyrotropes, lactotropes, and somatotropes. All of several reported *IGSF1* mutations cause either protein maturation or membrane trafficking defects. Impaired TRH signaling is suggested as a crucial cause of CoCH in patients with *IGSF1* mutations. Serum TSH and TH levels are decreased but detectable and TSH response to TRH administration is blunted. The severity of CoCH is usually mild to moderate. In most cases, neurological development is normal, even if the disease is diagnosed in adulthood. However, very recently a novel *IGSF1* insertion mutation was identified with mild neurological phenotypes including hypotonia, delayed psychomotor development, clumsy behavior, and attention deficit disorder (Tenenbaum-Rakover et al. 2016).

In all the described males, the main clinical manifestation was CoCH, occurring either in isolation or with concurrent hypoprolactinemia. Although *IGSF1* mutation carriers are usually born larger than mean standards for gestational age, transient partial GH deficiency in childhood was reported. Recently a novel frameshift *IGSF1* mutation in a male with CoCH and GH deficiency was described. Surprisingly, in some cases IGF-I level was increasing with age and even acromegalic features were described in adults. Pubertal development is usually impaired in boys with *IGSF1* mutations. Although testicular growth is normal, testosterone rise and pubertal growth spurt are delayed. Eventually, in adults, macroorchidism is often described. Interestingly, female *IGSF1* mutation carriers are not totally unaffected. CoCH occurs in one-third of them, sometimes hypoprolactinaemia and/or benign ovarian cysts were also reported. There is a huge variety of phenotypes among patients with

IGSF1 mutations; thus, the function of this gene can be determined by other genetic or environmental factors. CoCH caused by *IGSF1* mutation is usually described as an isolated form of CoCH, but increasingly reported concomitant hypoprolactinemia and GH deficiency suggest that this condition should rather be considered as a form of CPHD (Asakura et al. 2015; Schoenmakers et al. 2015).

Clinical manifestation of CoCH associated with CPHD depends on whether the mutant gene encodes an early or a late transcription factor. In patients with mutations in the early transcription factors, such as *HESX1, LHX3, LHX4, SOX2, SOX3, OTX2,* CoCH occurs with various concomitant developmental abnormalities, including septo-optic dysplasia (SOD), holoprosencephaly and midline defects, ocular defects, neurodevelopmental impairment, or skeletal anomalies. When the mutation affects the late transcription factors, such as *PROP1* or *POU1F1*, CoCH is not associated with any specific syndrome.

Septo-optic dysplasia is a syndrome comprising two of the following three disorders: optic nerve hypoplasia and/or pituitary hypoplasia and/or defects of midline forebrain, such as *corpus callosum* agenesis and lack of *septum pellucidum*. The severity of hypopituitarism is variable. In patients with hypopituitarism, GH deficiency is the most common, followed by TSH and adrenocorticotrophic hormone (ACTH) deficiencies. Gonadotropin secretion is often preserved. In patients with CoCH and SOD, mutations in *HESX1, SOX3*, and *OTX2* were reported.

HESX1 (HESX Homeobox 1; gene located in the short arm of chromosome 3 at position 14.3) is member of a family of homeobox genes, which control the early embryonic development, including early brain development. The HESX1 protein plays a crucial role in the formation of the pituitary and is also necessary for the development of the forebrain, including optic nerves. In patients with *HESX1* mutations, the anterior pituitary is hypoplastic or even absent. The posterior pituitary is usually ectopic, but sometimes its imaging may be normal (Figs. 1 and 2). CHPD

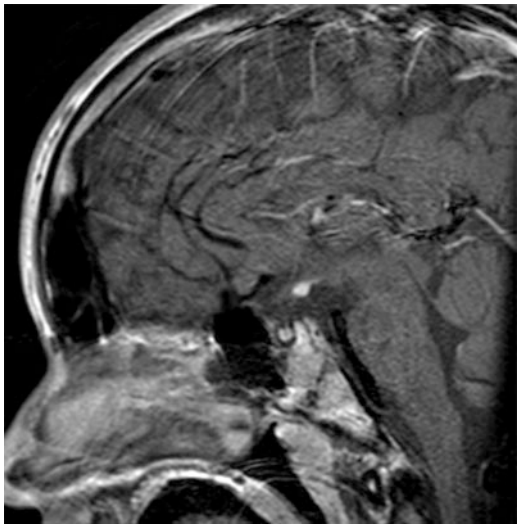


Fig. 1 Hypoplastic anterior pituitary with ectopic posterior pituitary in a boy with congenital CPHD (MRI scan from the authors' collections)

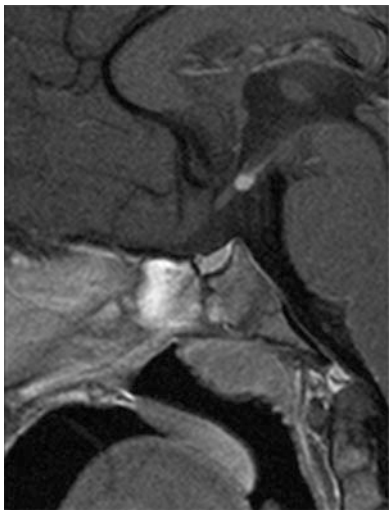


Fig. 2 Hypoplastic anterior pituitary with ectopic posterior pituitary in a girl with congenital CPHD (MRI scan from the authors' collections)

may include TSH, GH, ACTH, LH, and FSH deficiencies. SOD occurs often in those patients.

SOX3 (SRY-Box 3, Sex Determining Region Y-Box3) located in the X chromosome encodes a transcription factor required for the development of the hypothalamic-pituitary axis. This gene is also involved in craniofacial morphogenesis. Additionally, it plays an important role in initiating male sex determination by directing the development of supporting cell precursors towards Sertoli cells. Mutations in *SOX3* may result in several disorders, including X-linked hypopituitarism ranging from isolated GH deficiency to panhypopituitarism with CoCH. Concomitant X-linked mental retardation or learning difficulties often occur. MRI reveals various abnormalities including undescended posterior pituitary, hypoplasia of the anterior pituitary, or even persistent craniopharyngeal canal.

OTX2 (Orthodenticle homeobox 2; located in chromosome 14q22.3) encodes a transcription factor which plays an important role in the development of the brain, craniofacial structures, and sensory organs. Mutations in this gene lead to microphthalmia/anophthalmia and CPHD. In patients with heterozygous mutations in *OTX2,* pituitary manifestation is highly variable, from isolated GH insufficiency to deficiency of all anterior pituitary hormones. The posterior pituitary may be normal or ectopic.

CoCH is a part of CPHD in the cases of mutations in Lim homeodomain transcription factors (*LHX3, LHX4*). *LHX3* gene, located in chromosome 9q34.3, is one of the genes responsible for the anterior pituitary development. In patients with homozygous or compound heterozygous *LHX3* mutations, CPHD includes TSH, GH, FSH, LH, and PRL deficiencies. ACTH deficiency is rare, but such cases were also described. In MRI the pituitary may be hypoplastic or enlarged, or sometimes even the occurrence of microadenoma was reported. In patients with *LHX3* mutations, several concomitant disorders may occur, such as cervical

abnormalities with short/rigid cervical spine and/or vertebral abnormalities, and/or sensorineural hearing loss, and/or mental retardation. *LHX4* gene, located in chromosome 1q25.3, is also important for the anterior pituitary development. Heterozygous *LHX4* mutations lead to GH, TSH, ACTH, and FSH and/or LH deficiencies. The anterior pituitary is hypoplastic, sometimes the posterior pituitary is not descended or ectopic (as in the cases presented in the Figs. 1 and 2). *Sella turcica* may be poorly formed and other abnormalities, such as pointed cerebellar tonsils or Arnold-Chiari malformations (skull malformation leading to downward dislocation of the part of cerebellum through the foramen magnum into vertebral canal).

PROP1 (PROP paired-like homeobox 1, prophet of Pit1), located in chromosome 5q35.3, encodes a transcription factor essential for the pituitary gland development. *PROP1* expression is vital for normal expression of POU domain transcription factor 1 (*POU1F1, Pit-1*), which is responsible for differentiation of various types of the anterior pituitary cells. Mutations in *PROP1* are the most common reasons of CPHD with GH, TSH, PRL, LH, FSH, and ACTH deficiencies of variable onset and extent. In patients with recessive *PROP1* mutations, hormone deficiencies may manifest gradually, usually (as for example in the p.R120C mutation) beginning from GH deficiency in childhood with subsequent TSH, PRL, LH, and FSH deficiencies, occurring several years later. ACTH deficiency (if present) is usually delayed and occurs as the last of all pituitary hormone insufficiencies. The anterior pituitary may be hypoplastic or normal; however, in children it may even be initially enlarged. The posterior pituitary is eutopic.

POU1F1 (POU class 1 homeobox 1; POU Domain Class 1, Transcription Factor 1; Pit-1), located in chromosome 3p11.2, encodes transcription factor responsible for differentiation of lactotropes, somatotropes, and thyrotropes in the developing anterior pituitary. In patients with *POU1F1* mutations, CPHD is characterized by deficiencies of GH, PRL, and TSH with preserved production of ACTH and gonadotropins. GH and PRL deficiencies are usually present from a very early life, while TSH deficiency may sometimes occur later in childhood or even in adulthood. The anterior pituitary may be normal or hypoplastic. Other common abnormalities include prominent forehead, mid face hypoplasia, and depressed nose.

The major causes of CoCH are summarized in Table 1.

In rare cases, CPHD results from congenital brain tumors, mainly craniopharyngiomas, which lead to nongenetic congenital CPHD. In such cases, the prognosis is poor because of the brain destruction by tumor mass and frequently occurring deficiencies of all pituitary hormones during pregnancy and in the postpartum period (Fig. 3).

Acquired CH

More than a half of acquired CH cases is caused by pituitary macroadenomas which destroy or compress the pituitary itself, the pituitary stalk, or the hypothalamus. The most frequent pituitary adenomas are PRL or GH secreting tumors, as well as nonfunctioning adenomas (Fig. 4).

Table 1 The major causes and differential diagnosis of CoCH

Mutation	Inheritance/ chromosome	Hormone deficits	Typical clinical and biochemical features
Isolated TSH deficiency			
TSHβ	Autosomal recessive/1p13.2	TSH	Clinically overt profound hypothyroidism with severe mental retardation if treatment is delayed Normal or enlarged pituitary in MRI Increased αGSU level, impaired TSH response to TRH (with preserved PRL response)
TRHR	Autosomal recessive/8q23.1	TSH PRL slightly decreased	Usually apparent clinical manifestation of CoCH Mild severity of CoCH No extrapituitary abnormalities Normal pituitary in MRI Blunted TSH and PRL response to TRH
TBL1X	X-linked/Xp22.31	TSH	Mild severity of CoCH Normal pituitary and hypothalamus in MRI Concomitant hearing loss
CPHD with other specific abnormalities			
IGSF1	X-linked/Xq26.2	TSH Sometimes Prl and/or GH (GH deficiency may be transient)	Mild severity of CoCH Blunted TSH response to TRH Normal pituitary in MRI Delayed puberty Macroorchidism Ovarian cysts Sometimes acromegalic features in late adulthood
HESX1	Autosomal recessive or dominant/3p14.3	GH TSH ACTH LH, FSH	CoCH of variable severity SOD (anterior pituitary hypoplasia/aplasia, optic nerve hypoplasia, *corpus callosum* agenesis) Ectopic posterior pituitary in MRI
SOX3	X-linked/Xq27.1	GH TSH ACTH LH, FSH	Possible SOD MRI findings: Anterior pituitary hypoplasia Ectopic posterior pituitary Sometimes persistent craniopharyngeal canal X-linked mental retardation
OTX2	Autosomal dominant/14q22.3	GH TSH ACTH LH, FSH	Possible SOD Normal or hypoplastic anterior pituitary in MRI Ectopic or normal posterior pituitary Anophthalmia or microphthalmia Retinal dystrophy
LHX3	Autosomal recessive/9q34.3	GH TSH LH, FSH	Hypoplastic, normal or enlarged anterior pituitary in MRI Short cervical spine/reduced neck rotation

(*continued*)

Table 1 (continued)

Mutation	Inheritance/ chromosome	Hormone deficits	Typical clinical and biochemical features
		PRL Rarely ACTH	Hearing loss Mental retardation
LHX4	Autosomal dominant/1q25.3	GH TSH ACTH, Variable LH/ FSH	Hypoplastic anterior pituitary Normal or ectopic posterior pituitary Poorly formed *sella turcica* Cerebellar abnormalities
CPHD without other specific abnormalities			
PROP1	Autosomal recessive/5q35.3	GH TSH LH, FSH PRL ACTH	CPHD of variable extent and severity Hypoplastic, normal, or enlarged anterior pituitary in MRI Normal posterior pituitary
POU1F1	Autosomal recessive or dominant/3p11.2	GH TSH PRL	Hypoplastic anterior pituitary Normal posterior pituitary

SOD septo-optic dysplasia

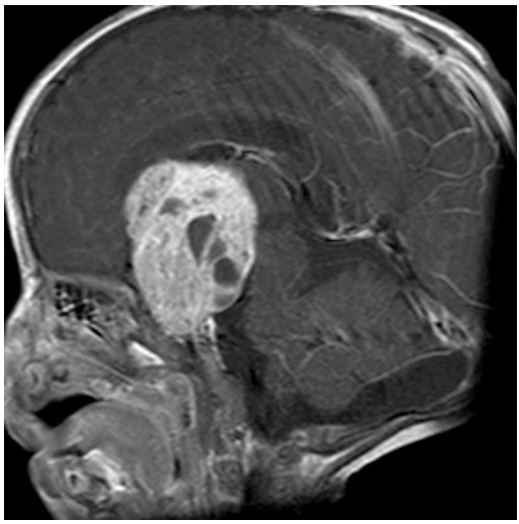


Fig. 3 A neonate with congenital craniopharyngioma with CPHD and diabetes insipidus (MR scan from the authors' collections)

The most frequent extrasellar brain tumors causing CH are craniopharyngiomas, which should be suspected especially in young patients. In patients with craniopharyngiomas, endocrine manifestations with pituitary hormone deficiencies and/or diabetes insipidus are usually the first symptoms of the tumor and may precede neurologic symptoms by months or even years (Fig. 5). These lesions have benign histology but malignant behavior with a tendency to invade surrounding structures and to recur after resection. Isolated CH or much more often CH with other pituitary

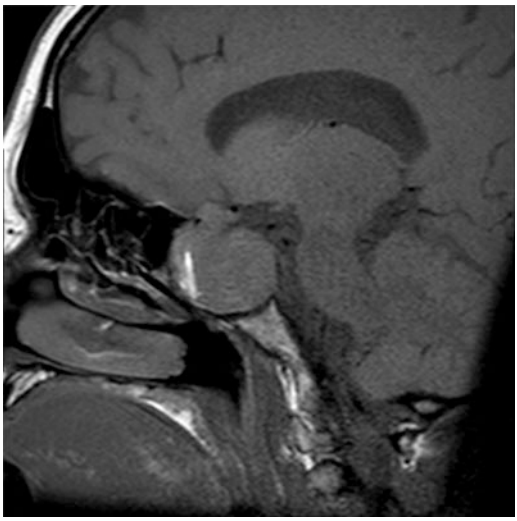


Fig. 4 MRI scan of pituitary macroadenoma which caused CH and gonadotropin deficiency (MR scan from the authors' collections)

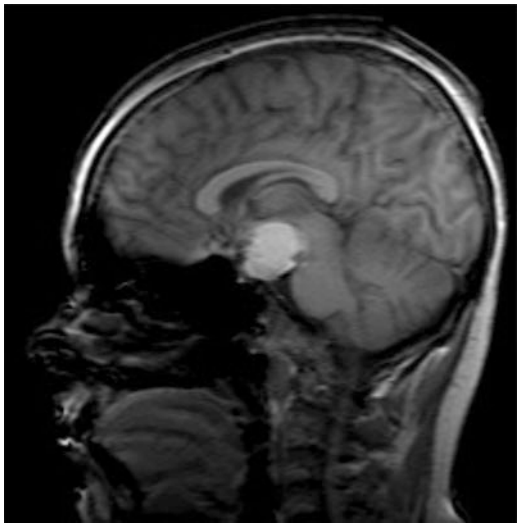


Fig. 5 MRI scan of craniopharyngioma in patient with CPHD and diabetes insipidus (MR scan from the authors' collections)

hormone deficiencies may result not only from the tumor mass effect, with the hypothalamus and/or the pituitary compression and destruction, but very commonly may be caused by tumor surgery, sometimes even repeated several times. Thus, a decision about surgical resection of craniopharyngioma should be made with caution after considering possible risk and potential benefits.

As it was mentioned above, iatrogenic CH may be a result of the pituitary or the brain surgery, as well as cranial irradiation due to the brain, nasopharyngeal, or paranasal sinus tumors. Sometimes, after head and neck irradiation, hypothyroidism is of both primary and central origin. In such cases, diagnosis and treatment are real

clinical challenges. The risk of CH after neurosurgery depends mainly on the extension of tumor mass and on the surgeon experience. To minimize the risk of CPHD, such operation should be performed by a high-volume surgeon.

Due to the improvement in medical care, the survival of patients after brain injuries, subarachnoid hemorrhage, or infarcts is constantly increasing, being more and more frequent cause of CH or CPDH. These patients require special attention as hypopituitarism may be transient in many cases; thus, regular follow up and re-evaluation of the pituitary function is mandatory in this group.

Formerly, postpartum pituitary necrosis (Sheehan's syndrome) was a common reason of CPDH and CH. Although now Sheehan's syndrome is rare in developed countries, it is still quite common in poor developing areas. Hypopituitarism in Sheehan's syndrome develops slowly and gradually. Characteristic is the postpartum lack of lactation and amenorrhea, but the concentration of TSH, which is often measured as the only marker of thyroid function, remains normal for many years. The diagnosis is often made 20–30 years after delivery, when there is actually no adrenal and thyroid reserve left. Thus, Sheehan's syndrome should always be considered in women with typical clinical signs and symptoms of CPHD who have still normal serum TSH concentration.

Empty sella syndrome is a condition when the subarachnoid space enters into sella turcica and fills it up with cerebrospinal fluid, leading to the anterior pituitary compression and flattering. Pituitary tumor apoplexy, not rarely occurring during delivery, may lead to the formation of arachnoid cysts compressing the residual pituitary (Fig. 6). Such cases of empty sella syndrome often occur with CH or CPHD. Similarly, CPHD of various extent is also common in the cases when empty sella is a result of neurosurgery, irradiation, or Sheehan's syndrome. In contrast, in patients with incidentally discovered empty sella in imaging studies

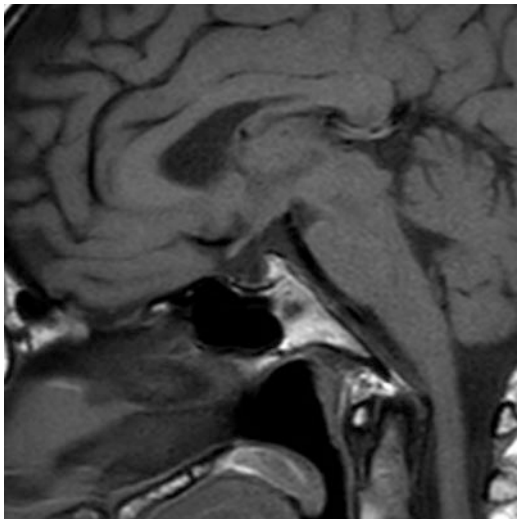


Fig. 6 MRI scan of empty sella syndrome in a 55-year-old women with newly diagnosed Sheehan's syndrome with CPHD (MR scan from the authors' collections)

(performed due to other indications), pituitary function is usually normal and the patients do not require any hormonal substitution.

Variable extent of CPHD including CH may be a result of lymphocytic hypophysitis which can be either isolated or a part of an autoimmune polyglandular syndrome (APS). Anterior pituitary insufficiencies and/or diabetes insipidus are common in such cases. Lymphocytic hypophysitis most often occurs during pregnancy and postpartum but in rare cases may affect both men and women of any age. Extensive infiltration of the pituitary results in the anterior pituitary cell destruction which – in turn – leads to hormone deficiencies of various degree. Isolated ACTH, PRL, or TSH deficiency may occur and, interestingly, women may suffer from CH or secondary adrenal insufficiency and, in spite of that, still menstruate. MRI usually demonstrates diffuse homogeneous sellar mass with the pituitary gland and pituitary stalk enlargement (typical "pear-shaped" appearance). Such MRI may be easily mistaken for pituitary adenoma or other pituitary tumors.

Infiltrative diseases such as sarcoidosis, hemochromatosis, or Langerhans cell histiocytosis are rare causes of CPHD. The hypothalamus and the pituitary are common intracranial sites of sarcoidosis. Neuroendocrine dysfunction is generally caused by the hypothalamic rather than the pituitary insufficiency, and pituitary responsiveness to synthetic hypothalamic releasing hormones is usually preserved. In such patients, gonadotropin deficiency is the most frequent endocrine disorder followed by TSH deficiency, diabetes insipidus, and hyperprolactinemia. CH can be isolated or more frequently occurs with concomitant LH and FSH deficiencies, and diabetes insipidus. Corticosteroid therapy significantly improves radiologic lesions, but the hypothalamic-pituitary insufficiency is usually irreversible despite adequate treatment of the disease. Similarly in the pituitary and hypothalamic hemochromatosis, gonadotropin deficiency is the most common endocrine manifestation of the disease. Hypogonadotropic hypogonadism is often the first clinical feature of iron excess. Hypogonadism can be reversible if the treatment is introduced early. CH or other pituitary deficiencies occur several years later and are irreversible even if the patient is treated properly. The most common endocrine manifestation of Langerhans cell histiocytosis is diabetes insipidus. Hypogonadotropic hypogonadism is often the first anterior pituitary disorder which can be reversible on treatment. Other hormone deficiencies, including CH or panhypopituitarism, occur frequently, sometimes with concomitant hyperprolactinemia.

Infectious diseases such as tuberculosis, syphilis, or mycotic infections can lead to CH or CPHD but – due to wide availability and widespread use of antimicrobial drugs – such cases are extremely rare.

TSH secretion can be decreased by many drugs, including glucocorticoids, dobutamine, dopamine, and its analogs or somatostatin analogs. However, such effect is not constant and after some time, the negative feedback restores the physiologic thyroid status. Antineoplastic drugs which bind to retinoid X nuclear receptor can cause severe inhibition of TSH secretion which lasts as long as the drug is administered.

In neonates, transient CH can be caused by corticosteroids or dopamine, given to pregnant women before or during a complicated and/or preterm delivery.

Decreased TSH level in neonates may sometimes result from prolonged TSH suppression caused by placental passage of large amounts of stimulating anti-TSH receptor antibodies (TRAbs) and – to less degree – thyroid hormones, from mother with severe hyperthyroidism to fetus. After delivery symptomatic thyrotoxicosis may be present in such neonates. Thyroid hormone concentration usually normalizes after some time, but low TSH level may persist even up to 6 months after birth.

Nonthyroidal severe diseases can lead to suppression of TRH synthesis and consequently to development of a biochemical variant of CH. Management of this nonthyroidal illness syndrome (NTIS) (or euthyroid sick syndrome) raises a lot of controversy, but it is commonly believed to be a defense mechanism against redundant metabolic stimulation by thyroid hormones. Thus, treatments of NTIS with L-thyroxine are generally not recommended.

Sometimes there is no evident cause of CH, and TSH deficiency remains idiopathic as all known potential causative factors are excluded. Constant surveillance of such patients is necessary as sometimes initially occult causative disorder may become overt later in time (Table 2).

Table 2 The major causes of acquired CH

Benign and malignant neoplastic lesions	Pituitary macroadenoma Craniopharyngioma Meningioma Glioma Chordoma Epidermoid or dermoid tumors Metastases (mainly breast carcinoma)
Iatrogenic	Neurosurgery Head irradiation Drugs (e.g., retinoid X receptor-selective ligands)
Injury	Head traumas
Non-neoplastic compressive lesions	Rathke cleft cysts Empty sella (due to various reasons) Parasellar aneurysm
Vascular factors	Pituitary apoplexy (usually associated with pituitary tumor) Subarachnoid hemorrhage Postpartum pituitary necrosis (Sheehan's syndrome)
Autoimmune disease	Lymphocytic hypophysitis
Infiltrative diseases	Sarcoidosis Hemochromatosis Langerhans cell histiocytosis
Infections	Tuberculosis Mycoses Syphilis
Idiopathic	?

Quoted after Persani (2012), with the authors' own modifications

Diagnosis

In most patients with acquired CH, symptoms of thyroid hormone deficiency are mild to moderate and, especially in CPHD, may be masked by other deficiencies, mainly secondary adrenal insufficiency. The diagnosis is based on laboratory test results of low FT4 with decreased or normal serum TSH concentration. As it was mentioned before, in some patients with CH of hypothalamic origin, serum immunoreactive TSH level may be elevated, but the TSH particle is devoid of full biological activity. In such cases, laboratory test results are similar to those in primary mild hypothyroidism, which may lead to wrong diagnosis.

Before introducing wide diagnostics of CH, several conditions which may decrease TSH secretion should be excluded, including drug administration (glucocorticoids, dobutamine, dopamine, and its analogs or somatostatin analogs, retinoids), starvation or NTIS due to critical illnesses.

Before establishing the final diagnosis of CH on the basis of laboratory test results, any interference in FT4 and TSH measurements should be excluded. To recognize CH properly, FT4 but not total T4 (TT4) serum concentration should be measured in order to avoid the influence of unstable concentrations of thyroid hormone binding globulin (TBG). Many factors can alter TBG level as its concentration decreases, for example, with age and due to androgen administration or physiologic pubertal testosterone rise and increases due to estrogen influence (in pregnancy, on contraception or tamoxifen). Every disease that leads to disruption of protein synthesis (e.g., malnutrition, hepatic diseases) alters TBG level. Measurement of FT4 is much more reliable than TT4 in diagnosis of thyroid hormone deficiency, and the most accurate method is equilibrium dialysis which – unfortunately – is not widely available in the routine practice. However, quite commonly employed two-step back-tritation assays usually allow to avoid interference with autoantibodies or altered binding globulins. Falsely low TSH level may be a result of the presence of heterophilic antibodies in patient's serum. If heterophilic (anti-animal) antibodies are directed against the same species as the assay antibodies, the heterophilic antibodies block TSH binding to assay antibodies which results in falsely low TSH result, suggesting CH. If such situation is suspected, TSH level should be reassessed by immunoassay with different antibody pair and/or by dilution test and/or by treatment with polyethylene glycol or protein G (Persani 2012, Persani et al. 2012).

If any possible interference is excluded, overt forms of CH can be easily confirmed by the findings of low FT4 together with low/normal TSH levels. However, hypothalamic CH after cranial irradiation may sometimes manifest as hidden CH with still normal FT4. This condition can be recognized only by demonstration of disturbed circadian TSH rhythm (lack of nocturnal surge) or abnormal TSH response to TRH stimulation. TRH test can allow not only to confirm mild forms of CH but also to differentiate CH of hypothalamic or pituitary origin. In hypothalamic CH, TSH response is exaggerated, delayed, and/or prolonged, while blunted TSH reaction is typical for pituitary CH. In many patients with CH, both the hypothalamus

and the pituitary are affected and in such cases the differential diagnosis based on TRH test can be impossible. One should always remember that normal TSH response to TRH does not imply exclusion of CH diagnosis. Thus, in clinical practice, application of TRH test should be limited to doubtful cases, when abnormal TSH response may confirm the presence of CH. In some patients, exaggerated and prolonged TSH response to stimulation by TRH does not go along with an increase in FT4 level, which is a typical feature of poor bioactivity of TSH molecules.

In patients with variety of pituitary diseases monitored by several years, an observation of time-related decrease in FT4 concentration exceeding 20% as compared to initial FT4 measurements performed by the same laboratory may be helpful in establishing CH diagnosis.

Markers of peripheral thyroid hormone action such as – among others – levels of sex hormone binding globulin (SHBG), lipids, or bone turnover markers cannot be used for support of CH diagnosis, especially in patients with CPHD which can significantly alter levels of those markers.

In every case of low thyroid hormone levels, primary thyroid disease should be excluded as CH may be a result of intermittent thyrotoxicosis but also because the hypothalamic CH may appear with slight TSH elevation at immunoassay. Besides hormonal tests, in every patient with suspected CH thyroid ultrasound (US) examination should be performed. Normal thyroid US and absent antithyroid antibodies support the suspicion of CH. Checking thyroid response to recombinant human TSH (rhTSH) may be helpful if the product is available. There are several characteristic features which strongly suggest hypothalamic and/or pituitary origin of hypothyroidism. Such features include other pituitary/hypothalamic disease, signs and symptoms of intrasellar or uppersellar lesions (headaches, visual defects), medical history of head trauma, cardiovascular event, cranial surgery, or irradiation.

The presence of other diseases that can lead to NTI should be excluded prior to further diagnostics of CH. Pituitary and hypothalamus MRI is required in every case when CH is suspected.

As it was mentioned before, in every case of CH, detailed investigation of other pituitary hormone secretion is mandatory as additional anterior pituitary hormone deficiencies are present in majority of patients.

A few syndromes of thyroid hormone resistance can mimic CH. Allan-Herndon-Dudley syndrome results from mutation of *MCT8* gene, encoding membrane transporter of thyroid hormones. In patients with this syndrome, low FT4 sometimes coexists with normal TSH level (TSH can be also slightly elevated). Distinguishing Allan-Herndon-Dudley syndrome form CH is not difficult because of severe clinical phenotype already visible in young children with significant cognitive and psychomotor retardation. Characteristically, triiodothyronine (T3) circulating levels are elevated 2- to 3-fold, comparing to healthy subjects. Heterozygous mutation of *THRA* gene, encoding thyroid hormone receptor α, is associated with similar biochemical findings as in Allan-Herndon-Dudley syndrome. This disease also has characteristic manifestation including severe constipation, mental retardation, delayed bone development, and growth retardation.

Special Remarks Concerning CoCH Diagnosis

In neonates, CoCH can be immediately diagnosed only by screening test based on concomitant assessments of TSH and TT4 on the blood spot. Screening programs based exclusively on TSH measurement fail to identify CH as TSH level is low or normal in such cases. Thus, the diagnosis of CH may be delayed which carries the risk of neurodevelopmental retardation (cretinism) (Léger et al. 2014; Schoenmakers et al. 2015). However, CoCH should be suspected in every case of characteristic clinical symptoms of congenital hypothyroidism, including jaundice, hypotonia, macroglossia, retarded growth, failure to thrive, coarse cry, etc. In neonates with inherited CH due to biallelic *TSHβ* mutations, manifestation of the disease is usually severe. In contrast, in CoCH caused by defects of the pituitary transcription factors, the onset of hormone deficiencies may be delayed, but the disease is usually associated with characteristic craniofacial abnormalities often visible even in prenatal US examinations. Hypoglycemia resulting from concomitant ACTH and/or GH deficiency is common in such cases.

Suspicion of *TSHβ* defect in patients with CH can be supported by high level of α-GSU and impaired TSH response to TRH with preserved normal PRL response.

Complete TRH resistance may be asymptomatic, especially in early childhood but can be recognized by blunted response of TSH and PRL after TRH administration.

Biochemical manifestation of CH includes low FT4 level with inappropriately normal or low TSH. The increased activity of type 2 deiodinase (DIO2) results in sometimes still normal circulating T3 levels.

In patients with CPHD in whom TSH deficiency evolves in time (e.g., *POU1F1* mutations), regular monitoring of serum FT4 level should be introduced because decreasing FT4 concentrations are considered as early markers of CH.

In every case of CoCH, further evaluation of other hormone pituitary function is mandatory as additional anterior pituitary hormone deficiencies may be present in the majority of patients. In most children with CoCH, concomitant GH deficiency is recorded (89%), followed by ACTH (78%) and gonadotropin (46%) deficiencies. Posterior pituitary dysfunction occurs in minority of patients, but most or even all of them have SOD. MRI reveals structural pituitary abnormalities, as well as other CNS defects and can guide further genetic evaluation.

In many patients with specific phenotype, target genetic screening should be performed (Schoenmakers et al. 2015). Genetic diagnosis is extremely important to ensure whether the patient is at risk of other pituitary hormone deficiencies (e.g., mutations of transcription factor genes). In such cases, long-term surveillance for other pituitary hormone deficiencies (including especially ACTH) is required. On the other hand, if the genetic defect is specific for TSH biosynthesis (*TSHβ* or *TRHR* mutations), other hormone deficiencies are unlikely.

Genetic screening is based on the clinical phenotype combined with radiological findings and extrapituitary abnormalities. In patients with clinically isolated CoCH with no other pathologic features, genetic tests depend on the severity of CoCH. In severe CoCH with normal PRL level and increased αGSU, mutation in *TSHβ* should

be suspected. If isolated TSH deficiency with normal secretion of other anterior pituitary hormones is combined with macroorchidism and seems to be X-linked, mutations in *IGSF1* are highly probable. In the cases of SOD with optic nerve and/or pituitary hypoplasia and/or midline defect, several genes should be checked for mutations, including *HESX1, SOX3, FGF8/FGFR1, KAL1, PROK2/PROKR2,* and *OTX2*. In CPHD with ectopic posterior pituitary, genetic testing for mutations in *HESX1, SOX2, PROK2/PROKR2, LHX3/4*, and *OTX2* should be performed. In patients with CHPD and normal or hypoplastic anterior pituitary, without any additional specific syndrome, genetic testing should include mutations in *PROP1, POU1F1*, and *IGSF1*. In the latter case, CoCH is associated with PRL and/or sometimes with GH deficiency. In patients with CPHD and ocular abnormalities, such as anophthalmia/microphthalmia and/or retinal dysplasia, mutations in *SOX2* and *OTX2* should be suspected.

Differential diagnosis of CoCH due to various mutations is presented in Table 1.

After diagnosis of CoCH in a young proband, family screening for undiagnosed CH should be performed to prevent cardiometabolic and other consequences of long-term undiagnosed hypothyroidism even if it remains only subclinical. Treatment with levothyroxine (L-T4) may significantly improve their health and quality of life.

Treatment

The aim of treatment is to restore normal serum concentrations of thyroid hormones. TSH concentration should be interpreted with caution or should not be measured at all, to avoid misinterpretation of usually low TSH level on treatment. Similarly to primarily hypothyroidism, oral daily dose of L-T4 is a treatment of choice in CH. Combination therapy with L-T4 and liothyronine (L-T3) is not recommended in routine clinical practice.

In patients with probably long-lasting hypothyroidism, especially elderly or/and with concomitant cardiovascular disease, treatment should be introduced with low daily dose of L-T4 (even 12.5 μg) which should be progressively increased for a couple of weeks. Before starting L-T4 concomitant secondary adrenal insufficiency in the course of CPHD should always be excluded. If present, secondary adrenal insufficiency should be substituted before L-T4 treatment to avoid exacerbation of cortisol deficiency, which may even precipitate adrenal crisis. If there is no possibility to exclude adrenal hypofunction, prophylactic treatment with glycocorticosteroids should be administered before starting L-T4. In such cases evaluation of adrenal function can be postpone and performed later when such diagnostics is available.

Establishing of adequate dose of L-T4 in CH is much more difficult than in primary hypothyroidism, in which TSH reflects the treatment efficacy. The value of TSH measurement in CH is limited, but unsuppressed TSH level on L-T4 is firmly indicative for undertreatment. Determination of FT4 concentration has the best accuracy in monitoring CH treatment, while FT3 level may be valuable in detecting

overtreatment. For treatment monitoring, blood should be withdrawn before the morning dose of L-T4. The target level of FT4 during treatment should be maintained within the upper half of the normal range. Majority of CH patients require on average 1.5–1.6 μg L-T4/kg body weight daily. Older patients need lower doses of L-T4 than the young (approximately 1.1 μg L-T4/kg body weight daily). Low FT4 level suggests undertreatment, while overtreatment is better reflected by elevated FT3. Undertreatment should be strongly suspected if TSH level is greater than 0.5 mIU/l and/or FT4 is below the lower tertile of normal range.

Biochemical markers of thyroid action on tissues, such as concentrations of SHBG, bone turnover markers, or lipids, are not useful in CH diagnosis but may be helpful in long-term treatment monitoring, provided that other pituitary/hypothalamus hormone deficiencies are excluded or adequately compensated with replacement therapy. Any potential disorder in adrenal, gonadal, or somatotrope pituitary function may itself alter those markers and make them useless in assessment of adequacy of L-T4 dose.

Concomitant treatment of CPHD significantly influences L-T4 requirement. Patients on recombinant human growth hormone (rhGH) replacement therapy usually require higher L-T4 doses. On the other hand, mild forms of CH may be concealed by GH deficiency, and subnormal values of thyroid hormones may appear only after rhGH substitution. Estrogen substitution increases L-T4 requirement due to increasing concentration of thyroxine binding globulin (TBG) and the L-T4 dose should be modified on the basis of FT4 concentration. On average, daily dose of L-T4 should be increased by 0.1–0.15 μg/kg body weight when estrogen or rhGH replacement therapy is introduced. On the contrary, androgens decrease serum TBG concentration and a decrease in L-T4 dose may be necessary. L-T4 dose should also be re-established in patients, in whom hydrocortisone therapy is introduced or modified during L-T4 treatment (Biondi and Wartofsky 2014; Garber et al. 2012; Jonklaas et al. 2014).

Treatment monitoring and appropriate L-T4 dose adjustment are difficult in patients with CH. Therefore, one should always remember about the principles of correct L-T4 administration and be aware of the potential factors affecting the effectiveness of the treatment. Co-administration of food and most beverages impair L-T4 absorption. Thus, the tablet should be taken with water, optimally 60 min (at least 30 min) before breakfast or at bedtime (at least 3 h after the last meal). It is important to have similar food for breakfasts and to avoid food which may interfere with L-T4 absorption, such as fiber rich products, soy, or espresso coffee (Garber et al. 2012).

Several commonly used medication can significantly reduce L-T4 absorption, including calcium carbonate, ferrous sulfate, proton pomp inhibitors, H2-receptor antagonists, aluminum-containing antiacids, sucralfate, bile acid sequestrants (cholestyramine or colesevelam) and phosphate binders (sevelamer, aluminum hydroxide), oral bisphosphonates, charcoal, chromium picolinate, and ciprofloxacin. Because many of these substances (e.g., ferrous sulfate or calcium carbonate) can be found in over-the-counter dietary supplements, the patient should always be advised to take such formulations at least 3–4 h after L-T4. It is important to

remember to adjust L-T4 dose after introduction of any additional treatment which potentially influence its absorption, binding with proteins or metabolism (Biondi and Wartofsky 2014; Garber et al. 2012; Jonklaas et al. 2014).

In a patient in whom L-T4 dose requirement suddenly significantly increases, gastrointestinal diseases, including mainly *Helicobacter pylori* induced gastritis, atrophic gastritis, or celiac disease should be suspected. After effective treatment of such disorders, L-T4 dose should be re-adjusted.

It is worth remembering that L-T4 absorption also decreases with age and is reduced in severe obesity.

Various L-T4 products available on the market are not equal in absorption and every change in the formulation of L-T4 (brand name or generic) should be followed by dose re-adjustment.

In patients with cirrhosis or renal failure, usually no adjustment in L-T4 dosing is required. In the cases of nephrotic syndrome, L-T4 dose re-evaluation is necessary due to excessive urinary thyroid hormone losses.

In patients with CH in whom adherence to L-T4 therapy is not possible to achieve due to several reasons including mental retardation, weekly oral administration of the full week dose of L-T4 can be considered (Biondi and Wartofsky 2014; Garber et al. 2012; Jonklaas et al. 2014).

Special Situation in Pediatric Patients with CH/CoCH

In infants and young children with CH, higher doses of L-T4 are required comparing to adults. In neonates and young children, treatment should always be started with full replacement dose to achieve adequate concentrations of circulating FT4 as soon as possible, to minimize neurological consequences of thyroid hormone deficiency. It is recommended to start treatment in neonates with 10–15 μg/kg body weight daily and modify the dose according to FT4 level every 1–2 weeks. Optimal outcome seems to be achieved when thyroid hormone levels reach target normal values within 2 weeks of therapy. Once a proper dose is established, FT4 concentration should be evaluated every 1–2 months during the first year of life. Between the ages of 1 and 3 years, thyroid status should be evaluated every 2–4 months, with less frequency in subsequent years. In neonates, infants and young children L-T4 should be administered at the same time every day. It should be crushed and mixed with water, nonsoy formula, or breast milk. Concurrent ingestion of soy, iron, calcium, or colic drops containing simethicone should be avoided as they can significantly decrease L-T4 absorption. If intravenous administration of L-T4 is required, the dose should not exceed 80% of the oral dose (Léger et al. 2014). Progressively lower L-T4 doses per kg of body weight are required in infants, young children, and adolescents. Commonly accepted doses are as follows: 6–8 μg/kg body weight for 6- to 12-month-old infants, 4–6 μg/kg body weight for 1–3 year old children, 3–5 μg/kg body weight for 3- to 10-year-old children, 2–4 μg/kg body weight for 10- to 16-year-old adolescents, and 1.6 μg/kg body weight when endocrine maturation is complete. L-T4 replacement therapy

promotes acceleration of growth velocity which allows the child to reach the expected target height (Biondi and Wartofsky 2014; Jonklaas et al. 2014).

Summary

- Secondary hypothyroidism is characterized by decreased thyroid hormone synthesis due to absent or insufficient thyroid stimulation by the pituitary thyrotropin (TSH). The disease results from anatomical and/or functional disorder of the pituitary and/or the hypothalamus.
- Isolated congenital or acquired CH are rare, and in most cases, secondary thyroid insufficiency is a part of combined pituitary hormone deficiency (CPHD).
- The typical biochemical test results include low FT4 level with decreased, low normal, or simply normal serum TSH concentration.
- Congenital CH is an important diagnostic challenge since routine newborn screening for thyroid function usually includes only TSH level, which can give misleading conclusion of normal thyroid function or even of hyperthyroidism.
- Isolated congenital TSH deficiency is a result of mutations in genes which control TSH synthesis, including *TSHβ, TRHR*, and *TBL1X*.
- Congenital CH being a part of CPHD can result from mutations in one of several transcription factors important for the entire pituitary development, such as *HESX1, LHX3, LHX4, SOX2, SOX3, OTX2, GATA2, ISL1,* PITX1/2, *PROP1*, or *POU1F1*.
- Acquired CH or CPHD of various extent and severity can be caused by intrasellar or extrasellar tumors (mainly pituitary adenomas and craniopharyngiomas), neurosurgery and/or head irradiation, brain injuries, pituitary apoplexy, empty sella syndrome, parasellar aneurysm, subarachnoid hemorrhage, lymphocytic hypophysitis, infiltrative, or infectious diseases.
- In every case of CH, detailed investigation of other pituitary hormone secretion and MRI of the pituitary and the hypothalamus are obligatory.
- CH should be treated with L-T4 given orally once a day. Treatment is challenging as TSH cannot guide L-T4 dose adjustment.
- Before starting L-T4 administration, concomitant secondary adrenal insufficiency in the course of CPHD should always be excluded. If present, secondary adrenal insufficiency should be substituted before L-T4 treatment to avoid exacerbation of cortisol deficiency which may even precipitate adrenal crisis.
- Majority of CH patients require on average 1.5–1.6 μg L-T4/kg body weight daily. The target level of FT4 during treatment should be maintained within the upper half of the normal range.
- In congenital CH, L-T4 should be administered as soon as possible after birth. It is recommended to start treatment in neonates with 10–15 μg/kg body weight daily and the dose should be decreased as the child gets older.
- Concomitant treatment of CPHD significantly influences L-T4 requirement, and the dose should be re-established when estrogens, androgens, hydrocortisone, or rhGH are introduced during L-T4 treatment.

Cross-References

- Physiopathology, Diagnosis, and Treatment of Diabetes Insipidus
- Physiopathology, Diagnosis, and Treatment of Nonfunctioning Pituitary Adenomas
- Physiopathology, Diagnosis, and Treatment of Secondary Female Hypogonadism

References

Asakura Y, Abe K, Muroya K, Hanakawa J, Oto Y, Narumi S, Hasegawa T, Adachi M. Combined growth hormone and thyroid-stimulating hormone deficiency in a Japanese patient with a novel frameshift mutation in IGSF1. Horm Res Paediatr. 2015;84:349–54.

Biondi B, Wartofsky L. Treatment with thyroid hormone. Endocr Rev. 2014;35:433–512.

Bonomi M, Busnelli M, Beck-Peccoz P, Costanzo D, Antonica F, Dolci C, Pilotta A, Buzi F, Persani L. A family with complete resistance to thyrotropin-releasing hormone. N Engl J Med. 2009;360:731–4.

Collu R, Tang J, Castagné J, Lagacé G, Masson N, Huot C, Deal C, Delvin E, Faccenda E, Eidne KA, Van Vliet GA. Novel mechanism for isolated central hypothyroidism: inactivating mutations in the thyrotropin-releasing hormone receptor gene. J Clin Endocrinol Metab. 1997;82:1561–5.

Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, Pessah-Pollack R, Singer PA, Woeber KA. American association of clinical endocrinologists and American Thyroid Association taskforce on hypothyroidism in adults. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Thyroid. 2012;22:1200–35.

Heinen CA, Losekoot M, Sun Y, Watson PJ, Fairall L, Joustra SD, Zwaveling-Soonawala N, Oostdijk W, van den Akker EL, Alders M, Santen GW, van Rijn RR, Dreschler WA, Surovtseva OV, Biermasz NR, Hennekam RC, Wit JM, Schwabe JW, Boelen A, Fliers E, van Trotsenburg ASP. Mutations in TBL1X are associated with central hypothyroidism. J Clin Endocrinol Metab. 2016;101(12):4564–73.

Hermanns P, Couch R, Leonard N, Klotz C, Pohlenz J. A novel deletion in the thyrotropin Beta-subunit gene identified by array comparative genomic hybridization analysis causes central congenital hypothyroidism in a boy originating from Turkey. Horm Res Paediatr. 2014;82:201–5.

Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS, Sawka AM. American Thyroid Association task force on thyroid hormone replacement. Guidelines for the treatment of hypothyroidism: prepared by the American thyroid association task force on thyroid hormone replacement. Thyroid. 2014;24:1670–751.

Koulouri O, Nicholas AK, Schoenmakers E, Mokrosinski J, Lane F, Cole T, Kirk J, Farooqi IS, Chatterjee VK, Gurnell M, Schoenmakers N. A novel thyrotropin-releasing hormone receptor missense mutation (P81R) in central congenital hypothyroidism. J Clin Endocrinol Metab. 2016;101:847–51.

Léger J, Olivieri A, Donaldson M, Torresani T, Krude H, van Vliet G, Polak M, Butler G. ESPE-PES-SLEP-JSPE-APEG-APPES-ISPAE; congenital hypothyroidism consensus conference group. European society for Paediatric Endocrinology consensus guidelines on screening, diagnosis, and management of congenital hypothyroidism. Horm Res Paediatr. 2014;81:80–103.

Persani L. Clinical review: central hypothyroidism: pathogenic, diagnostic, and therapeutic challenges. J Clin Endocrinol Metab. 2012;97:3068–78.

Persani L, Bonomi M, Radin R, Beck-Peccoz P. Diagnostic and therapeutic challenges of acquired thyrotropic deficiency. Ann Endocrinol (Paris). 2012;73:138–40.

Schoenmakers N, Alatzoglou KS, Chatterjee VK, Dattani MT. Recent advances in central congenital hypothyroidism. J Endocrinol. 2015;227:R51–71.

Tenenbaum-Rakover Y, Turgeon MO, London S, Hermanns P, Pohlenz J, Bernard DJ, Bercovich D. Familial central hypothyroidism caused by a novel IGSF1 gene mutation. Thyroid. 2016; 26(12):1693–700.

Physiopathology, Diagnosis, and Treatment of Secondary Hyperthyroidism

8

Paolo Beck-Peccoz, Claudia Giavoli, Giulia Rodari, and Andrea Lania

Contents

Abstract

Thyrotropin-secreting pituitary adenomas (TSHomas) are a rare cause of secondary hyperthyroidism and account for less than 2% of all pituitary adenomas. In the last 30 years, the recognition of TSHomas has been facilitated by the routine use of ultrasensitive TSH immunometric assays, i.e., methods clearly able to distinguish between TSH concentration in normal controls and undetectable TSH levels in hyperthyroid patients, as well as by the direct measurement of circulating

P. Beck-Peccoz (✉)
University of Milan, Milan, Italy
e-mail: paolo.beckpeccoz@unimi.it

C. Giavoli · G. Rodari
Endocrinology and Metabolic Diseases Unit, Department of Clinical Sciences and Community Health, University of Milan, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy
e-mail: claudia.giavoli@gmail.com; rodarigiulia@gmail.com

A. Lania
Department of Biomedical Sciences, Humanitas University and Endocrinology Unit, Humanitas Research Hospital, Rozzano, Italy
e-mail: andrea.lania@humanitas.it

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_8

free thyroid hormones (FT4 and FT3). TSHomas must be promptly diagnosed whenever measurable levels of TSH in the presence of high FT4 and FT3 concentrations are documented. As similar biochemical picture is found in patients with thyroid hormone resistance (RTH), a differential diagnosis between the two disorders must be performed. Therefore, a correct diagnosis is fundamental in order to prevent dramatic consequences, such as improper thyroidectomy in patients with secondary hyperthyroidism due to TSHoma or unnecessary pituitary surgery in patients with RTH. The differential diagnosis between TSHomas and RTH mainly rests on dynamic testing, such as T3 suppression test, TRH tests, as well as injection of long acting somatostatin for 2 or more months.

First-line therapeutical approach to TSHomas remains pituitary neurosurgery, though in particular cases medical treatment should be considered. The medical treatment of TSHomas mainly rests on the administration of somatostatin analogs, such as octreotide and lanreotide, which are effective in reducing TSH secretion in the majority of patients with consequent normalization of FT4 and FT3 levels and restoration of the euthyroid state.

Keywords

Thyroid hyperfunction · TSH-induced · TSH-secreting pituitary adenomas (TSHomas) · Resistance to thyroid hormones · Thyrotropin (thyroid-stimulating hormone, TSH) · Pituitary glycoprotein hormone α-subunit (α-GSU) · Somatostatin analogs (octreotide, lanreotide) · Dopaminergic drugs (cabergoline, bromocriptine)

Introduction

Hyperthyroidism due to TSH-secreting pituitary adenoma (TSHoma) is a very rare disorder. In the past, it was estimated that less than 2% of all pituitary tumors are TSHomas with a prevalence of one case per million (Beck-Peccoz et al. 1996). However, recent data show that the prevalence of TSHomas is about 2–3 cases per million inhabitants and the incidence is about 0.2–0.3 cases per million per year (Raappana et al. 2010; Ónnestam et al. 2013).

Such a rare disorder is due to two different clinical situations, i.e., TSHomas and resistance to thyroid hormone action (RTH) (Refetoff et al. 1993; Gurnell et al. 2016). The main difference between these two syndromes consists in the presence of signs and symptoms of hyperthyroidism in patients with TSHoma, while RTH patients are in general euthyroid (so-called generalized RTH, GRTH). However, in a minority of RTH patients, features of hyperthyroidism may be present involving some organs, e.g., heart (tachycardia) and brain (nervousness, insomnia, attention deficit, hyperactivity) and not others. This particular form of RTH is termed pituitary RTH (PRTH). Both TSHomas and RTH are characterized by elevated serum circulating free thyroid hormone levels in the presence of measurable (normal or high) serum TSH concentrations. TSH secretion from the tumor is autonomous, whereas

thyrotropes of patients with RTH are refractory to the action of high levels of thyroid hormones, so that in both situations negative feedback mechanism is not operating (Beck-Peccoz et al. 1996; Refetoff et al. 1993; Foppiani et al. 2007; Kienitz et al. 2007; Ness-Abramof et al. 2007; Clarke et al. 2008; Gurnell et al. 2016; Beck-Peccoz et al. 2016). Moreover, the secretion by TSHomas is characterized by increase pulse frequency, delayed circadian rhythm, and increased basal secretion (Roelfsema et al. 2008).

Many years ago, Gershengorn and Weintraub (1975) suggested calling the above situations inappropriate TSH secretion, where "inappropriate" refers to the fact that, contrary to what happens in the classical hyperthyroidism, TSH is not inhibited in these particular forms of thyroid hyperfunction. Currently, we propose to classify TSHomas and PRTH as the two different forms of "central hyperthyroidism."

In the past, many patients with TSHoma were misdiagnosed as having a Graves' disease, and this mistake was due to the lack of sensitivity of the various assays for TSH measurement. Nowadays, serum TSH is routinely measured by ultrasensitive immunometric assays and circulating free thyroid hormones by direct immunoassays, a fact that greatly improved the diagnostic workup of hyperthyroid patients, allowing the recognition of the cases with unsuppressed TSH secretion and increased levels of free thyroid hormones. Therefore, central hyperthyroidism is now more often diagnosed earlier, and an increased number of patients with normal or elevated TSH levels in the presence of high free thyroid hormone concentrations have been recognized (Refetoff et al. 1993; Beck-Peccoz et al. 2016; Gurnell et al. 2016). When the diagnosis of central hyperthyroidism has been made, the differential diagnosis between TSHoma and RTH, particularly PRTH, is mandatory (Beck-Peccoz et al. 2013; Gurnell et al. 2016). Early diagnosis and correct therapeutic approach to patients with TSHomas may prevent the occurrence of neurological and endocrine complications, such as headache, visual field defects, and hypopituitarism, and should improve the rate of cure. Conversely, improper thyroid ablation or unnecessary pituitary surgeries in patients with RTH are the distressing consequences of the failure to recognize these different disorders.

In the present chapter, we will focus on the pathophysiology, clinical features, diagnostic procedures, differential diagnosis, and treatment of thyroid hyperfunction due to the presence of TSH-secreting pituitary adenomas.

Pathophysiology

TSHomas arise from adenomatous transformation of thyrotroph cell type. They are benign tumors, and till now transformation of a TSHoma into a carcinoma with multiple metastases has been described in only three patients (Mixson et al. 1993; Brown et al. 2006; Lee et al. 2012). Loss of pituitary glycoprotein hormone alpha-subunit (α-GSU) has been reported in one patient (Mixson et al. 1993), while in another case TSH-secreting carcinoma developed from a previously nonfunctioning pituitary adenoma (Brown et al. 2006). The majority of benign TSHomas (74%) secretes TSH alone, though this is often accompanied by unbalanced hypersecretion

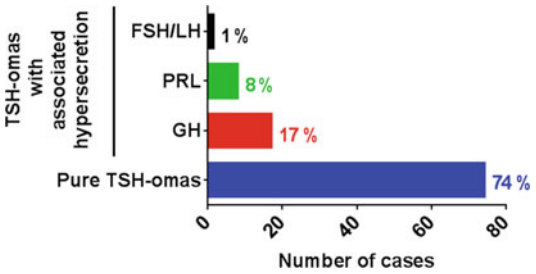


Fig. 1 Classification of TSH-secreting pituitary adenomas based on hormone secretion into circulation

of α-GSU, particularly in macroadenomas. Indeed, very few patients with TSH-secreting microadenomas present with hypersecretion of α-GSU (Socin et al. 2003).

About one-fourth of TSHomas are mixed adenomas and are characterized by concomitant hypersecretion of other anterior pituitary hormones, mainly growth hormone (GH) or prolactin (PRL), which are known to share with TSH common transcription factors, such as PROP-1, Pit-1 (POU1F1) and HESX-1 (Asa et al. 1993; Cohen and Radovick 2002; Mantovani et al. 2006; Pereira et al. 2016). Indeed, hypersecretion of TSH and GH is the most frequent association (17%), followed by hypersecretion of TSH and PRL (8%) and occasionally TSH and gonadotropins (Fig. 1). Interestingly, no mixed TSHomas were found to cosecrete ACTH molecules. Moreover, previous data have documented the existence of central hyperthyroidism due to a TSH-secreting adenoma, not associated with hypersecretion of other pituitary hormones, composed of two different cell types: one secreting α-GSU alone and another cosecreting α-GSU and TSH (Terzolo et al. 1991). Such an observation may explain the finding of an unbalanced release of α-subunit and TSH, responsible for very high α-subunit/TSH molar ratios, and why after therapy, certain thyrotroph adenomas recur only with α-GSU, and not TSH, hypersecretion (Kourides et al. 1977). Nonetheless, a positive immunohistochemistry for one or more pituitary hormone does not necessarily correlate with its or their hypersecretion in vivo (Bertholon-Grégoire et al. 1999; Pellegrini-Bouiller et al. 1997; Azzalin et al. 2016). Accordingly, clinically and biochemically silent thyrotropinomas have been reported (Banerjee et al. 2000; Lim et al. 2001; Rabbiosi et al. 2012; Tritos et al. 2013; Kirkman et al. 2014). Moreover, true TSH-secreting tumors associated with Hashimoto's thyroiditis and hypothyroidism have been documented (Iskandar et al. 2003; Losa et al. 2006; Ma et al. 2006; Beck-Peccoz et al. 2016).

In patients with confirmed biochemical findings of TSHoma and normal anterior pituitary gland, the presence of ectopic TSH secretion should be taken into consideration (Thompson et al. 2012). In fact, ectopic TSHoma occurring in the nasopharyngeal pituitary residue have been reported in six cases (Cooper and Wenig 1996; Pasquini et al. 2003; Collie and Collie 2005; Tong et al. 2013; Song et al. 2014; Wang et al. 2016).

Microadenomas (diameter < 1 cm) were recorded in less than 15% of the cases before 1996 (Socin et al. 2003), but their prevalence among the total number of TSHoma is progressively increasing due to improved testing of thyroid function and awareness among Endocrinologists and General Practitioners. Recently, the

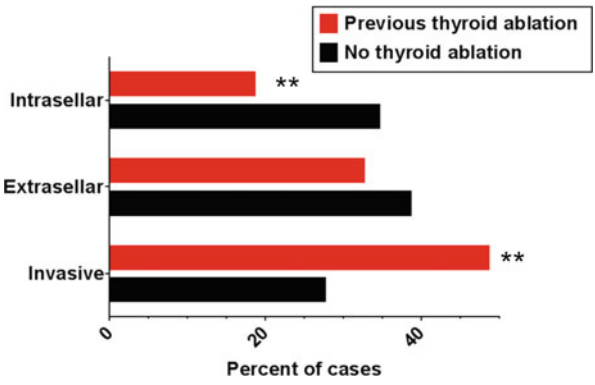


Fig. 2 Effects of previous thyroid ablation on the characteristic of TSHomas: "intrasellar" refers to both microadenomas and intrasellar macroadenomas, "extrasellar" to macroadenomas with suprasellar extension, and "invasive" to invasive macroadenomas. $^{**}P < 0.05$ by Fisher's exact test

percentage of microadenomas is ranging between 30% and 35% of all TSHomas (Malchiodi et al. 2014). Nonetheless, most TSHomas had been diagnosed at the stage of macroadenomas and showed localized or diffuse invasiveness into the surrounding structures, especially into the dura mater and bone (Brucker-Davis et al. 1999; Socin et al. 2002; Foppiani et al. 2007; Ness-Abramof et al. 2007; Clarke et al. 2008; Malchiodi et al. 2014; Beck-Peccoz et al. 2016). Extrasellar extension in the supra- and/or parasellar direction were present in the majority of cases. These findings highlight the deleterious effects of misdiagnosis and mistreatment of these adenomas, and the relevant action on tumor growth exerted by the reduction of circulating thyroid hormone levels through an altered feedback mechanism (Fig. 2).

The molecular mechanisms underlying the formation of TSHomas are presently unknown. Inactivation analysis of X-chromosome demonstrated that most pituitary adenomas, including the small number of TSHomas investigated, derive from the clonal expansion of a single initially transformed cell (Ma et al. 2003). Therefore, the presence of a transforming event providing gain of proliferative function followed by secondary mutations or alterations favoring tumor progression, presumably also apply to TSHomas. A number of proto-oncogenes, tumor suppressor genes, as well as pituitary specific genes, have been screened for mutations able to confer growth advantage to thyrotroph cells. As for other pituitary adenomas, no mutations in oncogenes commonly activated in human cancer, particularly *Ras*, have been reported in TSHomas. In contrast with GH-secreting adenomas in which the oncogene *gsp* is present in about 40% of cases, none of the screened TSHomas has been shown to express activating mutations of genes encoding for G protein subunits, such as αs, αq, α11, or αi2, or for TRH receptor (Dong et al. 1996). Negative results have also been reported when dopamine type 2 receptor gene was investigated (Friedman et al. 1994). Moreover, the transcription factor Pit-1 exerts a crucial role on cell differentiation and expression of PRL, GH, and TSH genes. Thus, Pit-1 gene has been studied and shown to be overexpressed, but not mutated, in 14 TSHomas (Pellegrini-Bouiller et al. 1997). As far as the possible loss of anti-oncogenes is concerned, no loss of *p53* was found in one TSHoma studied, while

loss of retinoblastoma gene (*Rb*) was not investigated in TSHomas. Another candidate gene is menin, whose mutations are responsible for multiple endocrine neoplasia type 1 (MEN1). In fact, about one-fourth of sporadic pituitary adenomas show loss of heterozygosity (LOH) on 11q13, where menin is located, and LOH on this chromosome seems to be associated with the transition from the noninvasive to the invasive phenotype. A recent screening study carried out on 13 TSHomas using polymorphic markers on 11q13 showed LOH in three, but none of them showed a menin mutation at sequence analysis (Asteria et al. 2001). Interestingly, hyperthyroidism due to TSHomas has been reported in five cases within a familial setting of MEN 1 (Beck-Peccoz et al. 1996; Taylor et al. 2000). Finally, germline mutations in the aryl hydrocarbon receptor interacting protein (AIP) are known to be involved in sporadic pituitary tumorigenesis, but mutations were found in a single patient with TSHoma (Barlier et al. 2007; Daly et al. 2010).

The extreme refractoriness of tumoral thyrotropes to the inhibitory action of thyroid hormones led to search for alterations in thyroid hormone receptor (TR) function (Gittoes et al. 1998; Tagami et al. 2011). Absence of TRα1, TRα2, and TRβ1 expression was reported in one TSHoma, but aberrant alternative splicing of TRβ2 mRNA encoding TRβ variant lacking T3 binding activity was recently shown as a mechanism for impaired T3-dependent negative regulation of both TSHβ and α-GSU in tumoral tissue (Ando et al. 2001a). Moreover, recent data suggest that somatic mutations of TRβ may be responsible for the defect in negative regulation of TSH secretion in some TSHomas (Ando et al. 2001b). Finally, it has recently been demonstrated that knock-in mutant mice harboring a mutation in the TRβ gene spontaneously develop TSHomas and that aberrant pituitary growth is due to activation of phosphatidylinositol 3-kinase signaling (Lu et al. 2008).

Finally, somatostatin receptor subtypes have been studied in some adenomas (Spada et al. 1985; Bertherat et al. 1992; Horiguchi et al. 2007; Gatto et al. 2011). The presence of subtypes 1, 2A, 3, and 5 were documented, a figure that may explain the high efficacy in blocking the hormone hypersecretion and tumor shrinkage during somatostatin analog treatment in the majority of patients with TSHoma (Horiguchi et al. 2007). Moreover, it has been shown that LOH and particular polymorphisms at the somatostatin receptor type 5 gene locus are associated with an aggressive phenotype and resistance to somatostatin analog treatment, possibly due to lack of somatostatin-induced inhibition of TSH secretion (Filopanti et al. 2004). Overexpression of basic fibroblast growth factor by some TSHomas suggests the possibility that it may play a role in the development of fibrosis and tumor cell proliferation of this unusual type of pituitary neoplasm (Sato et al. 1995; Ezzat et al. 1995). Moreover, the overexpression of basic fibroblast growth factor may explain the presence of some TSHomas defined “pituitary stone” (Webster et al. 1994).

Clinical Features

Signs and symptoms of hyperthyroidism are frequently associated with patients with TSHoma with those due to the compression of the surrounding anatomical structures, thus causing visual field defects, loss of vision, headache, and partial or

Table 1 Clinical features in patients with TSHoma (data from reports published until December 2016 and personal unpublished observations)

Clinical features	Patients with TSHoma
Age range (years)	8–86
Female/Male ratio	1.4
Previous thyroidectomy	28%
Severe thyrotoxicosis	15%
Goiter	85%
Thyroid nodule(s)	58%
Macroadenomas	72%
Visual field defects	30%
Headache	18%
Menstrual disorders	35%
Galactorrhea	25%
Acromegaly	17%

complete hypopituitarism (Table 1). Most patients have a long history of thyroid dysfunction, frequently misdiagnosed as Graves' disease, and about 30% of them had inappropriate thyroidectomy or radioiodine thyroid ablation (Beck-Peccoz et al. 1996; Brucker-Davis et al. 1999; Socin et al. 2003; Ness-Abramof et al. 2007; Varsseveld et al. 2014; Yamada et al. 2014; Beck-Peccoz et al. 2016). The deleterious effects of incorrect diagnosis are demonstrated by the finding that the occurrence of invasive macroadenomas is particularly high among patients with previous thyroid ablation by surgery or radioiodine (Fig. 2; Beck-Peccoz et al. 2016). This aggressive transformation of the tumor resembles that occurring in Nelson's syndrome after adrenalectomy for Cushing's disease.

TSHomas may be diagnosed at any age without sex preference (Nakayama et al. 2012; Yamada et al. 2014; Beck-Peccoz et al. 2016). The severity of hyperthyroidism is sometimes milder than expected on the basis of circulating thyroid hormone levels (Lim et al. 2001; Rabbiosi et al. 2012). In some acromegalic patients, signs and symptoms of hyperthyroidism may be clinically missed, as those of acromegaly overshadow them. Cardiotoxicosis with atrial fibrillation and/or cardiac failure and episodes of periodic paralysis are less frequent as compared to the frequency observed in patients with primary hyperthyroidism. The diagnosis of TSHoma may be delayed when autoimmune hypothyroidism is coexistent with the pituitary tumor (Langlois et al. 1996; Idiculla et al. 2001; Ma et al. 2003; Losa et al. 2006). In such a situation, the inadequate suppression of TSH during LT4 replacement therapy may suggest the presence of an autonomous TSH hypersecretion from the pituitary tumor.

Goiter is present in 85% of patients, even in those previously thyroidectomized, as thyroid residue may regrow as a consequence of TSH hyperstimulation (Table 1). Occurrence of uni- or multinodular goiter is frequent (about 58% of reported cases), whereas differentiated thyroid carcinomas were documented in a few cases (Gasparoni et al. 1998; Kishida et al. 2000; Ohta et al. 2001; Poggi et al. 2009; Ünlütürk et al. 2013). Progression towards toxic nodular goiter is very rare

(Abs et al. 1994). In contrast to what is observed Graves' disease, the occurrence of circulating antithyroid autoantibodies is similar to that found in the general population, being about 8%. However, Graves' hyperthyroidism may coexist with TSHoma in some patients (Kamoi et al. 1985; Kamoun et al. 2014). Bilateral exophthalmos occurred in a few patients who subsequently developed autoimmune thyroiditis, while unilateral exophthalmos due to orbital invasion by pituitary tumor was reported in three patients with TSHomas (Kourides et al. 1980; Yovos et al. 1981; Beck-Peccoz et al. 2016).

Disorders of the gonadal axis are frequent, menstrual disorders being present in all females with mixed TSH/PRL tumors and in one-third of those with pure TSHoma (Table 1). Central hypogonadism, delayed puberty, and decreased libido were also found in a number of males with TSHomas and/or mixed TSH/FSH adenomas.

Diagnostic Procedures

The finding of elevated levels of circulating thyroid hormones in the presence of measurable TSH concentrations is the biochemical hallmark of central hyperthyroidism. However, attention should be paid to the presence of circulating factors, such as antibodies against TSH or thyroid hormones, as well as abnormal forms of albumin or transthyretin that may interfere in the measurement methods of both TSH and thyroid hormones, giving spuriously high hormone levels and possibly simulating the biochemical characteristics of central hyperthyroidism (Koulouri et al. 2013; Koulouri and Gurnell 2013; Beck-Peccoz et al. 2016). Particular attention should be paid to the measurement of FT4, as only the "two-step" immunometric assays are to be employed, i.e., methods able to avoid possible interference due to the contact between serum interfering factors and the tracer (Schoenmakers et al. 2014).

No difference in basal values of TSH and free thyroid hormone levels was seen between patients with TSHoma and those with RTH (Fig. 3). However, an unbalanced hypersecretion of circulating free α-GSU levels and elevated α-GSU/TSH molar ratio are merely detected in patients with TSHoma (Table 1; Terzolo et al. 1991; Brucker-Davis et al. 1999; Socin et al. 2003). The calculation of α-GSU/TSH molar ratio increases the diagnostic sensitivity of hormone measurement, if such a calculation is done taking into account the serum levels of TSH and gonadotropins (Beck-Peccoz et al. 1992). Nevertheless, in a recent series of TSHomas, normal α-GSU levels and α-GSU/TSH molar ratio were observed in about 60% of the cases and more frequently in macroadenomas than in microadenomas (Socin et al. 2003). These findings are probably related to the fact that a higher number of microadenomas are diagnosed nowadays. Indeed, these data show a relationship between multiple hypersecretion and tumor volume: the bigger the tumor, the higher the number and the amount of hormones secreted in excess (Socin et al. 2003). It is worth noting that the bioactivity of secreted TSH may be enhanced or reduced in some patients with TSHoma (Beck-Peccoz and Persani 1994). These findings

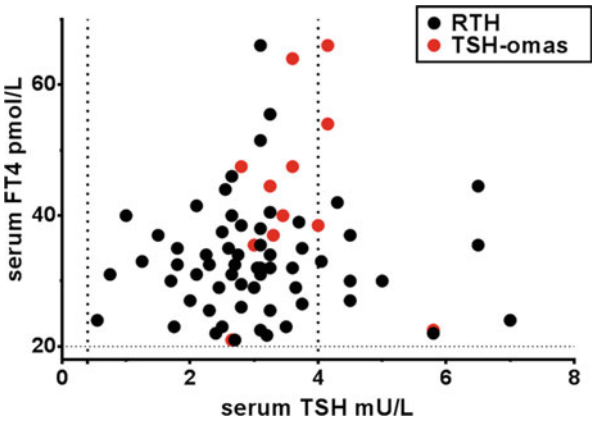


Fig. 3 Serum levels of Free Thyroxine (FT4) and TSH in patients with TSH-secreting pituitary adenoma (TSHomas) as compared to those with resistance to thyroid hormone action (RTH). Horizontal dashed line indicates the upper limit of FT4 normal range. The vertical dashed lines indicate the TSH normal range. No significant difference between RTH and TSHomas was found

explain the lack of correlation between serum TSH and FT3 circulating levels in patients with TSHoma.

In addition, measurements of several parameters of peripheral thyroid hormone action have been proposed to quantify the degree of tissue hyperthyroidism (Beck-Peccoz et al. 2016). In particular, bone (carboxyterminal cross-linked telopeptide of type I collagen, ICTP) and liver (sex-hormone binding globulin, SHBG) parameters may help in differentiating hyperthyroid patients with TSHoma from those with PRTH (Beck-Peccoz et al. 1990; Persani et al. 1997). In fact, as it occurs in the common forms of hyperthyroidism, such as Graves' disease or toxic goiter, patients with TSHoma have high ICTP and SHBG levels, while they are into the normal range in patients with hyperthyroidism due to PRTH (Table 2).

Dynamic testing is mandatory in the diagnosis of TSHoma, in particular the T3 suppression test (80–100 μg/day per 8–10 days). A complete inhibition of TSH secretion after T3 suppression test has never been recorded in patients with TSHoma (Table 2), particularly in those previously thyroidectomized (Brucker-Davis et al. 1999; Socin et al. 2003; Beck-Peccoz et al. 2016). In this latter condition, T3 suppression seems to be the most sensitive and specific test in assessing the presence of a TSHoma. However, this test is contraindicated in elderly patients or in those with coronary heart disease. Therefore, TRH test (200 μg iv) has been widely used in the work-up of these adenomas. In the vast majority of patients, TSH and α-GSU levels do not increase after TRH injection (Table 2). In patients with hyperthyroidism, discrepancies between TSH and α-GSU responses to TRH are pathognomonic of TSHomas cosecreting other pituitary hormones (Terzolo et al. 1991).

As most TSHomas maintain the sensitivity to native somatostatin and its analogs (octreotide and lanreotide) (Bertherat et al. 1992; Chanson et al. 1993; Gancel et al. 1994; Kuhn et al. 2000), we have recently treated a series of patients with TSHomas or PRTH with multiple injections of long-acting somatostatin analogs documenting a marked decrease of FT3 and FT4 levels in all patients but one with pituitary adenoma, while all patients with PRTH did not respond

Table 2 Parameters useful in differentiating patients with TSH-secreting pituitary adenomas (TSHomas) from those with resistance to thyroid hormones (RTH). Only patients with intact thyroid were taken into account. Data are obtained from patients followed at our Institution and are expressed as mean ± SE or percent

Parameter	TSHomas	RTH	*P*
	(n = 52)	(n = 84)	
Serum TSH mU/L	2.9 ± 0.8	2.4 ± 0.7	NS
High α-GSU levels	64%	3%	<0.0001
High α-GSU/TSH m.r.	78%	2%	<0.0001
Serum FT4 pmol/L	41.4 ± 5.3	36.2 ± 3.1	NS
Serum FT3 pmol/L	15.5 ± 1.7	13.9 ± 1.1	NS
Serum SHBG nmol/L	138 ± 25	54 ± 8	<0.0001
Serum ICTP μg/L	9.2 ± 5.2	3.1 ± 1.1	<0.001
Abnormal TSH response to			
T3 suppression[a]	100%	100%[b]	NS
Blunted TSH response			
To TRH test	90%	3%	<0.0001

Abbreviations: *α-GSU* pituitary glycoprotein hormone alpha-subunit, *SHBG* sex hormone-binding globulin, *ICTP* carboxyterminal cross-linked telopeptide of type I collagen

[a]T3 suppression test, i.e., Werner's test (80–100 μg T3 for 8–10 days). Quantitatively normal responses to T3, i.e., complete inhibition of both basal and TRH-stimulated TSH levels, have never been recorded in either group of patients

[b]Although abnormal in quantitative terms, TSH response to T3 suppression test was qualitatively normal in the majority of RTH patients

(Mannavola et al. 2005). Thus, administration of these long-acting analogs for at least 2 months can help in the differential diagnosis of problematic cases of central hyperthyroidism. Nevertheless, since none of these tests is of clear-cut diagnostic value, it is recommend the use of both T3 suppression and TRH test whenever possible, because the combination of their results increases the specificity and sensitivity of the diagnostic workup.

Finally, high-resolution computed tomography (CT) and nuclear magnetic resonance imaging (MRI) are nowadays the preferable tools for the visualization of a TSHoma. Most TSHomas were diagnosed at the stage of macroadenomas with frequent suprasellar extension or sphenoidal sinus invasion. Microadenomas are now reported with increasing frequency, accounting for 30–35% of all recorded cases in both clinical and surgical series (Socin et al. 2003; Kienitz et al. 2007). Recently, pituitary scintigraphy with radiolabeled octreotide (octreoscan) has been shown to successfully localize TSHomas expressing somatostatin receptors (Losa et al. 1997; Foppiani et al. 2007). However, the specificity of octreoscan is low, since positive scans can be seen in the case of a pituitary mass of different types, either secreting or nonsecreting. Such a procedure may be useful in the recognition of the possible ectopic localization of a TSHoma, as six cases of TSHomas were found in the nasopharyngeal region (Cooper and Wenig 1996; Pasquini et al. 2003; Collie and Collie 2005; Tong et al. 2013; Song et al. 2014; Wang et al. 2016).

Differential Diagnosis

The possible presence of Graves' disease, uni- or multinodular toxic goiter or activating mutations of TSH receptor, is ruled out by the finding of measurable levels of circulating TSH. Once the existence of central hyperthyroidism is confirmed and the presence of methodological interferences excluded (Koulouri et al. 2013; Koulouri and Gurnell 2013; Beck-Peccoz et al. 2016), several diagnostic steps have to be carried out to differentiate a TSHoma from RTH, particularly PRTH (Fig. 4). Indeed, the presence of neurological signs and symptoms (e.g., visual defects and headache) or clinical features of concomitant hypersecretion of other pituitary hormones (acromegaly, amenorrhea/galactorrhea) points to the presence of a TSHoma. Furthermore, an alteration of the pituitary gland at MRI or CT scan strongly supports the diagnosis of TSHoma. Nevertheless, the differential diagnosis with PRTH may be difficult when the pituitary adenoma is very small, or in the case of confusing lesions, such as ectopic tumors, empty sella, or pituitary incidentalomas, the latter lesion being frequently found in the general population. In these cases, elevated α-GSU concentrations or high α-GSU/TSH molar ratio, high circulating levels of parameters of peripheral thyroid hormone action (SHBG, ICTP),

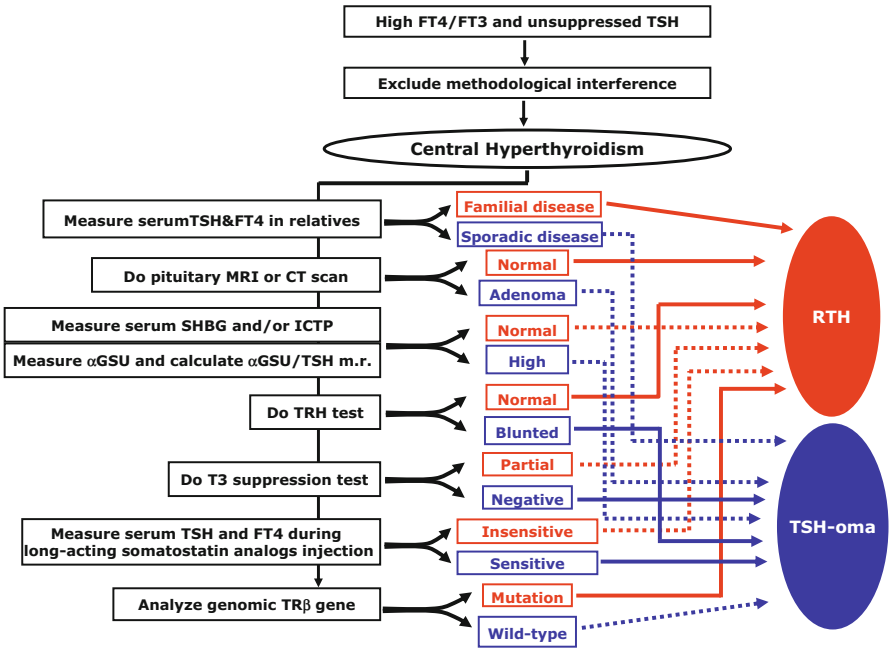


Fig. 4 Flow-chart for the differential diagnosis between resistance to thyroid hormone (RTH) and TSH-secreting pituitary adenoma (TSHoma). After exclusion of methodological interference, central hyperthyroidism is confirmed. A series of clinical, biochemical, and genetic tests may be necessary to reach the final diagnosis (modified from Beck-Peccoz et al. 2013)

TSH unresponsiveness to TRH stimulation or to T_3 suppression tests, decrease of TSH and free thyroid hormones during injection of long-acting analogs for at 2–3 months favor the presence of a TSHoma (Beck-Peccoz et al. 2013¸ Gurnell et al. 2016; Beck-Peccoz et al. 2016). Moreover, the finding of similar biochemical data in relatives definitely points to the presence of RTH, as familial cases of TSHomas have not been documented. Finally, an apparent association between TSHoma and RTH has been recently reported and somatic mutations in the thyroid-hormone receptor have been found in some tumors (Ando et al. 2001a; Ando et al. 2001b); thus, the occurrence of TSHoma in patients with RTH should be carefully taken into account (Watanabe et al. 1993; Safer et al. 2001; Teng et al. 2015).

Treatment

The therapeutical approach to TSHomas is the transsphenoidal or subfrontal adenomectomy, the choice of the route depending on the tumor volume and its suprasellar extension and invasiveness (Beck-Peccoz et al. 2013). The primary objectives of the treatment are in effect the removal of the pituitary tumor and the restoration of euthyroidism. The operation may be difficult as the tumor may present a marked fibrosis, possibly related to high expression of basic fibroblast growth factor (Ezzat et al. 1995). In addition, these tumors may be locally invasive involving the cavernous sinus, internal carotid artery, or other structures, thus rendering complete resection of the tumor either impractical or dangerous. Antithyroid drugs (methimazole or propylthiouracil, 20–30 and 200–300 mg/day, respectively) or somatostatin analogs, such as octreotide (100 μg sc, bid or tid), along with propranolol (80–120 mg/day orally) can be administered in order to restore euthyroidism before surgery (Wallace et al. 2015). However, this approach may cause TSH secretion from normal, nonadenomatous thyrotropes to be reactivated, so that one may lose a useful parameter to judge the complete removal of the adenoma, i.e., the unmeasurable levels of circulating TSH few days after successful surgery (Losa et al. 1996). If surgery is contraindicated or declined, pituitary radiotherapy (no less than 45 Gy fractionated at 2 Gy per day or 10–25 Gy in a single dose if a stereotactic Gamma Unit is available) should be considered (Mouslech et al. 2016). A successful experience of an invasive TSHoma associated with an unruptured aneurysm treated by two-stage operation and gamma knife has been reported (Ohki et al. 1999).

With the above therapeutic approaches, normalization of thyroid hormone circulating levels and apparent complete removal of tumor mass was observed in one-third of patients who may therefore be considered apparently cured (follow-up ranged from 2 to 121 months). Indeed, only the complete suppression of TSH secretion during T3 suppression test permits to document the total removal of the TSHoma (Fig. 5; Losa et al. 1996). An additional one-third of patients were judged improved, as normalization of thyroid hormone circulating levels was achieved in all, though there was no complete removal of the adenoma. Together these findings indicate that about two-thirds of TSHomas are under control with surgery and/or

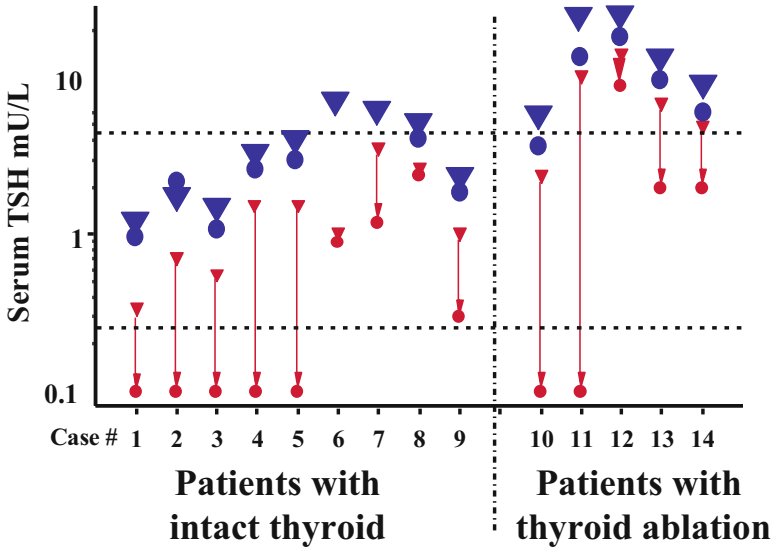


Fig. 5 Results of T3 suppression test carried out before (blue triangles and circles) and after (red triangles and circles) pituitary surgery in patients with TSH-secreting pituitary adenoma. Cases #1–9 had an intact thyroid, while patients #10–14 had a previous thyroid ablation. Horizontal dashed lines indicate serum TSH normal range. Note the lack of TSH suppression in all patients before pituitary tumor resection. Complete suppression of TSH levels, i.e., complete removal of the adenoma, was seen in about half of patients after surgery, independent of previous thyroid ablation

irradiation. In the remaining patients, TSH hypersecretion was unchanged after treatment, a fact that undoubtedly reflects the large size of the tumor and its invasiveness. Previous thyroid ablation or antithyroid drug treatments did not significantly affect the results of surgery and/or radiotherapy. Postsurgical deaths were reported in few cases. Evaluation of pituitary function, particularly ACTH secretion, should be carefully investigated soon after surgery and checked again every year, especially in patients treated with radiotherapy. In addition, in the case of surgical cure, postoperative TSH is undetectable and may remain low for weeks or even months, causing central hypothyroidism. A permanent central hypothyroidism may also occur due to the compression exerted by the tumor on the surrounding pituitary cells or the pituitary stalk or to surgical damage of the normal thyrotropes. Thus, transient or permanent L-T4 replacement therapy may be necessary. Finally, in few patients total thyroidectomy was performed after pituitary surgery failure, as the patients were at risk of thyroid storm.

Although the surgical cure rate of TSHomas is today improved due to an early diagnosis, some patients require medical therapy in order to control the hyperthyroidism or may be even treated medically as first-line therapy (Fliers et al. 2012; Gatto et al. 2015; Rimareix et al. 2015). Somatostatin analogs are highly effective in reducing TSH secretion by neoplastic thyrotropes (Beck-Peccoz et al. 1989; Orme et al. 1991; Bertherat et al. 1992; Chanson et al. 1993; Gancel et al. 1994; Kuhn et al. 2000;

Tayloret al. 2000; Horiguchi et al. 2007; Rabbiosi et al. 2012; Rimareix et al. 2015; Gatto et al. 2015), thus supporting the fact that the inhibitory pathway mediated by somatostatin receptors appears to be intact in such adenomas. Consistently, there is a good correlation between SRIH binding capacity and maximal biological response, as quantified by inhibition of TSH secretion and in vivo restoration of euthyroid state (Bertherat et al. 1992; Horiguchi et al. 2007). The presence of dopamine receptors in TSHomas was the rationale for therapeutic trials with dopaminergic agonists, such as bromocriptine or cabergoline (Chanson et al. 1984; Zuniga et al. 1997; Mulinda et al. 1999). Several studies have shown a large heterogeneity of TSH responses to dopaminergic agents both in primary cultures and in vivo, the best results having been achieved in mixed TSH/PRL adenomas (Spada et al. 1985). Effects of these two inhibitory agents should be nowadays re-evaluated in light of the demonstration of possible heterodimerization of somatostatin receptor type 5 and dopamine D2 receptor (Rocheville et al. 2000). Nonetheless, the medical treatment of TSHomas today rests on long-acting somatostatin analogs, such as octreotide LAR or lanreotide SR or lanreotide Autogel. Treatment with these analogs leads to a reduction of TSH and α-GSU secretion in almost all cases, with restoration of the euthyroid state in the majority of them. Circulating thyroid hormone levels normalized in more than 95% of patients. Goiter size was significantly reduced by somatostatin analog therapy in one-fifth of cases. Vision improvement was documented in two-third of patients and pituitary tumor mass shrinkage occurred in about 40% of them. Resistance to somatostatin analog treatment, true escape of TSH secretion from the inhibitory effects of the drugs or discontinuation of treatment due to side effects was documented in a minority of cases. Of interest are the findings of octreotide treatment in pregnant women, which was effective in restoring euthyroidism in the mother and had no side effects on development and thyroid function of the fetuses (Blackhurst et al. 2002; Chaiamnuay et al. 2003). Moreover, in almost all patients with mixed TSH/GH hypersecretion, signs and symptoms of acromegaly concomitantly disappeared. Patients on somatostatin analogs have to be carefully monitored, as untoward side effects, such as cholelithiasis and carbohydrate intolerance, may become manifest. The administered dose should be tailored for each patient, depending on therapeutic response and tolerance (including gastrointestinal side effects). The tolerance is usually very good, as gastrointestinal side effects are transient with long-acting analogs. The marked somatostatin-induced suppression of TSH secretion and consequent biochemical hypothyroidism seen in some patients may require a reduction of somatostatin analog doses or even an L-T4 substitution. Finally, no data have been reported on somatostatin analog treatment of TSHomas in patients who underwent thyroid ablation by thyroidectomy or radioiodine. Since aggressive and invasive macroadenomas are more frequently found in these patients (Beck-Peccoz et al. 2016), it is mandatory to treat them in order to block further growth of pituitary tumor mass.

The recurrence rate of TSHomas appears to be uncommon (Socin et al. 2003; Brucker-Davis et al. 1999; Losa et al. 1996, Malchiodi et al. 2014). Clinical and biochemical evaluation should be done two or three times after operation and then

every year. Pituitary imaging and visual field should be performed every 2–3 years, but should be done quickly if serum TSH and free thyroid hormone levels increase (Beck-Peccoz et al. 2013).

Summary

Patients with TSHoma present with a characteristic biochemical picture: high levels of circulating free thyroid hormones in the presence of normal/high concentrations of TSH. Such a biochemical picture may be caused by methodological interference in the measurement methods of both TSH and free thyroid hormones. Therefore, it is mandatory to check the results using different methods of measurement and to establish a close collaboration with the Institution laboratory.

The clinical appearance of hyperthyroidism may be mild, sometimes overshadowed by signs and symptoms of concomitant acromegaly or by neurological symptoms (headache, visual field defect) due to compression on the surrounding anatomical structures by the pituitary tumor.

T3 suppression and TRH tests, as well as injection for 2 or 3 months of long acting somatostatin, appear to be useful in the differential diagnosis between TSHomas and syndromes of thyroid hormone resistance. In addition, the findings of several parameters of peripheral thyroid hormone action in the hyperthyroid range may help in differentiating TSHoma from RTH.

Since the primary objectives of the treatment are the removal of the pituitary tumor, the restoration of euthyroidism and the prevention of neurological symptoms, such as headache, visual field defects, and hypopituitarism, the first approach to TSHomas remains the surgical removal of the adenoma. If surgery is contraindicated or declined, as well as in the case of surgical failure, the medical treatment is indicated and based on long acting somatostatin analog administration, such as octreotide or lanreotide, which are successful in the majority of patients with TSHoma.

References

Abs R, Stevenaert A, Beckers A. Autonomously functioning thyroid nodules in a patient with a thyrotropin-secreting pituitary adenoma: possible cause-effect relationship. Eur J Endocrinol. 1994;131:355–8.

Ando S, Sarlis NJ, Krishnan J, Feng X, Refetoff S, Zhang MQ, Oldfield EH, Yen PM. Aberrant alternative splicing of thyroid hormone receptor in a TSH-secreting pituitary tumor is a mechanism for hormone resistance. Mol Endocrinol. 2001a;15:1529–38.

Ando S, Sarlis NJ, Oldfield EH, Yen PM. Somatic mutation of TRbeta can cause a defect in negative regulation of TSH in a TSH-secreting pituitary tumor. J Clin Endocrinol Metab. 2001b;86:5572–6.

Asa SL, Puy LA, Lew AM, Sundmark VC, Elsholtz HP. Cell type-specific expression of the pituitary transcription activator pit-1 in the human pituitary and pituitary adenomas. J Clin Endocrinol Metab. 1993;77:1275–80.

Asteria C, Anagni M, Persani L, Beck-Peccoz P. Loss of heterozigosity of the MEN1 gene in a large series of TSH-secreting pituitary adenomas. J Endocrinol Investig. 2001;24:796–801.

Azzalin A, Appin CL, Schniederjan MJ, Constantin T, Ritchie JC, Veledar E, Oyesiku NM, Ioachimescu AG. Comprehensive evaluation of thyrotropinomas: single-center 20-year experience. Pituitary. 2016;19:183–93.

Banerjee AK, Sharma BS, Kak VK. Clinically and biochemically silent thyrotroph adenoma with oncocytic change. Neurol India. 2000;48:374–7.

Barlier A, Vanbellinghen JF, Daly AF, Silvy M, Jaffrain-Rea ML, Trouillas J, Tamagno G, Cazabat L, Bours V, Brue T, Enjalbert A, Beckers A. Mutations in the aryl hydrocarbon receptor interacting protein gene are not highly prevalent among subjects with sporadic pituitary adenomas. J Clin Endocrinol Metab. 2007;92:1952–5.

Beck-Peccoz P, Persani L. Variable biological activity of thyroid-stimulating hormone. Eur J Endocrinol. 1994;131:331–40.

Beck-Peccoz P, Mariotti S, Guillausseau PJ, Medri G, Piscitelli G, Bertoli A, Barbarino Rondena AM, Chanson P, Pinchera A. Treatment of hyperthyroidism due to inappropriate secretion of thyrotropin with the somatostatin analog SMS 201-995. J Clin Endocrinol Metab. 1989;68:208–14.

Beck-Peccoz P, Roncoroni R, Mariotti S, Medri G, Marcocci C, Brabant G, Forloni F, Pinchera A, Faglia G. Sex hormone-binding globulin measurement in patients with inappropriate secretion of thyrotropin (IST): evidence against selective pituitary thyroid hormone resistance in non-neoplastic IST. J Clin Endocrinol Metab. 1990;71:19–25.

Beck-Peccoz P, Persani L, Faglia G. Glycoprotein hormone alpha-subunit in pituitary adenomas. Trends Endocrinol Metab. 1992;3:41–5.

Beck-Peccoz P, Brucker-Davis F, Persani L, Smallridge RC, Weintraub BD. Thyrotropin-secreting pituitary tumors. Endocr Rev. 1996;17:610–38.

Beck-Peccoz P, Lania A, Beckers A, Chatterjee K, Wemeau JL. European thyroid association guidelines for the diagnosis and treatment of thyrotropin-secreting pituitary tumors. Eur Thyroid J. 2013;2:76–82.

Beck-Peccoz P, Lania A, Persani L. TSH-producing adenomas. In: Jameson JL, DeGroot LJ, editors. Endocrinology adult and pediatric. 7th ed. Philadelphia: Elsevier Saundres; 2016. p. 266–74.

Bertherat J, Brue T, Enjalbert A, Gunz G, Rasolonjanahary R, Warnet A, Jaquet P, Epelbaum J. Somatostatin receptors on thyrotropin-secreting pituitary adenomas: comparison with the inhibitory effects of octreotide upon in vivo and in vitro hormonal secretions. J Clin Endocrinol Metab. 1992;75:540–6.

Bertholon-Grégoire M, Trouillas J, Guigard MP, Loras B, Tourniaire J. Mono- and plurihormonal thyrotropic pituitary adenomas: pathological, hormonal and clinical studies in 12 patients. Eur J Endocrinol. 1999;140:519–27.

Blackhurst G, Strachan MW, Collie D, Gregor A, Statham PF, Seckl JE. The treatment of a thyrotropin-secreting pituitary macroadenoma with octreotide in twin pregnancy. Clin Endocrinol. 2002;57:401–4.

Brown RL, Muzzafar T, Wollman R, Weiss RE. A pituitary carcinoma secreting TSH and prolactin: a non-secreting adenoma gone awry. Eur J Endocrinol. 2006;154:639–43.

Brucker-Davis F, Oldfield EH, Skarulis MC, Doppman JL, Weintraub B. Thyrotropin-secreting pituitary tumors: diagnostic criteria, thyroid hormone sensitivity, and treatment outcome in 25 patients followed at the National Institutes of Health. J Clin Endocrinol Metab. 1999;84:476–86.

Chaiamnuay S, Moster M, Katz MR, Kim YN. Successful management of a pregnant woman with a TSH secreting pituitary adenoma with surgical and medical therapy. Pituitary. 2003;6:109–13.

Chanson P, Orgiazzi J, Derome PJ, Bression D, Jedynak CP, Trouillas J, Legentil P, Racadot J, Peillon F. Paradoxical response of thyrotropin to L-dopa and presence of dopaminergic receptors in a thyrotropin-secreting pituitary adenoma. J Clin Endocrinol Metab. 1984;59:542–6.

Chanson P, Weintraub BD, Harris AG. Octreotide therapy for thyroid stimulating-secreting pituitary adenomas. A follow-up of 52 patients. Ann Intern Med. 1993;119:236–40.

Clarke MJ, Erickson D, Castro MR, Atkinson JLD. Thyroid-stimulating pituitary adenomas. J Neurosurg. 2008;109:17–22.

Cohen LE, Radovick S. Molecular bases of pituitary hormone deficiencies. Endocr Rev. 2002;23:431–42.

Collie RB, Collie MJ. Extracranial thyroid-stimulating hormone-secreting ectopic pituitary adenoma of the nasopharynx. Otolaryngol Head Neck Surg. 2005;133:453–4.

Cooper DS, Wenig BM. Hyperthyroidism caused by an ectopic TSH-secreting pituitary tumor. Thyroid. 1996;6:337–43.

Daly AF, Tichomirowa MA, Petrossians P, Heliövaara E, Jaffrain-Rea ML, Barlier A, Naves LA, Ebeling T, Karhu A, Raappana A, Cazabat L, De Menis E, Montañana CF, Raverot G, Weil RJ, Sane T, Maiter D, Neggers S, Yaneva M, Tabarin A, Verrua E, Eloranta E, Murat A, Vierimaa O, Salmela PI, Emy P, Toledo RA, Sabaté MI, Villa C, Popelier M, Salvatori R, Jennings J, Longás AF, Labarta Aizpún JI, Georgitsi M, Paschke R, Ronchi C, Valimaki M, Saloranta C, De Herder W, Cozzi R, Guitelman M, Magri F, Lagonigro MS, Halaby G, Corman V, Hagelstein MT, Vanbellinghen JF, Barra GB, Gimenez-Roqueplo AP, Cameron FJ, Borson-Chazot F, Holdaway I, Toledo SP, Stalla GK, Spada A, Zacharieva S, Bertherat J, Brue T, Bours V, Chanson P, Aaltonen LA, Beckers A. Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. J Clin Endocrinol Metab. 2010;95:E373–83.

Dong Q, Brucker-Davis F, Weintraub BD, Smallridge RC, Carr FE, Battey J, Spiegel AM, Shenker A. Screening of candidate oncogenes in human thyrotroph tumors: absence of activating mutations of the Gαq, Gα11, Gαs, or thyrotropin-releasing hormone receptor genes. J Clin Endocrinol Metab. 1996;81:1134–40.

Ezzat S, Horvath E, Kovacs K, Smyth HS, Singer W, Asa SL. Basic fibroblast growth factor expression by two prolactin and thyrotropin-producing pituitary adenomas. Endocr Pathol. 1995;6:125–34.

Filopanti M, Ballaré E, Lania AG, Bondioni S, Verga U, Locatelli M, Zavanone LM, Losa M, Gelmini S, Peri A, Orlando C, Beck-Peccoz P, Spada A. Loss of heterozygosity at the SS receptor type 5 locus in human GH- and TSH-secreting pituitary adenomas. J Endocrinol Investig. 2004;27:937–42.

Fliers E, van Furth WR, Bisschop PH. Cure of a thyrotrophin (TSH)-secreting pituitary adenoma by medical therapy. Clin Endocrinol. 2012;77:788–90.

Foppiani L, Ruelle A, Cavazzani P, Del Monte P. TSH-secreting adenomas: rare pituitary tumors with multifaceted clinical and biological features. J Endocrinol Investig. 2007;30:603–9.

Friedman E, Adams EF, Höög A, Gejman PV, Carson E, Larsson C, De Marco L, Werner S, Fahlbusch R, Nordenskjöld M. Normal structural dopamine type 2 receptor gene in prolactin-secreting and other pituitary tumors. J Clin Endocrinol Metab. 1994;78:568–74.

Gancel A, Vuillermet P, Legrand A, Catus F, Thomas F, Kuhn JM. Effets of a slow-release formulation of the new somatostatin analogue lanreotide in TSH-secreting pituitary adenomas. Clin Endocrinol. 1994;40:421–8.

Gasparoni P, Rubello D, Persani L, Beck-Peccoz P. Unusual association between a thyrotropin-secreting pituitary adenoma and a papillary thyroid carcinoma. Thyroid. 1998;8:181–3.

Gatto F, Barbieri F, Castelletti L, Arvigo M, Pattarozzi A, Annunziata F, Saveanu A, Minuto F, Castellan L, Zona G, Florio T, Ferone D. In vivo and in vitro response to octreotide LAR in a TSH-secreting adenoma: characterization of somatostatin receptor expression and role of subtype 5. Pituitary. 2011;14:141–7.

Gatto F, Grasso LF, Nazzari E, Cuny T, Anania P, Di Somma C, Colao A, Zona G, Weryha G, Pivonello R, Ferone D. Clinical outcome and evidence of high rate post-surgical anterior hypopituitarism in a cohort of TSH-secreting adenoma patients: might somatostatin analogs have a role as first-line therapy? Pituitary. 2015;18:583–91.

Gershengorn MC, Weintraub BD. Thyrotropin-induced hyperthyroidism caused by selective pituitary resistance to thyroid hormone. A new syndrome of "inappropriate secretion of TSH". J Clin Invest. 1975;56:633–42.

Gittoes NJ, McCabe CJ, Verhaeg J, Sheppard MC, Franklyn JA. An abnormality of thyroid hormone receptor expression may explain abnormal thyrotropin production in thyrotropin-secreting pituitary tumors. Thyroid. 1998;8:9–14.

Gurnell M, Visser TJ, Beck-Peccoz P, Chatterjee VK. Resistance to thyroid hormone. In: Jameson JL, DeGroot LJ, editors. Endocrinology adult and pediatric. 7th ed. Philadelphia: Elsevier Saundres; 2016. p. 1648–65.

Horiguchi K, Yamada M, Umezawa R, Satoh T, Hashimoto K, Tosaka M, Yamada S, Mori M. Somatostatin receptor subtypes mRNA in TSH-secreting pituitary adenomas: a case showing a dramatic reduction in tumor size during short octreotide treatment. Endocr J. 2007;54:371–8.

Idiculla JM, Beckett G, Statham PF, Ironside JW, Atkin SL, Patrick AW. Autoimmune hypothyroidism coexisting with a pituitary adenoma secreting thyroid-stimulating hormone, prolactin and a-subunit. Ann Clin Biochem. 2001;38:566–71.

Iskandar SB, Supit E, Jordan RM, Peiris AN. Thyrotropin-secreting pituitary tumor and Hashimoto's disease: a novel association. South Med J. 2003;96:933–6.

Kamoi K, Mitsuma T, Sato H, Yokoyama M, Washiyama K, Tanaka R, Arai O, Takasu N, Yamada T. Hyperthyroidism caused by a pituitary thyrotrophin-secreting tumour with excessive secretion of thyrotrophin-releasing hormone and subsequently followed by Graves' disease in a middle-aged woman. Acta Endocrinol. 1985;110:373–82.

Kamoun M, d'Herbomez M, Lemaire C, Fayard A, Desailloud R, Huglo D, Wemeau JL. Coexistence of thyroid-stimulating hormone-secreting pituitary adenoma and Graves' hyperthyroidism. Eur Thyroid J. 2014;3:60–4.

Kienitz T, Quinkler M, Strasburger CJ, Ventz M. Long-term management in five cases of TSH-secreting pituitary adenomas: a single center study and review of the literature. Eur J Endocrinol. 2007;157:39–46.

Kirkman MA, Jaunmuktane Z, Brandner S, Khan AA, Powell M, Baldeweg SE. Active and silent thyroid-stimulating hormone-expressing pituitary adenomas: presenting symptoms, treatment, outcomes, and recurrence. World Neurosurg. 2014;82:1224–31.

Kishida M, Otsuka F, Kataoka H, Yokota K, Oishi T, Yamauchi T, Doihara H, Tamiya T, Mimura Y, Ogura T, Makino H. Hyperthyroidism in a patient with TSH-producing pituitary adenoma coexisting with thyroid papillary adenocarcinoma. Endocr J. 2000;47:731–8.

Koulouri O, Gurnell M. How to interpret thyroid function tests. Clin Med (Lond). 2013;13:282–6.

Koulouri O, Moran C, Halsall D, Chatterjee K, Gurnell M. Pitfalls in the measurement and interpretation of thyroid function tests. Best Pract Res Clin Endocrinol Metab. 2013;27:745–62.

Kourides IA, Ridgway EC, Weintraub BD, Bigos ST, Gershengorn MC, Maloof F. Thyrotropin-induced hyperthyroidism: use of alpha and beta subunit levels to identify patients with pituitary tumors. J Clin Endocrinol Metab. 1977;45:534–43.

Kourides IA, Pekonen F, Weintraub BD. Absence of thyroid-binding immunoglobulins in patients with thyrotropin-mediated hyperthyroidism. J Clin Endocrinol Metab. 1980;51:272–4.

Kuhn JM, Arlot S, Lefebvre H, Caron P, Cortet-Rudelli C, Archambaud F, Chanson P, Tabarin A, Goth MI, Blumberg J, Catus F, Ispas S, Beck-Peccoz P. Evaluation of the treatment of thyrotropin-secreting pituitary adenomas with a slow release formulation of the somatostatin analog lanreotide. J Clin Endocrinol Metab. 2000;85:1487–91.

Langlois MF, Lamarche JB, Bellabarba D. Long-standing goiter and hypothyroidism: an unusual presentation of a TSH-secreting adenoma. Thyroid. 1996;6:329–35.

Lee W, Cheung AS, Freilich R. TSH-secreting pituitary carcinoma with intrathecal drop metastase. Clin Endocrinol. 2012;76:604–6.

Lim EM, Bhagat CI, Walsh J. Asymptomatic thyrotropin-secreting pituitary microadenoma. Intern Med J. 2001;31:428–9.

Losa M, Giovanelli M, Persani L, Mortini P, Faglia G, Beck-Peccoz P. Criteria of cure and follow-up of central hyperthyroidism due to thyrotropin-secreting pituitary adenomas. J Clin Endocrinol Metab. 1996;81:3086–90.

Losa M, Magnani P, Mortini P, Persani L, Acerno S, Giugni E, Songini C, Fazio F, Beck-Peccoz P, Giovanelli M. Indium-111 pentetreotide single-photon emission tomography in patients with TSH-secreting pituitary adenomas: correlation with the effect of a single administration of octreotide on serum TSH levels. Eur J Nucl Med. 1997;24:728–31.

Losa M, Mortini P, Minelli R, Giovanelli M. Coexistence of TSH-secreting pituitary adenoma and autoimmune hypothyroidism. J Endocrinol Investig. 2006;29:555–9.

Lu C, Willingham MC, Furuya F, Cheng SY. Activation of phosphatidylinositol 3-kinase signaling promotes aberrant pituitary growth in a mouse model of thyrois-stimulating hormone-secreting pituitary tumors. Endocrinology. 2008;149:3339–45.

Ma W, Ikeda H, Watabe N, Kanno M, Yoshimoto T. A plurihormonal TSH-producing pituitary tumor of monoclonal origin in a patient with hypothyroidism. Horm Res. 2003;59:257–61.

Malchiodi E, Profka E, Ferrante E, Sala E, Verrua E, Campi I, Lania AG, Arosio M, Locatelli M, Mortini P, Losa M, Motti E, Beck-Peccoz P, Spada A, Mantovani G. Thyrotropin-secreting pituitary adenomas: outcome of pituitary surgery and irradiation. J Clin Endocrinol Metab. 2014;99:2069–76.

Mannavola D, Persani L, Vannucchi G, Zanardelli M, Fugazzola L, Verga U, Facchetti M, Beck-Peccoz P. Different response to chronic somatostatin analogues in patients with central hyperthyroidism. Clin Endocrinol. 2005;62:176–81.

Mantovani G, Asteria C, Pellegrini C, Bosari S, Alberti L, Bondioni S, Peverelli E, Spada A, Beck-Peccoz P. HESX1 expression in human normal pituitaries and pituitary adenomas. Mol Cell Endocrinol. 2006;247:135–9.

Mixson AJ, Friedman TC, Katz DA, Feuerstein IM, Taubenberger JK, Colandrea JM, Doppman JL, Oldfield EH, Weintraub BD. Thyrotropin-secreting pituitary carcinoma. J Clin Endocrinol Metab. 1993;76:529–33.

Mouslech Z, Somali M, Sakali AK, Savopoulos C, Mastorakos G, Hatzitolios AI. TSH-secreting pituitary adenomas treated by gamma knife radiosurgery: our case experience and a review of the literature. Hormones (Athens). 2016;15:122–8.

Mulinda JR, Hasinski S, Rose LI. Successful therapy for a mixed thyrotropin-and prolactin-secreting pituitary macroadenoma with cabergoline. Endocr Pract. 1999;5:76–9.

Nakayama Y, Jinguji S, Kumakura S, Nagasaki K, Natsumeda M, Yoneoka Y, Saito T, Fujii Y. Thyroid-stimulating hormone (thyrotropin)-secretion pituitary adenoma in an 8-year-old boy: case report. Pituitary. 2012;15:110–5.

Ness-Abramof R, Ishay A, Harel G, Sylvetzky N, Baron E, Greenman Y, Shimon I. TSH-secreting pituitary adenomas. Pituitary. 2007;10:307–10.

Ohki M, Sato K, Tuchiya D, Sato S, Saito S, Kinjo T, Kayama T, Jokura H, Yoshimoto T. A case of TSH-secreting pituitary adenoma associated with an unruptured aneurysm: successful treatment by two-stage operation and gamma knife. No To Shinkei. 1999;51:895–9.

Ohta S, Nishizawa S, Oki Y, Namba H. Coexistence of thyrotropin-producing pituitary adenoma with papillary adenocarcinoma of the thyroid: a case report and surgical strategy. Pituitary. 2001;4:271–4.

Ónnestam L, Berinder K, Burman P, Dahlqvist P, Engström BE, Wahlberg J, Nyström HF. National incidence and prevalence of TSH-secreting pituitary adenomas in Sweden. J Clin Endocrinol Metab. 2013;98:626–35.

Orme SM, Lamb JT, Nelson M, Belchetz PE. Shrinkage of thyrotrophin secreting pituitary adenoma treated with octreotide. Postgrad Med J. 1991;67:466–8.

Pasquini E, Faustini-Fustini M, Sciarretta V, Saggese D, Roncaroli F, Serra D, Frank G. Ectopic TSH-secreting pituitary adenoma of the vomerosphenoidal junction. Eur J Endocrinol. 2003;148:253–7.

Pellegrini-Bouiller I, Morange-Ramos I, Barlier A, Gunz G, Enjalbert A, Jaquet P. Pit-1 gene expression in human pituitary adenomas. Horm Res. 1997;47:251–8.

Pereira BD, Raimundo L, Mete O, Oliveira A, Portugal J, Asa SL. Monomorphous plurihormonal pituitary adenoma of pit-1 lineage in a giant adolescent with central hyperthyroidism. Endocr Pathol. 2016;27:25–33.

Persani L, Preziati D, Matthews CH, Sartorio A, Chatterjee VK, Beck-Peccoz P. Serum levels of carboxyterminal cross-linked telopeptide of type I collagen (ICTP) in the differential diagnosis of the syndromes of inappropriate secretion of TSH. Clin Endocrinol. 1997;47:207–14.

Poggi M, Monti S, Pascucci C, Toscano V. A rare case of follicular thyroid carcinoma in a patient with thyrotropin-secreting pituitary adenoma. Am J Med Sci. 2009;337:462–5.

Raappana A, Koivukangas J, Ebeling T, Pirilä T. Incidence of pituitary adenomas in Northern Finland in 1992–2007. J Clin Endocrinol Metab. 2010;95:4268–75.

Rabbiosi S, Peroni E, Tronconi GM, Chiumello G, Losa M, Weber G. Asymptomatic thyrotropin-secreting pituitary macroadenoma in a 13-year-old girl: successful first-line treatment with somatostatin analogs. Thyroid. 2012;22:1076–9.

Refetoff S, Weiss RE, Usala SJ. The syndromes of resistance to thyroid hormone. Endocr Rev. 1993;14:348–99.

Rimareix F, Grunenwald S, Vezzosi D, Rivière LD, Bennet A, Caron P. Primary medical treatment of thyrotropin-secreting pituitary adenomas by first-generation somatostatin analogs: a case study of seven patients. Thyroid. 2015;25:877–82.

Rocheville M, Lange DC, Kumar U, Patel SC, Patel RC, Patel YC. Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science. 2000;288:154–7.

Roelfsema F, Pereira AM, Keenan DM, Veldhuis JD, Romijn JA. Thyrotropin secretion by thyrotropinomas is characterized by increased pulse frequency, delayed diurnal rhythm, enhanced basal secretion, spikiness, and disorderliness. J Clin Endocrinol Metab. 2008;93:4052–7.

Safer JD, Colan SD, Fraser LM, Wondisford FE. A pituitary tumor in a patient with thyroid hormone resistance: a diagnostic dilemma. Thyroid. 2001;11:281–91.

Sato S, Kanai N, Kanai H, Sugase T, Hanada M, Hayakawa T, Saito Y, Oonishi T, Oda Y, Miyagawa J. TSH-secreting fibrous pituitary adenoma showing calcification: a case report. No Shinkei Geka. 1995;23:259–63.

Socin HV, Chanson P, Delemer B, Tabarin A, Rohmer V, Mockel J, Stevenaert A, Beckers A. The changing spectrum of TSH-secreting pituitary adenomas: diagnosis and management in 43 patients. Eur J Endocrinol. 2003;148:433–42.

Song M, Wang H, Song L, Tian H, Ge Q, Li J, Zhu Y, Li J, Zhao R, Ji HL. Ectopic TSH-secreting pituitary tumor: a case report and review of prior cases. BMC Cancer. 2014;14:544–7.

Spada A, Bassetti M, Martino E, Giannattasio G, Beck-Peccoz P, Sartorio A, Vallar L, Baschieri L, Pinchera A, Faglia G. In vitro studies on TSH secretion and adenylate cyclase activity in a human TSH-secreting pituitary adenoma. Effects of somatostatin and dopamine. J Endocrinol Investig. 1985;8:193–8.

Schoenmakers N, Moran C, Campi I, Agostini M, Bacon O, Rajanayagam O, Schwabe J, Bradbury S, Barrett T, Geoghegan F, Druce M, Beck-Peccoz P, O' Toole A, Clark P, Bignell M, Lyons G, Halsall D, Gurnell M, Chatterjee K. A novel albumin gene mutation (R222I) in familial dysalbuminemic hyperthyroxinemia. J Clin Endocrinol Metab. 2014;99:E1381–6.

Tagami T, Usui T, Shimatsu A, Beniko M, Yamamoto H, Moriyama K, Naruse M. Aberrant expression of thyroid hormone receptor beta isoform may cause inappropriate secretion of TSH in a TSH-secreting pituitary adenoma. J Clin Endocrinol Metab. 2011;96:E948–52.

Taylor TJ, Donlon SS, Bale AE, Smallridge RC, Francis TB, Christensen RS, Burma KD. Treatment of a thyrotropinoma with octreotide-LAR in a patient with multiple endocrine neoplasia-1. Thyroid. 2000;10:1001–7.

Teng X, Jin T, Brent GA, Wu A, Teng W, Shan Z. A patient with a thyrotropin-secreting microadenoma and resistance to thyroid hormone (P453T). J Clin Endocrinol Metab. 2015;100:2511–4.

Terzolo M, Orlandi F, Bassetti M, Medri G, Paccotti P, Cortelazzi D, Angeli A, Beck-Peccoz P. Hyperthyroidism due to a pituitary adenoma composed of two different cell types, one secreting alpha-subunit alone and another cosecreting alpha-subunit and thyrotropin. J Clin Endocrinol Metab. 1991;72:415–21.

Thompson LD, Seethala RR, Muller S. Ectopic sphenoid sinus pituitary adenoma (ESSPA) with normal anterior pituitary gland: a clinicopathologic and immuno-phenotypic study of 32 cases with a comprehensive review of the english literature. Head Neck Pathol. 2012;6:75–100.

Tong A, Xia W, Qi F, Jin Z, Yang D, Zhang Z, Li F, Xing X, Lian X. Hyperthyroidism caused by an ectopic thyrotropin-secreting tumor of the nasopharynx: a case report and review of the literature. Thyroid. 2013;23:1172–7.

Tritos NA, Eppakayala S, Swearingen B, Hedley-Whyte ET, Miller KK, Nachtigall LB, Grinspoon SK, Biller BM, Klibanski A. Pathologic and clinical features of pituitary adenomas showing TSH immunoreactivity. Pituitary. 2013;16:287–93.

Ünlütürk U, Sriphrapradang C, Erdoğan MF, Emral R, Güldiken S, Refetoff S, Güllü S. Management of differentiated thyroid cancer in the presence of resistance to thyroid hormone and TSH-secreting adenomas: a report of four cases and review of the literature. J Clin Endocrinol Metab. 2013;98:2210–7.

Varsseveld NC, Bisschop PH, Biermasz NR, Pereira AM, Fliers E, Drent ML. A long-term follow-up study of eighteen patients with thyrotrophin-secreting pituitary adenomas. Clin Endocrinol. 2014;80:395–402.

Wallace IR, Healy E, Cooke RS, Ellis PK, Harper R, Hunter SJ. TSH-secreting pituitary adenoma: benefits of pre-operative octreotide. Endocrinol Diabetes Metab Case Rep. 2015;2015:150007.

Wang Q, Lu XJ, Sun J, Wang J, Huang CY, Wu ZF. Ectopic suprasellar thyrotropin-secreting pituitary adenoma: case report and literature review. World Neurosurg. 2016;95:617.e13–8.

Watanabe K, Kameya T, Yamauchi A, Yamamoto N, Kuwayama A, Takei I, Maruyama H, Saruta T. Thyrotropin-producing microadenoma associated with pituitary resistance to thyroid hormone. J Clin Endocrinol Metab. 1993;76:1025–30.

Webster J, Peters JR, John R, Smith J, Chan V, Hall R, Scanlon MF. Pituitary stone: two cases of densely calcified thyrotropin-secreting pituitary adenomas. Clin Endocrinol. 1994;40:137–43.

Yamada S, Fukuhara N, Horiguchi K, Yamaguchi-Okada M, Nishioka H, Takeshita A, Takeuchi Y, Ito J, Inoshita N. Clinicopathological characteristics and therapeutic outcomes in thyrotropin-secreting pituitary adenomas: a single-center study of 90 cases. J Neurosurg. 2014;121:1462–73.

Yovos JG, Falko JM, O'Dorisio TM, Malarkey WB, Cataland S, Capen CC. Thyrotoxicosis and a thyrotropin-secreting pituitary tumor causing unilateral exophthalmos. J Clin Endocrinol Metab. 1981;53:338–43.

Zuniga S, Mendoza V, Espinoza IF, Zarate A, Mason M, Mercado M. A plurihormonal TSH-secreting pituitary microadenoma: report of a case with an atypical clinical presentation and transient response to bromocriptine therapy. Endocr Pathol. 1997;8:81–6.

Physiopathology, Diagnosis, and Treatment of Secondary Female Hypogonadism

9

Athanasios Antoniou-Tsigkos, Djuro Macut, and George Mastorakos

Contents

A. Antoniou-Tsigkos (✉) · G. Mastorakos
Endocrine Unit, ARETAIEION University Hospital, National and Kapodistrian University of Athens, Athens, Greece
e-mail: thanasisantsi@gmail.com; mastorakg@gmail.com

D. Macut
Clinic of Endocrinology, Diabetes and Metabolic Diseases, University of Belgrade, Belgrade, Serbia
e-mail: djmacut@gmail.com

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_9

Abstract

Female hypogonadism is a state characterized by absent or decreased ovarian function. It results from a gonadal (primary hypogonadism) or an extragonadal (secondary hypogonadism) princeps defect. In secondary hypogonadism, hypothalamic gonadotropin-releasing hormone or/and pituitary-secreted gonadotropins (follicle-stimulating hormone, luteinizing hormone) are either deficient or inactive leading to decreased secretion of gonadal steroids and subsequent amenorrhea. In certain conditions, both hypothalamic and pituitary dysfunctions are present. The genetic causes of secondary hypogonadism manifest mainly as congenital genetic syndromes (i.e., Kallmann syndrome) while some of them have been attributed to recognized single gene mutations and others have been characterized as idiopathic forms. Acquired causes of secondary hypogonadism include reversible causes such as functional hypothalamic amenorrhea, drugs, chronic illnesses, and irreversible causes such as central nervous system insults (trauma, irradiation, and intracranial tumors). Diagnosis should take in consideration the age at the clinical presentation (prepubertal or postpubertal), the physical findings as well as biochemical and imaging findings. Genetic investigation can be employed for more precise diagnosis. Finally, treatment should focus upon *the treatment of the causal factor* wherever possible and *the hormone replacement therapy*. The latter is adapted to the age of diagnosis of secondary female hypogonadism (prepubertal vs. postpubertal).

Keywords

Secondary hypogonadism · Hypogonadotropic hypogonadism · Hypogonadism · Kisspeptin · Hypothalamic amenorrhea · Kallman syndrome · Idiopathic hypogonadotropic hypogonadism · Hormone replacement therapy

List of Abbreviations

ACTH	Adrenocorticotropic hormone
ADH	Antidiuretic hormone
cAMP	cyclic adenosine monophosphate
AVP	Antidiuretic hormone
AMH	Anti-Mullerian hormone
hCG	Human chorionic gonadotropin
CBT	Cognitive behavioral therapy
CDGP	Constitutional delay of growth and puberty
CRH	Corticotropin-releasing hormone
DHEA	Dehydroepiandrosterone
FHA	Functional hypothalamic amenorrhea

FSH	Follicle-stimulating hormone
GABA	Gamma-Aminobutyric Acid
GnRH	Gonadotropin-releasing hormone
GH	Growth hormone
HPO	Hypothalamic-pituitary-ovarian
IHH	Isolated hypogonadotrophic hypogonadism
IGF-1	Insulin growth factor 1
KDNY	Kisspeptin-Neurokinin B-Dynorphin
KS	Kallmann syndrome
LDL	Low-density lipoprotein
LH	Luteinizing hormone
α-MSH	α-Melanocyte-Stimulating hormone
NPY	Neuropeptide Y
PTH	Parathyroid hormone
PVN	paraventricular nucleus
POA	Preoptic area
PRL	Prolactin
POMC	Proopiomelanocortin
SHBG	Sex hormone binding globulin
TSH	Thyroid stimulating hormone
TRH	Thyrotropin-releasing hormone

Introduction

Female hypogonadism is a state characterized by absent or decreased ovarian function. It results from a gonadal (primary hypogonadism) or an extragonadal (secondary hypogonadism) princeps defect. In secondary hypogonadism, hypothalamic gonadotropin-releasing hormone (GnRH) or/and pituitary-secreted gonadotropins (follicle-stimulating hormone, FSH; luteinizing hormone, LH) are either deficient or inactive leading to decreased secretion of gonadal steroids and subsequent amenorrhea. Throughout the medical literature, the term secondary hypogonadism may be encountered also as GnRH deficiency or gonadotropin deficiency depending on the level of the defect (suprapituitary or pituitary, respectively); as central; or as hypogonadotropic hypogonadism given that, independently of the level of the abnormality (suprapituitary or/and pituitary), the result is the same, i.e., decreased gonadotropins levels.

Anatomy, Embryology, and Physiology of the Female Gonadal Axis

The origins of the female gonadal axis must be sought at the emergence of embryonic GnRH neurons. The control of GnRH release is complex depending on multiple neurotransmitters and neurohormones, which include catecholamines, opiates,

neuropeptide Y, neurotensin, gamma-aminobutyric acid (GABA), kisspeptin, neurokinin B, dynorphin, corticotropin-releasing hormone (CRH), and prolactin (PRL), as well as gonadal steroids.

Olfactory Bulb and the Migration of Embryonic GnRH Neurons

In the developing embryo, hypothalamic GnRH neurons arise from an epithelial cluster of cells in the olfactory placode outside the central nervous system. However, some studies have shown that they may arise from the anterior pituitary placode and the cranial neural crest, and then transiently associate with the olfactory placode (Whitlock 2005). Regardless of their site of origin, fetal cells in the olfactory area can respond to odorant stimuli and secrete GnRH (Barni et al. 1999). Subsequently, these neurons migrate into the olfactory bulb and olfactory tract before continuing to move into the mediobasal hypothalamus in the preoptic area (POA) and the arcuate nucleus (Gibson et al. 1984). This migratory process is regulated by a number of factors such as anosmin-1 (the product of ANOS 1 gene), neuropilins, leukemia inhibitory factor, and fibroblast growth factor (FGF) receptor (R) 1. The association of GnRH neurons with the olfactory bulb and tract might explain the high frequency (men are the overwhelming majority) of anosmia (lack of smell) in patients with GnRH deficiency.

Hypothalamus, GnRH Neurons and the Kisspeptin-Neurokinin B-Dynorphin Neuronal System

The hypothalamus represents 0.3% of the total brain, measures 4 by 3 cm, and weighs approximately 10 g. Its nomenclature results from its anatomical position under the thalamus. It contains many nuclei responsible for endocrine regulation, reproduction, metabolism, temperature regulation, emotional responses, and electrolyte balance (Mancall and Brock 2011). On one side, it is bordered by the anterior part of the subthalamus, the internal capsule, and the optic tract while on the other side it forms the lateral wall and floor of the third ventricle. The median eminence of the hypothalamus extends to the anterior pituitary and via its neurosecretory neurons affects hormone production from the anterior pituitary. The hypothalamus comprises three zones: lateral, medial, and periventricular. Within each zone lie several nuclei. The cell bodies of the neurosecretory neurons which produce GnRH, a hormone pertinent to reproduction, are located in the median POA and the arcuate/infundibular nucleus of the hypothalamus, forming a neuronal network with projections to the median eminence (Fig. 1). In humans, the number of GnRH neurons is estimated to range between 1000 and 1500. From there, GnRH is secreted into the fenestrated capillaries of portal circulation and therein carried to the anterior pituitary where it enhances the release of FSH and LH.

GnRH, a decapeptide, can be detected in the fetal hypothalamus as early as 9–10 weeks of gestation (Grumbach and Kaplan 1990). To date, three types of

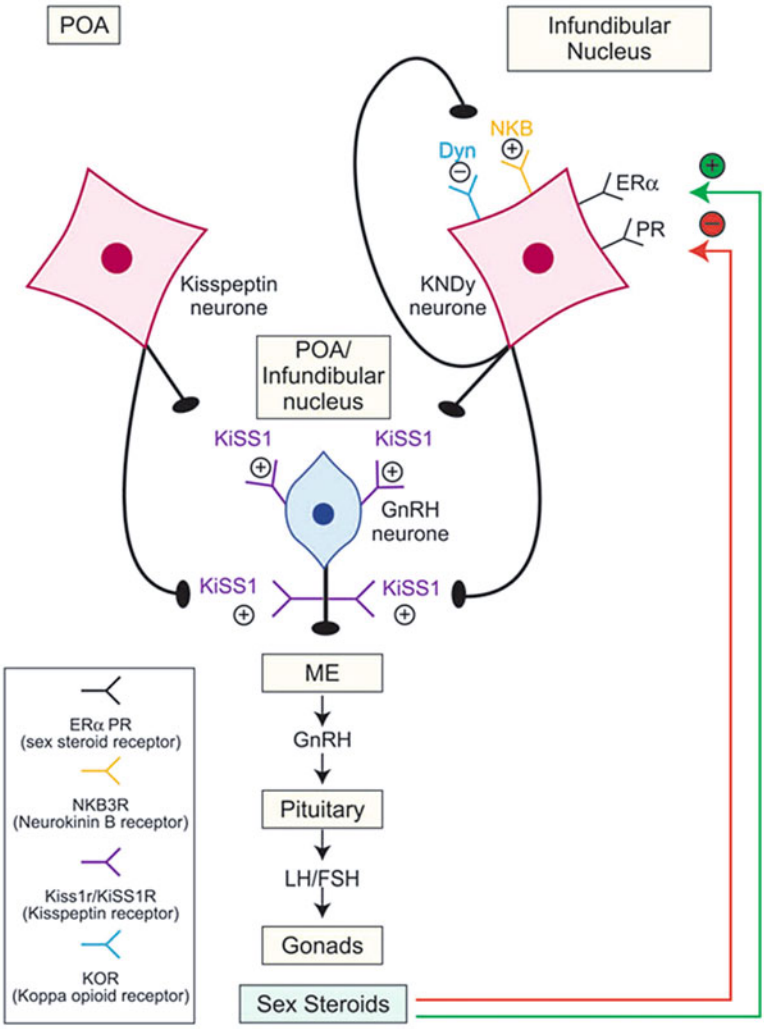


Fig. 1 Neuroanatomy of kisspeptin-GnRH pathway and the control of HPG axis in humans. Kisspeptin signals directly to GnRH neurons, which express KISS1R. Kisspeptin neurons are found within the hypothalamus in the preoptic area (POA) and the infundibular nucleus. Kisspeptin neurons coexpress neurokinin B and dynorphin (KNDy neurons), which autosynaptically regulate kisspeptin secretion (via neurokinin B receptor and kappa opioid peptide receptor). In humans, infundibular KNDy neurons relay negative (*red*) and positive (*green*) feedback (Adapted from Skorupskaite et al. 2014)

GnRH (GnRH-I, GnRH-II, and GnRH-III) have been detected in humans (Yahalom et al. 1999; White et al. 1998). GnRH-I is the classic hypothalamic hormone responsible for the regulation, synthesis, storage, and secretion of pituitary gonadotropins (Yao et al. 2011). It is synthesized from a larger, 92 amino acid precursor

molecule (Nikolics et al. 1985). Its half-life is 2–4 min, and it is difficult to be detected in the periphery because of its rapid cleavage. GnRH-II was first described in brain tissue but, unlike GnRH-I, it is produced mainly in peripheral tissues, such as the endometrium, the ovaries, the breast (Fister et al. 2009; Leung et al. 2003), and the human placenta (Chou et al. 2004). It differs from GnRH-I by three amino acids at positions 5, 7, and 8. GnRH-III was detected in hypothalamic neurons (Yahalom et al. 1999) although its role in humans is unclear.

Hypothalamic GnRH is secreted in two distinct modes (Maeda et al. 2010): *pulsatile* and *surge* modes. In the *pulsatile* mode of secretion, there are distinct pulses (episodic) of GnRH secretion into the portal circulation. GnRH concentrations are undetectable during interpulse intervals. The pulsatile GnRH release is subject to pulse frequency and intensity modifications during the menstrual cycle. Pulsatile GnRH secretion is followed by pulsatile LH and FSH release (Dierschke et al. 1970; Naftolin et al. 1972) (Fig. 2). In women, the frequency and amplitude of LH pulses depend on the menstrual cycle phase, with pulses every 1–2 h during the early follicular phase, merging into a continuous midcycle surge and to decreased pulse frequency down to 1 pulse every 4 h during the luteal phase (Yen and Tsai 1971). In

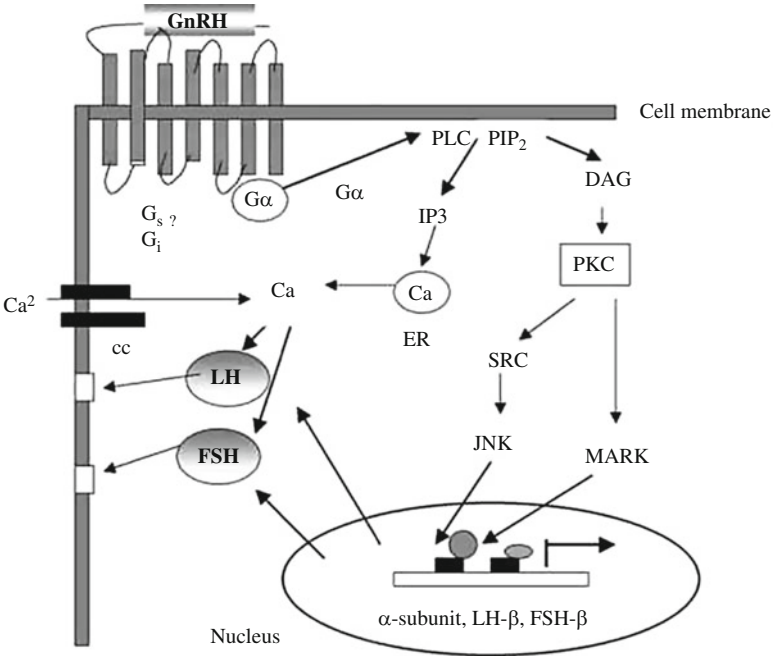


Fig. 2 Representation of the GnRH receptor pathway depicting the G protein-coupled transmembrane receptor. Activation of the GnRH-IR leads to the activation of phospholipase C, which in its turn generates the second messengers inositol triphosphate and diacyl glycerol. The subsequent stimulation of protein kinase and cyclic adenosine monophosphate (cAMP) leads to release of calcium ions which in turn release the gonadotropines LH and FSH.

humans, LH pulse frequency is used as a surrogate marker of GnRH pulsatility, as ethical considerations and technical challenges preclude sampling of hypophyseal blood or cerebrospinal fluid to measure GnRH concentrations directly (Sauder et al. 1984; Reame et al. 1985). Higher frequencies of pulsatile GnRH release lead to increased LH and decreased FSH production, thus increasing the LH/FSH ratio, while lower frequencies lead to decreased ratio (Wildt et al. 1981a). Continuous GnRH secretion leads to the suppression of FSH and LH release as well as suppression of FSH and LH gene transcription by the anterior pituitary. The *surge* mode of GnRH secretion is observed in females, during the preovulatory phase, in which the presence of GnRH in the portal circulation appears to be persistently elevated. However, it remains to be proven if this represents constant GnRH secretion or is the reflection of a high-frequency pulsatile secretion (Maeda et al. 2010; Moenter et al. 2003). The difficulties that high-frequency sampling presents keep this question unanswered.

In humans, there are two types of GnRH receptors: the GnRH-IR and the GnRH-IIR. The former is a G protein-coupled transmembrane receptor (Cui et al. 2000; Sealfon et al. 1997). Activation of the GnRH-IR leads to the activation of phospholipase C, which in its turn generates the second messengers inositol triphosphate and diacylglycerol (Fig. 2). The subsequent stimulation of protein kinase and cyclic adenosine monophosphate leads to the release of calcium ions (Shacham et al. 2001). GnRH-IR is found in the brain, the human placenta (Wolfahrt et al. 2001), ovarian follicles (Choi et al. 2006; Bramley et al. 1987), in myometrium, and leiomyomata (Kobayashi et al. 1997) as well as human pancreas, liver, heart, skeletal muscle, kidney, placenta, and peripheral blood (Chen et al. 1999; Kakar and Jennes 1995; Cheung and Wong 2008). GnRH-IIR is also a G protein-coupled transmembrane receptor. Unlike the GnRH-IR, it has a C-terminal cytoplasmic tail (Neil et al. 2001). nRH-IIR can be found the pituitary, placenta, ovary, uterus, prostate, mature sperm, pancreas, small and large intestines, kidney, and liver (Choi et al. 2006; Neill 2002; Eicke et al. 2005; van Biljon et al. 2002).

The vicinity of GnRH neurons with other central neurohormonal regulators submits the GnRH hypothalamic network to a range of neuroendocrine and metabolic inputs. KiSS1, the gene encoding kisspeptins, encodes a precursor peptide consisted of 145 amino acids, which in its turn is cleaved to a 54 amino acid peptide. The latter can be truncated to 14, 13, and 10 amino acid peptides, all sharing the same C-terminal sequence (Kotani et al. 2001; Pasquier et al. 2014). These peptides are collectively referred to as kisspeptins. The suggested abbreviations for these molecules are Kp-10, Kp-13, Kp-14, and Kp-54 (Gottsch et al. 2009). Accumulated scientific evidence has revealed a reproductive role for kisspeptin, neurokinin B, and dynorphin regarding hypothalamic regulation. Kisspeptin, neurokinin B, and dynorphin are colocalized in the same hypothalamic neuronal population in the sheep as well as in the human (Fig. 1). These neurons are termed KNDy (Kisspeptin-Neurokinin B-Dynorphin) neurons. This interconnected neuronal system seems to be involved in the control of GnRH and gonadotropin secretion (Cheng et al. 2010; Hrabovszky et al. 2010). In humans, kisspeptin neurons are distributed in the rostral POA and in the infundibular nucleus in the hypothalamus (Hrabovszky et al. 2010; Rometo et al. 2007) (Fig. 1).

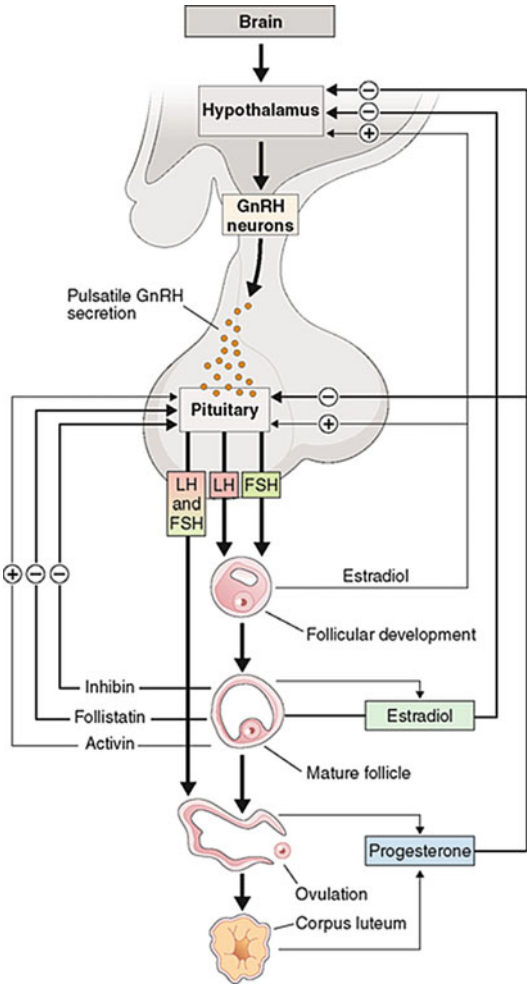


Fig. 3 Schematic representation of the major components of the hypothalamic-pituitry-ovarian axis depicting the major hormonal feedbacks and interplays

Kisspeptin axons form dense plexuses in the human infundibular stalk, where the secretion of GnRH occurs (Hrabovszky et al. 2010). Kisspeptin and GnRH networks are in close proximity as proven by the axosomatic, axodendritic and axoaxonal contacts between kisspeptin and GnRH axons (Hrabovszky et al. 2010; Uemoyama et al. 2011) (Fig. 1). GnRH neurons express the mRNA of kisspeptin receptor (Kiss1r) as well as kisspeptin receptors, suggesting the kisspeptin involvement in GnRH secretion (Irwig et al. 2004; Han et al. 2005; Messager et al. 2005). Kisspeptin signals directly to the hypothalamic GnRH neurons via kisspeptin receptor to release GnRH into the portal circulation. Neurokinin B and dynorphin (κ opioid peptide) but not kisspeptin receptors are found within the KNDy cells (Lehman et al. 2010; Pinilla et al.; 2012; Burke et al. 2006). These neuropeptides coordinate pulsatile GnRH and LH secretion through the stimulatory effects of neurokinin B and kisspeptin, and the inhibitory action of

dynorphin (Navarro et al. 2009). Kisspeptin-mediated GnRH secretion is sex steroid dependent. In humans, KNDy neurons in the infundibular nucleus alone are involved in negative and positive sex-steroid feedback (Skorupskaite et al. 2014; Rometo et al. 2007; Smith et al. 2005a). Kisspeptin is a potent stimulator of the hypothalamic-pituitary-ovarian (HPO) axis being the most potent GnRH secretagogue currently known. Apparently, the role of kisspeptin in normal pubertal development is crucial (Pinilla et al. 2012). The response to kisspeptin is different in men and women. In the former, kisspeptin stimulates potently LH release, while in the latter its effect varies depending on the phase of the menstrual cycle (Skorupskaite et al. 2014).

Other Neuroregulators

Endogenous opiates (opioids): These are natural narcotics produced by the brain. There are three classes of opiates: enkephalins, endorphins, and dynorphins. Endorphin levels increase throughout the menstrual cycle being at their lowest during menstruation and at their highest in the luteal phase. An increase in endorphin release results to decreased LH pulse frequency due to suppressed hypothalamic GnRH release. Thus, opioid receptors blockers, such as naltrexone, lead to enhancement of LH pulse frequency (Evans et al. 1992). Apparently, stress-related amenorrhea is probably the result of GnRH suppression by endogenous opiates (Goodman et al. 1995) (Fig. 4). Women suffering from stress-related amenorrhea demonstrate higher hypothalamic CRH, which controls proopiomelanocortin (POMC) production, the precursor to endorphins (Brundu et al. 2006; Nepomnaschy et al. 2007). Sex steroids appear to play a role in endorphin secretion.

Excitatory amino acids, catecholamines, NPY, and neurotensin: These are neural enhancers of the estradiol (E2) stimulatory effect on GnRH (Smith and Jennes 2001). Glutamate and aspartate directly or indirectly stimulate GnRH secretion (Brann and Mahesh 1994). These amine acids are important in regulating the GnRH pulse generator and in synchronizing the estrous cycle. Noradrenergic and adrenergic neurons acting via the α-1 adrenergic receptor stimulate both pulsatile and preovulatory release of GnRH.

Neuropeptide Y, a 36 amino acid peptide, is present in high concentrations in the hypothalamus. The interactions between NPY and the reproductive axis are complex and depend upon the sex steroid environment. A potent stimulation of LH secretion by NPY has been reported in sex steroid primed animals, whereas in castrated animals, central NPY administration results in an inhibition of LH release (Kalra and Crowley 1984a, b; Kaynard et al. 1990; Khorram et al. 1987; McDonald et al. 1989). NPY exerts positive, rather than negative, effect on GnRH and LH secretion in ovary-intact or estrogen-treated animals (Sabatino et al. 1989) (Figs. 4 and 5). This might be due, in part, to the ability of E2 to stimulate the expression of NPY1 receptors (Hill et al. 2004), while E2 stimulates also NPY expression (Bauer-Dantoin et al. 1992), particularly when followed by progesterone treatment (Crowley et al. 1985). It is this stimulatory effect that appears to be important in the positive

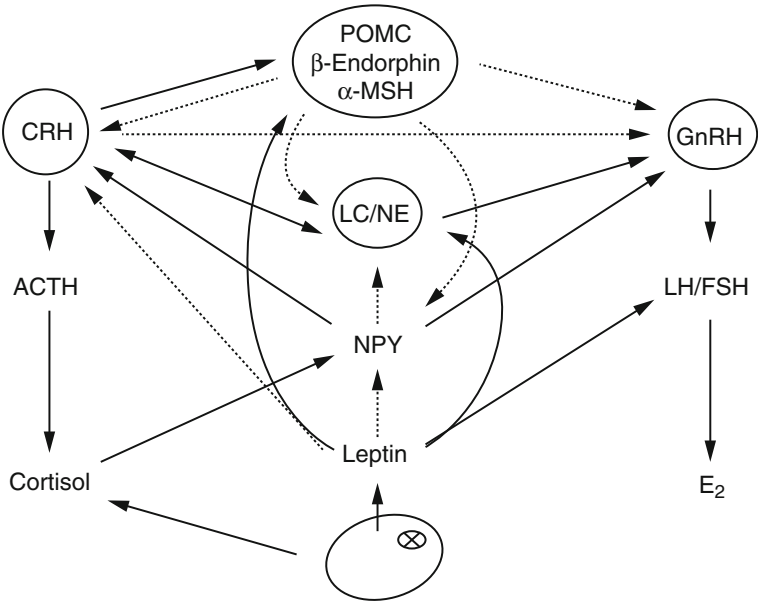


Fig. 4 Interactions of leptin with the HPA axis and the female reproductive axis. In general, leptin inhibits the HPA axis and stimulates the reproductive system. Leptin provides positive input to the female reproductive axis indirectly via inhibition of the HPA axis and the arcuate nucleus POMC and activation of the locus ceruleus-norepinephrine system (LC/NE). NPY exerts positive effect on GnRH and LH secretion in ovary-intact or estrogen-treated animals (solid line). However, when infused chronically, NPY consistently induces a profound inhibition of the gonadotrope axis suggesting that the increased expression of hypothalamic NPY observed in fasting or unfavorable metabolic conditions may account for the tonic inhibition of pulsatile GnRH release. In this case, the increase of leptin can lead to HPO axis activation via inhibition (dotted line) of the inhibitor (NPY). Solid line = stimulation; dotted line = inhibition (Chrousos et al. 1998)

feedback actions of ovarian steroids. Indeed, spontaneous and E2-induced LH surges are blunted in female NPY-KO mice (Xu et al. 2000) while NPY gene expression increases in association with preoovulatory LH surges (Bauer-Dantoin et al. 1992). Thus, the E2-induced preovulatory midcycle gonadotropin surge is exerted, at least in part, via NPY expression and release, which in turn contributes to the preoovulatory GnRH surge into the hypophyseal portal vessels. However, when infused chronically, NPY consistently induces a profound inhibition of the gonadotrope axis (Catzeflis et al. 1993; Pierroz et al. 1996; Pierroz et al. 1995) suggesting that the increased expression of hypothalamic NPY observed in fasting or unfavorable metabolic conditions (Kalra et al. 1991) may account for the tonic inhibition of pulsatile GnRH release (Aubert et al. 1998). Along this line of physiology, NPY seems to play an important role in the onset of puberty. Specifically, it appears to have an inhibitory role on the GnRH neuron and possibly to other interneurons to GnRH. This inhibitory role is removed at the beginning of puberty (Terasawa and Fernandez 2001; El Majdoubi et al. 2000).

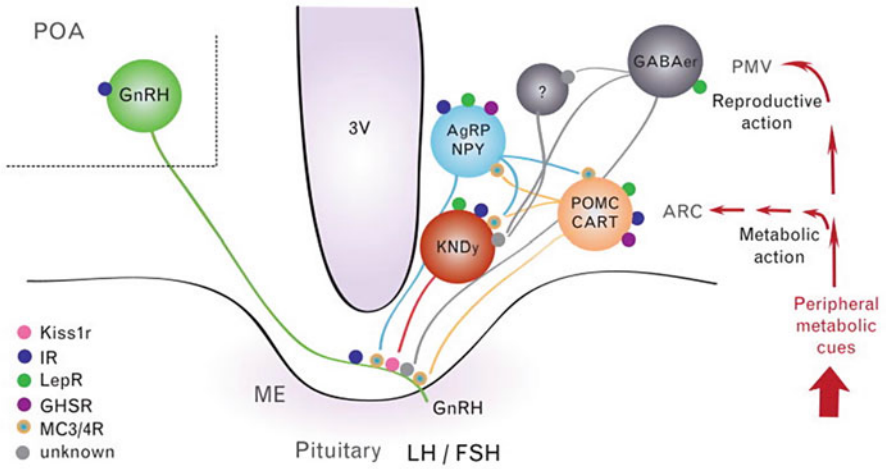


Fig. 5 Schematic representation of neural interactions among various neurons mediating peripheral metabolic and reproductive cues to the GnRH neuron. *3 V*, third ventricle; *AgRP*, agouti-related peptide; *ARC*, arcuate nucleus; *CART*, cocaine- and amphetamine-regulated transcript; *GABA*, g-aminobutyric acid; *GHSR*, growth hormone secretagogue receptor; *IR*, insulin receptor; *Kiss1r*, kisspeptin receptor; *KNDy*, kisspeptin/neurokinin B/dynorphin; *LepR*, leptin receptor; *MC3r*, melanocortin receptor 3; *MC4r*, melanocortin receptor 4; *ME*, median eminence; *NPY*, neuropeptide Y; *PMV*, ventral premammillary nucleus; *POA*, preoptic area; *POMC*, pro-opiomelanocortin (Navarro and Kaiser 2013)

Neurotensin expressed by POA neurons mediates estrogen-induced release of GnRH mostly through the NT1 receptor subtype. The NT neurons express E2 receptors (ER), while neurotensin synthesis is estrogen-induced. Other excitatory inputs are mediated by VIP, catecholamines, and possibly vasopressin, whereas nitric oxide and gonadotropin-inhibitory hormone/RFamide-related peptide-3 act likely in an inhibitory way (Christian and Moenter 2008, 2010; Lee et al. 2000; Miller et al. 2006; Ducret et al. 2009).

GABA: Although GABA is classically an inhibitory neurotransmitter in the central nervous system, most mature GnRH neurons are stimulated directly by GABA, allocating GABA an excitatory role regarding the HPO axis (Fig. 5). The precise physiology of this mechanism is still unclear (Watanabe et al. 2014; Mijiddorj et al. 2015; Moore et al. 2015). GABA has been reported to have a role in mediating puberty possibly following a reduction of the tonic GABA inhibition upon GnRH release which is, at least in part, mediated through kisspeptin neurons (Watanabe et al. 2014).

Substances linking energy balance and reproductive function: The role of these substances should be to negate animals their reproductive potential (resulting to parturition, lactation, and other parental behaviors) when they are in negative energy balance (Schneider 2004). Kisspeptin signaling links nutrition/metabolic status and reproduction by sensing energy stores and deciphering this information into GnRH secretion (Fig. 5). These physiologic relationships are found behind the

physiopathologic mechanisms leading to diseases combining disturbances of both reproduction and metabolism such as *diabetes*, *obesity*, *functional hypothalamic amenorrhea* (FHA), etc. (Skorupskaite et al. 2014; Dandona and Dhindsa 2011). Periods of fasting and calorie restriction decrease LH pulse frequency and increase pulse amplitude (Schneider 2004; Schreihofer et al. 1993; Loucks et al. 1998; Loucks and Thuma 2003). Food deprivation impairs GnRH and gonadotropin secretion. Leptin (a satiety hormone secreted by adipose tissue) plays an instrumental role in reproduction (Macut et al. 1998) and the regulation of LH release (Skorupskaite et al. 2014; Bergendahl and Veldhuis 1995; Bergendahl et al. 1998; Weigle et al. 1997) (Figs. 4 and 5). Humans with mutations in leptin or in leptin receptor present with hypogonadism. Patients suffering FHA present with lower serum concentrations of leptin, suggesting a role of this hormone in their low gonadotropin secretion as compared with similar-weight eumenorrheic women (Chan and Mantzoros 2005; Welt et al. 2004). Leptin is also involved in the decrease of food intake by its action on the supraoptic, paraventricular (PVN), and arcuate nucleus and lateral hypothalamus via activation of the neurons that synthesize anorexigenic peptides namely POMC and cocaine- and amphetamine- regulated transcript (CART). α-Melanocyte-stimulating hormone (α-MSH), a posttranslational product of POMC by cleavage, is a potent inhibitor of food intake. At the same time, leptin suppresses the activity of orexigenic neurons which express agouti-related peptide (AgRP) and NPY (Fig. 4). The action of leptin action at the hypothalamus is also responsible for counterbalancing the effect of ghrelin, which is a major orexigenic hormone. In addition to the hypothalamus, leptin acts on the mesolimbic dopamine system, which is part of the brain reward system and through its action on the neurons of the solitary tract also promotes satiety (Laughlin et al. 1998). Leptin receptors are also found on galanin-expressing neurons, which may also participate in the anorexigenic effect of leptin (Warren et al. 1999). Leptin stimulates GnRH neurons indirectly as they do not express leptin receptors (Quennell et al. 2009). Lately, it is believed that leptin stimulates kisspeptin neurons directly, as 40% of the arcuate kisspeptin neurons express leptin receptors; or indirectly. Then kisspeptin neurons, in their turn, stimulate GnRH release (Chou and Mantzoros 2014). Primary leptin targets for mediation of its reproductive effects include probably nitric oxide-producing neurons in the preoptic hypothalamus (Bellefontaine et al. 2014) and neuronal circuits in the ventral premammillary nucleus (Donato et al. 2011), which might transmit metabolic information to GnRH neurons through kisspeptin dependent and/or independent pathways. Leptin concentrations may also play a role in the CRH increase in the brain during stress-related amenorrhea and weight-loss amenorrhea (Kelesidis et al. 2010). Progressive fasting results in a rapid decline in leptin concentration, which occurs before any loss of fat mass (Boden et al. 1996), thereby triggering an adaptive mechanism to conserve energy (Chan et al. 2003). In mice and humans, the neuroendocrine response to food deprivation besides other includes an increase of adrenocorticotropic hormone (ACTH) and cortisol levels (Ahima et al. 1996; Miller et al. 1998; Vance and Thorner 1989). Additionally, ob/ob mice exhibit increased adrenal stimulation by ACTH (Bray and York 1979), and leptin administration blunts the stress-mediated increase in ACTH and cortisol in normal mice

(Heiman et al. 1997). From analyses of the pulse parameters of leptin, an inverse relationship was identified between circulating leptin and ACTH in healthy men (Licinio et al. 1997). However, studies in humans with mutations in the leptin or leptin receptor genes reveal that, despite abnormal leptin function, normal adrenal function is maintained (Clement et al. 1998; Farooqi et al. 1999; Farooqi et al. 2007). Open-label studies of relative leptin deficiency in men with acute energy deprivation (i.e., starvation) and women with chronic energy deprivation (i.e., FHA) (Chan et al. 2003; Welt et al. 2004) showed no major effect of leptin replacement on the hypothalamic-pituitary-adrenal (HPA) axis but detailed studies were not performed. Similar results were reported in women with lipoatrophy and hypoleptinemia (Oral et al. 2002) studied in the context of an open-label study although there was a small but statistically significant decrease in cortisol concentration after leptin replacement in a recent randomized, placebo-controlled trial (Chou et al. 2011).

Pituitary and Gonadotropic Cells

The pituitary gland measures 12 × 8 mm and weighs approximately 500 mg (Mancall and Brock 2011). It is located beneath the third ventricle and above the sphenoidal sinus within the sella turcica. The adult pituitary gland is distinguished in the adenohypophysis (anterior lobe) and the neurohypophysis (posterior lobe). The former is an ectodermal derivative of the stomatodeum while the latter is a diencephalic downgrowth connected with the hypothalamus. The infundibulum extends from the hypothalamus to the pituitary gland and it is continuous with the median eminence. The communication between the hypothalamus and the anterior pituitary is vascular, whereas the connection between the hypothalamus and the posterior pituitary is neuronal. The anterior pituitary contains several cell types: thyrotropes (~5%, they secrete thyroid stimulating hormone, TSH); adrenocorticotropes (~15%, they secrete ACTH); somatotropes (~10%, they secrete growth hormone, GH); lactotropes (~15%, they secrete PRL); and gonadotropes. The latter represent approximately 10% of the anterior pituitary cell population, secrete FSH and LH, are scattered throughout the pars distalis and are the major constituent of the pars tuberalis. In addition to all these hormones, the anterior pituitary secretes activin, inhibin, and follistatin, which play a role in menstrual cycle regulation (Fig. 3). The posterior pituitary lobe contains two cell types that secrete, respectively, antidiuretic hormone (ADH) and oxytocin.

FSH, a glycoprotein dimer, consists of two subunits: α (alpha) and β (beta). The α-subunit is common in FSH, LH, TSH, and human chorionic gonadotropin (hCG). The β-subunit is distinct and hormone-specific, conferring each hormone its distinct function. The α-subunit consists of 92 amino acids, while the FSH β-subunit consists of 118 amino acids and five sialic acid residues. These residues are responsible for the half-life of the hormone. The higher the sialic acid content, the longer the half-life of that molecule (Morell et al. 1971). FSH has a half-life of several hours. The rate-limiting step in gonadotropin production is the availability of β-subunits. Apart GnRH stimulation, the FSH β-subunit synthesis depends also on activin

(Besecke et al. 1996). Both FSH and LH exert their biological effects through G-protein coupled receptors. Follicle-stimulating hormone concentrations start to rise a few days prior to the onset of menses. This gonadotropin is responsible for the recruitment of a cohort of ovarian follicles as well as for the selection of the dominant follicle; it induces granulosa cell growth and activates aromatase activity. The progressively increased production of estrogen and inhibin-B by the growing follicular granulosa cells leads to FSH suppression. Despite this drop in the FSH level, the dominant follicle continues to grow due to the high concentration of FSH receptors on the increased in number surrounding granulosa cells (Amsterdam and Rotmensch 1987). The decrease of FSH concentrations results to a higher androgenic microenvironment in the nondominant follicles. The FSH concentrations decrease after ovulation of the dominant follicle. LH is a glycoprotein dimer consisting of two subunits: α and β. The LH β-subunit consists of 121 amino acids and one to two sialic acid residues, contributing to its short half-life of approximately 20 min. Its pulses are, typically, higher in amplitude than FSH. LH starts to rise prior to the onset of menses, as well. The gradual LH increase throughout the follicular phase is followed by an LH surge prior to ovulation. This surge is due to a positive-feedback of E2 produced by the dominant follicle. LH levels decline during the secretory phase of the cycle.

FSH and LH receptors both belong to the G-protein coupled receptors family. The FSH receptors exist exclusively on the membrane of granulosa cells, while LH receptors are found on membranes of theca cells. In the presence of E2, FSH induces LH receptors on granulosa cells. LH receptor activity primarily stimulates androstenedione production from theca cells. Then, this hormone is transported to neighboring granulosa cells, aromatized to E1, and eventually converted to E2.

In infancy, LH pulsatile secretion is enhanced postnatally (minipuberty of the neonate), but in a few weeks it becomes quiescent (Waldhauser et al. 1981). Greater basal and GnRH-stimulated LH concentrations are observed in early childhood (<5 years). This activity is subdued at mid-childhood (5–11 years) and increases thereafter followed by pubertal development (Conte et al. 1980). A steady acceleration in LH pulsatility, starting at night, announces the onset of puberty (Marshall et al. 1991). In women, the pattern of GnRH secretion defines the physiology of the menstrual cycle (Marshall et al. 1991, 1993). The LH pulse frequency is slow in the luteal phase and increasingly speeds up during the follicular and the preovulatory phases (Yen et al. 1972). Abnormalities in GnRH – and hence LH pulse frequency – are associated with a number of reproductive endocrine disorders.

Prolactin: It is a well-known inhibitor of GnRH release and a suppressor of the HPO axis. Hyperprolactinemia induces reproductive dysfunction associated with secondary amenorrhea, hypogonadotropic hypogonadism, and infertility (Bohnet et al. 1976). The neuroendocrine pathway by which PRL inhibits GnRH pulse frequency is not yet fully elucidated.

The HPA axis: Physical and psychosocial stress is associated with hypothalamic amenorrhea, apparently via activation of the HPA axis (Chrousos et al. 1998; Vrekoussis et al. 2010; Brundu et al. 2006). The inhibitory effect of cortisol on the HPO axis occurs at suprahypothalamic, hypothalamic, and pituitary levels (Fig. 4).

Finally, glucocorticoids can directly modulate ovarian function, through the presence of its receptor in ovarian cell types, mainly through regulation of steroid hormone production.

Ovary and Uterus

The ovaries are located in the pelvis along the sides of the uterus, and in reproductive-age women, they measure approximately 2.5 × 3 × 1.5 cm in size. The ovary consists of an outer cortex, where the ovarian follicles are found, and an inner medulla which contains mainly fibromuscular tissue and vasculature. The ovarian follicle consists of an oocyte surrounded by layers of granulosa (responsible for E2 production in the growing follicle) and theca cells (Fig. 3). After ovulation, the remnant cells (both of granulosa and thecal origin) of the follicle luteinize and start secreting progesterone. Inhibin and anti-Müllerian hormone (AMH) are also secreted by the granulosa cells. The uterus is a fibromuscular organ, largely receptive to all steroid hormones. The endometrium normally proliferates in response to the rising E2 levels in the first half of the menstrual cycle and is converted to a secretory layer in response to progesterone produced by the corpus luteum in the second half of the menstrual cycle.

Estrogens are 18-carbon steroid hormones. In humans, they include estrone (E1), E2, and estriol (E3). Estradiol, the most potent estrogen, is the product of the ovary. Serum E2 levels rise during the follicular phase of the menstrual cycle in parallel to the growth of the follicle. In the bloodstream, it is bound to carrier proteins such as albumin (binds 60% of E2) and sex hormone binding globulin (SHBG; binds 38% of E2). The remaining 2% is active and found free in the bloodstream. Estradiol levels drop after ovulation followed by a second rise in the midluteal phase. Estrone is mainly the product of peripheral androstenedione conversion while it is also generated in the liver via 17β-hydroxysteroid dehydrogenase conversion of E2. ERα and ERβ are the two known estrogen receptors (Kuiper et al. 1996; Mosselman et al. 1996). A biphasic effect of E2 on gonadotropin secretion is well described. An initial negative feedback upon FSH secretion is critical for follicle selection while the subsequent positive feedback upon LH secretion is critical for the ovulation (Knobil et al. 1980). Progesterone, a steroid hormone, is a 21-carbon molecule produced by the corpus luteum while its peak occurs in the midluteal phase (>5 ng/mL). In the bloodstream, it is bound to albumin (80%), to corticosteroid-binding globulin (18%), and to SHBG (0.5%). The unbound fraction of progesterone circulates free in the circulation. There are three progesterone receptors: progesterone receptor (PR)-A, PR-B, and PR-C. At high concentrations, progesterone inhibits FSH and LH secretion through negative feedback upon the hypothalamus and the pituitary (Wildt et al. 1981b). In luteal phase, the increased levels of progesterone lead to decreased GnRH pulse frequency. Progesterone receptors, besides being present in a small number on GnRH neurons (Fox et al. 1990; King et al. 1995; Skinner et al. 2001), are colocalized with dynorphin in the KNDy neurons (Foradori et al. 2002) while progesterone administration increases dynorphin concentrations (Foradori et al. 2005).

Ovarian androgens [androstenedione, testosterone, and dehydroepiandrosterone (DHEA)] are produced from the theca cells. They are 19-carbon steroids. The main androgen secreted by theca cells is androstenedione. Most of testosterone is derived from the conversion of androstenedione via the enzymatic activity of 17β-hydroxysteroid dehydrogenase. FSH leads androstenedione and testosterone to aromatization to estrogens in the neighboring granulosa cells. Testosterone exerts negative feedback on gonadotropin secretion. Few GnRH neurons express ARs (Herbison et al. 1996). It is suggested that GnRH neurons rely upon an intermediary neuronal population to mediate testosterone feedback, such as KNDy neurons which express androgen receptors (AR) (Smith et al. 2005b) In addition, studies have shown that the androgen feedback is also mediated by aromatization of testosterone (Smith et al. 2005b).

Ovarian peptide hormones: Inhibin, activin, and AMH play a role in the menstrual cycle by modulating central nervous system hormone release (Fig. 3). Inhibin is a polypeptide secreted mainly by granulosa cells. It is also found in pituitary gonadotropes (Bauer-Dantoin et al. 1995; Blumenfeld 2001). Two molecules of inhibin have been identified: inhibin-A and inhibin-B. Activin is also secreted by the granulosa cells. It augments the secretion of FSH by enhancing GnRH receptor formation (Kaiser et al. 1997; Norwitz et al. 2002). It participates in androgen synthesis by enhancing the action of LH in the ovary. The effects of activin are blocked by inhibin and follistatin (Bilezikjian et al. 1996). Follistatin also known as activin-binding glycoprotein in humans is expressed in nearly all tissues of higher animals (Tortoriello et al. 2001). The main role of follistatin in the ovary, so far, appears to be the progression of the follicle from early antral to antral/dominant.

AMH being the product of the granulosa cells of small antral and preantral follicles reflects their quantity (Durlinger et al. 2002). Because its amount depends on the size of primordial follicles, it reflects the ovarian reserve in human primordial follicles. Estradiol acting via ER-β leads to interaction with the AMH promoter region (Grynberg et al. 2012). Thus, AMH acting as a regulator of E2 production from small antral follicles, which produce small amounts of E2 prior to selection, contributes to the ovarian/pituitary dialogue which regulates the development of the selected follicle to ovulation (Jeppesen et al. 2013). In vitro studies of neonatal ovaries and ovarian cortical strips of various species, including human, revealed the inhibitory effect of AMH on primordial to primary follicle transition. It is also believed that AMH inhibits FSH-stimulated preantral follicle growth by reducing follicle sensitivity to FSH, contributing thus, to the emergence of the dominant follicle (Durlinger et al. 2001). The relationship among AMH, the follicular pool, and recruitment throughout the reproductive life cycle is complex and is dependent on the stage of sexual development (Fleming et al. 2012). It seems that AMH exerts a physiological effect on human antral follicles before final selection. The timing of ovulation is secured by a balanced feed-forward system between E2 (and inhibin) plasma concentration resulting from the preovulatory follicle secretion and gonadotropin plasma concentration resulting from the pituitary secretion (Baird and Smith 1993) (Fig. 3). Finally, it should be stated that CRH and CRH receptors are detected in human thecal, stromal cells, and in follicular fluid. Ovarian CRH is involved in the

regulation of ovarian steroidogenesis as well as follicular maturation, ovulation, and luteolysis (Mastorakos et al. 1994; Ghizzoni et al. 1997; Kiapekou et al. 2010).

Etiology and Physiopathology of Secondary Female Hypogonadism

Etiology

Secondary hypogonadism is a group of disorders resulting from an abnormality in GnRH secretion from the hypothalamus (or higher) or an abnormality in gonadotropins secretion from the pituitary. In certain conditions, both hypothalamic and pituitary dysfunctions are present. The genetic causes of secondary hypogonadism manifest mainly as congenital genetic syndromes while some of them have been attributed to recognized single gene mutations and others have been characterized as idiopathic forms (Table 1). Acquired causes of secondary hypogonadism include reversible causes, such as FHA, drugs, chronic illnesses; and irreversible causes, such as central nervous system (CNS) insults (brain tumors, traumatic brain injuries, infections, brain/pituitary irradiation, and infiltrative disorders and pituitary apoplexy) (Table 2).

Genetic Causes of Secondary Female Hypogonadism

In Olfactory-Hypothalamic Dysfunction

Based on the presence or absence of dysfunction of the olfaction, the secondary hypogonadism due to genetic condition is distinguished in two groups, one with anosmia (*Kallman syndrome*) and one without anosmia (normosmic *isolated or idiopathic hypogonadotropic hypogonadism, IHH*).

Table 1 Genetic causes of secondary female hypogonadism

1. Hypothalamic dysfunction
Kallman syndrome **(genes involved)** ANOS1, NELF, CHD7, HS6ST1, WDR11, SEMA3A FGFR1, PROK2R, PROK2 genes **Isolated or idiopathic hypogonadotropic hypogonadism, IHH** **(genes involved)** Kiss1/Kiss1R, GnRH1/ GnRHR, FGFR1, FGF8, PROK2, PROKR2, CHD7, WDR11, TAC3/ TACR3 genes
2. Pituitary dysfunction (genes involved)
PROP1, HESX1, LHX4, SOX 2 genes
3. Other
Genes involved: LEP/LEPR, DAX-1, SF-1 genes, FSH/LH β subunits, **Syndromes:** Prader-Willi, Boucher-Neuhauser, Bardet-Biedl, Noonan syndromes pseudohypoparathyroidism type 1A

Table 2 Acquired causes of secondary female hypogonadism

Reversible	Irreversible
Functional hypothalamic amenorrhea	**Brain tumors:** Craniopharyngioma, teratoma, meningioma, hamartoma, etc.
Drugs: GnRH agonists/antagonists, glucocorticoids, narcotics, chemotherapy	Traumatic brain injury
Chronic illnesses: Eating disorders, hypothyroidism, hyperprolactinemia, diabetes mellitus, Cushing disease	Brain/pituitary irradiation
	Infection
	Infiltrative disorders: Sarcoidosis, Langerhans cell histiocytosis, haemochromatosis, leukaemia, lymphoma, Wegener granulomatosis, lymphoma, metastases
	Pituitary apoplexy

A defective GnRH neuron migration leads to *Kallmann syndrome (KS)*, which is characterized by secondary hypogonadism due to GnRH deficiency and anosmia. By magnetic resonance imaging (MRI), an aplasia of the olfactory bulb is described in individuals with KS (Vogl et al. 1994). The X-linked form is due to mutation in the ANOS1 gene (an X chromosome-located gene), and it manifests as a defect in the migration of GnRH and olfactory neurons and is transmitted via an X-linked recessive pattern of inheritance. Thus, it appears predominantly in males. ANOS1 gene encodes for anosmin-1, a glycoprotein essential for neuronal migration and growth (Franco et al. 1991). In one study, ANOS 1 gene defects accounted for only 14% of cases with familial KS. All other causative genetic defects may appear both in males and females. Indeed, mutations in the genes NELF, CHD7, HS6ST1, WDR11, and SEMA3A are also associated with defects in neuronal migration, leading to KS (Silveira et al. 2010; Young et al. 2012; Semple and Topaloglu 2010). On the other hand, autosomal forms in KS may be more prevalent than previously thought. Mutations in unidentified autosomal genes were postulated to cause the remainder. In as many as 10% of KS cases, mutations of the FGFR1 have been described (Sato et al. 2004). Of note, mutations in the genes encoding for prokineticin (PROK)2 signaling peptides lead to secondary hypogonadism without anosmia, indicating that factors other than suboptimal migration can also lead to functional deficiencies in GnRH. Furthermore, mutations in the PROK2 and PROK2 receptor (PROK2R) genes have been identified in individuals with either KS or *normosmic isolated or IHH* (Pitteloud et al. 2007). Surprisingly, mature GnRH neurons do not express PROKR2 (Martin et al. 2011). Additional phenotypic abnormalities such as craniofacial defects (cleft lip/palate, high-arched palate, ocular hypertelorism, dental agenesis), neurosensory deafness, digital anomalies (clinodactyly, syndactyly, camptodactyly), unilateral renal agenesis, and neurological defects (oculomotor abnormalities, bimanual synkinesis or mirror hand movements, cerebellar ataxia) may be present in KS patients.

The term *isolated or IHH* describes the partial or complete absence of pubertal development, secondary to dysfunctional GnRH secretion without anatomical abnormalities in the hypothalamic and pituitary region while all pituitary hormones other than gonadotropins function normally (Seminara et al. 1998). Despite important recent advances, the genetic basis of congenital IHH has been identified in approximately 30% of patients. Mutations in Kiss1 and Kiss1 receptor (Kiss1R *alias* GPR54), which has a critical role in hypothalamic GnRH signaling and release, have been identified in IHH (de Roux et al. 2003). Defects in FGFR1, FGF8, CHD7, and WDR11 have also been associated with normosmic IHH, although in a lower frequency (Silveira et al. 2010; Semple and Topaloglu 2010). Mutations in tachykinin (TAC)3/TACR3, and GNRH1/GNRHR genes that interfere in the secretion and action of GnRH are described exclusively in patients with normosmic IHH (Silveira et al. 2010; Pfaffle et al. 1999). Loss of function mutations of the GnRHR has been associated with normosmia and secondary hypogonadism (at least eight mutations of this receptor have been identified in seven families). Due to incomplete activation of GnRHR function, there is notable genotype-phenotype variation even within members of the same kindred (de Roux and Milgrom 2001). Females typically present with primary amenorrhea (Seminara et al. 1998).

In Pituitary Dysfunction

Pituitary gonadotropin synthesis may be defective due to mutations in a variety of transcription factors such as Prop-1, an important transcription factor involved in the developmental cascade of pituitary gonadotrope cells. PROP1 is the prophet of the pituitary transcription factor Pit1, a paired-like homeodomain transcription factor responsible for early embryonic pituitary development. Prop-1 gene mutations can result in familial combined pituitary hormone deficiencies of variable phenotypic expression, including GH deficiency, central hypothyroidism, and secondary hypogonadism (hypogonadotropic) (Pfaffle et al. 1999). Deficiencies in HESX1, a transcription factor needed for normal pituitary development (Dasen et al. 2001), initially identified in 1998, cause a rare cause of septo-optic dysplasia (Dattani et al. 1998), which may be associated with hypogonadotropic hypogonadism (Haddad and Eugster 2005). Other transcription factors implicated in rare cases of hypogonadotropic hypogonadism include LHX4 (Pfaeffle et al. 2008) and SOX 2 (Kelberman et al. 2006), while all patients with hypopituitarism, including idiopathic forms, are at risk for hypogonadotropic hypogonadism.

Others

Congenital leptin deficiency results from loss of function mutations of the LEP gene, which encodes for the leptin protein. Leptin receptor belongs to the interleukin-6 family of receptors. The interaction of leptin with its receptor stimulates the Jak-Stat pathway and leads to activation of downstream target genes. In patients with leptin deficiency, clinical manifestations include hyperphagia, obesity, and secondary hypogonadism. Administration of leptin seemingly rectifies these abnormalities (Farooqi et al. 2002). Leptin receptor (LEPR) abnormalities have a similar phenotype to congenital leptin deficiency. Females with this mutation have secondary

hypogonadism. They present with delayed puberty, lack of a pubertal growth spurt, and reduced expression of secondary sexual characteristics. Eventually, irregular menses appear due to aromatization of subcutaneous fat to estrogen (Farooqi et al. 2007).

Nuclear receptors influence gene transcription at multiple levels and exert their effects in a time- and dosage-specific fashion. The DAX-1 gene encodes an orphan nuclear receptor expressed in the hypothalamus, the pituitary, the adrenals, and the gonads (Zanaria et al. 1994). Its mutations have been identified at the origin of the X-linked congenital adrenal hypoplasia mainly encountered in males. Atypical presentations have included hypothalamic-pituitary hypogonadism in a female with homozygous DAX-1 mutation (Merke et al. 1999), delayed puberty in heterozygous females (Seminara et al. 1999), and the delayed onset of adrenal insufficiency and hypogonadism in adolescence (Okuhara et al. 2008) and adulthood in a few affected individuals (Ozisik et al. 2003). Steroidogenic factor-1 (SF-1) encodes an orphan nuclear receptor expressed in the hypothalamus, the pituitary, the adrenals, and the gonads (Ingraham et al. 1994). The GnRHR and the LH β subunit are among its targets.

To date, no mutations of the LH/FSH α subunit have been described while mutations of the FSH and LH β subunits as well as inactivating mutations of the gonadotropin-receptor have been identified mainly as causes of delayed puberty, hypogonadism, primary or secondary amenorrhea, and abnormal reproductive function in females (Themmen and Huhtaniemi 2000). In certain cases, LH receptor mutations in females result in amenorrhea with normal secondary sexual development and elevation of LH and FSH (Latronico et al. 1996). Given that follicles of all stages of development are present suggests that although LH is necessary for ovulation, follicular development may occur under the influence of FSH and ovarian paracrine factors alone (Toledo et al. 1996).

Numerous syndromes include in their description neuroendocrine dysfunction of the HPO axis. Perhaps the best known is Prader-Willi syndrome caused by a genetic defect involving paternal chromosome 15, usually in the form of a microdeletion within the long arm or maternal unipaternal disomy (Wharton and Loechner 1996) (refers to the situation in which two copies of a chromosome come from the same parent, instead of one copy coming from the mother, and one copy coming from the father). In these patients, hypothalamic dysfunction is evidenced by their hypotonia, hyperphagia, intermittent temperature instability, and hypogonadism attributed to an absence of or abnormal location of GnRH neurons. Patients with Prader-Willi syndrome present with low circulating serum gonadotropins (Jeffcoate et al. 1980), hypoplasia of the clitoris or labia minora, primary amenorrhea, and delayed puberty (Crino et al. 2003). Because the spectrum of hypogonadism is wide in Prader-Willi syndrome, some women have achieved fertility without hormone replacement therapy (Schulze et al. 2001; Akefeldt et al. 1999). Hypogonadism due to hypothalamic-pituitary dysfunction is also encountered in Boucher-Neuhauser, Bardet-Biedl, and Noonan syndromes while pseudohypoparathyroidism type 1A is associated with multiple hormone resistance due to loss-of-function mutation in the GNAS1 gene.

Of note, *Constitutional Delay of Growth and Puberty (CDGP)* is a variation of normal development sometimes difficult to differentiate from pathologic secondary hypogonadism. In this condition with strong familial pattern (Sedlmeyer and Palmert 2002), puberty and the pubertal growth spurt occur at or later than the extreme upper end of the normal age. In fact, puberty progresses through the normal stages but starts at a later time. Subsequently, children with CDGP achieve their genetic potential for height (von Kalckreuth et al. 1991).

Acquired Causes of Secondary Female Hypogonadism

The acquired causes of secondary hypogonadism are due to structural or functional abnormalities of the hypothalamic-pituitary axis. Usually they present with multiple pituitary hormone deficiencies. Their etiology includes: *CNS or pituitary tumors*, *infiltrative diseases*, *infection*, *brain/pituitary radiation*, *pituitary apoplexy*, *head trauma*, *drugs* (GnRH agonists/antagonists, glucocorticoids, narcotics, chemotherapy), functional deficiency resulting from *chronic systemic illness*, *eating disorders*, *hypothyroidism*, *hyperprolactinemia, diabetes mellitus*, and *Cushing's disease* (Table 2).

Reversible Acquired Causes (Including Functional)

Secondary hypogonadism may be present in hypothyroidism. In hypothyroid children, pituitary sensitivity to GnRH may be reduced. Menstrual irregularities are frequent in severe hypothyroidism (Krassas et al. 1999), while elevated TSH concentrations are found in adolescent girls presenting with oligomenorrhea (Nezi et al. 2016). Patent primary hypothyroidism is often accompanied by elevated thyrotropin-releasing hormone (TRH)-driven functional hyperprolactinemia which, in its turn, leads to secondary hypogonadism. Overt hypogonadism occurs when PRL is significantly increased. The latter leads to suppression of GnRH release and subsequently to low gonadotropin levels. LH pulse frequency is decreased, while LH response to GnRH is normal or increased (Sauder et al. 1984). The normalization of PRL is associated with recovery of LH pulse frequency and disappearance of the hypogonadism. The dysfunction in the HPO axis leads to impaired follicular maturation and development of corpora lutea in postpubertal hypothyroid rats and their absence in prepubertal ones (Armada-Dias et al. 2001).

Drugs that can reversibly suppress the HPO axis at its different levels include opiates, glucocorticoid, and psychotropic agents such as phenothiazines.

Irreversible Acquired Causes (Including Tumors)

Acquired central nervous system insults such as tumor, trauma, irradiation infection, or infiltrations by systemic diseases may be responsible for irreversible secondary hypogonadism.

Hypothalaminc lesions due to masses can lead to disrupted GnRH secretion, even alteration of the hypothalamic–pituitary portal circulation resulting to secondary hypogonadism. Other hypothalamic-pituitary hormonal routes can be affected as

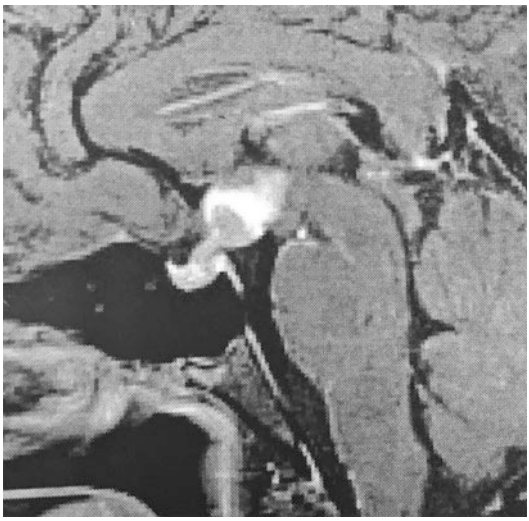


Fig. 6 Magnetic resonance imaging of a small-cell lung carcinoma metastasis at the suprasellar area above the pituitary stalk and behind the optic chiasm projecting into the third ventricle (Personal case)

well. *Craniopharyngiomas* and *germ cell tumors* are typical examples of tumors that can cause secondary hypogonadism. The former are rare epithelial tumors arising from remnants of the craniopharyngeal duct occurring with an incidence of 0.13 cases per 100,000 person-years (Fig. 6). They are usually cystic or mixed (84–99%) and of benign histology. They are rarely detected when only intracellar and usually they have a suprasellar component. Craniopharyngiomas are followed by significant pathologies, including the endocrine, visual (when they attain the optic chiasm), and neuropsychological comorbidities. Regarding the endocrine pathologies that accompany them, a significant variability of individual pituitary hormone deficits is noted (88–100% for GH, 80–95% for FSH and LH, 55–88% for ACTH, 39–95% for TSH, and 25–86% for AVP). However, in craniopharyngiomas, the consequences of hypothalamic damage appear to be more disabling and occur commonly in these patients (Yen et al. 1999). Regarding germ cell tumors, they are believed to result from malignant transformation and abnormal migration of primordial germ cells. These tumors occur most commonly in the pineal region (50%) and in the anterior hypothalamus (30%), and they can cause hypopituitarism. A paradoxical case, in children, is that of intracranial lesions which cause *central precocious puberty* (CPP), the most common of which is a hypothalamic hamartoma, a heterotopic mass located typically in the region of the tuber cinereum. This congenital malformation consists of GnRH-secreting neurons or transforming growth factor α–producing astroglial cells, both of which are believed to function as ectopic triggers on the hypothalamic pulse generator, resulting in an escape from the normal central nervous system inhibitory constraint on the pubertal onset.

Traumatic brain injury is an insult to the brain that could result in significant neurocognitive, neuropsychological, and neuroendocrine dysfunctions and/or sequelae (Morton and Wehman 1995; Kelly et al. 2000). In this situation, both anterior and posterior pituitary hormone insufficiencies are observed (Lieberman et al. 2001).

Although diabetes insipidus is easily recognized in the acute postinjury phase, subtle insufficiencies of other pituitary hormones may be escaping diagnosis for months or years. All patients with a history of traumatic brain injury require ongoing surveillance for pituitary insufficiency and particularly secondary hypogonadism.

Irradiation can be a cause of secondary hypogonadism in patients who receive 40 Gy or more of radiation in the head region and delayed puberty may occur if irradiation is administered before normal initiation of puberty (Mills et al. 1997). Secondary hypogonadism may appear even years after irradiation, with rates of total incidence ranging from 20% to 50% (Constine et al. 1993; Rappaport et al. 1982).

An important but rare cause of secondary hypogonadism is *infiltrating diseases* causing hypothalamic dysfunction including *sarcoidosis*, *Langerhans cell histiocytosis*, *hemochromatosis*, *leukemia*, *lymphoma*, and *Wegener's granulomatosis*. Of course, the possibility of metastases from other organs (i.e. lung cancer) should be always included in the differential diagnosis.

Clinical Presentation

The clinical presentation of secondary hypogonadism depends on the age of the patient and more specifically with regard to its occurrence before or after the initiation of puberty. Thus, the clinical classification of secondary hypogonadism is based upon the cardinal symptom of female hypogonadism, which is the absence of menses (amenorrhea). Subsequently, secondary hypogonadism is distinguished depending on whether it occurs before (primary amenorrhea) or after (secondary amenorrhea) the initiation of menses. In secondary amenorrhea, menstrual cessation occurs after its normal initiation. Sometimes this distinction is unclear because partial progression through puberty is commonly encountered in a subset of affected individuals in many conditions, leading to clinical heterogeneity even within the same kindred. In primary amenorrhea, the absence of menarche (first menstrual bleeding) is usually accompanied by the delay of the pubertal development (breast development, height, axillary hair) although some patients undergo some degree of pubertal development that then ceases. In case of prolactinoma occurring before puberty, no milky breast discharge should be expected due to the absence of breast development and/or low estrogen levels.

Of note, the clinical phenotype of isolated GnRH deficiency in women is broader than previously thought. In light of this phenotypic variability, dismissing the diagnosis of GnRH deficiency in women with spontaneous thelarche and isolated menses is not appropriate, as GnRH function may change over time and/or adrenarche may provide the substrate for early breast and endometrial development. In women with isolated GnRH deficiency, rare sequence variants in all of the genes implicated in this disorder are found, including ANOS1 (Shaw et al. 2011). Anosmia is a clinical symptom that facilitates differential diagnosis and diagnosis per se.

The clinical consequences of E2 deficiency in postpubertal women with secondary hypogonadism are similar to those seen in women with postpubertally occurring

primary hypogonadism (primary ovarian insufficiency or premature ovarian failure). Findings in premenopausal women presenting secondary hypogonadism include irregular periods or amenorrhea, anovulatory infertility, vaginal atrophy, and hot flashes. Obviously, the diagnosis of secondary hypogonadism during premenopause is extremely difficult as it is confounded with the climacteric clinical picture. No physical findings of hypogonadism are detectable initially, but in time, breast tissue decreases and bone mineral density (BMD) declines. Finally it should be stated that depending on the cause of the secondary hypogonadism the clinical presentation may include headaches or vision loss (in case of tumor), milky breast discharge (in case of prolactinoma), symptoms of other hormonal deficiencies (such as hypothyroidism), and congenital anomalies (in congenital GnRH deficiency).

Diagnosis

Diagnosis of Secondary Female Hypogonadism

The evaluation of a child with delayed puberty begins with a careful history and physical examination. Eventually, the distinction between *IHH* and *constitutional delay of puberty* is a difficult one. Time is a critical factor in distinguishing between these two conditions. In IHH spontaneous puberty never occurs while in constitutional delay of puberty, spontaneous and otherwise normal puberty eventually occurs. Evidence suggests that either of these conditions are not discrete clinical entities but rather part of a common phenotypic spectrum. In families with members suffering IHH, delayed puberty occurs at a much higher frequency in otherwise "normal" members of the same family compared to the general population, suggesting that constitutional delay of puberty may represent a milder clinical variant of the IHH phenotype (Waldstreicher et al. 1996; Pitteloud et al. 2006). The distinction between constitutional delay of puberty and IHH cannot be reliably made at any age. Thus, the age of 18 years has been suggested as the age at which IHH can be diagnosed. However, the recent description of IHH "reversal" occurring in persons in their 20s and older raises the possibility that such individuals may have a severe form of constitutional delay of puberty (Raivio et al. 2007). The presence of other clinical features associated with IHH (i.e., anosmia, synkinesia) may lead to the diagnosis of this entity before the age of 18 years. No clinically available test can reliably differentiate between these two entities. It seems that the mean serum concentrations of LH and sex hormones after GnRH or hCG stimulation vary significantly between individuals with constitutional delay of puberty and those with IHH. However, the clinical utility of GnRH stimulation test is limited by the significant variation in individual LH and sex hormone serum concentrations, resulting in considerable overlap between groups (Degros et al. 2003). A peak-to-basal ratio of free α subunit after GnRH administration has been proposed for the differentiation between them with a sensitivity and specificity in the 95% range and an overlap rate of 10% (Mainieri and Elnecave 2003). In addition, more recently, the combination of a 19-day hCG test with a conventional GnRH test has also been

proposed to improve this differentiation (Segal et al. 2009). Given the relatively small number of individuals studied and the limited follow-up in both studies, prospective validation is required to determine the true diagnostic reliability of the above tests.

In adult women, hypogonadism expressed as amenorrhea is diagnosed by the absence of menses for more than 3 months in females (adolescents or women) who previously had regular menstrual cycles or 6 months for those who previously had irregular menses (Deligeoroglou et al. 2010).

Finally, it should be stated that menopause is also a state of hypogonadism. Commonly, the postmenopausal woman is not called hypogonadal if she is of typical menopausal age. This is because hypogonadism is an abnormality, whereas menopause is considered a normal endocrine change. Natural menopause is defined as the permanent cessation of menstrual periods, determined retrospectively after a woman has experienced 12 months of amenorrhea not due to any other obvious pathological or physiological cause. It occurs at a median age of approximately 51.5 years of age in normal women, depending on race and geographical latitude. It is a reflection of complete, or near complete, ovarian follicular depletion, with resulting hypoestrogenemia and high FSH concentrations. Menopause before the age of 40 is considered to be abnormal and is referred to as *premature ovarian failure*. The menopausal transition, or perimenopause, occurs after the years of regular reproductive function, but before menopause, and is characterized by irregular menstrual cycles, endocrine changes, and symptoms such as hot flashes. If gonadotropins, measured for any other reason, are found low or undetectable during menopause (particularly the first decade after menopause) the suspicion of causes of hypogonadotropinism (secondary hypogonadism) should be set and investigated. Clinical signs and symptoms from the other hormonal axes should be sought.

Differential Diagnosis between Primary and Secondary Female Hypogonadism

Physicians should carry out a comprehensive patient history and a thorough physical examination of patients with amenorrhea.

Before initiation of puberty, the differential diagnosis between primary and secondary female hypogonadism is extremely difficult if diagnosis of hypogonadism per se is suspected. Gonadotropins are not elevated in either situation (van Vliet 1988). Only specific characteristics of distinct etiologies can be of help (i.e., anosmin in KS, clinical presentation in genetic syndromes, information from the karyotype, etc.).

Postpubertally, to categorize hypogonadism with regard to the defective level of the HPO, gonadotropins are measured. In primary hypogonadism, where the defect is gonadal, LH and FSH are usually elevated, whereas in secondary hypogonadism, both are either inappropriately normal or low, suggesting that the defect is found in the hypothalamo-pituitary region.

Differential Diagnosis between Pituitary and Suprapituitary Secondary Female Hypogonadism

In a clinical context, the GnRH stimulation test is often performed to differentiate between causes emerging from the hypothalamus or the pituitary. However, its discriminatory power is challenged because it might take several days of GnRH stimulation to prime otherwise normal pituitary gonadotrope cells which are not normally stimulated by GnRH in case of secondary hypogonadism of suprapituitary origin (Segal et al. 2009). Thus, a single GnRH injection for a test might lead to false negative results. Imaging investigation is of major importance for the differentiation.

Differential Diagnosis between Genetic and Acquired Causes of Secondary Female Hypogonadism

Clinical presentation of IHH depends on the time of onset (i.e., congenital vs acquired), the severity of the defect, and the presence of associated conditions. Typically the diagnosis of congenital IHH is made during the second or early third decade of life, when the patients present with delayed pubertal onset, absent or poorly developed secondary sexual characteristics, primary amenorrhea, eunuchoid proportions, or infertility. Newborn girls have no obvious abnormal stigmata that might provide clues to the diagnosis. Most commonly, however, the diagnosis cannot be confirmed until the expected time of puberty onset, except in the neonatal period, when during minipuberty gonadotropin and sexual steroid levels are expected to be elevated and, in this case, they are not. The presence of anosmia during childhood is suggestive of KS. If the child is too young to undergo olfaction tests, MRI scan showing absent or abnormal olfactory bulbs or sulci strongly suggests the diagnosis. Of note, normal MRI does not rule out the disease because normal olfactory bulbs can be present in up to 20% of KS patients (Silveira et al. 2002; Mitchell et al. 2011).

Adult-onset IHH in women is characterized by secondary amenorrhea, decreased libido, infertility, and osteoporosis. Low/normal gonadotropin levels (usually less than 4–5 IU/L) in association with low concentrations of E2 (less than 20 pg/mL) evokes the diagnosis. Rarely, selective deficiencies of LH or FSH can occur due to inactivating mutations of the specific β subunits. Anterior pituitary function must be investigated to rule out a more complex endocrine disorder with multiple hormone deficiencies. Although widely used, the practical value of the GnRH test has been questionable because of its low cost-effectiveness. Indeed, the GnRH test provides no extra diagnostic information relative to baseline gonadotropin levels. In patients with IHH, the response to GnRH test is highly variable and depends on the severity of the gonadotropin deficiency, which is often reflected by the clinical phenotype. Similarly, the pituitary function can be first evaluated by basal hormonal levels (measured by ultrasensitive assays). Thyroid function should be assessed by TSH combined with free T4. IGF-I can be used to evaluate the somatotropic axis, whereas secondary adrenal deficiency can be assessed by measuring a morning cortisol and

ACTH. The stimulatory tests should be reserved for the situations in which the basal hormone measurements are not helpful or if there is strong clinical evidence of a multiple pituitary hormone deficiency. If KS is suspected, anosmia can be easily diagnosed by questioning the patient, whereas olfactometry, such as University of Pennsylvania Smell Identification Test, is necessary to determine reliably whether olfaction is normal or partially defective. Accurate olfactory phenotyping in these subjects can inform for the pathophysiology of this condition and guide genetic testing.

MRI of the hypothalamopituitary region is very useful. It can demonstrate a malformation, an expansive or infiltrative disorder of the hypothalamo-pituitary region. However, the cost-effectiveness of MRI scan to exclude pituitary and/or hypothalamic tumors is unknown according to the recent clinical practice guideline. Pituitary and/or hypothalamic tumors should be investigated by MRI in patients with multiple pituitary hormone deficiency, persistent hyperprolactinemia, or symptoms of tumor mass effect (headache, visual impairment, or visual field defect). In the presence of suspected functional causes of secondary amenorrhea, such as severe obesity (Di Carlo et al. 1999), nutritional disorders, and drugs, MRI may be a second-line diagnostic test. Renal ultrasound examination is usually recommended to patients with secondary hypogonadism because it is known that unilateral kidney agenesis may be more prevalent in patients with ANOS1 defects.

The genetic study is usually the last step in the congenital IHH investigation, and complete clinical characterization could certainly help in the gene selection. Bone mineral density of the lumbar spine, femoral neck, and hip is recommended at the initial diagnosis of HH and after 1–2 years of sex steroid therapy in hypogonadal patients with osteoporosis or low trauma fracture.

If an acquired cause of secondary hypogonadism is suspected, the appropriate diagnostic approach for the revelation of each pathological cause should be adopted.

Treatment of Secondary Female Hypogonadism

It comprises (1) *the treatment of the causal factor* and (2) *the hormone replacement therapy.*

Regarding the former, one should address the causative agent of the hypogonadism followed by the appropriate therapeutic intervention, e.g., surgery or irradiation for the tumor, drug treatment for the infective chronic disease, and discontinuation of the offensive drug treatment. As far as the symptomatic treatment, the main goal prepubertally should be the induction and maintenance of puberty by hormone treatment with sex steroids. Special care should be given to achievement of the genetic potential for height.

Postpubertally, in women with secondary hypogonadism, there are three goals to satisfy: (1) correction of the causal factor when feasible, (2) installation or improvement of fertility with ultimate result a healthy take-home baby, and (3) prevention of the complications related to the pathophysiology of the disease process (e.g., estrogen replacement to prevent osteoporosis).

Etiologic Treatment of Secondary Female Hypogonadism

Treatment of Reversible Acquired Causes (Including Functional)

The treatment should be focused on controlling the causative agent if it is a disease, or termination of the offending drug use, with the possibility of reversing their effects on the HPO axis.

In particular, the treatment of *FHA* involves correcting the energy imbalance to improve the HPO axis function. This includes *cognitive behavioral therapy* (CBT) and/or other forms of psychological support, increased caloric consumption, and/or improved nutrition, even if this often requires weight gain and/or decreased exercise activity. Additionally in cases of FHA with severe bradycardia, hypotension, orthostasis, and/or electrolyte imbalance, an inpatient evaluation is recommended. Combined oral contraceptives are not recommended for the sole purpose of regaining menses or improving BMD as, although they will restore menses, they may mask the return of spontaneous menses and will not correct bone density, particularly if patients maintain an energy deficit. In the case of adolescents and women who have not had return of menses after 6–12 months, a short-term use of transdermal E2 replacement therapy with cyclic progestin (not oral contraceptives or ethinyl E2) is recommended. The use of bisphosphonates, denosumab, testosterone, and leptin for improvement of BMD in adolescents and women with FHA has not been proven beneficial but the short-term use of recombinant 1–34 parathyroid hormone (rPTH) is an option in the rare adult FHA cases with delayed fracture healing and very low BMD. For the treatment of fertility in women with FHA, the options are either treatment with pulsatile GnRH, wherever this is possible, as a first line followed by gonadatropin therapy; induction of ovulation with the recommended protocols when GnRH is not available. A trial of treatment with clomiphene citrate, if they have sufficient endogenous estrogen level or a trial of CBT, can be considered as the latter has the potential to restore ovulatory cycles and fertility without the need for medical intervention. The use of kisspeptin and leptin for treating infertility has not proven its efficacy. Lastly, due to the increased risk for fetal loss, small for gestational age babies, preterm labor, and delivery by Cesarean section for extreme low weight, particularly in the setting of eating disorders, it is recommended that the induction of ovulation in women with FHA should be limited to women with BMI of at least 18.5 kg/m2.

Treatment of Irreversible Acquired Causes (Including Tumors)

Regarding the acquired causes of secondary hypogonadism, if the nature of the damage is permanent, treatment is the same as for the genetic causes described above.

It should be stated that caution should be exerted in the case of hormone replacement therapy as studies have shown that there is an increased risk of developing brain tumors, meningiomas in particular. Confirmatory molecular and immunohistochemical studies showed that meningioma is a hormone sensitive tumor, with approximately 70% of meningiomas expressing progesterone receptors and approximately 30% expressing estrogen receptors.

Hormone Replacement Therapy

Treatment of Prepubertal Secondary Female Hypogonadism

Induction of puberty: To allow optimal breast development, initial treatment should consist of unopposed estrogen replacement via oral or topical preparations. Many formulations of estrogens are available; a suggested oral regimen is using conjugated estrogens (wherever available) 0.3 mg daily to be increased gradually to an adult replacement dose of 1–1.25 mg daily. Equivalent adult doses of oral therapy are micronized E2, 2 mg; esterified estrogen, 1.25 mg; ethinyl E2, 8–10 μg; and conjugated estrogens, 1.25 mg. Once breast development is in progress a progestin should be added for endometrial protection (usually after 24 months of treatment), e.g., cyclical micronized progesterone 200 mg daily for 10–14 days. Although the informed preference of the individual plays an important role in the choice of the treatment plan, low-estrogen formulations should be considered in women with clotting abnormalities. Limitations of oral estrogen therapy include variable bioavailability due to first-pass metabolism within the liver, which subsequently might affect liver function and clotting factors (Mauras et al. 2007; Ankarberg-Lindgren et al. 2001). As a result, transdermal estrogen formulations are gaining in popularity even for the induction of puberty. Pubertal induction can be accomplished with transdermal E2 at a dose as low as 3.1–6.2 μg E2/24 h (Ankarberg-Lindgren et al. 2001). Puberty can then be mimicked with subsequent doubling of the dose of E2 after a median duration of 8 months and addition of progesterone 2 years after estrogen initiation. A transdermal estrogen dose of 100 μg/24 h is equivalent to an adult regimen. Significantly greater serum concentrations of 17β-E2 were noted with oral estrogen as compared to transdermal estrogen. However, no differences have been noted between short-term transdermal vs. oral estrogen therapy regarding metabolic effects including lipolysis, lipid, and carbohydrate oxidation as well as resting energy expenditure (Mauras et al. 2007). In contrast, a pilot study comparing treatment of girls with Turner syndrome with transdermal E2 versus oral conjugated estrogens found better bone mineral accrual and uterine development in the group treated with transdermal E2 (Nabhan et al. 2009). Percutaneous E2 gel has also been investigated for pubertal induction in girls with Turner syndrome at a starting dose of 100 μg nightly with increases of 100 μg for each additional year up to 5 years. Local skin irritation is a side effect of percutaneous gel therapy (Piippo et al. 2004). For hypogonadal women, estrogen replacement is needed throughout reproductive life.

Treatment of Postpubertal (Adult) Secondary Female Hypogonadism

Hormone replacement therapy: Estrogen transdermal patches are widely used in adult women, and doses of 0.625 and 1.25 mg of oral conjugated estrogens have been reported to be similar to those of 50 and 100 μg of transdermal E2 per 24 h (Chetkowski et al. 1986). Optimal calcium and vitamin D intake should be encouraged and specific treatment for decreased bone mass with bisphosphonates should be considered depending on the degree of bone mineralization.

Fertility treatment: In women with IHH, pulsatile GnRH stimulation and exogenous gonadotropins are approved for folliculogenesis. Either therapy should be

administered with close supervision by physicians specialized in ovulation induction. Intravenous administration of GnRH at various frequencies throughout the menstrual cycle closely mimics normal menstrual cycle resulting in ovulation of a single follicle (Santoro et al. 1986). This therapy results in lower rates of both multiple gestation and ovarian hyperstimulation syndrome as compared to the traditional treatment with exogenous gonadotropins. However, the rate of conception for either approach is approximately 30% per ovulatory cycle (Martin et al. 1990). If spontaneous conception is not achieved with these ovulation induction methods, IVF may be an option.

Genetic counseling: Finally, in addition to the above therapeutic measures, genetic counseling should be offered to patients and their families, including information on the nature, inheritance, and implications of the genetic disorder. Genetic counseling includes genetic risk assessment for the patient while by employing family history and genetic testing the genetic status for family members should be clarified.

Treatment in menopause does not differ from the suggested treatment for menopausal women.

Summary

Female hypogonadism is a state characterized by absent or decreased ovarian function. It results from a gonadal (primary hypogonadism) or an extragonadal (secondary hypogonadism) princeps defect. In secondary hypogonadism, hypothalamic GnRH or/and pituitary-secreted gonadotropins are either deficient or inactive leading to decreased secretion of gonadal steroids and subsequent amenorrhea. Throughout the medical literature, the term secondary hypogonadism may be encountered also as GnRH deficiency or gonadotropin deficiency depending on the level of the defect (hypothalamic or pituitary, respectively); as central; or as hypogonadotropic hypogonadism given that, independently of the level of the abnormality (hypothalamic or/and pituitary) the result is the same, i.e., decreased gonadotropins levels. Secondary hypogonadism is a group of disorders resulting from an abnormality in GnRH secretion from the hypothalamus or an abnormality in gonadotropins secretion from the pituitary. In certain conditions, both suprapituitary and pituitary dysfunctions are present. The genetic causes of secondary hypogonadism manifest mainly as congenital genetic syndromes (i.e., KS) while some of them have been attributed to recognized single gene mutations and others have been characterized as idiopathic forms. The acquired causes of secondary hypogonadism are due to structural or functional abnormalities of the hypothalamic-pituitary axis. Usually they present with multiple pituitary hormone deficiencies. Their etiology includes: *CNS or pituitary tumors*, *infiltrative diseases*, *infection*, *brain/pituitary irradiation*, *pituitary apoplexy*, *head trauma*, *drugs* (GnRH agonists/antagonists, glucocorticoids, narcotics, chemotherapy), functional deficiency resulting from *chronic systemic illness*, *eating disorders*, *hypothyroidism*, *hyperprolactinemia*, *diabetes mellitus*, and *Cushing disease*. Diagnosis should take in consideration the age at the clinical presentation (prepubertal or postpubertal), the

physical findings as well as biochemical and imaging findings. Genetic investigation can be employed for more precise diagnosis. The treatment of secondary female hypogonadism should include *the treatment of the causal factor* and *the hormone replacement therapy.* One should address the causative agent of the hypogonadism followed by the appropriate therapeutic intervention, e.g., surgery or irradiation for the tumor, drug treatment for the infective, chronic disease, and discontinuation of the offensive drug treatment. Regarding the hormone replacement therapy, the main goal prepubertally should be the induction and maintenance of puberty, by hormone treatment with sex steroids. Special care should be given to achievement of the genetic potential for height. Postpubertally, in women with secondary hypogonadism, treatment should include correction of the causal factor when feasible, installation or improvement of fertility with ultimate result a healthy take-home baby and finally, prevention of the complications related to the pathophysiology of the disease process (e.g., estrogen replacement to prevent osteoporosis).

References

Ahima RS, Prabakaran D, Mantzoros C, et al. Role of leptin in the neuroendocrine response to fasting. Nature. 1996;382:250–2.

Akefeldt A, Tornhage CJ, Gillberg C. A woman with Prader-Willi syndrome gives birth to a healthy baby girl. Dev Med Child Neurol. 1999;41(11):789–90.

Amsterdam A, Rotmensch S. Structure-function relationships during granulosa cell differentiation. Endocr Rev. 1987;8(3):309–37.

Ankarberg-Lindgren C, Elfving M, Wikland KA, Norjavaara E. Nocturnal application of transdermal estradiol patches produces levels of estradiol that mimic those seen at the onset of spontaneous puberty in girls. J Clin Endocrinol Metab. 2001;86(7):3039–44.

Armada-Dias L, Carvalho JJ, Breitenbach MMD, Franci CR, Moura EG. Is the infertility in hypothyroidism mainly due to ovarian or pituitary functional changes? Braz J Med Biol Res. 2001;34(9):1209–15.

Aubert ML, Pierroz DD, Gruaz NM, et al. Metabolic control of sexual function and growth: role of neuropeptide Y and leptin. Mol Cell Endocrinol. 1998;140(1–2):107–13. Review

Baird DT, Smith KB. Inhibin and related peptides in the regulation of reproduction. Oxford RevReprod. Biol. 1993;15:191–232.

Barni T, Maggi M, Fantoni G, et al. Sex steroids and odorants modulate gonadotropin-releasing hormone secretion in primary cultures of human olfactory cells. J Clin Endocrinol Metab. 1999;84(11):4266.

Bauer-Dantoin AC, McDonald JK, Levine JE, Neuropeptide Y. Potentiates luteinizing hormone (LH)-releasing hormone-induced LH secretion only under conditions leading to preovulatory LH surges. Endocrinology. 1992;131(6):2946–52.

Bauer-Dantoin AC, Weiss J, Jameson JL. Roles of estrogen, progesterone, and gonadotropin-releasing hormone (GnRH) in the control of pituitary GnRH receptor gene expression at the time of the preovulatory gonadotropin surges. Endocrinology. 1995;136(3):1014–9.

Bellefontaine N, Chachlaki K, Parkash J, et al. Leptin-dependent neuronal NO signaling in the preoptic hypothalamus facilitates reproduction. J Clin Invest. 2014;124:2550–9.

Bergendahl M, Veldhuis JD. Altered pulsatile gonadotropin signaling in nutritional deficiency in the male. Trends Endocrinol Metab. 1995;6(5):145–59.

Bergendahl M, Aloi JA, Iranmanesh A, Mulligan TM, Veldhuis JD. Fasting suppresses pulsatile luteinizing hormone (LH) secretion and enhances orderliness of LH release in young but not older men. J Clin Endocrinol Metab. 1998;83(6):1967–75.

Besecke LM, Guendner MJ, Schneyer AL, Bauer-Dantoin AC, Jameson JL, Weiss J. Gonadotropin-releasing hormone regulates follicle-stimulating hormone-beta gene expression through an activin/follistatin autocrine or paracrine loop. Endocrinology. 1996;137(9):3667–73.

Bilezikjian LM, Corrigan AZ, Blount AL, Vale WW. Pituitary follistatin and inhibin subunit messenger ribonucleic acid levels are differentially regulated by local and hormonal factors. Endocrinology. 1996;137(10):4277–84.

Blumenfeld Z. Response of human fetal pituitary cells to activin, inhibin, hypophysiotropic and neuroregulatory factors in vitro. Early Pregnancy. 2001;5(1):41–2.

Boden G, Chen X, Mozzoli M, Ryan I. Effect of fasting on serum leptin in normal human subjects. J Clin Endocrinol Metab. 1996;81:3419–23.

Bohnet HG, Dahlén HG, Wuttke W, Schneider HP. Hyperprolactinemic Anovulatory syndrome. J Clin Endocrinol Metab. 1976;42(1):132–43.

Bramley TA, Stirling D, Swanston IA, Menzies GS, McNeilly AS, Baird DT. Specific binding sites for gonadotrophin-releasing hormone, LH/chorionic gonadotrophin, low-density lipoprotein, prolactin and FSH in homogenates of human corpus luteum. II: concentrations throughout the luteal phase of the menstrual cycle and early pregnancy. J Endocrinol. 1987;113(2):317–27.

Brann DW, Mahesh VB. Excitatory Amino Acids. Their Role in Neuroendocrine Function. Front Neuroendocrinol. 1994;15:3–49.

Bray GA, York DA. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol Rev. 1979;59:719–809.

Brundu B, Loucks TL, Adler LJ, Cameron JL, Berga SL. Increased cortisol in the cerebrospinal fluid of women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab. 2006;91 (4):1561–5.

Burke MC, Letts PA, Krajewski SJ, Rance NE. Coexpression of dynorphin and neurokinin B immunoreactivity in the rat hypothalamus: morphologic evidence of interrelated function within arcuate nucleus. J Comp Neurol. 2006;5:712–26.

Catzeflis C, Pierroz DD, Rohner-Jeanrenaud F, Rivier JE, Sizonenko PC, Aubert ML. Neuropeptide Y administered chronically into the lateral ventricle profoundly inhibits both the gonadotropic and the somatotropic axis in intact adult female rats. Endocrinology. 1993;132(1):224–34.

Chan JL, Mantzoros CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. Lancet. 2005;366(9479):74.

Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. J Clin Invest. 2003;111:1409–21.

Chen HF, Jeung EB, Stephenson M, Leung PC. Human peripheral blood mononuclear cells express gonadotropin-releasing hormone (GnRH), GnRH receptor, and interleukin-2 receptor gamma-chain messenger ribonucleic acids that are regulated by GnRH in vitro. J Clin Endocrinol Metab. 1999;84(2):743–50.

Cheng G, Coolen LM, Padmanabhan V, Goodman RL, Lehman MN. The kisspeptin/neurokinin B/dynorphin (KDNy) cell population of the arcuate nucleus: sex differences and effects of prenatal testosterone in sheep. Endocrinology. 2010;1:301–11.

Chetkowski RJ, Meldrum DR, Steingold KA, et al. Biologic effects of transdermal estradiol. N Engl J Med. 1986;314(25):1615–20.

Cheung LW, Wong AS. Gonadotropin-releasing hormone: GnRH receptor signaling in extra-pituitary tissues. FEBS J. 2008;275(22):5479–95.

Choi JH, Gilks CB, Auersperg N, Leung PC. Immunolocalization of gonadotropin-releasing hormone (GnRH)-I, GnRH-II, and type I GnRH receptor during follicular development in the human ovary. J Clin Endocrinol Metab. 2006;91(11):4562–7.

Chou, Mantzoros. 20 years of leptin: role of leptin in human reproductive disorders. J Endocrinol. 2014;223(1):T49–62.

Chou CS, Beristain AG, MacCalman CD, Leung PC. Cellular localization of gonadotropin-releasing hormone (GnRH) I and GnRH II in first-trimester human placenta and decidua. J Clin Endocrinol Metab. 2004;89(3):1459–66.

Chou SH, Chamberland JP, Liu X, et al. Leptin is an effective treatment for hypothalamic amenorrhea. Proc Natl Acad Sci U S A. 2011;108:6585–90.

Christian CA, Moenter SM. Vasoactive intestinal polypeptide can excite gonadotropin-releasing hormone neurons in a manner dependent on estradiol and gated by time of day. Endocrinology. 2008;149:3130–6.

Christian CA, Moenter SM. The neurobiology of preovulatory and estradiol-induced gonadotropin-releasing hormone surges. Endocr Rev. 2010;31(4):544–77.

Chrousos GP, Torpy DJ, Gold PW. Interactions between the hypothalamic-pituitary-adrenal Axis and the female reproductive system: clinical implications. Ann Intern Med. 1998;129(3):229–40.

Clement K, Vaisse C, Lahlou N, et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature. 1998;392:398–401.

Constine LS, Woolf PD, Cann D, et al. Hypothalamic-pituitary dysfunction after radiation for brain tumors. N Engl J Med. 1993;328(2):87–94.

Conte FA, Grumbach MM, Kaplan SL, Reiter EO. Correlation of luteinizing hormone-releasing factor-induced luteinizing hormone and follicle-stimulating hormone release from infancy to 19 years with the changing pattern of gonadotropin secretion in Agonadal patients: relation to the restraint of puberty. J Clin Endocrinol Metab. 1980;50(1):163–8.

Crino A, Schiaffini R, Ciampalini P, et al. Hypogonadism and pubertal development in PraderWilli syndrome. Eur J Pediatr. 2003;162(5):327–33.

Crowley WR, Tessel RE, O'Donohue TL, Adler BA, Kalra SP. Effects of ovarian hormones on the concentrations of immunoreactive neuropeptide Y in discrete brain regions of the female rat: correlation with serum luteinizing hormone (LH) and median eminence LH-releasing hormone. Endocrinology. 1985;117(3):1151–5.

Cui J, Smith RG, Mount GR, et al. Identification of Phe313 of the gonadotropin-releasing hormone (GnRH) receptor as a site critical for the binding of nonpeptide GnRH antagonists. Mol Endocrinol. 2000;14(5):671–81.

Dandona P, Dhindsa S. Update: hypogonadotropic hypogonadism in type 2 diabetes and obesity. J Clin Endocrinol Metab. 2011;96(9):2643–51.

Dasen JS, Barbera JP, Herman TS, et al. Temporal regulation of a paired-like homeodomain repressor/TLE corepressor complex and a related activator is required for pituitary organogenesis. Genes Dev. 2001;15(23):3193–207.

Dattani MT, Martinez-Barbera JP, Thomas PQ, et al. Mutations in the homeobox gene HESX1/Hesx1 associated with septooptic dysplasia in human and mouse. Nat Genet. 1998;19(2):125–33.

de Roux N, Milgrom E. Inherited disorders of GnRH and gonadotropin receptors. Mol Cell Endocrinol. 2001;179(1–2):83–7.

de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A. 2003;100(19):10972–6.

Degros V, Cortet-Rudelli C, Soudan B, Dewailly D. The human chorionic gonadotropin test is more powerful than the gonadotropin-releasing hormone agonist test to discriminate male isolated hypogonadotropic hypogonadism from constitutional delayed puberty. Eur J Endocrinol. 2003;149:23–9.

Deligeoroglou E, Athanasopoulos N, Tsimaris P, Dimopoulos KD, Vrachnis N, Creatsas G. Evaluation and management of adolescent amenorrhea. Ann N Y Acad Sci. 2010;1205:23–32.

Di Carlo C, Palomba S, De Fazio M, Gianturco M, Armellino M, Nappi C. Hypogonadotropic hypogonadism in obese women after biliopancreatic diversion. Fertil Steril. 1999;72(5):905–9.

Dierschke DJ, Bhattacharya AN, Atkinson LE, Knobil E. Circhoral oscillations of plasma LH levels in the Ovariectomized rhesus monkey. Endocrinology. 1970;87(5):850–3.

Donato J Jr, Cravo RM, Frazão R, et al. Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons. J Clin Invest. 2011;121:355–68.

Ducret E, Anderson GM, Herbison AE. RFamide-related peptide-3, a mammalian gonadotropin-inhibitory hormone ortholog, regulates gonadotropin-releasing hormone neuron firing in the mouse. Endocrinology. 2009;150:2799–804.

Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001;142:4891–9.

Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology. 2002;143(3):1076–84.

Eicke N, Gunthert AR, Viereck V, et al. GnRH-II receptor-like antigenicity in human placenta and in cancers of the human reproductive organs. Eur J Endocrinol. 2005;153(4):605–12.

El Majdoubi M, Sahu A, Ramaswamy S, Plant TM. Neuropeptide Y: a hypothalamic brake restraining the onset of puberty in primates. Proc Natl Acad Sci U S A. 2000;97(11):6179–84.

Evans WS, Weltman JY, Johnson ML, Weltman A, Veldhuis JD, Rogol AD. Effects of opioid receptor blockade on luteinizing hormone (LH) pulses and interpulse LH concentrations in normal women during the early phase of the menstrual cycle. J Endocrinol Investig. 1992;15(7):525–31.

Farooqi IS, Jebb SA, Langmack G, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med. 1999;341:879–84.

Farooqi IS, Matarese G, Lord GM, et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest. 2002;110:1093–103.

Farooqi IS, Wangensteen T, Collins S, et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. N Engl J Med. 2007;356:237–47.

Fister S, Gunthert AR, Aicher B, Paulini KW, Emons G, Grundker C. GnRH-II antagonists induce apoptosis in human endometrial, ovarian, and breast cancer cells via activation of stress-induced MAPKs p38 and JNK and proapoptotic protein Bax. Cancer Res. 2009;69(16):6473–81.

Fleming R, Kelsey TW, Anderson RA, Wallace WH, Nelson SM. Interpreting human follicular recruitment and antimullerian hormone concentrations throughout life. Fertil Steril. 2012;98:1097–102.

Foradori CD, Coolen LM, Fitzgerald ME, Skinner DC, Goodman RL, Lehman MN. Colocalization of progesterone receptors in Parvicellular Dynorphin neurons of the ovine preoptic area and hypothalamus. Endocrinology. 2002;143(11):4366–74.

Foradori CD, Goodman RL, Adams VL, Valent M, Lehman MN. Progesterone increases Dynorphin a concentrations in cerebrospinal fluid and Preprodynorphin messenger ribonucleic acid levels in a subset of Dynorphin neurons in the sheep. Endocrinology. 2005;146(4):1835–42.

Fox SR, Harlan RE, Shivers BD, Pfaff DW. Chemical characterization of neuroendocrine targets for progesterone in the female rat brain and pituitary. Neuroendocrinology. 1990;51(3):276–83.

Franco B, Guioli S, Pragliola A, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature. 1991;353(6344):529–36.

Ghizzoni L, Mastorakos G, Vottero A, et al. Corticotropin-releasing hormone (CRH) inhibits steroid biosynthesis by cultured human granulosa-lutein cells in a CRH and interleukin-1 receptor-mediated fashion. Endocrinology. 1997;138(11):4806–11.

Gibson MJ, Krieger DT, Charlton HM, Zimmerman EA, Silverman AJ, Perlow MJ. Mating and pregnancy can occur in genetically hypogonadal mice with preoptic area brain grafts. Science. 1984;225(4665):949.

Goodman RL, Parfitt DB, Evans NP, Dahl GE, Karsch FJ. Endogenous opioid peptides control the amplitude and shape of gonadotropin-releasing hormone pulses in the ewe. Endocrinology. 1995;136(6):2412–20.

Gottsch ML, Navarro VM, Zhao Z, et al. Regulation of Kiss1 and Dynorphin gene expression in the murine brain by classical and nonclassical Estrogen receptor pathways. J Neurosci. 2009;29(29):9390–5.

Grumbach M, Kaplan S. The neuroendocrinology of human puberty: an ontogenetic perspective. In: Grumbach M, Sizonenko P, Aubert M, editors. Control of the onset of puberty. Baltimore: Williams & Wilkins; 1990.

Grynberg M, Pierre A, Rey R, et al. Differential regulation of ovarian anti-mullerian hormone (AMH) by estradiol through a- and b- estrogen receptors. J Clin Endocrinol Metab. 2012;97: E1649–57.

Haddad NG, Eugster EA. Hypopituitarism and neurodevelopmental abnormalities in relation to central nervous system structural defects in children with optic nerve hypoplasia. J Pediatr Endocrinol Metab. 2005;18(9):853–8.

Han SK, Gottsch ML, Lee KJ, et al. Activation of gonadotropin-releasing hormone neurons by Kisspeptin as a neuroendocrine switch for the onset of puberty. J Neurosci. 2005;25(49):11349–56.

Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS. Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinology. 1997;138:3859–63.

Herbison AE, Skinner DC, Robinson JE, King IS. Androgen receptor-immunoreactive cells in ram hypothalamus: distribution and co-localization patterns with gonadotropin-releasing hormone, somatostatin and tyrosine hydroxylase. Neuroendocrinology. 1996;63(2):120–31.

Hill JW, Urban JH, Xu M, Levine JE. Estrogen induces neuropeptide Y (NPY) Y1 receptor gene expression and responsiveness to NPY in Gonadotrope-enriched pituitary cell cultures. Endocrinology. 2004;145(5):2283–90.

Hrabovszky E, Ciofi P, Vida B, et al. The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. Eur J Neurosci. 2010;31(11):1984–98.

Ingraham HA, Lala DS, Ikeda Y, et al. The nuclear receptor steroidogenic factor 1 acts at multiple levels of the reproductive axis. Genes Dev. 1994;8(19):2302–12.

Irwig MS, Fraley GS, Smith JT, et al. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. Neuroendocrinology. 2004;80 (4):264–72.

Jeffcoate WJ, Laurance BM, Edwards CR, Besser GM. Endocrine function in the Prader-Willi syndrome. Clin Endocrinol. 1980;12(1):81–9.

Jeppesen JV, Anderson RA, Kelsey TW, et al. Which follicles make the most anti-Mullerian hormone in humans? Evidence for an abrupt decline in AMH production at the time of follicle selection. Mol Hum Reprod. 2013;19:519–27.

Kaiser UB, Conn PM, Chin WW. Studies of gonadotropin-releasing hormone (GnRH) action using GnRH receptor-expressing pituitary cell lines. Endocr Rev. 1997;18(1):46–70.

Kakar SS, Jennes L. Expression of gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor mRNAs in various non-reproductive human tissues. Cancer Lett. 1995;98(1):57–62.

Kalra SP, Crowley WR. Differential effects of pancreatic polypeptide on luteinizing hormone release in female rats. Neuroendocrinology. 1984a;38(6):511–3.

Kalra SP, Crowley WR. Norepinephrine-like effects of neuropeptide Y on LH release in the rat. Life Sci. 1984b;35(11):1173–6.

Kalra SP, Dube MG, Sahu A, Phelps CP, Kalra PS. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proc Natl Acad Sci U S A. 1991;88(23):10931–5.

Kaynard AH, Pau KY, Hess DL, Spies HG. Third-ventricular infusion of neuropeptide Y suppresses luteinizing hormone secretion in ovariectomized rhesus macaques. Endocrinology. 1990;127(5):2437–44.

Kelberman D, Rizzoti K, Avilion A, et al. Mutations within Sox2/SOX2 are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans. J Clin Invest. 2006;116(9):2442–55.

Kelesidis T, Kelesidis I, Chou S, Mantzoros CS. Narrative review: the role of leptin in human physiology: emerging clinical applications. Ann Intern Med. 2010;152(2):93–100.

Kelly DF, Gonzalo IT, Cohan P, Berman N, Swerdloff R, Wang C. Hypopituitarism following traumatic brain injury and aneurysmal subarachnoid hemorrhage: a preliminary report. J Neurosurg. 2000;93(5):743–52.

Khorram O, Pau KY, Spies HG. Bimodal effects of neuropeptide Y on hypothalamic release of gonadotropin-releasing hormone in conscious rabbits. Neuroendocrinology. 1987;45(4):290–7.
Kiapekou E, Zapanti E, Mastorakos G, Loutradis D. Update on the role of ovarian corticotropin-releasing hormone. Ann N Y Acad Sci. 2010;1205:225–9.
King JC, Tai DW, Hanna IK, et al. A subgroup of LHRH neurons in guinea pigs with progestin receptors is centrally positioned within the total population of LHRH neurons. Neuroendocrinology. 1995;61(3):265–75.
Knobil E, Plant TM, Wildt L, Belchetz PE, Marshall G. Control of the rhesus monkey menstrual cycle: permissive role of hypothalamic gonadotropin-releasing hormone. Science. 1980;207 (4437):1371–3.
Kobayashi Y, Zhai YL, Iinuma M, Horiuchi A, Nikaido T, Fujii S. Effects of a GnRH analogue on human smooth muscle cells cultured from normal myometrial and from uterine leiomyomal tissues. Mol Hum Reprod. 1997;3(2):91–9.
Kotani M, Detheux M, Vandenbogaerde A, et al. The metastasis suppressor gene KiSS-1 encodes Kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J Biol Chem. 2001;276(37):34631–6.
Krassas GE, Pontikides N, Kaltsas T, et al. Disturbances of menstruation in hypothyroidism. ClinEndocrinol. 1999;50:655–9.
Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A. 1996;93(12):5925–30.
Latronico AC, Anasti J, Arnhold IJ, et al. Brief report: testicular and ovarian resistance to luteinizing hormone caused by inactivating mutations of the luteinizing hormone-receptor gene. N Engl J Med. 1996;334(8):507–12.
Laughlin GA, Dominguez CE, Yen SS. Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab. 1998;83(1):25.
Lee EJ, Moore CT, Hosny S, Centers A, Jennes L. Expression of estrogen receptor-α and c-Fos in adrenergic neurons of the female rat during the steroid-induced LH surge. Brain Res. 2000;875:56–65.
Lehman MN, Coolen LM, Goodman RL. Minireview: Kisspeptin/neurokinin B/Dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology. 2010;151(8):3479–89.
Leung PC, Cheng CK, Zhu XM. Multi-factorial role of GnRH-I and GnRH-II in the human ovary. Mol Cell Endocrinol. 2003;202(1–2):145–53.
Licinio J, Mantzoros C, Negrao AB, et al. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat Med. 1997;3:575–9.
Lieberman SA, Oberoi AL, Gilkison CR, Masel BE, Urban RJ. Prevalence of neuroendocrine dysfunction in patients recovering from traumatic brain injury. J Clin Endocrinol Metab. 2001;86(6):2752–6.
Loucks AB, Thuma JR. Luteinizing hormone Pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. J Clin Endocrinol Metab. 2003;88(1):297–311.
Loucks AB, Verdun M, Heath EM. Low energy availability, not stress of exercise alters LH pulsatility in exercising women. J Appl Physiol. 1998;84(1):37–46.
Macut D, Micic D, Pralong FP, Bischof P, Campana A. Is there a role for leptin in human reproduction? Gynecol Endocrinol. 1998;12(5):321–6.
Maeda K, Ohkura S, Uenoyama Y. Neurobiological mechanisms underlying GnRH pulse generation by the hypothalamus. Brain Res. 2010;1364:103–15.
Mainieri AS, Elnecave RH. Usefulness of the free alpha-subunit to diagnose hypogonadotropic hypogonadism. Clin Endocrinol. 2003;59:307–13.
Mancall EL, Brock DG, editors. Gray's clinical neuroanatomy: the anatomic basis for clinical neuroscience. 1st ed. Philadelphia: Elsevier Saunders; 2011.
Marshall JC, Dalkin AC, Haisenleder DJ, Paul SJ, Ortolano GA, Kelch RP. Gonadotropin-releasing hormone pulses: regulators of gonadotropin synthesis and ovulatory cycles. Recent Prog Horm Res. 1991;47:155–87.

Marshall JC, Dalkin AC, Haisenleder DJ, Griffin ML, Kelch RP. GnRH pulses – the regulators of human reproduction. Tans Am Clin Climatol Assoc. 1993;104:31–46.
Martin K, Santoro N, Hall J, Filicori M, Wierman M, Crowley WF Jr. Clinical review 15: management of ovulatory disorders with pulsatile gonadotropin-releasing hormone. J Clin Endocrinol Metab. 1990;71:1081A–G.
Martin C, Balasubramanian R, Dwyer AA, et al. The role of the prokineticin 2 pathway in human reproduction: evidence from the study of human and murine gene mutations. Endocr Rev. 2011;32(2):225–46.
Mastorakos G, Scopa CD, Vryonidou A, et al. Presence of immunoreactive corticotropin-releasing hormone in normal and polycystic human ovaries. J Clin Endocrinol Metab. 1994;79 (4):1191–7.
Mauras N, Shulman D, Hsiang HY, Balagopal P, Welch S. Metabolic effects of oral versus transdermal estrogen in growth hormone-treated girls with turner syndrome. J Clin Endocrinol Metab. 2007;92(11):4154–60.
McDonald JK, Lumpkin MD, DePaolo LV. Neuropeptide-Y suppresses pulsatile secretion of luteinizing hormone in ovariectomized rats: possible site of action. Endocrinology. 1989;125 (1):186–91.
Merke DP, Tajima T, Baron J, Cutler GB Jr. Hypogonadotropic hypogonadism in a female caused by an X-linked recessive mutation in the DAX1 gene. N Engl J Med. 1999;340(16):1248–52.
Messager S, Chatzidaki EE, Ma D, et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Natl Acad Sci U S A. 2005;102(5):1761–6.
Mijiddorj T, Kanasaki H, Sukhbaatar U, Oride A, Kyo S. DS1, delta subunit-containing GABA(a) receptor agonist, increases gonadotropin subunit gene expression in mouse pituitary gonadotrophs. Biol Reprod. 2015;92(2):45.
Miller KK, Parulekar MS, Schoenfeld E, et al. Decreased leptin levels in normal weight women with hypothalamic amenorrhea: the effects of body composition and nutritional intake. J Clin Endocrinol Metab. 1998;83:2309–12.
Miller BH, Olson SL, Levine JE, Turek FW, Horton TH, Takahashi JS. Vasopressin regulation of the proestrous luteinizing hormone surge in wild-type and clock mutant mice. Biol Reprod. 2006;75:778–84.
Mills JL, Fears TR, Robison LL, Nicholson HS, Sklar CA, Byrne J. Menarche in a cohort of 188 long-term survivors of acute lymphoblastic leukemia. J Pediatr. 1997;131(4):598–602.
Mitchell AL, Dwyer A, Pitteloud N, Quinton R. Genetic basis and variable phenotypic expression of Kallmann syndrome: towards a unifying theory. Trends Endocrinol Metab. 2011;22:249–58.
Moenter SR, DeFazio A, Pitts GR, Nunemaker CS. Mechanisms underlying episodic gonadotropin-releasing hormone secretion. Front Neuroendocrinol. 2003;24(2):79–93.
Moore AM, Prescott M, Marshall CJ, Yip SH, Campbell RE. Enhancement of a robust arcuate GABAergic input to gonadotropin-releasing hormone neurons in a model of polycystic ovarian syndrome. Proc Natl Acad Sci U S A. 2015;112(2):596–601.
Morell AG, Gregoriadis G, Scheinberg IH, Hickman J, Ashwell G. The role of sialic acid in determining the survival of glycoproteins in the circulation. J Biol Chem. 1971;246(5):1461–7.
Morton MV, Wehman P. Psychosocial and emotional sequelae of individuals with traumatic brain injury: a literature review and recommendations. Brain Inj. 1995;9(1):81–92.
Mosselman S, Polman J, Dijkema RER. Beta: identification and characterization of a novel human estrogen receptor. FEBS Lett. 1996;392(1):49–53.
Nabhan ZM, DiMeglio LA, Qi R, Perkins SM, Eugster EA. Conjugated oral versus transdermal estrogen replacement in girls with turner syndrome: a pilot comparative study. J Clin Endocrinol Metab. 2009;94(6):2009–14.
Naftolin F, Yen SS, Tsai CC. Rapid cycling of plasma gonadotrophins in normal men as demonstrated by frequent sampling. Nat New Biol. 1972;236(64):92–3.
Navarro VM, Kaiser UB. Metabolic influences on neuroendocrine regulation of reproduction. Curr Opin Endocrinol Diabetes Obes. 2013;20(4):335–41.

Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA. Regulation of gonadotropin-releasing hormone secretion by Kisspeptin/Dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J Neurosci. 2009;29(38):11859–66.

Neill JD. GnRH and GnRH receptor genes in the human genome. Endocrinology. 2002;143(3):737–43.

Neill JD, Duck LW, Sellers JC, Musgrove LC. A gonadotropinreleasing hormone (GnRH) receptor specific for GnRH II in primates. Biochem Biophys Res Commun. 2001;282(4):1012–8.

Nepomnaschy PA, Sheiner E, Mastorakos G, Arck PC. Stress, immune function, and women's reproduction. Ann N Y Acad Sci. 2007;1113:350–64.

Nezi M, Christopoulos P, Paltoglou G, et al. Focus on BMI and subclinical hypothyroidism in adolescent girls first examined for amenorrhea or oligomenorrhea. The emerging role of polycystic ovary syndrome. J Pediatr Endocrinol Metab. 2016;29(6):693–702.

Nikolics K, Mason AJ, Szonyi E, Ramachandran J, Seeburg PH. A prolactin-inhibiting factor within the precursor for human gonadotropin-releasing hormone. Nature. 1985;316 (6028):511–7.

Norwitz ER, Xu S, Jeong KH, et al. Activin a augments GnRH-mediated transcriptional activation of the mouse GnRH receptor gene. Endocrinology. 2002;143(3):985–97.

Okuhara K, Abe S, Kondo T, et al. Four Japanese patients with adrenal hypoplasia congenita and hypogonadotropic hypogonadism caused by DAX-1 gene mutations: mutant DAX-1 failed to repress steroidogenic acute regulatory protein (StAR) and luteinizing hormone beta-subunit gene promoter activity. Endocr J. 2008;55(1):97–103. Epub 2008 Jan 17

Oral EA, Ruiz E, Andewelt A, et al. Effect of leptin replacement on pituitary hormone regulation in patients with severe lipodystrophy. J Clin Endocrinol Metab. 2002;87:3110–7.

Ozisik G, Mantovani G, Achermann JC, et al. An alternate translation initiation site circumvents an amino-terminal DAX1 nonsense mutation leading to a mild form of X-linked adrenal hypoplasia congenita. J Clin Endocrinol Metab. 2003;88(1):417–23.

Pasquier J, Kamech N, Lafont AG, Vaudry H, Rousseau K, Dufour S. Molecular evolution of GPCRs: Kisspeptin/kisspeptin receptors. J Mol Endocrinol. 2014;52:T101–17.

Pfaeffle RW, Hunter CS, Savage JJ, et al. Three novel missense mutations within the LHX4 gene are associated with variable pituitary hormone deficiencies. J Clin Endocrinol Metab. 2008;93(3):1062–71.

Pfaffle RW, Blankenstein O, Wuller S, Kentrup H. Combined pituitary hormone deficiency: role of Pit-1 and Prop-1. Acta Paediatr Suppl. 1999;88(433):33–41.

Pierroz DD, Gruaz NM, d'Alièves V, Aubert ML. Chronic administration of neuropeptide Y into the lateral ventricle starting at 30 days of life delays sexual maturation in the female rat. Neuroendocrinology. 1995;61(3):293–300.

Pierroz DD, Catzeflis C, Aebi AC, Rivier JE, Aubert ML. Chronic administration of neuropeptide Y into the lateral ventricle inhibits both the pituitary-testicular axis and growth hormone and insulin-like growth factor I secretion in intact adult male rats. Endocrinology. 1996;137(1):3–12.

Piippo S, Lenko H, Kainulainen P, Sipilä I. Use of percutaneous estrogen gel for induction of puberty in girls with turner syndrome. J Clin Endocrinol Metab. 2004;89(7):3241–7.

Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev. 2012;92:1235–316.

Pitteloud N, Meysing A, Quinton R, et al. Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. Mol Cell Endocrinol. 2006;254-255:60–9.

Pitteloud N, Zhang C, Pignatelli D, et al. Loss-of-function mutation in the prokineticin 2 gene causes Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci U S A. 2007;104(44):17447–52.

Quennell JH, Mulligan AC, Tups A, et al. Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. Endocrinology. 2009;150(6):2805–12.

Raivio T, Falardeau J, Dwyer A, et al. Reversal of idiopathic hypogonadotropic hypogonadism. N Engl J Med. 2007;357(9):863–73.

Rappaport R, Brauner R, Czernichow P, et al. Effect of hypothalamic and pituitary irradiation on pubertal development in children with cranial tumors. J Clin Endocrinol Metab. 1982;54(6):1164–8.

Reame NE, Sauder SE, Case GD, Kelch RP, Marshall JC. Pulsatile gonadotropin secretion in women with hypothalamic amenorrhea: evidence that reduced frequency of gonadotropin-releasing hormone secretion is the mechanism of persistent anovulation. J Clin Endocrinol Metab. 1985;61(5):851–8.

Rometo AM, Krajewski SJ, Voytko ML, Rance NE. Hypertrophy and increased Kisspeptin gene expression in the hypothalamic infundibular nucleus of postmenopausal women and Ovariectomized monkeys. J Clin Endocrinol Metab. 2007;92(7):2744–50.

Sabatino FD, Collins P, McDonald JK. Neuropeptide-Y stimulation of luteinizing hormone-releasing hormone secretion from the median eminence in vitro by estrogen-dependent and extracellular Ca2+−independent mechanisms. Endocrinology. 1989;124(5):2089–98.

Santoro N, Filicori M, Crowley WF Jr. Hypogonadotropic disorders in men and women: diagnosis and therapy with pulsatile gonadotropin-releasing hormone. Endocr Rev. 1986;7:11–23.

Sato N, Katsumata N, Kagami M, et al. Clinical assessment and mutation analysis of Kallmann syndrome 1 (KAL1) and fibroblast growth factor receptor 1 (FGFR1, or KAL2) in five families and 18 sporadic patients. J Clin Endocrinol Metab. 2004;89(3):1079–88.

Sauder SE, Frager M, Case GD, Kelch RP, Marshall JC. Abnormal patterns of pulsatile luteinizing hormone secretion in women with hyperprolactinemia and amenorrhea: responses to bromocriptine. J Clin Endocrinol Metab. 1984;59(5):941–8.

Schneider JE. Energy balance and reproduction. Physiol Behav. 2004;81(2):289–317.

Schreihofer D, Amico J, Cameron JL. Reversal of fasting-induced suppression of luteinizing hormone (LH) secretion in male rhesus monkeys by intragastric nutrient infusion: evidence for rapid stimulation of LH by nutritional signals. Endocrinology. 1993;132(5):1890–7.

Schulze A, Mogensen H, Hamborg-Petersen B, Graem N, Ostergaard JR, Brøndum-Nielsen K. Fertility in Prader-Willi syndrome: a case report with Angelman syndrome in the offspring. Acta Paediatr. 2001;90(4):455–9.

Sealfon SC, Weinstein H, Millar RP. Molecular mechanisms of ligand interaction with the gonadotropin-releasing hormone receptor. Endocr Rev. 1997;18(2):180–205.

Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an academic center. J Clin Endocrinol Metab. 2002;87(4):1613–20.

Segal TY, Mehta A, Anazodo A, Hindmarsh PC, Dattani MT. Role of gonadotropin-releasing hormone and human chorionic gonadotropin stimulation tests in differentiating patients with hypogonadotropic hypogonadism from those with constitutional delay of growth and puberty. J Clin Endocrinol Metab. 2009;94:780–5.

Seminara SB, Hayes FJ, Crowley WF Jr. Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): pathophysiological and genetic considerations. Endocr Rev. 1998;19:521–39.

Seminara SB, Achermann JC, Genel M, Jameson JL, Crowley WF Jr. X-linked adrenal hypoplasia congenita: a mutation in DAX1 expands the phenotypic spectrum in males and females. J Clin Endocrinol Metab. 1999;84(12):4501–9.

Semple RK, Topaloglu AK. The recent genetics of hypogonadotrophic hypogonadism – novel insights and new questions. Clin Endocrinol. 2010;72:427–35.

Shacham S, Harris D, Ben-Shlomo H, et al. Mechanism of GnRH receptor signaling on gonadotropin release and gene expression in pituitary gonadotrophs. Vitam Horm. 2001;63:63–90.

Shaw ND, Seminara SB, Welt CK, et al. Expanding the phenotype and genotype of female GnRH deficiency. J Clin Endocrinol Metab. 2011;96(3):E566–76.

Silveira LF, MacColl GS, Bouloux PM. Hypogonadotropic hypogonadism. Semin Reprod Med. 2002;20:327–38.

Silveira LF, Trarbach EB, Latronico AC. Genetics basis for GnRH dependent pubertal disorders in humans. Mol Cell Endocrinol. 2010;324:30–8.

Skinner DC, Caraty A, Allingham R. Unmasking the progesterone receptor in the preoptic area and hypothalamus of the ewe: no Colocalization with gonadotropin-releasing neurons. Endocrinology. 2001;142(2):573–9.
Skorupskaite K, George JT, Anderson RA. The kisspeptin-GnRH pathway in human reproductive health and disease. Hum Reprod Update. 2014;20(4):485–500.
Smith MJ, Jennes L. Neural signals that regulate GnRH neurons directly during the oestrous cycle. Reproduction. 2001;122:1):1–10.
Smith JT, Cunningham MJ, Rissman EF, Clifton DK, Steiner RA. Regulation of Kiss1 gene expression in the brain of the female mouse. Endocrinology. 2005a;146(9):3686–92.
Smith JT, Dungan HM, et al. Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. Endocrinology. 2005b;146(7):2976–84.
Terasawa E, Fernandez DL. Neurobiological mechanisms of the onset of puberty in primates. Endocr Rev. 2001;22:111−151.
Themmen APN, Huhtaniemi IT. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocr Rev. 2000;21(5):551–83.
Toledo SP, Brunner HG, Kraaij R, et al. An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46,XX female. J Clin Endocrinol Metab. 1996;81(11):3850–4.
Tortoriello DV, Sidis Y, Holtzman DA, Holmes WE, Schneyer AL. Human follistatin-related protein: a structural homologue of follistatin with nuclear localization. Endocrinology. 2001;142(8):3426–34.
Uenoyama Y, Inoue N, Pheng V. Ultrastructural evidence of kisspeptin-gonadotrophin-releasing hormone (GnRH) interaction in the median eminence of female rats: implication of axo-axonal regulation of GnRH release. J Neuroendocrinol. 2011;10:863–70.
van Biljon W, Wykes S, Scherer S, Krawetz SA, Hapgood J, Type II. Gonadotropin-releasing hormone receptor transcripts in human sperm. Biol Reprod. 2002;67(6):1741–9.
van Vliet G. Hormonal changes during development in Turner's syndrome. Acta Paediatr Scand Suppl. 1988;343:31–7.
Vance ML, Thorner MO. Fasting alters pulsatile and rhythmic cortisol release in normal man. J Clin Endocrinol Metab. 1989;68:1013–8.
Vogl TJ, Stemmler J, Heye B, et al. Kallman syndrome versus idiopathic hypogonadotropic hypogonadism at MR imaging. Radiology. 1994;191(1):53–7.
von Kalckreuth G, Haverkamp F, Kessler M, Rosskamp RH. Constitutional delay of growth and puberty: do they really reach their target height? Horm Res. 1991;35(6):222–5.
Vrekoussis T, Kalantaridou SN, Mastorakos G, et al. The role of stress in female reproduction and pregnancy: an update. Ann N Y Acad Sci. 2010;1205:69–75.
Waldhauser F, Weissenbacher G, Frisch H, Pollak A. Pulsatile secretion of gonadotropins in early infancy. Eur J Pediatr. 1981;137(1):71–4.
Waldstreicher J, Seminara SB, Jameson JL, et al. The genetic and clinical heterogeneity of gonadotropin-releasing hormone deficiency in the human. J Clin Endocrinol Metab. 1996;81:4388–95.
Warren MP, Voussoughian F, Geer EB, Hyle EP, Adberg CL, Ramos RH. Functional hypothalamic amenorrhea: hypoleptinemia and disordered eating. J Clin Endocrinol Metab. 1999;84(3):873.
Watanabe M, Fukuda A, Nabekura J. The role of GABA in the regulation of GnRH neurons. Front Neurosci. 2014;8:387.
Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. J Clin Endocrinol Metab. 1997;82(2):561–5.
Welt CK, Chan JL, Bullen J, et al. Recombinant human leptin in women with hypothalamic amenorrhea. N Engl J Med. 2004;351(10):987.
Wharton RH, Loechner KJ. Genetic and clinical advances in Prader-Willi syndrome. Curr Opin Pediatr. 1996;8(6):618–24.
White RB, Eisen JA, Kasten TL, Fernald RD. Second gene for gonadotropin-releasing hormone in humans. Proc Natl Acad Sci U S A. 1998;95(1):305–9.

Whitlock KE. Origin and development of GnRH neurons. Trends Endocrinol Metab. 2005;16(4):145.

Wildt L, Häusler A, Marshall G, et al. Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. Endocrinology. 1981a;109(2):376–85.

Wildt L, Hutchison JS, Marshall G, Pohl CR, Knobil E. On the site of action of progesterone in the blockade of the estradiol-induced gonadotropin discharge in the rhesus monkey. Endocrinology. 1981b;109(4):1293–4.

Wolfahrt S, Kleine B, Jarry H, Rossmanith WG. Endogenous regulation of the GnRH receptor by GnRH in the human placenta. Mol Hum Reprod. 2001;7(1):89–95.

Xu M, Hill JW, Levine JE. Attenuation of luteinizing hormone surges in neuropeptide Y knockout mice. Neuroendocrinology. 2000;72:263–71.

Yahalom D, Chen A, Ben-Aroya N, et al. The gonadotropin-releasing hormone family of neuropeptides in the brain of human, bovine and rat: identification of a third isoform. FEBS Lett. 1999;463(3):289–94.

Yao B, Liu HY, YC G, et al. Gonadotropin releasing hormone positively regulates steroidogenesis via extracellular signal-regulated kinase in rat Leydig cells. Asian J Androl. 2011;13(3):438–45.

Yen SS, Tsai CC. The biphasic pattern in the feedback action of Ethinyl Estradiol on the release of pituitary FSH and LH. J Clin Endocrinol Metab. 1971;33(6):882–7.

Yen SS, Tsai CC, Naftolin F, Vandenberg G, Ajabor L. Pulsatile patterns of gonadotropin release in subjects with and without ovarian function. J Clin Endocrinol Metab. 1972;34(4):671–5.

Yen SSC, Jaffe RB, Barbieri RL. Reproductive endocrinology. Philadelphia/London/Toronto/Montreal/Sidney/Tokyo: W.B. Saunders Company; 1999.

Young J, Metay C, Bouligand J, et al. SEMA3A deletion in a family with Kallmann syndrome validates the role of semaphorin 3A in human puberty and olfactory system development. Hum Reprod. 2012;27:1460–5.

Zanaria E, Muscatelli F, Bardoni B, et al. An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature. 1994;372 (6507):635–41.

Central Hypogonadism in the Male: Physiopathology, Diagnosis, and Treatment

10

Julia Rohayem and Eberhard Nieschlag

Contents

Abstract

The hypothalamic-pituitary-gonadal (HPG) axis coordinates two functions essential for male reproductive capacity: synthesis and secretion of sex steroid hormones, primarily testosterone, and production of spermatozoa. Our understanding of the physiologic principles of male reproductive health have substantially increased in recent years, enabling physicians not only to identify genetic causes of hypogonadism among a wide spectrum of possible disorders, but also to establish therapeutic strategies. Men with hypothalamic or pituitary disorders

J. Rohayem (✉) · E. Nieschlag
Center of Reproductive Medicine and Andrology, University of Münster, Münster, Germany
e-mail: Julia.Rohayem@ukmuenster.de; Eberhard.Nieschlag@ukmuenster.de

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_10

nowadays have an excellent prognosis for fertility restoration and for paternity by natural intercourse or using assisted reproductive techniques.

Keywords
Central hypogonadism · Secondary hypogonadism · Tertiary hypogonadism · Functional hypogonadism · GnRH · LH · FSH · GnRH replacement · hCG/rFSH replacement · Gonadotropin replacement

Physiology of the Hypothalamo-Pituitary-Gonadal (HPG) Axis

The male gonads have two major functions: the synthesis of androgens and the production of male gametes. Testicular function is regulated by the hypothalamo-pituitary-gonadal axis: gonadotropin-releasing hormone (GnRH), a decapeptide produced in hypothalamic GnRH-neurons, is released into the portal blood via discrete pulses and acts on specific receptors on gonadotropin-secreting cells in the anterior pituitary. The glycoprotein hormones LH and FSH are secreted into the bloodstream upon GnRH stimulation, then binding to their target cells in the male gonads. While LH binds to the LHCG receptor of Leydig cells, thereby stimulating testosterone synthesis and secretion, FSH acts on its receptor in Sertoli cells, thereby inducing spermatid maturation (spermiogenesis) during spermatogenesis. For induction and maintenance of quantitatively and qualitatively normal spermatogenesis, both gonadotropins are required (Nieschlag et al. 1999b).

Negative feedback on GnRH neurons is provided by gonadal sex steroids (testosterone, its bioactive form, dihydrotestosterone, and its derivative, estradiol) and the Sertoli cell product inhibin B. The negative feedback of androgens and of other regulatory signals (such as stress or starvation) to the hypothalamus is conveyed by KNDy cells in the hypothalamic arcuated nucleus, via the neuropeptides kisspeptin, dynorphin, and neurokinin (Lehman et al. 2010). In addition, a dual inhibitory effect at the hypothalamic and pituitary level is exerted by estrogens that are generated in adipose tissues through the aromatization of androgens (Hayes et al. 2000; Rochira et al. 2006; Pitteloud et al. 2008) (Fig. 1).

Classification, Pathophysiology of Hypogonadism in the Male

The term **hypogonadism** is used to describe testicular dysfunction in men. Due to the dual function of the gonads, testicular disturbances may induce both testosterone deficiency and impaired fertility. While disturbed endocrine gonadal dysfunction disrupts spermatogenesis, infertility itself does not impair testosterone production.

If the functional disturbance affects the testes, this is indicated by a compensatory rise in serum gonadotropin levels. This condition is referred to as **primary or hypergonadotropic hypogonadism**. If **central** structures, i.e., either **the hypothalamus or the pituitary** function are affected, resulting in a decrease of serum

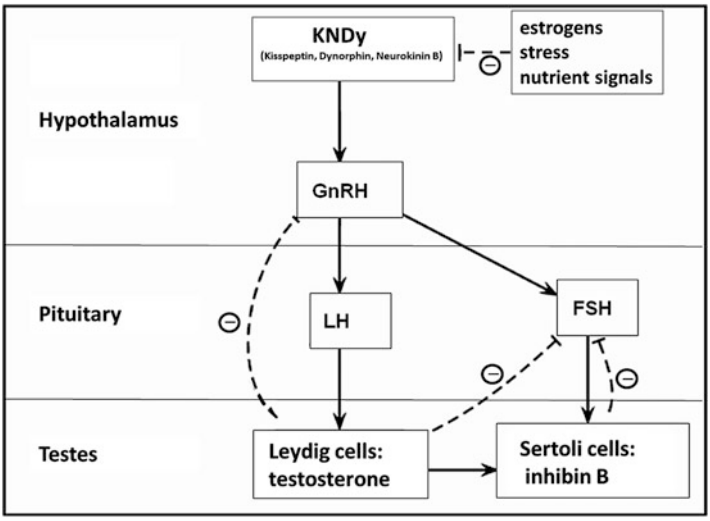


Fig. 1 Regulation of the hypothalamo-pituitary-gonadal (HPG) axis

luteinizing hormone (LH) and follicle-stimulation hormone (FSH) levels, this is called **hypogonadotropic hypogonadism.** While the term **secondary hypogonadism** should be reserved for conditions with exclusive impairment of pituitary function, the term **tertiary hypogonadism** applies to conditions with an underlying hypothalamic disturbance, that is, with defective GnRH secretion, with the consequence of inadequate pituitary stimulation to secrete gonadotropins.

Late-onset-hypogonadism refers to conditions where both testicular and central functional defects are involved**.**

Symptoms of hypogonadism may also appear if LH, FSH, and testosterone serum levels are in the normal range. This is the case if the effect of gonadal sex steroids is hampered at the level of androgen receptors on target organs (**androgen insensitivity**).

This chapter deals with **central, i.e., secondary and tertiary hypogonadism**. Central hypogonadism can be either congenital or acquired.

Tertiary/Hypothalamic Hypogonadism

Congenital Tertiary/Hypothalamic Hypogonadism

Isolated Congenital HH (CHH) and Kallmann Syndrome

Conditions with congenital hypothalamic hypogonadism (CHH) are characterized by absent or inadequate pulsatile secretion of GnRH by hypothalamic neurons (Belchetz et al. 1978), i.e., by an impaired function of the "GnRH pulse generator." The term CHH includes two major conditions: Kallmann syndrome and isolated congenital hypogonadotropic hypogonadism.

While isolated CHH is defined by HH with normosmia, patients with Kallmann syndrome have an associated inability to smell. Their anosmia is a consequence of a defective development of the olfactory bulbs (Takeda et al. 1992). Kallmann patients are not always aware of their inability to perceive aromatic substances (such as soap or coffee) due to their uncompromised perception of substances that stimulate the trigeminus nerve (such as ammonia or vinegar). Therefore, it may be necessary to perform semiquantitative olfactometry to reliably identify impaired olfaction (Dunkel and Quinton 2014).

Both Kallmann syndrome and CHH may be associated with other phenotypic disorders such as dental and digital anomalies, cleft lips and palate, iris coloboma, and kidney agenesis. Patients may also exhibit neurologic features such as synkinesis (mirror movements) and cerebral ataxia.

CHH remains undiagnosed in most cases of HH until late childhood. The clinical suspicion of CHH is usually raised in boys with absent pubertal development after age 14.

As HPG axis dysfunction is already present during fetal development, maldescended testes and/or micropenis (with a stretched penile length below 2.5 cm) in the male newborn may offer a valuable clue to underlying CHH. Detection of an absent physiologic gonadotropin surge during the first 6 months of life (the so-called minipuberty) represents another opportunity for early diagnosis of the condition (Grinspon and Rey 2010).

If not recognized upon these features during infancy, CHH becomes obvious at an age when normal puberty is expected: as a consequence of insufficient increases of LH and FSH, serum levels of testosterone remain low, i.e., in the prepubertal range. Depending on the severity of hormonal deficiencies, pubertal development may either be delayed, arrested, or totally absent. The definition of "pubertal delay" is based upon pubertal onset at an age that is 2–2.5 SD later than the population mean which is accepted as 14 years in Caucasian boys. In cases with absent puberty, testicular volumes remain prepubertal (i.e., < 4 ml each side) after age 14. In boys with spontaneous pubertal onset with early arrest, testicular volumes increase slightly, but do not reach normal adult sizes (i.e., over 10–12 ml each side) and sperm do not appear in seminal fluids. Failure of penile enlargement, absence of pubertal body hair, absence of beard growth and of voice mutation, and nocturnal ejaculations are additional typical clinical features of pubertal failure (Table 1).

Of note, pubic and axillary hair do develop at the expected age, as they result from maturation of the adrenal glands (adrenarche) with the secretion of adrenal androgens, a process that is usually uncompromised in HH. However, psychosexual maturation (with awakening of libido), being dependent on a normal pubertal increase in androgen serum levels, does not occur.

Late epiphyseal closure in the long bones of adolescents affected by CHH is a consequence of low estrogen levels that result from low rates of testosterone aromatization in adipose tissues. Late maturation and fusion of the growth plates give rise to eunuchoid body proportions with long legs and arms (with an arm span longer than body height).

Table 1 Symptoms of hypogonadism due to testosterone deficiency relative to age at manifestation

Affected organ/ function	Onset of testosterone deficiency	
	Before completed puberty	After completed puberty
Hair	Straight frontal hairline, no androgenic hair loss/no baldness	Previous androgenic hair loss possible, arrested loss
Beard growth	No beard growth	Beard growth diminishing
Body hair	Horizontal pubic hairline	Male body hair decreasing
Larynx	No voice mutation	Accomplished voice mutation (unchanged)
Skin	Prepubertal sebum production, lack of acne, pallor	Diminished sebum production, wrinkling, hot flashes
Bones	Eunuchoid body proportions	Osteopenia ➔ osteoporosis
Bone marrow	Mild anaemia	Mild anaemia
Skeletal muscles	Underdeveloped	Hypotrophic
Prostate	Prepubertal size	Volume reduction
Penis	Prepubertal size	Adult size (unchanged)
Testes	Prepubertal volumes, maldescended testes possible	Decrease in volume and consistency
Spermatogenesis	Not initiated	Arrested: secondary oligo−/ azoospermia
Ejaculate	No emissions	Decrease in volume
Libido	Prepubertal	Decreased /lost
Potency	Spontaneous erections possible, Not sexually stimulated	Reduced erectile capacity

The genetic basis of congenital tertiary/hypogonadotropic hypogonadism could be elucidated in less than half of the patients over the past decades.

CHH and Kallmann syndrome may occur sporadically or clustered in families. Inheritance is either autosomal-dominant, autosomal-recessive, or X-linked (Costa-Barbosa et al. 2013) and may be either monogenic or digenic (Pitteloud et al. 2007; Quaynor et al. 2011). More than 25 gene loci have been identified to date (Dwyer et al. 2015). Recently, targeted multiplex next-generation sequencing has become available, allowing for simultaneous mutation analysis of all genes known to be associated with CHH or Kallmann syndrome (Table 2).

Mutations in *KAL1* are the most common genetic cause of **anosmic CHH = Kallmann syndrome** with an X-linked (recessive) inheritance. *KAL1* is located on Xp22.3. It codes for anosmin, a protein responsible for the migration of GnRH neurons to the hypothalamus and the concomitant migration of olfactory axons to the olfactory epithelium during embryonic development. Further mutations known to cause Kallmann syndrome genes concern *SEMA3A, SOX10, FEZF1, HESX, ILI7RD*, and *NLF*.

Genes currently recognized to be involved in **both Kallmann syndrome and normosmic CHH** include *KAL2 (=FGFR1); FGF8; KAL3 (=PROK2); KAL4 (=PROKR2), HS6ST, AXL,* and *WDR11*. Heterozygous *FGFR1* mutations with an

Table 2 Genes potentially mutated in patients with central hypogonadism

Congenital hypothalamic disorder/ congenital tertiary HH	***Possibly affected genes***
(anosmic) Kallmann syndrome	*KAL1, SEMA3A, SOX10, FEZF1, HESX1, IL17RD, NELF*
Kallmann syndrome and CHH	*KAL2 = FGFR1; FGF8;* *KAL3 = PROK2; KAL4 = PROKR2,* *HS6ST, AXL, WDR11*
(normosmic) CHH	*KISS1, KISS1R = GRP54,* *TAC3, TAC3R,* *GNRH1,* *LEP, LEPR*
Syndromic tertiary CHH	
CHARGE syndrome	*CHD7*
Bardet-Biedl syndrome	*BBS 1–19*
Prader Willi syndrome (combined tertiary and primary HH)	Lack of gene expression from the paternal chromosome 15q-11-q13
Gordon-Holmes syndrome **Boucher-Neuhauser syndrome**	*RNF216, OTUD4, stub, PNPLA6* *PNPLA6*
Combined congenital tertiary, secondary and primary HH	
CHH with associated adrenal insufficiency	*DAX1*
Congenital pituitary disorder/ Congenital secondary HH	
Congenital isolated LH deficiency (Pasqualini syndrome	*LHß*
Congenital isolated FSH deficiency	*FSHß*
Congenital combined LH and FSH-deficiency	*GnRHR*
CHH with associated multiple pituitary deficiencies	*PROP1, PIT1 (POU1F1), LHX3, LHX4, GLI2, FGF8, KAL4 = PROKR2, HESX1*

autosomal dominant mode of inheritance account for about 15% of (familial or sporadic) CHH or Kallmann syndrome (Dodé et al. 2003). *FGFR1,* as well as its ligand, the fibroblast growth factor 8 (*FGF8*) is involved in organogenesis (Falardeau et al. 2008); therefore patients with *FGFR1* or *FGF8* mutations may have digital bone anomalies, such as polydactyly and camptodactyly in association with CHH (Costa-Barbosa et al. 2013). *PROK2(KAL3)* codes for prokineticin 2, a chemoattractant for neural precursor cells. The gene coding for its receptor, *PROKR2*, is also referred to as *KAL4* (Dodé et al. 2006).

Mutations of the following genes have been described **exclusively in normosmic patients with CHH**: *KISS1, KISS1R (=GRP54), TAC3, TAC3R, GNRH1, GNRHR, LEP* (Strobel et al. 1998), and *LEPR* (Clement et al. 1998).

GPR54 and its ligands, the Kisspeptins, play an essential role in GnRH secretion (De Roux et al. 2003; Seminara et al. 2003). Likewise, the neuropeptide neurokinin B

has been recognized as a critical central regulator of GnRH secretion, since mutations in its coding gene *TAC3* or its receptor *TACR* cause CHH (Topaloglu et al. 2009).

An obvious candidate gene for CHH, the *GNRH1* gene, has been identified as a rare cause of normosmic isolated GnRH deficiency in humans, with an autosomal recessive trait. The *GNRH1* gene encodes the pre-prohormone of GnRH (Bouligand et al. 2009; Chan et al. 2009). Mutations may result in both mild and severe forms of GnRH deficiency.

(For *GnRHR* gene mutations see chapter "Congenital Combined LH and FSH-Deficiency")

In patients with normosmic congenital hypogonadotropic hypogonadism and with extreme obesity, inactivating mutations in *LEP* or the gene coding for its receptor *LEPR* have been detected (Clément et al. 1998; Strobel et al. 1998; Fischer-Posovszky et al. 2010). Leptin is a fat-derived hormone that regulates food intake, energy expenditure, and hypothalamic reproductive function. Treatment with recombinant leptin has been shown to restore LH pulses and normalize testosterone levels, besides normalizing weight in morbidly obese homozygous leptin-deficient men (Licinio et al. 2004).

Tertiary HH Due to Syndromic Disorders

Patients with the **CHARGE syndrome** (**c**oloboma, **h**eart failure, **a**tresia choanae, **r**etarded growth and development, **g**enital and **e**ar malformations) also suffer from hypogonadotropic hypogonadism. The syndrome may be caused by an autosomal-dominant mutation of the *CHD7* gene (Janssen et al. 2012).

Patients with a **Prader-Labhart-Willi syndrome** are affected by a combination of tertiary and primary hypogonadotropic hypogonadism (Eiholzer et al. 2006). The syndrome is caused by a reduced expression of genes inherited from the paternal chromosome 15q-11-q13 (Butler et al. 2015) (Table 2). Newborns and infants often come to clinical attention because of a generalized muscular hypotonia. Delayed motor development, moderate mental retardation, and early obesity are characteristic features. If not treated with growth hormone, male patients hardly reach an adult height of 160 cm. Male genitalia remains hypoplastic at a pubertal age, and pubertal development does not progress spontaneously with resulting infertility.

Patients affected by the **Bardet-Biedl syndrome** may have hypogonadotropic hypogonadism. This autosomal-recessive multisystemic syndrome is characterized by obesity, mental retardation, kidney malformations, polydactylia, retinal degeneration, and hypogonadism. Nineteen gene mutations on various chromosomes BBS1–19 have been described to date (Khan et al. 2016).

Gordon-Holmes syndrome refers to a condition with hypogonadotropic hypogonadism combined with cerebellar ataxia. The syndrome is thought to be associated with mutations in genes involved in regulation of autophagy of the cells' own cytosolic components (or protein aggregates), a cellular process concerned with cellular homeostasis. So far, mutations in the following genes have been described: *RNF216, OTUD4, STUB1,* and *PNPLA6* (Alqwaifly and Bohlega 2016).

Boucher-Neuhauser syndrome is a neurodegenerative disorder, characterized by an association of progressive cerebellar degeneration (with early onset ataxia),

hypogonadotropic hypogonadism and chorioretinal dystrophy. It has recently been linked to autosomal-recessive mutations in the *PNPLA6* gene (Tarnutzer et al. 2015). PNPLA6 codes for a precursor for the synthesis of acetylcholine.

Tertiary HH Combined with Adrenal Insufficiency: X-Linked Adrenal Hypoplasia

Congenita (AHC)

If central hypogonadotropic hypogonadism is **associated with adrenal insufficiency**, DAX1 mutations may be the cause (Habiby et al. 1996; Achermann et al. 2001; Jadhav et al. 2011). The rare hypogonadotropic condition named X-linked adrenal hypoplasia congenita (AHC) results from a combined and variable deficiency of hypothalamic GnRH secretion and/or impaired pituitary responsiveness to GnRH. The dosage sensitive sex-reversal-1 (DAX-1) gene is located on the short arm of the X-chromosome in the region Xp21.3–21.2 (Table 2).

The majority of affected newborn patients are diagnosed within the first 2 months because of a life-threatening adrenal insufficiency crisis. Some patients have residual cortisol or mineralocorticoid production (Mantovani et al. 2002), giving rise to hypogonadotropic hypogonadism without or with subclinical adrenal insufficiency. Replacement of pulsatile GnRH does not induce adequate LH- and FSH stimulation and treatment with hCG and FSH may not sufficiently stimulate testosterone production and spermatogenesis, revealing a further testicular defect, in addition to the secondary and tertiary defect of the HPG axis. In line with this clinical observation, animal models indicate that *DAX1* plays a critical role in testis development and function. DAX1 functions as a transcriptional repressor, particularly of pathways regulated by other nuclear receptors, such as steroidogenic factor 1 (SF1). It also acts as a negative coregulator of the estrogen receptor (ER, *NR3A1–2*), the liver receptor homologue-1 (LRH-1, *NR5A2*), the androgen receptor (AR, *NR3C4*), and the progesterone receptor (PR, *NR3C3*), each by distinct repression mechanisms. However, how disruption of DAX1 leads to adrenal, hypothalamic, and pituitary developmental defects similar to SF1 disruption remains to be clarified. The treatment of choice of this complex HPG axis impairment, in addition to gluco- and mineralocorticoid replacement, is therefore testosterone. Most patients are primarily azoospermic and testicular histology reveals a Sertoli-cell-only syndrome. However, in patients with residual hormonal activity, isolated foci with spermatogenesis may be found. In these cases, single sperm may be extracted from biopsied tissue and intraplasmatic sperm injection (ICSI) may result in pregnancy and live birth (Frapsauce et al. 2011).

Acquired Tertiary/Hypothalamic Hypogonadism

Functional Hypogonadotropic Hypogonadism

In this potentially reversible condition, hypothalamic GnRH pulsatility is downregulated. The phenomenon can be observed in men with severe malnutrition, such as in restrictive eating disorders (anorexia nervosa or starvation due to poverty or malignant diseases) (Wabitsch et al. 2001, with malabsorption (in inflammatory

bowel diseases, celiac disease, or cystic fibrosis), or in patients with chronic diseases. Functional HH may also be caused by excessive obesity with insulin resistance or type 2 diabetes (Dhindsa et al. 2010). Downregulation of GnRH pulse generator activity is also observed in athletes performing extreme exercise or overtraining, resulting in decrease of testosterone and sperm production (Nieschlag and Vorona 2015; Tenforde et al. 2016). Rarely is it due to serious stress, including severe social deprivation or depression. The indication for central hormone replacement has to be based upon the chances for resolution of the underlying condition with causal treatment.

Drug-Induced Hypogonadotropic Hypogonadism

Prolonged abuse of anabolic androgenic steroids (AAS) (including testosterone preparations) suppresses the HPG axis (Nieschlag and Vorona 2015). As shown in clinical trials of testosterone-based hormonal male contraception, suppression is reversible if androgen application is ceased (Nieschlag 2010); however, reactivation of the GnRH pulse generator may require 3–24 months (Liu et al. 2006). In these men, cessation of exogenous hormone administration or abuse is indicated, rather than initiation of gonadotropin replacement to overcome the AAS-induced hypogonadism (Nieschlag and Vorona 2015).

Inhibition of GnRH secretion occurs with the use of morphine, heroin, and methadone, all activating inhibitory neurons to the hypothalamus (Daniell 2002; Rajagopal et al. 2004). Iatrogenic HPG axis downregulation also occurs as a result of GnRH agonist treatment (for precocious puberty in boys and during antiandrogenic treatment of metastatic prostate cancer in adults). Therefore, inquiries on the recent intake of medications and on drug abuse are essential to recognize potentially reversible causes of HH.

Congenital Adrenal Hyperplasia (CAH) with Hypogonadotropic Hypogonadism

Undiagnosed or untreated classic congenital adrenal hypoplasia (CAH) due to 21-hydroxylase deficiency results in ACTH hypersecretion, giving rise to adrenal androgen hypersecretion. Adrenal androgens or their metabolites (e.g., estrone, originating from the aromatization of androstenedione) may suppress the hypothalamic GnRH-pulse generator and thus the secretion of pituitary LH and FSH. As a consequence, spermatogenesis is inhibited, resulting in oligozoospermia or azoospermia. However, adrenally derived testosterone in the blood steam is indistinguishable from testicularly derived testosterone, and near normal serum levels prevent symptoms of androgen deficiency. Therefore, the diagnosis has to be made upon measurement of 17-hydroxyprogesterone levels (in serum or saliva).

In males with salt-wasting CAH and tertiary hypogonadism, the cause of infertility is nonadherence or insufficient adherence to treatment. In milder/simple virilizing forms of CAH, the diagnosis of CAH may not have been made unless infertility, combined with suppressed LH and FSH levels, but low-normal serum testosterone levels, elevated 17-OHP serum levels, and hyperplasia of "testicular adrenal rests" in the testes (TART) are detected. The latter may be mistaken for

malign testicular tumors. However, as TART develop bilaterally, due to hyperplasia of adrenal cells that physiologically surround the rete testis, these tumors are always benign and do not require resection.

Adequate replacement of glucocorticoids in CAH men with hypogonadotropic hypogonadism may restore GnRH pulsatility and consequently resume gonadotropin suppression via normalization of ACTH-drive on the adrenal glands. In rare cases, LH and FSH suppression is prolonged. In men wishing paternity and without recovery of gonadotropin secretion on corticoid- (and mineralocorticoid) replacement, gonadotropin substitution with hCG and rFSH is a therapeutic option to induce spermatogenesis (Rohayem et al. 2014). Surgical resection of TART has been shown to be unsuccessful for improving semen quality (Claahsen -van der Grinten et al. 2007).

Secondary/Pituitary Hypogonadism

Congenital Secondary/Pituitary Hypogonadism

Isolated deficiencies of LH or FSH, each resulting from mutations of the corresponding genes, are very rare causes of defective pubertal maturation. *LHB* and *FSHB* are the genes coding for the unique ß-subunits that confer specificity of action of the heterodimeric glycoprotein-hormones LH and FSH on their corresponding gonadotropin receptors, in contrast to the common alpha-subunit of these glycoprotein hormones (Table 2).

Congenital Isolated LH Deficiency (Pasqualini Syndrome)

In this condition, **homozygous or compound heterozygous mutations of the LHß gene** cause the absence or functional deficiency of LH (Lofrano-Porto et al. 2007). Typically, affected male patients have absent puberty, small testes, a prepubertal penis size with LH serum levels below the detection limit, accompanied by prepubertally low serum testosterone levels, but normal or elevated FSH levels and normal inhibin B levels. Spermatogenesis may be reduced or arrested as Leydig cells are not developed (Matthews et al. 1993; Phillip et al. 1998; Lofrano-Porto et al. 2007; Valdes-Socin et al. 2014). Treatment with hCG causes the normalization of testosterone serum levels and spermatogenesis. Testosterone replacement may result in a slight increase of testicular volumes (to 6–7 ml) in the presence of high FSH-levels (Lofrano-Porto et al. 2007).

Congenital Isolated FSH Deficiency

Patients with **isolated FSH deficiency** have a normal pubertal development and are normally virilized men, but their testes remain small/of prepubescent size and their ejaculates are azoospermic. Their FSH serum levels are undetectable, while LH levels are normal or increased.

The lack of FSH is caused by inactivating **homozygous mutations in the FSH-β gene**. Treatment with rFSH over 6 months may increase testicular volumes (Simşek

et al. 2016). However, it is not clear from the literature whether spermatogenesis can be fully developed to produce sperm sufficient for paternity (Siegel et al. 2013).

Congenital Combined LH and FSH-Deficiency Due to GnRHR Mutations

Homozygous *GnRHR* mutations have been found to cause pituitary GnRH insensitivity by impairing GnRH receptor function in pituitary gonadotropin-secreting cells, with ensuing deficiencies in LH/FSH (De Roux et al. 1997; Layman et al. 1998; Beneduzzi et al. 2014). Depending on the degree of functional receptor impairment, patients may experience absence of pubertal development or pubertal arrest.

Congenital Pituitary Defects Causing Multiple Pituitary Hormone Deficiencies (MPHD)

If multiple pituitary hormonal deficiencies are associated with gonadotropin deficiency, mutations of *PROP1, PIT1 (=POU1F1), LHX3* (Netchine et al. 2000)*, LHX4, GLI2, FGF8, KAL4 (=PROKR2)*, and *HESX1* may be involved.

The *PROP-1* gene encodes the transcription factor PROP-1 (acronym for "prophet of Pit") that is required for the expression of *PIT1*. Pit1-dependent cell lines in the anterior pituitary include somatotrophs, lactotrophs and thyrotrophs and variably gonadotrophs and corticotrophs (Wu et al. 1998).

Acquired Secondary/Pituitary Hypogonadism

Acquired forms of HH are mostly due to pituitary tumors (craniopharyngeoma, pituitary adenoma, glioma) or surgery in the sella region. Pituitary failure with multiple pituitary hormone deficiencies may also result from cranial radiotherapy. Likewise, inflammatory processes (hypophysitis), iron overload (due to hemochromatosis or repetitive erythrocyte transfusions), granulomatosis, and vascular disorders can lead to pituitary insufficiency (Behre et al. 2010). Trauma from accidents, sports (e.g., boxing, football, soccer), or military activities are often overlooked as cause of hypopituitarism and especially when secondary hypogonadism occurs as an isolated posttraumatic sequela (Karaca et al. 2016; Kelly et al. 2014).

Endocrine deficiencies are to be expected if more than 75% of the anterior pituitary tissue is destroyed. Partial forms of HH with residual testosterone secretion and less severe symptoms of androgen deficiency and residual spermatogenesis may result from all above-mentioned conditions (Spratt et al. 1987; Waldstreicher et al. 1996).

In addition to endocrine deficiencies, pituitary tumors may cause headache and visual field defects due to their location and size.

Prolactin-secreting pituitary micro- or macroadenomas cause hypogonadism by a dual mechanism: prolactin itself induces a suppression of pituitary gonadotropin secretion; in addition, the adenoma displaces and compresses normal pituitary tissue, thereby impairing hormone secretion.

Hyperprolactinemia may also be caused pharmacologically by various prescribed drugs (e.g., H2-blockers, metoclopramide, imipramine, alpha-methyldopa, neuroleptic agents) Pharmacological hyperprolactinemia is best treated by eliminating the

respective drug. Surgical or radiologic removal of a prolactin-secreting macroadenoma has become necessary only in rare cases, as adenoma-caused hyperprolactinemia is primarily treated by dopamine agonists such as bromocriptine, cabergoline, quinagolide, or metergoline. These drugs are effective in suppressing hyperprolactinemia and adenoma growth so that additional testosterone or gonadotropin treatment is rarely required. Rarely, dopamine agonist treatment of prolactinomas may lead to hypersexuality. This condition, named "Dopa-testotoxicosis" is assumed to be induced by synergy between reward pathway stimulation and restoration of the eugonadal state after prolonged hypogonadism (De Sousa et al. 2017).

Diagnosis of Central Hypogonadism in the Male

During adolescence, hypogonadotropic hypogonadism is suspected in boys with delayed, absent, or partial and then arrested puberty and prepubescent gonadotropin serum levels. In adulthood, suspicion is raised by symptoms of testosterone deficiency (Table 1) and/or infertility, combined with low LH and FSH levels.

Suspicion of permanent HH has to be based upon meticulous pretreatment diagnostic work-up.

Diagnostic Work-Up

Decreased energy levels, fatigue and loss of libido are the first objective **signs of testosterone deficiency**. Depressive mood, lack of concentration, sleep disturbance, and erectile dysfunction are additional typical complaints. However, the patient may not spontaneously report erectile dysfunction due to reduction of his sexual desire. Therefore, the question regarding sexuality has to be explored explicitly. A standardized hypogonadism symptom questionnaire may help reveal this and other sensitive anamnestic items (Gelhorn et al. 2016). Further symptoms of androgen deficiency include the arrest of spermatogenesis, reduction of seminal fluid, ejaculatory frequency, and sperm count in semen. In the long term, reduction of body hair and beard growth, muscle and bone mineral mass loss (osteoporosis), and anemia will develop.

Family history should assess the parents' age at entering puberty and age at maternal menarche and the presence of signs of hypogonadism or infertility in relatives.

Previous medical history assesses the presence of undescended testes at birth (with age at possible orchidopexy) and previous diseases, including malignancies with chemo- and/or radiotherapy, inflammatory diseases (meningitis, orchitis), and testicular trauma or surgery. Inquiries on the ability to smell and on specific congenital features (that may be associated with congenital HH or Kallmann syndrome), including renal agenesis, dental and digital anomalies, cleft lip and palate, coloboma, synkinesia/mirror movements, and ataxia are similarly important.

Physical examination includes auxological measurements and pubertal Tanner staging with measurement of testicular volumes using a **Prader orchiometer**.

Scrotal ultrasound provides information on testicular and epididymal morphology, in addition to the (more accurate) assessment of testicular volumes.

While **hormone investigations** of the HPG axis focus on LH, FSH, and testosterone levels, measurement of TSH, T3, fT4, IGF1, IGFBP3, prolactin, and cortisol enables investigations of associated pituitary and adrenal hormone deficiencies.

Other laboratory analyses and function tests with the measurement of inhibin B levels and GnRH agonist (buserelin) testing may help differentiate HH from CDGP.

Further diagnostic work-up includes imaging procedures, such as **magnetic resonance imaging** (**MRI)** of the hypothalamo-pituitary region to rule out intracranial malformations, neoplasms, or infiltrating diseases. **Perimetry of visual fields** is helpful for detecting scotomas, specifically bitemporal hemianopsia in case of compression of the chiasma opticum by neoplasms in the sella region. Determination of bone age according to Greulich and Pyle (1959) by a **left-hand carpo-radiogram** is used to estimate residual longitudinal growth potential in adolescents with absent or arrested puberty. **Semiquantitative olfactometry** by University of Pennsylvania Smell Identification Test (UPSIT) (Doty et al. 1995) or Sniffin'sticks (Burghart Messtechnik GmbH, Wedel, Germany) (Hummel et al. 2007) can be performed to reliably distinguish between normal, partially, or totally defective olfaction.

Semen analysis is relevant to evaluate exocrine testicular function, especially if paternity is desired and should be performed according to the "WHO Laboratory Manual for Semen Analysis" (2010) under quality control.

Karyotyping (+ fluorescence in situ hybridization (FISH)) may be undertaken to rule out associated chromosomal anomalies. **Sequencing of candidate genes or performance of targeted multiplex NGS** in subjects with CHH with the description of mutations or polymorphisms is indicated for investigations on the genetic origin of HH. Results are also helpful for genetic counseling of a couple affected by male CHH prior to hormone replacement for spermatogenic induction.

Differential Diagnosis of Tertiary Hypogonadism: Constitutional Delay of Growth and Puberty (CDGP)

In boys presenting for delayed puberty or growth retardation, the most important differential diagnosis to congenital normosmic HH is the normal variant of a constitutional (self-limited) delay of growth and puberty (CDGP). CDGP is by far the most common cause of delayed puberty (pubertas tarda): up to 65% of boys with pubertal delay have CDGP (Sedlmeyer and Palmert 2002).

Once testosterone or gonadotropin replacement has been started, this measure will suppress the endogenous GnRH pulse generator activity and gonadotropin secretion, thereby impeding proper diagnosis. Therefore, it is imperative to previously establish the differential diagnosis between self-limited/potentially reversible hypogonadotropic states and permanent disorders.

Hormone profiles in CDGP are infantile and therefore indistinguishable from hypogonadotropic hypogonadism. A family history of late pubertal development can be found in 50–75% of subjects with CDGP (Wehkalampi et al. 2008; Sedlmeyer and Palmert 2002). Other indicators of CDGP are short stature for chronologic age, in concert with delayed bone age. This is due to a transitory partial growth hormone deficiency which requires no treatment.

A variety of physiological and stimulation tests have been proposed to differentiate between CHH and CDGP (Harrington and Palmert 2012), such as gonadotropin response to GnRH, testosterone response to hCG (Bang et al. 2017), assessment of LH pulsatility by frequent sampling, first morning-voided urinary LH and FSH, and prolactin response to various provocations.

Low-serum levels of the Sertoli cell marker inhibin B may be the best indicator of HH in prepubertal boys. Inhibin B levels below cut-off values of 35 pg/mL (Coutant et al. 2010) or 28.5 pg/mL (Rohayem et al. 2015) are indicative of HH. In addition, pituitary functional testing with the GnRH agonist buserelin, resulting in stimulated LH levels above 4 U/L after 4 h may be helpful to identify boys with CDGP among those with pubertal delay (Wilson et al. 2006). However, due to overlap in the results of all tests between the two entities, the diagnostic process remains challenging.

While reassurance and a "wait-and-see" approach may suit many boys with CDGP, in case of remaining diagnostic uncertainty, "priming" with testosterone may be performed, using three (to six)-monthly 50–100 mg testosterone enanthate i.m. injections every 4 weeks. A rise in LH serum levels on consequent reassessment of HPG axis function after additional 3 months without treatment, together with testicular growth above 4 ml each side would indicate pubertal activation of the GnRH pulse generator, thus CDGP, with no need for further hormone substitution. The above-mentioned procedure can be repeated if necessary (Soliman and De Sanctis 2012).

Hormonal Treatment of Central Hypogonadism in the Male

In all HH men, symptoms of androgen deficiency are the most prevalent complaints. Consequently, substitution with testosterone appears to be the first choice of treatment. Indeed, due to the rapid onset of testosterone action, this treatment is satisfying for the patient as well as for the attending physician. However, besides androgen deficiency, infertility is or will be a problem for many afflicted men. Therefore, induction of spermatogenesis should be prospectively discussed and possibly initiated even in patients without immediate wish for paternity. Hormone replacement strategies in adults with hypogonadotropic hypogonadism differ from those used in adolescents concerning initial dosages.

Generally, adults have already attained normal adult stature, thus eliminating the therapeutic conflicts between maximizing final height (according to genetic target height) and promptness of pubertal induction.

In boys with HH, however, one major therapeutic goal is to induce the pubertal growth spurt. Of note, structural changes of the brain during **adolescence** are

dynamic and protracted, occurring over the course of a decade or more and encompass not only reproductive maturation, but also cognitive, emotional, and social maturation. These behavioral maturational processes may and may not be influenced by gonadal steroid hormones (Sisk and Zehr 2005).

Independent of age, hormonal substitution aims at inducing and maintaining secondary sexual characteristics (penile growth, beard and body hair growth, voice mutation, and muscle and bone mass acquisition) and psychosexual maturation/activation (awakening of libido with sexually stimulated erections and regular ejaculations), thereby improving self-esteem and well-being.

If puberty is induced at an adult age, after psychosocial adaption to the undervirilized/juvenile appearance has occurred, psychological changes affecting partnership may ensue. It remains to be seen whether late hormone substitution in adulthood reverses all effects of low androgen levels during adolescence, as differences in spatial ability and of some reproductive behavioral aspects of psychosocial development have been reported in small patient cohorts (Hier and Crowley 1982; Gooren 1988).

The therapeutic goal of normalizing serum testosterone levels according to age references can be achieved by replacement of testosterone, GnRH, or gonadotropins. In the following, the indications for these therapies are described.

Pulsatile GnRH Substitution

In patients with HH due to hypothalamic GnRH deficiency, but normal pituitary function, GnRH is the most physiological but cumbersome treatment: As continuous application of GnRH results in downregulation of pituitary GnRH receptors, GnRH has to be substituted in a pulsatile fashion. This is performed by a portable minipump which has to be worn for at least one to 2 years and sometimes longer to stimulate gonadotropin secretion in the pituitary and consequently testosterone secretion and spermatogenesis in the testes. Only few patients are motivated to be compliant with this treatment modality for longer periods (Delemarre-van de Waal 2004). A recent study from China demonstrated that those patients who adhere to GnRH replacement may be rewarded by faster testicular growth and appearance of sperm (Mao et al. 2016) compared to gonadotropin replacement regimens, in contrast to earlier observations with no differences in outcome (Schopohl 1993; Büchter et al. 1998).

The needle of the catheter linked to the portable GnRH mini pump is placed into the patient's abdominal subcutaneous tissue and has to be changed every 2 days. The pump is programmed to deliver GnRH boluses every 120 minutes, as this frequency was shown to be most effective for gonadotropin stimulation. After a starting dose of 4 μg per pulse, increases of 2 μg may be performed every 4 weeks, aiming at testosterone levels in the normal adult range after 3–12 months (depending on the maturation progress of the testes). Response to treatment is monitored by the assessment of testicular growth, testosterone serum levels, and appearance of sperm in the ejaculate. The doses necessary to induce spermatogenesis vary between

HH patients, ranging 5–20 μg GnRH per bolus or 25–600 ng/kg GnRH per bolus. One to 3 years of treatment may be required to induce spermatogenesis sufficient to induce a pregnancy. Patients with previously undescended testes may require considerably longer treatment for induction of spermatogenesis than patients with eutopic testes (Büchter et al. 1998).

It has recently been shown that infusion of kisspeptin-54 is efficient in restoring GnRH and LH-pulsatility in patients with a mutation in *TAC3* or *TAC3R* with a loss of signaling by neurokinin B (Young et al. 2013). These data have had implications for the development of kisspeptin 54 as an efficacious trigger of oocyte maturation in women at high risk of ovarian hyperstimulation syndrome (OHSS) during in vitro fertilization (IVF) therapy (Abbara et al. 2015). Currently kisspeptin is undergoing testing for diagnosis of hypogonadotropic hypogonadism (Chan et al. 2014).

Gonadotropin Substitution

General Considerations

While GnRH treatment is reserved for patients with hypogonadotropic hypogonadism due to lack of GnRH synthesis or action, but with preserved capacity for gonadotropin secretion, gonadotropin substitution can be applied to all hypogonadotropic patients (Boehm et al. 2015) (Table 3). This applies to those men, who, besides virilization, aim for fertility and/or testicular growth. The subcutaneous administration of human chorionic gonadotropin (hCG) serves as a substitute for LH and stimulates Leydig cells to secrete testosterone (Büchter et al. 1998; Liu et al. 1988). This effect of hCG is enhanced in combination with FSH.

Follicle-stimulating hormone (FSH) is required for spermatid maturation (spermiogenesis) during initiation, and for maintenance of quantitatively normal spermatogenesis at puberty and thereafter (Matsumoto et al. 1986, 2009).

While available hCG preparations are derived from the urine of pregnant women, FSH preparations are either urinary-derived from postmenopausal women (hMG)

Table 3 Therapeutic options licensed for puberty induction and fertility treatment in male hypogonadotropic hypogonadism

Drug	*Trade name*	*Application*	*Dose*
Human chorionic gonadotropin (hCG)	Brevactid®[a] Pregnyl®[b] Novarel®[b]	s.c. or i.m.	1000–2500 IU s.c. twice weekly (on Mondays and Fridays)
Recombinant FSH (rFSH)	Gonal F®[a], Puregon®[a] Follistim®[b]	s.c. or i.m.	75–150 IU s.c. three times weekly (on Mondays, Wednesdays, Fridays)
Pulsatile GnRH	LutrePulse®[a]	Minipump s.c.	4–20 μg per pulse every 120 min

[a]Trade names of drugs marketed in Europe
[b]Trade names of drugs marketed in the USA

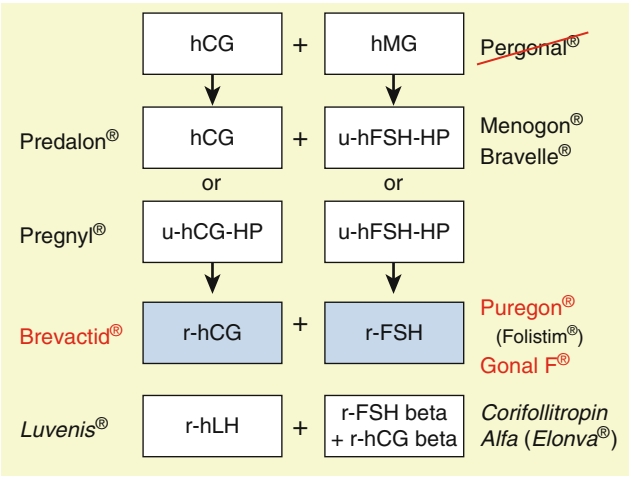


Fig. 2 Historical development of gonadotropin treatment for secondary or tertiary hypogonadism. The blue fields signify the preparations in use, the lower white fields indicate preparations under development

and purified, or recombinant (*rFSH*) (Kliesch et al. 1995; Bouloux et al. 2003; Warne et al. 2009). The long-acting recombinant FSH-CTP (Corifollitropin alfa/MK 8962) has to be subcutaneously injected only every second week. It has already been licensed for female indications and is at this time (2017) under investigation for use in the treatment of male hypogonadotropic hypogonadism (Nieschlag et al. 2017) (Fig. 2).

Induction of spermatogenesis and achievement of fertility in males can be attained within 6 months to 2–2.5 years, depending on previous testicular maturation before initiation of treatment. Subjects with initially absent puberty require longer replacement to achieve maximum testicular growth and sperm concentrations in their ejaculate than those with pubertal arrest or postpubertal onset of hypogonadotropic hypogonadism. In these patients, final testicular volumes often plateau at a subnormal level (Fig. 3) and maximal seminal sperm counts do not reach normal values. However, quality of spermatozoa seems to be high, as most patients are nevertheless able to impregnate their partner spontaneously (Burris et al. 1988; Pitteloud et al. 2002; Liu et al. 2002; Rohayem et al. 2016) (Fig. 4).

Once a gonadotropin treatment cycle has been completed, this reduces time to reinduction of fertility to 6–10 months by repeating this treatment in later years (Liu et al. 1988; Büchter et al. 1998). Spermatogenesis can be maintained at a lower level with hCG alone for some time (Depenbusch et al. 2002).

Maldescended testes, although a negative predictor of GnRH and gonadotropin response, do not preclude chances of attaining fertility, but require longer stimulation (Büchter et al. 1998).

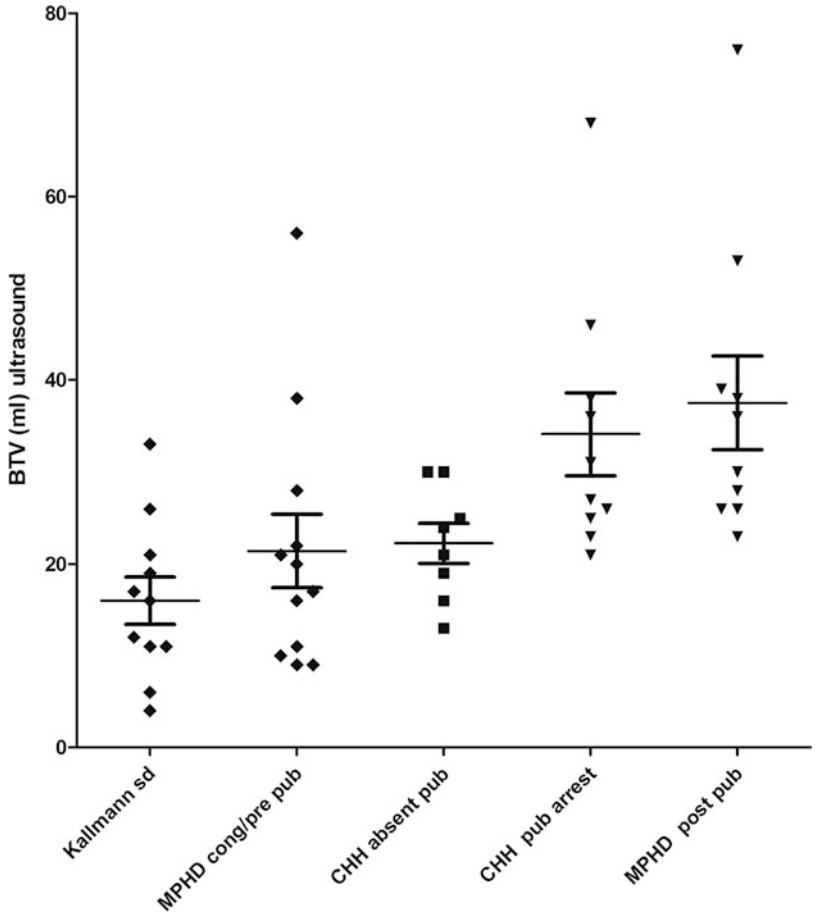


Fig. 3 Final bitesticular volumes (BTV) in 38 adult patients with hypogonadotropic hypogonadism under hCG/rFSH treatment correlate with onset of disease (Rohayem et al. 2016). Kallmann sd: Kallmann syndrome; MPHD cong/pr pub: congenital or pre pubertally acquired multiple pituitary hormone deficiencies; CHH absent pub: congenital hypogonadotropic hypogonadism with absent puberty; CHH pub arrest: congenital hypogonadotropic hypogonadism with spontaneously initiated puberty, with consequent pubertal arrest; MPHD post pub: postpubertally acquired multiple pituitary hormone deficiencies (by surgery or tumour in the sella region)

Hormone Replacement in Prepubertal Onset HH

A treatment regimen beginning with testosterone for induction of puberty stimulates normal linear growth, pubertal virilization, and psychosexual maturation in congenital HH. This is achieved by increasing doses of testosterone enanthate i.m., starting with 50 mg every 4 weeks. The dose is increased to 125 mg after 6–12 months and further increased to the full replacement dose for adult men: 250 mg after 1.5–2 years. If the adolescent feels that the effect of testosterone vanishes before the next injection is due, the interval of shots can be reduced to 3 weeks.

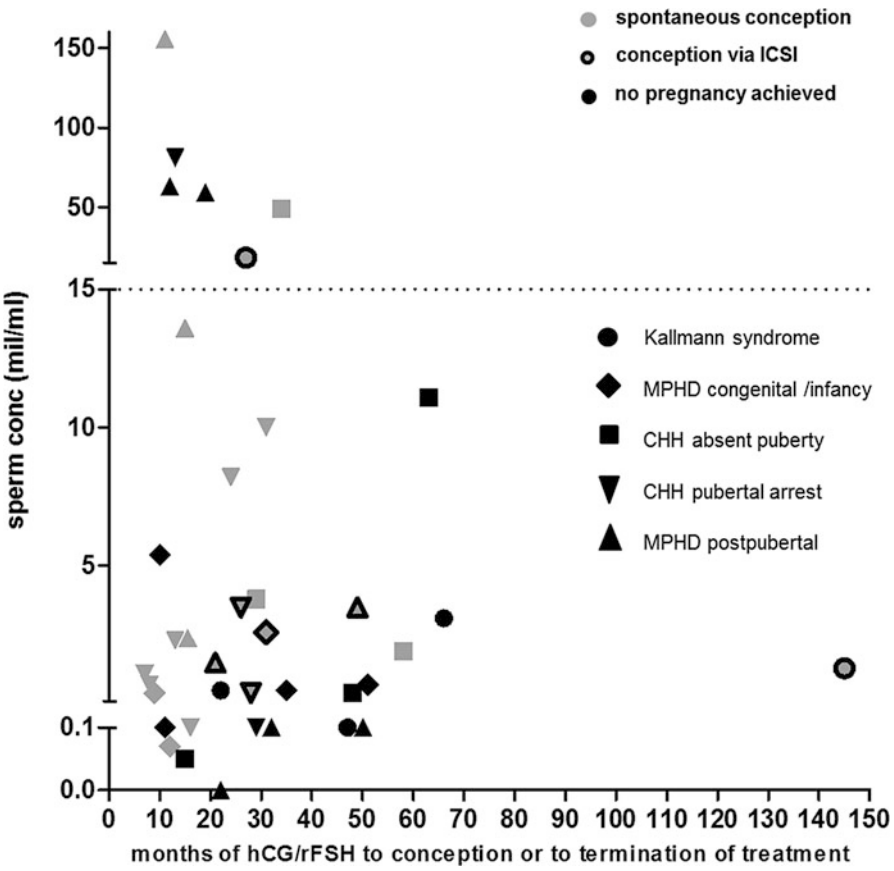


Fig. 4 Sperm concentrations at conception or termination of hCG/rFSH from 38 adult men with hypogonadotropic hypogonadism of different ethiology (Rohayem et al. 2016)

However, this traditional approach neglects testicular growth and the acquisition of fertility as components of normal puberty. The testes remain immature and small (< 4 ml each side), i.e., in a prepubertal state, and spermatogenesis is not initiated. Meanwhile, there is sufficient evidence that complete male puberty, comprising pubertal virilization in concert with testicular growth and initiation of spermatogenesis can be successfully achieved during adolescence by replacing gonadotropins (hCG/FSH) (Liu et al. 1988; Schopohl 1993; Barrio et al. 1999; Sinisi et al. 2008; Zacharin et al. 2012; Rohayem et al. 2017).

Gonadotropin treatment may be suggested for pubertal induction even if fertility is a matter for the future, and even though rFSH is costly, as normalization of testicular size and initiation of spermatogenesis may positively affect body image, thus providing self-assurance and confidence for the future in teenage boys with HH (Shiraishi et al. 2014, Rohayem et al. 2017). In addition, spermatogenesis, once driven to full maturation, can be restimulated much faster when fertility is desired

later in life (Büchter et al. 1998; Liu et al. 2002). Subsequent treatment with testosterone does not seem to jeopardize the outcome of later stimulation therapy (Rohayem et al. 2017). This is important for a few prepubertal boys who are unwilling to commit to five s.c. injections per week for 2–3 years. However, some of these young patients may be more agreeable to gonadotropin stimulation once pubertal induction with testosterone is accomplished (Rogol 2005).

In adolescent patients with open epiphyses (i.e., with a bone age <16 years), hCG treatment ought to be performed in small increments over the first year (until serum testosterone levels are in the normal adult range) to induce pubertal virilization. Thereby a pubertal growth spurt and attainment of final height in the range of midparental expectations can be achieved. Adverse pubertal effects including premature epiphyseal fusion, severe acne, or gynecomastia can successfully be avoided by this regimen. Subsequent combined hCG/rFSH replacement over 2–3 years is required to accomplish testicular growth and fully activate the individual's spermatogenic potential. Over 70% of adolescents will thereby reach normal adult testicular sizes and in more than 90% sperm will develop in semen (Zacharin et al. 2012; Rohayem et al. 2017).

In view of the challenging differential diagnosis of constitutional delay of puberty (CDGP), this regimen bears an important further advantage: low initial hCG doses have only weak suppressive properties at the hypothalamic or pituitary level, therefore activation of the pubertal GnRH pulse generator in cases of unrecognized CDGP is possible. Patients wrongly diagnosed with HH may thus be recognized if special attention to a potential rise in LH levels is given during the first phase of gonadotropin replacement.

In patients with pubertal failure but a late diagnosis of CHH in adulthood, or those with previous testosterone treatment, final height may already have been attained. In these adult subjects, higher initial hCG replacement doses and faster dose escalations can be applied than in HH boys with open epiphyses/immature growth plates.

Success rates concerning spermatogenic induction in adults are slightly below those of adolescents, ranging from 65% to 90% (Büchter et al. 1998; Burgués and Calderon 1997; EMHSG 1998; Bouloux et al. 2002).

Treatment Protocol for Testosterone-Naïve Prepubertal Adolescents

A starting dose of (250-) 500 IU hCG, injected subcutaneously on Mondays and Fridays, with incremental increases of 250–500 IU hCG per injection every 6 months to a maximum of 3 × 2500 IU hCG s.c./week is recommended. The aim is to achieve pubertal levels of serum testosterone (≥1.5 ng/ml, [5.2 nmol/l]) after around 6 months, and levels in the midnormal adult range (testosterone >3.5 ng/ml, [12 nmol/l]) by 1 year. rFSH (follitropin alpha) 3 × (75-)150 IUs.c/week (injected Mondays, Wednesdays, and Fridays) is added when pubertal serum testosterone levels (>5.2 nmol/l) are reached. Subsequent rFSH dosage modifications above 150 IU per injection are not recommended as they are not beneficial for enhancement of spermatogenesis.

Treatment Protocol for Testosterone-Virilized Adolescents with Prepubertal Onset HH

These patients can be treated like post- pubertal adult patients (see section "Gonadotropin Replacement in Postpubertal Onset/Adult HH").

Gonadotropin Replacement in Postpubertal Onset/Adult HH

In patients with postpubertally acquired HH, testicular maturation has already been completed, as indicated by normal adult testicular size. However, if gonadotropin stimulation has been lacking for a prolonged period, paused spermatogenesis may have led to a (slight) reduction in testicular volume. Since gonadotropins are costly, this therapeutic investment is justified only if paternity is desired. In all other cases, testosterone is the preferred modality for replacement. When stimulation therapy for fertility induction is initiated in patients with previously accomplished testicular maturation, Leydig cell response (as indicated by a rise in serum testosterone levels to the adult range) to hCG occurs within 3 months. In rare cases, this is sufficient for initiation of spermatogenesis (Finkel et al. 1985). However, prolonged treatment with hCG alone will suppress endogenous residual FSH secretion. Therefore the addition of FSH is required.

Protocol: A full hCG starting dose of 1500 IU s.c. is applied twice weekly. The HCG dose should be reduced in cases of erythrocytosis, gynecomastia, or excessive acne. If testosterone levels remain below the normal adult range (<12 nmol/l) after 6–9 months, the hCG dose can be increased by increments of 500–1000 IU per injection every (3)–6 months to a maximum of 3 × 2500 IU s.c./week.

A full dose of rFSH (follitropin alpha) 3 × 150 IU is additionally injected thrice weekly, without subsequent dose modifications.

Reawakening of spermatogenesis occurs (with first spermatozoa appearing in semen) after 3–13 months of combined hCG/FSH replacement (Liu et al. 2009; Rohayem et al. 2016). Stimulation of spermatogenesis will not proceed further when the sperm concentration in the ejaculate reaches a plateau that is indicative of the individual's spermatogenic capacity.

Thereafter, sperm production may be maintained with hCG alone for several months (in patients with residual endogenous gonadotropin secretion) (Johnson 1978; Depenbusch et al. 2002).

Monitoring Gonadotropin Substitution

Measurement of serum LH, FSH, testosterone levels, and testicular volumes (via Prader orchiometer and/or ultrasound) ought to be performed at 3 to 6 monthly intervals during gonadotropin substitution, along with annual bone age estimation in adolescents, until a bone age of 16 years (and thus near final height) is reached. Special attention should be given to LH levels, as a spontaneous increase into a range above >1 U/L would indicate spontaneous HPG axis activation during gonadotropin substitution, which would question the diagnosis of HH. In such cases of CDGP, total cessation of hormone replacement is indicated.

Regular measurement of serum testosterone levels (e.g., every 3 months) is useful not only to monitor the Leydig cell response, but also, along with FSH levels,

adherence to treatment. Once maturity for semen is attained in previously prepubertal subjects, ejaculates can be collected and analyzed according to WHO guidelines (WHO 2010) after at least 48 h of sexual abstinence. Thereafter, semen analyses may be repeated every 3 months until a plateau of sperm concentrations is documented in at least two follow-up visits or until pregnancy is achieved. Sperm cryopreservation may be offered before gonadotropin replacement is stopped and patients are switched to testosterone replacement. However, as restimulation of testes with gonadotropins by a second treatment cycle is known to be successful in most subjects, even after a longer period of testosterone substitution (Büchter et al. 1998), sperm cryopreservation in adolescent patients is recommended only if primary spermatogenic response to treatment is very poor. In adult men with no wish for immediate fatherhood, cryostorage of semen may be indicated.

Misdiagnosis of CDGP and CHH Reversal

With respect to the clinical and genetic overlap between CHH patients and those with constitutionally delayed puberty, erroneous diagnosis of CDGP as HH cannot be excluded (Zuh et al. 2015). Therefore, interruption of treatment of HH is discussed with the patient after achievement of the primary treatment goals. Such interruption allows the physician to determine whether a spontaneous reactivation of the HPG axis occurs or whether substitution therapy has to continue life-long. Around 10% of patients with Kallmann syndrome or CHH (with *CHD7, FGFR1, GNRHR, TACR* mutations) bear potential for reversal of GnRH deficiency (Quinton et al. 1999; Raivio et al. 2007; Laitinen et al. 2012; Dwyer et al. 2016). Even with a replacement strategy with testosterone (enanthate or gel), continuous attention should be given to LH levels (the parameter reflecting the patient's endogenous HPG axis activity), in order to recognize potential spontaneous GnRH pulse generator activation. If reversal of HH occurs, it may not be sustained (Tommiska et al. 2013; Sidhoum et al. 2014). This vulnerability and plasticity of the reproductive axis emphasize the need for lifelong attention to symptoms of androgen deficiency and surveillance of HPG axis hormones in patients with spontaneous HH reversal.

Testosterone Substitution

Criteria for Testosterone Substitution

All patients with hypogonadism including central hypogonadism will eventually require testosterone replacement as hypogonadism implies testosterone deficiency. However, in patients with central hypogonadism, testosterone substitution should only be initiated once the patient's current or future fertility perspectives have been discussed. If the patient desires paternity in the immediate future, stimulation therapy by GnRH or gonadotropins should be the first choice as it stimulates spermatogenesis *as well as* testosterone production in the Leydig cells (see above).

If testosterone substitution is the treatment of choice, the question arises at which level of testosterone serum concentrations substitution is indicated.

Two factors contribute to controversial opinions and explain why the lower limit of normal and the limits for initiating testosterone substitution vary in various countries (Nieschlag et al. 2004) and in different guidelines (Bhasin et al. 2010; Dohle et al. 2012; Swerdloff and Wang 2012, Flaseriu et al. 2016):

First, serum testosterone undergoes a clear diurnal rhythm with higher levels in the morning and about 25% lower levels in the evening. Therefore, and for the sake of comparability, therapeutic decisions should be based on serum testosterone measurements during the morning hours.

Second, various testosterone symptoms have different threshold levels, loss of libido, and vigor may already occur below 12–15 nmol/L. Below 10 nmol/L depressive moods, sleep disturbances, lack of concentration, and diabetes type 2 are observed, while erectile dysfunction and hot flashes are reported below 8 nmol/L (Zitzmann et al. 2006).

Often perception of the physician rather than the patient's complaints and strain may govern prescribing behavior. Loss of libido is the most frequent and an early symptom demanding treatment and occurring below 12 nmol/L and provides a distinct signal for beginning testosterone substitution. Several large epidemiological studies from the USA (Bhasin et al. 2011), Australia (Sartorius et al. 2012), and Europe (Huhtaniemi et al. 2012) show that 12 nmol/L is the lowest value for serum testosterone of healthy men.

If several measurements provide ambivalent results, free testosterone in serum may aid the therapeutic decision. Free testosterone should be measured based on total testosterone and SHBG by using a generally available formula (http://issam.ch/freetesto.htm). Values below 225 pmol/L are considered subnormal.

Determination of serum testosterone varies depending on laboratory methodology and still provides a problem as wide scatters in external quality control programs show. Meanwhile, mass spectrometry methods (LLC-MS/MS) are the gold standard for measuring testosterone. Some guidelines and journals require measurements by these techniques. However, mass spectrometry may not be available everywhere. It has been shown that immune assays correlate well with results from mass spectrometry in the normal and upper subnormal range (Bhasin et al. 2011; Huhtaniemi et al. 2012; Haring et al. 2013), but are less reliable in in the low hypogonadal range.

Testosterone Preparations and Modes of Application

Testosterone was synthesized in 1935 and has remained in clinical use since that time (Nieschlag and Nieschlag 2014). Until the 1990s only preparations resulting in mostly unphysiologic serum levels existed. Today intramuscular, subdermal, transdermal, oral, and buccal testosterone preparations are available (Behre and Nieschlag 2012) (Table 4). WHO, NIH, and FDA have jointly formulated general principles of testosterone therapy as "Guidelines for the use of androgens in men" (Nieschlag et al. 1992) which are still valid today. The following are the most important:

- Only preparations of natural testosterone should be used for substitution therapy in hypogonadism since the full spectrum of testosterone action in the body can

Table 4 Testosterone preparations available for substitution

Intramuscular testosterone	**testosterone enanthate** 250 mg every 2–3 weeks **testosterone cypionate** 200 mg every 2–3 weeks **testosterone undecanoate** 1000 mg, first and after 6, then every 12 weeks: 750 mg first and after 4, then every 10 weeks	Testoviron® Depot 250 Testosteron Depot® Delatestryl® Nebido® Aveed® (USA)
Transdermal Testosterone	One system daily 50–100 mg in 5–10 g gel daily 62.5 mg in 5 g gel daily 30–120 mg in 1.5–4.5 ml	Testopatch® Androgel® / Testim ® Testotop® / Tostran® Axiron®
Oral Testosterone	Testosterone undecanoate 3–4 capsules à 40 mg daily	Andriol® / Testocaps®
Buccal Testosterone	Testosterone 2 × 30 mg tablets daily	Striant®

only be achieved if testosterone is aromatized to estradiol and 5α-reduced to dihydrotestosterone (DHT). As conversion to these metabolites occurs at physiologically well-balanced rates for natural testosterone secreted by the testes, and not with synthetic androgens, testosterone remains the only choice for substitution in central hypogonadism. The well-balanced synergism between testosterone and estradiol is specifically important for sexual function, bone metabolism, and body composition, while synergism with DHT is important for accessory sex organs and action on the skin (hair pattern, sebum production). Nor can the full spectrum of testosterone action be achieved by androgenic anabolic steroids or by selective androgen receptor modulators (SARMs).

- Testosterone substitution should result in circulating serum levels as close to physiology as possible. Testosterone treatment of hypogonadal males should avoid supraphysiologically high testosterone serum levels as well as subnormally low levels.

These demands are not achieved by intramuscular testosterone enanthate or cypionate resulting in a roller coaster pharmacokinetic pattern. Patients dislike the ups and downs of well-being, vigor, sexual activity, and emotional stability. Because these preparations are cheap, they are still in use but have been replaced, wherever affordable, by transdermal gels and intramuscular testosterone undecanoate resulting in physiologic serum testosterone levels.

In the following, testosterone substitution in the adult HH patient is described, for testosterone treatment in the adolescent HH patient see section "Hormone Replacement in Prepubertal Onset HH."

Oral Testosterone

Testosterone applied orally is readily absorbed by the intestine, but is also quickly eliminated by the first-pass effect in the liver. In order to overcome this effect a

modified synthetic testosterone molecule, **17α-alkylated methyltestosterone** was used for some time, but became obsolete because of its liver toxicity. However, esterified **testosterone undecanoate** administered orally, due to its long aliphatic side chain absorbed via the lymph, reaches circulation and target organs before the liver. Capsules of 40 mg (Andriol®) have to be taken 3 or 4 times/day for the treatment of hypogonadism. Pharmacokinetic analysis shows high intra- and interindividual variability in serum testosterone concentrations. Therefore this preparation is best suited as a supplement for low, but still ongoing testosterone production.

Buccal Testosterone

Testosterone incorporated into polyethylene matrices can be **formed into mucoadhesive tablets**. These tablets adhere to the gingiva above the incisors for many hours and slowly release testosterone into the circulation. Twice daily applications result in constant serum levels. However, up to 15% of patients experience irritation, inflammation, or gingivitis, but those who become accustomed to the tablets tolerate them well. Care must be taken that the tablets do not become disattached, e.g., during meals (Dinsmore and Willie 2012).

Intramuscular Testosterone

Testosterone, when injected, is very quickly degraded and therefore not suitable for substitution, the testosterone molecule has to be esterified to produce longer-acting substances. The most widely used preparations are **testosterone enanthate** and **cypionate,** of which 200–250 mg need to be injected in 2–3 week intervals for substitution purposes. **Testosterone propionate** is a shorter-acting ester. 50–100 mg last for only 2–3 days and are therefore not suited for long-term substitution.

The same *orally* effective testosterone undecanoate can also be used for ***intramuscular*** injection when administered in castor oil (Nieschlag et al. 1999a; Nieschlag and Nieschlag 2014). When injected in doses of 1000 mg in 4 ml castor oil (Nebido®) and then after 6 weeks, followed by further injections every 12 weeks, this preparation results in stable adult serum levels lasting for 10–14 weeks. After the initial loading dose, the patient on average requires injections only every 3 months (Zitzmann and Nieschlag 2007; Behre and Nieschlag 2012). In the USA, this preparation is available in doses of 750 mg in 3 ml castor oil (Aveed®) requiring further injections after 4 and then at 10 week intervals (Wang et al. 2010).

While the injectable preparations are generally considered very safe, pulmonary oil micro embolism (POME) characterized by brief respiratory symptoms (including cough, urge to cough, and dyspnea) immediately after the injection has been observed in rare cases (Mackey et al. 1995). Therefore special care has to be observed when injecting these oily solutions intramuscularly to avoid intravenous injections.

Transdermal Testosterone

Transdermal testosterone preparations mimic physiological diurnal variations and their kinetic profiles are closest to ideal substitution. They may be used as the first choice and are especially well suited for patients with fluctuating symptoms caused

by other preparations. In addition, upon removal, testosterone is immediately eliminated if any adverse health effects should occur (e. g. polycythemia).

Scrotal patches consisting of a thin film soaked with testosterone were the earliest transdermal preparations for clinical use (Behre et al. 1999). Later, non-scrotal transdermal systems were developed but had the disadvantage of skin irritation occurring in a high percentage of patients.

These preparations were soon superceded by **testosterone gels** needing to be applied to sufficiently large skin areas in order to allow enough testosterone to be resorbed. Physiological levels result when these gels, applied in the morning to the upper arms, shoulders and abdomen, are left to dry for 5–10 minutes. Some manufactures suggest covering the skin area with underwear and others recommend washing the skin after evaporation of the alcohol in order to avoid unintended transfer to children or women (DeRonde 2009; Nelson et al. 2013). Long-term use showed good results (Wang et al. 2004; McNicholas and Ong 2006; Behre et al. 2012; Kühnert et al. 2005), if the appropriate dose of the gel is administered; serum levels, although variable, remain within the normal range (Swerdloff et al. 2015).

Subdermal Testosterone Implants

Testosterone pellet implants were among the first modalities applied for testosterone replacement therapy; however, as a tunneling technique is required for implantation in the abdominal wall by a trocar, extrusion of the pellet and infection of the implantation site may occur. Three to six implants are inserted, resulting in initially supraphysiological levels and then slowly declining to the normal range for up to 4–6 months. In a cross-over study of pellets versus injectable testosterone undecanoate most patients preferred injections, mainly because of the simpler delivery (Fennell et al. 2010). Such implants have lost popularity and are only used in a few places (Kelleher et al.1999).

Monitoring of Testosterone Substitution

Once testosterone substitution has been initiated, the patient should be carefully monitored at regular intervals. As a general rule, the patient should be seen after 3, 6, and 12 months and then at least annually. Intervals may be more frequent if certain symptoms prevail or comorbidities occur requiring immediate attention. Often, the patients feel badly instructed about the purpose and schedule of the treatment regimen, and then do not spontaneously contact their physicians (Dwyer et al. 2016). Mechanisms should be installed by the attending physician to remind patients of necessary control visits and to encourage additional visits if required.

Inquiries on present complaints should include information on mental and physical activity, libido and sexual activity, and satisfaction.

Physical examination should assess body weight, waist circumference and fat distribution, beard growth, and hair pattern as well as possible development of breast tissue.

Evaluation of size and surface of the prostate (by digital rectal examination preferably supplemented by transrectal ultrasonography) should be performed annually. Laboratory work-up includes the measurement of red blood cell counts and

hematocrit to detect and avoid polycythemia and its sequelae, e.g., apoplexy and thromboembolism.

Bone density should be measured prior to the commencement of testosterone substitution and then at regular intervals of about 2 years.

Taking into consideration the pharmacokinetics of the respective testosterone preparation, serum testosterone should always be measured before the next dose is applied and visits should be scheduled accordingly to allow for dosage adjustments.

Of note: On replacement with testosterone, the prostate of the hypogonadal patient will grow to its normal size. PSA will also increase, but should not exceed the normal range. At present there is no compelling evidence that testosterone treatment increases the incidence of prostate carcinoma (Debruyne et al. 2017).

References

Abbara A, Jayasena CN, Christopoulos G, Narayanaswamy S, Izzi-Engbeaya C, Nijher GM, Comninos AN, Peters D, Buckley A, Ratnasabapathy R, Prague JK, Salim R, Lavery SA, Bloom SR, Szigeti M, Ashby DA, Trew GH, Dhillo WS. Efficacy of kisspeptin-54 to trigger oocyte maturation in women at high risk of ovarian hyperstimulation syndrome (OHSS) during in vitro fertilization (IVF) therapy. J Clin Endocrinol Metab. 2015;100:3322–31.

Achermann JC, Meeks JJ, Jameson JL. Phenotypic spectrum of mutations in DAX-1 and SF-1. Mol Cell Endocrinol. 2001;185:17–25.

Alqwaifly M, Bohlega S. Ataxia and hypogonadotropic hypogonadism with intrafamilial variability caused by RNF216 mutation. Neurol Int. 2016;8:6444.

Bang AK, Nordkap L, Almstrup K, Priskorn L, Petersen JH, Rajpert-De Meyts E, Andersson AM, Juul A, Jørgensen N. Dynamic GnRH and hCG testing: establishment of new diagnostic reference levels. Eur J Endocrinol. 2017;176:379–91.

Barrio R, De Luis D, Alonso M, Lamas A, Moreno JC. Induction of puberty with human chorionic gonadotropin and follicle-stimulating hormone in adolescent males with hypogonadotropic hypogonadism. Fertil Steril. 1999;71:244–8.

Behre HM, Nieschlag E. Testosterone preparations for clinical use in males. In: Nieschlag E, Behre HM, Nieschlag S, editors. Testosterone: action, deficiency, substitution. 4th ed. Cambridge: Cambridge University Press; 2012. p. 309–35.

Behre HM, Nieschlag E, Partsch CJ, Wieacker P, Simoni M. Diseases of the hypothalamus and the pituitary gland. In: Nieschlag E, Behre HM, editors. Andrology: male reproductive health and dysfunction. 3rd ed. Berlin: Springer; 2010. p. 169–92.

Behre HM, von Eckardstein S, Kliesch S, Nieschlag E. Long-term substitution therapy of hypogonadal men with transscrotal testosterone over 7–10 years. Clin Endocr. 1999;50:629–35.

Behre HM, Tammela TL, Arver S, Tolrá JR, Bonifacio V, Lamche M, Kelly J, Hiemeyer F, European Testogel® Study Team, Giltay EJ, Gooren LJ. A randomized, double-blind, placebo-controlled trial of testosterone gel on body composition and health-related quality-of-life in men with hypogonadal to low-normal levels of serum testosterone and symptoms of androgen deficiency over 6 months with 12 months open-label follow-up. Aging Male. 2012;15:198–207.

Behre HM, Zitzmann M. Imaging diagnosis. In: Nieschlag E, Behre HM, editors. Andrology: male reproductive health and dysfunction. 3rd ed. Berlin: Springer; 2010. p. 101–7.

Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophyseal responses to continuous and intermittent delivery of hypothalamic gonadotropin releasing hormone. Science. 1978;202:631–3.

Beneduzzi D, Trarbach EB, Min L, Jorge AA, Garmes HM, Renk AC, Fichna M, Fichna P, Arantes KA, Costa EM, Zhang A, Adeola O, Wen J, Carroll RS, Mendonça BB, Kaiser UB,

Latronico AC, Silveira LF. Role of gonadotropin-releasing hormone receptor mutations in patients with a wide spectrum of pubertal delay. Fertil Steril. 2014;102:838–46.

Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM, Task force, Endocrine Society. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010;6:2536–59.

Bhasin S, Pencina M, Jasuja GK, Travison TG, Coviello A, Orwoll E, Wang PY, Nielson C, Wu F, Tajar A, Labrie F, Vesper H, Zhang A, Ulloor J, Singh R, D'Agostino R, Vasan RS. Reference ranges for testosterone in men generated using liquid chromatography tandem mass spectrometry in a community-based sample of healthy nonobese young men in the Framingham heart study and applied to three geographically distinct cohorts. J Clin Endocrinol Metab. 2011;96:2430–9.

Boehm U, Bouloux PM, Dattani MT, de Roux N, Dodé C, Dunkel L, Dwyer AA, Giacobini P, Hardelin JP, Juul A, Maghnie M, Pitteloud N, Prevot V, Raivio T, Tena-Sempere M, Quinton R, Young J. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism–pathogenesis, diagnosis and treatment. Nat Rev Endocrinol. 2015;11:547–64.

Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombès M, Millar RP, Guiochon-Mantel A, Young J. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. N Engl J Med. 2009;360:2742–8.

Bouloux P, Warne DW, Loumaye E. Efficacy and safety of recombinant human follicle-stimulating hormone in men with isolated hypogonadotropic hypogonadism. Fertil Steril. 2002;77:270–3.

Bouloux PM, Nieschlag E, Burger HG, Skakkebaek NE, FC W, Handelsman DJ, Baker GH, Ochsenkuehn R, Syska A, McLachlan RI, Giwercman A, Conway AJ, Turner L, van Kuijk JH, Voortman G. Induction of spermatogenesis by recombinant follicle-stimulating hormone (puregon) in hypogonadotropic azoospermic men who failed to respond to human chorionic gonadotropin alone. J Androl. 2003;24:604–11.

Büchter D, Behre HM, Kliesch S, Nieschlag E. Pulsatile GnRH or human chorionic gonadotropin/human menopausal gonadotropin as effective treatment for men with hypogonadotropic hypogonadism: a review of 42 cases. Eur J Endocrinol. 1998;139:298–303.

Burgués S, Calderón MD. Subcutaneous self-administration of highly purified follicle stimulating hormone and human chorionic gonadotrophin for the treatment of male hypogonadotrophic hypogonadism. Hum Reprod. 1997;12:980–6.

Burris AS, Rodbard HW, Winters SJ, Sherins RJ. Gonadotropin therapy in men with isolated hypogonadotropic hypogonadism: the response to human chorionic gonadotropin is predicted by initial testicular size. J Endocrinol Metab. 1988;66:1144–51.

Butler MG, Wang K, Marshall JD, Naggert JK, Rethmeyer JA, Gunewardena SS, Manzardo AM. Coding and noncoding expression patterns associated with rare obesity-related disorders: Prader-Willi and Alström syndromes. Adv Genomics Genet. 2015;2015:53–75.

Chan YM, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsiaras S, Gaspert A, Lavoie HB, Wu CH, Crowley WF Jr, Amory JK, Pitteloud N, Seminara SB. GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci U S A. 2009;106:11703–8.

Chan YM, Lippincott MF, Butler JP, Sidhoum VF, Li CX, Plummer L, Seminara SB. Exogenous kisspeptin administration as a probe of GnRH neuronal function in patients with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2014;99:E2762–71.

Claahsen-van der Grinten HL, Otten BJ, Takahashi S, Meuleman EJ, Hulsbergen-van de Kaa C, Hermus AR. Testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia: evaluation of pituitary-gonadal function before and after successful testis-sparing surgery in eight patients. J Clin Endocrinol Metab. 2007;92:612–5.

Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougnères P, Lebouc Y, Froguel P, Guy-Grand B. A mutation in the

human leptin receptor gene causes obesity and pituitary dysfunction. Nature. 1998;392:398–401.

Coutant R, Biette-Demeneix E, Bouvattier C, Bouhours-Nouet N, Gatelais F, Dufresne S, Rouleau S, Lahlou N. Baseline inhibin B and anti-Mullerian hormone measurements for diagnosis of hypogonadotropic hypogonadism (HH) in boys with delayed puberty. J Clin Endocrinol Metab. 2010;95:5225–32.

Costa-Barbosa FA, Balasubramanian R, Keefe KW, Shaw ND, Al-Tassan N, Plummer L, Dwyer AA, Buck CL, Choi JH, Seminara SB, Quinton R, Monies D, Meyer B, Hall JE, Pitteloud N, Crowley WF Jr. Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotypes. J Clin Endocrinol Metab. 2013;98:E943–53.

Daniell HW. Hypogonadism in men consuming sustained-action oral opioids. The J Pain. 2002;3:377–84.

Debruyne FM, Behre HM, Roehrborn CG, Maggi M, Wu FC, Schröder FH, Jones TH, Porst H, Hackett G, Wheaton OA, Martin-Morales A, Meuleman E, Cunningham GR, Divan HA, Rosen RC, Investigators RHYME. Testosterone treatment is not associated with increased risk of prostate cancer or worsening of lower urinary tract symptoms: prostate health outcomes in the registry of hypogonadism in men. BJU Int. 2017;119:216–24.

Delemarre-van de Waal HA. Application of gonadotropin releasing hormone in hypogonadotropic hypogonadism-diagnostic and therapeutic aspects. Eur J Endocrinol. 2004;151(Suppl 3): U89–94.

Depenbusch M, von Eckardstein S, Simoni M, Nieschlag E. Maintenance of spermatogenesis in hypogonadotropic hypogonadal men with hCG alone. Eur J Endorinol. 2002;147:617–24.

de Ronde W. Hyperandrogenism after transfer of topical testosterone gel: case report and review of published and unpublished studies. Hum Reprod. 2009;24:425–8.

de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A. 2003;100:10972–6.

de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, Milgrom E. A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. N Engl J Med. 1997;337:1597–602.

De Sousa SM, Chapman IM, Falhammar H, Torpy DJ. Dopa-testotoxicosis: disruptive hypersexuality in hypogonadal men with prolactinomas treated with dopamine agonists. Endocrine. 2017;55:618–24.

Dhindsa S, Miller MG, McWhirter CL, Mager DE, Ghanim H, Chaudhuri A, Dandona P. Testosterone concentrations in diabetic and nondiabetic obese men. Diabetes Care. 2010;33:1186–92.

Dinsmore WW, Wyllie MG. The long-term efficacy and safety of a testosterone mucoadhesive buccal tablet in testosterone-deficient men. Br J Urol Int. 2012;110:162–9.

Dodé C, Levilliers J, Dupont JM, De Paepe A, Le Dû N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, Pêcheux C, Le Tessier D, Cruaud C, Delpech M, Speleman F, Vermeulen S, Amalfitano A, Bachelot Y, Bouchard P, Cabrol S, Carel JC, Delemarre-van de Waal H, Goulet-Salmon B, Kottler ML, Richard O, Sanchez-Franco F, Saura R, Young J, Petit C, Hardelin JP. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat Genet. 2003;33:463–5.

Dodé C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, Morgan G, Murat A, Toublanc JE, Wolczynski S, Delpech M, Petit C, Young J, Hardelin JP. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. PLoS Genet. 2006;2:e175.

Dohle GR, Arvers S, Bettocchi C (2012) Guidelines on male hypogonadism. European Association of Urology. www.uroweb.org.

Doty RL, Bromley SM, Stern MB. Olfactory testing as an aid in the diagnosis of Parkinson's disease: development of optimal discrimination criteria. Neurodegeneration. 1995;4:93–7.

Dunkel L, Quinton R. Transition in endocrinology: induction of puberty. Eur J Endocrinol. 2014;170:229–39.

Dwyer AA, Phan-Hug F, Hauschild M, Elowe-Gruau E, Pitteloud N. TRANSITION IN ENDOCRINOLOGY: Hypogonadism in adolescence. Eur J Endocrinol. 2015;173:R15–24.

Dwyer AA, Raivio T, Pitteloud N. MANAGEMENT OF ENDOCRINE DISEASE: Reversible hypogonadotropic hypogonadism. Eur J Endocrinol. 2016;174:R267–74.

Eiholzer U, l'Allemand D, Rousson V, Schlumpf M, Gasser T, Girard J, Grüters A, Simoni M. Hypothalamic and gonadal components of hypogonadism in boys with Prader-Labhart-Willi syndrome. J Clin Endocrinol Metab. 2006;91:892–8.

European Metrodin HP Study Group EMHSG. Efficacy and safety of highly purified urinary follicle-stimulating hormone with human chorionic gonadotropin for treating men with isolated hypogonadotropic hypogonadism. European Metrodin HP Study Group. Fertil Steril. 1998;70:256–62.

Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, Sidis Y, Jacobson-Dickman EE, Eliseenkova AV, Ma J, Dwyer A, Quinton R, Na S, Hall JE, Huot C, Alois N, Pearce SH, Cole LW, Hughes V, Mohammadi M, Tsai P, Pitteloud N. Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. J Clin Invest. 2008;118:2822–31.

Fennell C, Sartorius G, Ly LP, Turner L, Liu PY, Conway AJ, Handelsman DJ. Randomized cross-over clinical trial of injectable vs. implantable depot testosterone for maintenance of testosterone replacement therapy in androgen deficient men. Clin Endocrinol. 2010;73:102–9.

Finkel DM, Phillips BA, Snyder PJ. Stimulation of spermatogenesis by gonadotropins in men with hypogonadotropic hypogonadism. N Engl J Med. 1985;313:651–5.

Fischer-Posovszky P, von Schnurbein J, Moepps B, Lahr G, Strauss G, Barth TF, Kassubek J, Mühleder H, Möller P, Debatin KM, Gierschik P, Wabitsch M. A new missense mutation in the leptin gene causes mild obesity and hypogonadism without affecting T cell responsiveness. J Clin Endocrinol Metab. 2010;95:2836–40.

Fleseriu M, Hashim IA, Karavitaki N, Melmed S, Murad MH, Salvatori R, Samuels MH. Hormonal replacement in hypopituitarism in adults: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2016;101:3888–921.

Frapsauce C, Ravel C, Legendre M, Sibony M, Mandelbaum J, Donadille B, Achermann JC, Siffroi JP, Christin-Maitre S. Birth after TESE-ICSI in a man with hypogonadotropic hypogonadism and congenital adrenal hypoplasia linked to a DAX-1 (NR0B1) mutation. Hum Reprod. 2011;26:724–8.

Gelhorn HL, Vernon MK, Stewart KD, Miller MG, Brod M, Althof SE, DeRogatis LR, Dobs A, Seftel AD, Revicki DA. Content validity of the hypogonadism impact of symptoms questionnaire (HIS-Q): a patient-reported outcome measure to evaluate symptoms of hypogonadism. Patient. 2016;9:181–90.

Gooren L. Hypogonadotropic hypogonadal men respond less well to androgen substitution treatment than hypergonadotropic hypogonadal men. Arch Sex Behav. 1988;17:265–70.

Greulich WW, Pyle SI, editors. Radiographic atlas of skeletal development of hand and wrist. 2. ed. California: Stanford University Press; 1959.

Grinspon RP, Rey RA. Anti-mullerian hormone and sertoli cell function in paediatric male hypogonadism. Horm Res Paediatr. 2010;73:81–92.

Habiby RL, Boepple P, Nachtigall L, Sluss PM, Crowley WF Jr, Jameson JL. Adrenal hypoplasia congenita with hypogonadotropic hypogonadism: evidence that DAX-1 mutations lead to combined hypothalmic and pituitary defects in gonadotropin production. J Clin Invest. 1996;98:1055–62.

Haring R, Baumeister SE, Nauck M, Völzke H, Keevil BG, Brabant G, Wallaschofski H. Testosterone and cardiometabolic risk in the general population – the impact of measurement method on risk associations: a comparative study between immunoassay and mass spectrometry. Eur J Endocrinol. 2013;169:463–70.

Harrington J, Palmert MR. Clinical review: Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. J Clin Endocrinol Metab. 2012;97:3056–67.

Hayes FJ, Seminara SB, Decruz S, Boepple PA, Crowley WF Jr. Aromatase inhibition in the human male reveals a hypothalamic site of estrogen feedback. J Clin Endocrinol Metab. 2000;85:3027–35.

Hier DB, Crowley WF Jr. Spatial ability in androgen-deficient men. N Engl J Med. 1982;306:1202–5.

Huhtaniemi IT, Tajar A, Lee DM, O'Neill TW, Finn JD, Bartfai G, Boonen S, Casanueva FF, Giwercman A, Han TS, Kula K, Labrie F, Lean ME, Pendleton N, Punab M, Silman AJ, Vanderschueren D, Forti G, Wu FC, EMAS Group. Comparison of serum testosterone and estradiol measurements in 3174 European men using platform immunoassay and mass spectrometry; relevance for the diagnostics in aging men. Eur J Endocrinol. 2012;166:983–91.

Hummel T, Kobal G, Gudziol H, Mackay-Sim A. Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur Arch Otorhinolaryngol. 2007;264:237–43.

Jadhav U, Harris RM, Jameson JL. Hypogonadotropic hypogonadism in subjects with DAX1 mutations. Mol Cell Endocrinol. 2011;346:65–73.

Janssen N, Bergman JE, Swertz MA, Tranebjaerg L, Lodahl M, Schoots J, Hofstra RM, van Ravenswaaij-Arts CM, Hoefsloot LH. Mutation update on the CHD7 gene involved in CHARGE syndrome. Hum Mutat. 2012;33:1149–60.

Johnsen SG. Maintenance of spermatogenesis induced by hMG treatment by means of continuous hCG treatment in hypogonadotropic men. Acta Endocrinol. 1978;89:763–9.

Karaca Z, Tanrıverdi F, Ünlühızarcı K, Kelestimur F. GH and pituitary hormone alterations after traumatic brain injury. Prog Mol Biol Transl Sci. 2016;138:167–91.

Kelleher S, Turner L, Howe C, Conway AJ, Handelsman DJ. Extrusion of testosterone pellets: a randomized controlled clinical study. Clin Endocrinol. 1999;51:469–71.

Kelly DF, Chaloner C, Evans D, Mathews A, Cohan P, Wang C, Swerdloff R, Sim MS, Lee J, Wright MJ, Kernan C, Barkhoudarian G, Yuen KC, Guskiewicz K. Prevalence of pituitary hormone dysfunction, metabolic syndrome, and impaired quality of life in retired professional football players: a prospective study. J Neurotrauma. 2014;31:1161–71.

Khan SA, Muhammad N, Khan MA, Kamal A, Rehman ZU, Khan S. Genetics of human Bardet-Biedl syndrome, an update. Clin Genet. 2016;90:3–15.

Kliesch S, Behre HM, Nieschlag E. Recombinant human follicle-stimulating hormone and human chorionic gonadotropin for induction of spermatogenesis in a hypogonadotropic male. Fertil Steril. 1995;63:1326–8.

Kühnert B, Byrne M, Simoni M, Köpcke W, Gerss J, Lemmnitz G, Nieschlag E. Testosterone substitution with a new transdermal, hydroalcoholic gel applied to scrotal or non-scrotal skin: a multicentre trial. Eur J Endocrinol. 2005;153:317–26.

Laitinen EM, Tommiska J, Sane T, Vaaralahti K, Toppari J, Raivio T. Reversible congenital hypogonadotropic hypogonadism in patients with *CHD7, FGFR1* or *GNRHR* mutations. PLoS One. 2012;7:e39450.

Layman LC, Cohen DP, Jin M, Xie J, Li Z, Reindollar RH, Bolbolan S, Bick DP, Sherins RR, Duck LW, Musgrove LC, Sellers JC, Neill JD. Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism. Nat Genet. 1998;18:14–5.

Lehman MN, Coolen LM, Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology. 2010;151:3479–89.

Licinio J, Caglayan S, Ozata M, Yildiz BO, de Miranda PB, O'Kirwan F, Whitby R, Liang L, Cohen P, Bhasin S, Krauss RM, Veldhuis JD, Wagner AJ, DePaoli AM, McCann SM, Wong ML. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc Natl Acad Sci U S A. 2004;101:4531–6.

Liu L, Banks SM, Barnes KM, Sherins RJ. Two-year comparison of testicular responses to pulsatile gonadotropin-releasing hormone and exogenous gonadotropins from the inception of therapy in men with isolated hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 1988;67:1140–5.

Liu PY, Gebski VJ, Turner L, Conway AJ, Wishart SM, Handelsman DJ. Predicting pregnancy and spermatogenesis by survival analysis during gonadotrophin treatment of gonadotrophin-deficient infertile men. Hum Reprod. 2002;17:625–33.

Liu PY, Swerdloff RS, Christenson PD, Handelsman DJ, Wang C. Hormonal Male Contraception Summit Group. Rate, extent, and modifiers of spermatogenic recovery after hormonal male contraception: an integrated analysis. Lancet. 2006;367:1412–20.

Liu PY, Baker HW, Jayadev V, Zacharin M, Conway AJ, Handelsman DJ. Induction of spermatogenesis and fertility during gonadotropin treatment of gonadotropin-deficient infertile men: predictors of fertility outcome. J Clin Endocrinol Metab. 2009;94:801–8.

Lofrano-Porto A, Barra GB, Giacomini LA, Nascimento PP, Latronico AC, Casulari LA, da Rocha Neves Fde A. Luteinizing hormone beta mutation and hypogonadism in men and women. N Engl J Med. 2007;357:897–904.

Mackey MA, Conway AJ, Handelsman DJ. Tolerability of intramuscular injections of testosterone ester in oil vehicle. Hum Reprod. 1995;108:862–5.

Mantovani G, Ozisik G, Achermann JC, Romoli R, Borretta G, Persani L, Spada A, Jameson JL, Beck-Peccoz P. Hypogonadotropic hypogonadism as a presenting feature of late-onset X-linked adrenal hypoplasia congenita. J Clin Endocrinol Metab. 2002;87:44–8.

Mao JF, Liu ZX, Nie M, Wang X, HL X, Huang BK, Zheng JJ, Min L, Kaiser UB, Pulsatile WXY. Gonadotropin-releasing hormone therapy is associated with earlier spermatogenesis compared to combined gonadotropin therapy in patients with congenital hypogonadotropic hypogonadism. Asian J Androl. 2016. https://doi.org/10.4103/1008-682X.193568.

Matthews CH, Borgato S, Beck-Peccoz P, Adams M, Tone Y, Gambino G, Casagrande S, Tedeschini G, Benedetti A, Chatterjee VK. Primary amenorrhoea and infertility due to a mutation in the beta-subunit of follicle-stimulating hormone. Nat Genet. 1993;5:83–6.

Matsumoto AM, Karpas AE, Bremner WJ. Chronic human chorionic gonadotropin administration in normal men: evidence that follicle-stimulating hormone is necessary for the maintenance of quantitatively normal spermatogenesis in man. J Clin Endocrinol Metab. 1986;62:1184–92.

Matsumoto AM, Snyder PJ, Bhasin S, Martin K, Weber T, Winters S, Spratt D, Brentzel J, O'Dea L. Stimulation of spermatogenesis with recombinant human follicle-stimulating hormone (follitropin alfa; GONAL-f): long-term treatment in azoospermic men with hypogonadotropic hypogonadism. Fertil Steril 2009;92:979–990.

McNicholas T, Ong T. Review of Testim gel. Expert Opin Pharmacother. 2006;7:477–84.

Nelson D, Ho J, Pacaud D, Stephure D. Virilization in two pre-pubertal children exposed to topical androgen. J Pediatr Endocrinol Metab. 2013;24:1–5.

Netchine I, Sobrier ML, Krude H, Schnabel D, Maghnie M, Marcos E, Duriez B, Cacheux V, Moers A, Goossens M, Gruters A, Amselem S. Mutations in LHX3 result in a new syndrome revealed by combined pituitary hormone deficiency. Nat Genet. 2000;25:182–6.

Nieschlag E. Clinical trials in male hormonal contraception. Contraception. 2010;82:457–70.

Nieschlag E, Nieschlag S. Testosterone deficiency: a historical perspective. Asian J Androl. 2014;16:161–8.

Nieschlag E, Vorona E. Medical consequences of doping with anabolic androgenic steroids: effects on reproductive functions. Eur J Endocrinol. 2015;173:47–58.

Nieschlag E, Behre HM, Bouchard P, Corrales JJ, Jones TH, Stalla GK, Webb SM, Wu FC. Testosterone replacement therapy: current trends and future directions. Hum Reprod Update. 2004;10:409–19.

Nieschlag E, Bouloux P-MG, Stegmann BJ, Shankar RR, Guan Y, Tzontcheva A, McCrary Sisk C, Behre H. Efficacy and safety of the long-acting Corifollitropin Alfa in combination with hCG in adult men with hypogonadotropic hypodonadism. Reprod Endocr Biol. 2017;15:17.

Nieschlag E, Büchter D, Von Eckardstein S, Abshagen K, Simoni M, Behre HM. Repeated intramuscular injections of testosterone undecanoate for substitution therapy in hypogonadal men. Clin Endocrinol. 1999a;51:757–63.

Nieschlag E, Simoni M, Gromoll J, Weinbauer GF. Role of FSH in the regulation of spermatogenesis: clinical aspects. Clin Endocrinol. 1999b;51:139–46.

Nieschlag E, Wang C, Handelsman DJ, Swerdloff RS, Wu FCW, Einer-Jensen N, Waites GMH, editors. Guidelines for the use of androgens. Geneva: WHO Special Programme of Research/ Development and Research Training in Human Reproduction; 1992.

Phillip M, Arbelle JE, Segev Y, Parvari R. Male hypogonadism due to a mutation in the gene for the beta-subunit of follicle-stimulating hormone. N Engl J Med. 1998;338:1729–32.

Pitteloud N, Dwyer AA, DeCruz S, Lee H, Boepple PA, Crowley WF Jr, Hayes FJ. Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab. 2008;93:784–91.

Pitteloud N, Hayes FJ, Dwyer A, Boepple PA, Lee H, Crowley WF Jr. Predictors of outcome of long-term GnRH therapy in men with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2002;87:4128–36.

Pitteloud N, Quinton R, Pearce S, Raivio T, Acierno J, Dwyer A, Plummer L, Hughes V, Seminara S, Cheng YZ, Li WP, Maccoll G, Eliseenkova AV, Olsen SK, Ibrahimi OA, Hayes FJ, Boepple P, Hall JE, Bouloux P, Mohammadi M, Crowley W. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. J Clin Invest. 2007;117:457–63.

Quaynor SD, Kim HG, Cappello EM, Williams T, Chorich LP, Bick DP, Sherins RJ, Layman LC. The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. Fertil Steril. 2011;96:1424–30.

Quinton R, Cheow HK, Tymms DJ, Bouloux PM, Wu FC, Jacobs HS. Kallmann's syndrome: is it always for life? Clin Endocrinol. 1999;50:481–5.

Raivio T, Falardeau J, Dwyer A, Quinton R, Hayes FJ, et al. Reversal of idiopathic hypogonadotropic hypogonadism. N Engl J Med. 2007;357:863–73.

Rajagopal A, Vassilopoulou-Sellin R, Palmer JL, Kaur G, Bruera E. Symptomatic hypogonadism in male survivors of cancer with chronic exposure to opioids. Cancer. 2004;100:851–8.

Rochira V, Zirilli L, Genazzani AD, Balestrieri A, Aranda C, Fabre B, Antunez P, Diazzi C, Carani C, Maffei L. Hypothalamic-pituitary-gonadal axis in two men with aromatase deficiency: evidence that circulating estrogens are required at the hypothalamic level for the integrity of gonadotropin negative feedback. Eur J Endocrinol. 2006;155:513–22.

Rogol AD. Pubertal androgen therapy in boys. Pediatr Endocrinol Rev. 2005;3:383–90.

Rohayem J, Hauffa BP, Zacharin M, Kliesch S, Zitzmann M. The "German Adolescent Hypogonadotropic Hypogonadism Study Group". Testicular growth and spermatogenesis: new goals for pubertal hormone replacement in boys with hypogonadotropic hypogonadism? -a multicentre prospective study of hCG/rFSH treatment outcomes during adolescence. Clin Endocrinol (Oxford). 2017;86:75–87.

Rohayem J, Nieschlag E, Kliesch S, Zitzmann M. Inhibin B, AMH, but not INSL3, IGF1 or DHEAS support differentiation between constitutional delay of growth and puberty and hypogonadotropic hypogonadism. Andrology. 2015;3:882–7.

Rohayem J, Sinthofen N, Nieschlag E, Kliesch S, Zitzmann M. Causes of hypogonadotropic hypogonadism predict response to gonadotropin substitution in adults. Andrology. 2016;4:87–94.

Rohayem J, Tüttelmann F, Mallidis C, Nieschlag E, Kliesch S, Zitzmann M. Restoration of fertility by gonadotropin replacement in a man with hypogonadotropic azoospermia and testicular adrenal rest tumors due to untreated simple virilizing congenital adrenal hyperplasia. Eur J Endocrinol. 2014;170:K11–7.

Sartorius G, Spasevska S, Idan A, Turner L, Forbes E, Zamojska A, Allan CA, Ly LP, Conway AJ, McLachlan RI, Handelsman DJ. Serum testosterone, dihydrotestosterone and estradiol

concentrations in older men self-reporting very good health: the healthy man study. Clin Endocrinol. 2012;77:755–63.
Schopohl J. Pulsatile gonadotrophin releasing hormone versus gonadotrophin treatment of hypothalamic hypogonadism in males. Hum Reprod. 1993;8:175–9.
Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an academic center. J Clin Endocrinol Metab. 2002;87:1613–20.
Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH. The GPR54 gene as a regulator of puberty. N Engl J Med. 2003;349:1614–27.
Shiraishi K, Oka S, Matsuyama H. Assessment of quality of life during gonadotrophin treatment for male hypogonadotrophic hypogonadism. Clin Endocrinol. 2014;81:259–65.
Sidhoum VF, Chan YM, Lippincott MF, Balasubramanian R, Quinton R, Plummer L, Dwyer A, Pitteloud N, Hayes FJ, Hall JE, Martin KA, Boepple PA, Seminara SB. Reversal and relapse of hypogonadotropic hypogonadism: resilience and fragility of the reproductive neuroendocrine system. J Clin Endocrinol Metab. 2014;99:861–70.
Siegel ET, Kim HG, Nishimoto HK, Layman LC. The molecular basis of impaired follicle-stimulating hormone action: evidence from human mutations and mouse models. Reprod Sci. 2013;20:211–33.
Şimşek E, Montenegro LR, Binay C, Demiral M, Acıkalin MF, Latronico AC. Clinical and hormonal features of a male adolescent with congenital isolated follicle-stimulating hormone deficiency. Horm Res Paediatr. 2016;85:207–12.
Sinisi AA, Esposito D, Maione L, Quinto MC, Visconti D, De Bellis A, Bellastella A, Conzo G, Bellastella G. Seminal anti-Mullerian hormone level is a marker of spermatogenic response during long-term gonadotropin therapy in male hypogonadotropic hypogonadism. Hum Reprod. 2008;23:1029–34.
Sisk CL, Zehr JL. Pubertal hormones organize the adolescent brain and behavior. Front Neuroendocrinol. 2005;26:163–74.
Soliman AT, De Sanctis V. An approach to constitutional delay of growth and puberty. Indian J Endocrinol Metab. 2012;16:698–705.
Spratt DI, Carr DB, Merriam GR, Scully RE, Rao PN, Crowley WF. The spectrum of abnormal patterns of gonadotropin-releasing hormone secretion in men with idiopathic hypogonadotropic hypogonadism: clinical and laboratory correlations. J Clin Endocrinol Metab. 1987;64:283–91.
Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet. 1998;18:213–5.
Swerdloff RS, Wang CL. Review of guidelines on diagnosis and treatment of testosterone deficiency. In: Nieschlag E, Behre HM, Nieschlag S, editors. Testosterone: action, deficiency, substitution. 4th ed. Cambridge: Cambridge University Press; 2012. p. 408–20.
Swerdloff RS, Pak Y, Wang C, Liu PY, Bhasin S, Gill TM, Matsumoto AM, Pahor M, Surampudi P, Snyder PJ. Serum testosterone (T) level variability in T-gel-treated older hypogonadal men: treatment monitoring implications. J Clin Endocrinol Metab. 2015;100:3280–7.
Takeda T, Takasu N, Yamauchi K, Komiya I, Ohtsuka H, Nagasawa Y, Ohara N, Yamada T. Magnetic resonance imaging of the rhinencephalon in a patient with Kallmann's syndrome. Intern Med. 1992;31:394–6.
Tarnutzer AA, Gerth-Kahlert C, Timmann D, Chang DI, Harmuth F, Bauer P, Straumann D, Synofzik M. Boucher-Neuhäuser syndrome: cerebellar degeneration, chorioretinal dystrophy and hypogonadotropic hypogonadism: two novel cases and a review of 40 cases from the literature. J Neurol. 2015;262:194–202.
Tenforde AS, Barrack MT, Nattiv A, Fredericson M. Parallels with the female athlete triad in male athletes. Sports Med. 2016;46:171–82.
Tommiska J, Jørgensen N, Christiansen P, Juul A, Raivio T. A homozygous R262Q mutation in the gonadotropin-releasing hormone receptor presenting as reversal of hypogonadotropic hypogonadism and late-onset hypogonadism. Clin Endocrinol. 2013;78:316–7.

Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for neurokinin B in the central control of reproduction. Nat Genet. 2009;41:354–8.

Valdes-Socin H, Rubio Almanza M, Tomé Fernández-Ladreda M, Debray FG, Bours V, Beckers A. Reproduction, smell, and neurodevelopmental disorders: genetic defects in different hypogonadotropic hypogonadal syndromes. Front Endocrinol (Lausanne). 2014;5:109.

Wabitsch M, Ballauff A, Holl R, Blum WF, Heinze E, Remschmidt H, Hebebrand J. Serum leptin, gonadotropin, and testosterone concentrations in male patients with anorexia nervosa during weight gain. J Clin Endocrinol Metab. 2001;86:2982–8.

Waldstreicher J, Seminara SB, Jameson JL, Geyer A, Nachtigall LB, Boepple PA, Holmes LB, Crowley WF Jr. The genetic and clinical heterogeneity of gonadotropin-releasing hormone deficiency in the human. J Clin Endocrinol Metab. 1996;81:4388–95.

Wang C, Harnett M, Dobs AS, Swerdloff RS. Pharmacokinetics and safety of long-acting testosterone undecanoate injections in hypogonadal men: an 84-week phase III clinical trial. J Androl. 2010;31:457–65.

Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, Snyder PJ, Weber T, Berman N, Hull L, Swerdloff RS. Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. J Clin Endocrinol Metab. 2004;89:2085–98.

Warne DW, Decosterd G, Okada H, Yano Y, Koide N, Howles CM. A combined analysis of data to identify predictive factors for spermatogenesis in men with hypogonadotropic hypogonadism treated with recombinant human follicle-stimulating hormone and human chorionic gonadotropin. Fertil Steril. 2009;92:594–604.

Wehkalampi K, Widén E, Laine T, Palotie A, Dunkel L. Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care. J Clin Endocrinol Metab. 2008;93:723–8.

World Health Organization (WHO). WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: World Health Organization; 2010.

Wilson DA, Hofman PL, Miles HL, Unwin KE, McGrail CE, Cutfield WS. Evaluation of the buserelin stimulation test in diagnosing gonadotropin deficiency in males with delayed puberty. J Pediatr. 2006;148:89–94.

Wu W, Cogan JD, Pfaffle RW, Dasen JS, Frisch H, O'Connell SM, Flynn SE, Brown MR, Mullis PE, Parks JS, Phillips JA 3rd, Rosenfeld MG. Mutations in PROP1 cause familial combined pituitary hormone deficiency. Nat Genet. 1998;18:147–9.

Young J, George JT, Tello JA, Francou B, Bouligand J, Guiochon-Mantel A, Brailly-Tabard S, Anderson RA, Millar RP. Kisspeptin restores pulsatile LH secretion in patients with neurokinin B signaling deficiencies: physiological, pathophysiological and therapeutic implications. Neuroendocrinology. 2013;97:193–202.

Zacharin M, Sabin MA, Nair VV, Dagabdhao P. Addition of recombinant follicle-stimulating hormone to human chorionic gonadotropin treatment in adolescents and young adults with hypogonadotropic hypogonadism promotes normal testicular growth and may promote early spermatogenesis. Fertil Steril. 2012;98:836–42.

Zhu J, Choa REY, Guo MH, Plummer L, Buck C, Palmert MR, Hirschhorn JN, Seminara SB, Chan YM. A shared genetic basis for self-limited delayed puberty and idiopathic hypogonadotropic hypogonadism. The Journal of Clinical Endocrinology & Metabolism 2015;100:E646–E654.

Zitzmann M, Faber S, Nieschlag E. Association of specific symptoms and metabolic risks with serum testosterone in older men. J Clin Endocrinol Metab. 2006;91:4335–43.

Zitzmann M, Nieschlag E. Androgen receptor gene CAG repeat length and body mass index modulate the safety of long-term intramuscular testosterone undecanoate therapy in hypogonadal men. J Clin Endocrinol Metab. 2007;92:3844–53.

Physiopathology, Diagnosis, and Treatment of Hypercortisolism

11

Rosario Pivonello, Davide Iacuaniello, Chiara Simeoli, Maria Cristina De Martino, and Annamaria Colao

Contents

Abstract

Cushing's syndrome (CS) is a serious clinical condition caused by endogenous or exogenous cortisol excess. Endogenous CS is a rare endocrine disorder caused by chronic excessive cortisol secretion. In approximately 80% of cases, endogenous CS is a consequence of an adrenocorticotropin (ACTH) hypersecretion

R. Pivonello (✉) · D. Iacuaniello · C. Simeoli · M. C. De Martino · A. Colao
Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università Federico II di Napoli, Naples, Italy
e-mail: rosario.pivonello@unina.it; davide.iacuaniello@gmail.com; simeolichiara@gmail.com; demartino.mc@gmail.com; colao@unina.it

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_12

(ACTH-dependent CS), generally due to a ACTH-secreting pituitary tumor (Cushing's disease, CD, 70%), and, rarely, to an ACTH-secreting or corticotrophin releasing hormone (CRH)-secreting extra-pituitary tumor (Ectopic CS, ECS, 10%). In the remaining 20% of cases, CS is a direct consequence of autonomous cortisol overproduction by the adrenal glands (ACTH-independent CS), because of unilateral or bilateral adrenal diseases. This chapter reviews the physiopathology, the diagnostic algorithm required to establish the diagnosis and the main currently available treatments of CS.

Keywords
Cushing's syndrome · Cushing's disease · Ectopic Cushing's syndrome · Adrenal Cushing's syndrome · Cortisol · Adrenocorticotropin · Glucocorticoids

Introduction

Cushing's syndrome (CS) is a serious clinical condition caused by endogenous or exogenous cortisol excess, known as hypercortisolism. Endogenous CS is a rare endocrine disorder caused by chronic excessive cortisol secretion from the adrenal glands, with an estimated prevalence of 40 cases per million people and incidence of 0.7–2.4 cases per million people per year (Lacroix et al. 2015; Pivonello et al. 2008, 2016a). Endogenous CS has a higher prevalence in women and is more frequent during the fourth to sixth decades of life, although occurring at any age (Lacroix et al. 2015; Pivonello et al. 2008, 2016a). In approximately 80% of cases, endogenous CS is a consequence of an adrenocorticotropin (ACTH) hypersecretion (ACTH-dependent CS), generally due to a ACTH-secreting pituitary tumor (pituitary-dependent CS, or Cushing's disease, CD, 70%), and, rarely, to an ACTH-secreting or corticotrophin releasing hormone (CRH)-secreting extra-pituitary tumor (Ectopic CS, ECS, 10%) (Lacroix et al. 2015; Pivonello et al. 2008). In the remaining 20% of cases, CS is independent from ACTH hypersecretion (ACTH-independent CS), and a direct consequence of autonomous cortisol overproduction by the adrenal glands, because of unilateral benign or malignant adrenocortical tumors, bilateral macronodular adrenal hyperplasia (BMAH), or bilateral micronodular adrenal hyperplasia (BmAH) (Lacroix et al. 2015; Pivonello et al. 2008).

Clinical picture of CS has a variable expression resulting from a constellation of different signs, including weight gain, moon face, facial plethora, buffalo hump, supraclavicular and dorsal fat pads, purple striae, diffuse bruising, skin thinning, proximal myopathy, hirsutism, acne and alopecia, as well as symptoms, mainly including asthenia, fatigue, and mood disorders (Lacroix et al. 2015; Pivonello et al. 2008, 2015a, 2016a). This clinical picture is complicated by several comorbidities including metabolic syndrome, characterized by visceral obesity, impairment of glucose metabolism and dyslipidemia, strictly associated with systemic arterial hypertension and cardiovascular diseases, including vascular atherosclerosis and cardiac damage, which, together with thromboembolism, contribute to the increase in cardiovascular risk. Additional clinical complications include

musculoskeletal diseases, such as osteoporosis, skeletal fractures, and myopathy; neuropsychiatric diseases, such as impairment of cognitive function, depression, anxiety, and bipolar disorders; immune disorders, characterized by increased susceptibility to infections and possibly complicated by sepsis; impairment of reproductive and sexual function with consequent infertility or sexual disturbances (Lacroix et al. 2015; Pivonello et al. 2005b, 2008, 2015a, 2016a, b, 2017). These clinical complications negatively impact on quality of life (QoL) and increase the mortality, mainly consequence of cardiovascular events and sepsis. Therefore, a prompt screening, a confirmatory diagnosis, and an effective multidisciplinary therapeutic approach are mandatory in the attempt to improve clinical picture, morbidity, QoL, and mortality (Lacroix et al. 2015; Pivonello et al. 2008, 2015a, 2016a, b, 2017).

This chapter focuses on endogenous CS; however, it is important to mention the existence of the exogenous CS, or iatrogenic CS, caused by the prolonged exogenous exposure to glucocorticoids (GCs). Iatrogenic CS is the most common form of CS, although the lack of specific trials and proper registries and the surreptitious use of GCs make its epidemiological estimation undetectable (Hopkins and Leinung 2005). However, the oral, topic, and parenteral GCs use for several medical disorders, mainly including neoplastic, inflammatory, and autoimmune diseases, should be promptly excluded through accurate pharmacological history, before starting the diagnostic process of CS (Hopkins and Leinung 2005).

Physiopathology

In physiological conditions, cortisol, the main GC in humans, is produced from the adrenal glands under the control of the hypothalamus-pituitary-adrenal (HPA) axis. The hypothalamus, in response to changes in the external and internal environment, including the stimulation from stress exposure, the regulation of biological clocks and nutrients balance, produces CRH and arginine vasopressin (AVP), which, binding to their specific receptors (CRH-R1 and AVPR1B receptor, respectively), stimulate the corticotroph cells of the anterior pituitary to produce ACTH through the cleavage of the proopiomelanocortin (POMC). In turn, ACTH binds to its specific receptor (MC2-R) on cells of the adrenal cortex, stimulating the production of cortisol through an increase in cyclic AMP (cAMP) intracellular levels. The main regulation mechanisms of HPA axis include: the circadian rhythm of ACTH and cortisol secretion, the negative feedback of cortisol on CRH and ACTH production, and the response to stress (Papadimitriou and Priftis 2009; Jacobson 2005). These regulation mechanisms are generally compromised in CS (Newell-Price et al. 2006; Raff et al. 2014).

The *ACTH-dependent CS* is characterized by an increased ACTH stimulation of fasciculata and reticularis layers of the adrenal gland that mainly leads to hypercortisolism, but can also be responsible for an increased production of adrenal androgens, and bilateral adrenal hypertrophy and hyperplasia with possible micro/macronodular modifications (Newell-Price et al. 2006; Raff et al. 2014). In the

majority of cases the reasons of the abnormal ACTH secretion is CD, generally caused by ACTH-secreting pituitary tumors, although corticotroph pituitary hyperplasia (not associated to an ectopic CRH-secreting tumor) or ACTH-secreting pituitary carcinomas might rarely occur (Newell-Price et al. 2006; Biller et al. 1992; Mampalam et al. 1988; Young et al. 1988; Holthouse et al. 2001; Colao et al. 2010). The majority of ACTH-secreting pituitary tumors are microadenomas (less than 10 mm of maximal diameter), whereas macroadenomas (higher than 10 mm of maximal diameter) are less frequent (about 10%) and might present extrasellar extension (Pivonello et al. 2015a; Newell-Price et al. 2006; Raff et al. 2014; Hofmann et al. 2008). These tumors secrete an increased amount of ACTH, and, particularly macroadenomas, can also secrete large amounts of unprocessed POMC. Rarely, ACTH-secreting pituitary tumors can be silent corticotroph tumors being unable to secrete large amounts of ACTH, but capable to secrete unprocessed POMC, and potentially causing tumor mass effects (Newell-Price et al. 2006). The majority of ACTH-secreting pituitary tumors express CRH and vasopressin receptors and secrete ACTH in response to CRH and vasopressin, but have a reduced sensitivity to the negative feedback of cortisol on CRH and ACTH production (Newell-Price et al. 2006; Raff et al. 2014). These features represent the rational of the diagnostic biochemical tests currently used in the clinical setting. The pathogenesis of ACTH-secreting pituitary tumors remains still unclear, although, recently, advances have been made in understanding the genetic background of these tumors (Lacroix et al. 2015; Newell-Price et al. 2006; Raff et al. 2014; Perez-Rivas and Reincke 2016). ACTH-secreting pituitary tumors are generally sporadic and arise from the clonal expansion of a single cell containing one or few mutations which confer adaptive advantages; nevertheless, rarely, ACTH-secreting pituitary tumors arise in the context of familial syndromes caused by germline gene alterations (Lacroix et al. 2015; Perez-Rivas and Reincke 2016). Among these syndromes, CD can be part of the multiple endocrine neoplasia type 1 (MEN1), an autosomal dominant disease caused by germline mutations in the *MEN1* gene (encoding menin); multiple endocrine neoplasia type 4 (MEN4), a very rare condition caused by germline mutations in *CDKN1B* gene (encoding p27/KIP1); and familial isolated pituitary adenomas (FIPA), a syndrome characterized by the familial occurrence of isolated pituitary tumors and caused, in about 20% of cases, by germline mutation in *AIP* gene (encoding aryl hydrocarbon receptor interacting protein) (Lacroix et al. 2015; Perez-Rivas and Reincke 2016). Recently, in ACTH-secreting pituitary tumors, a high prevalence (35–62%) of somatic mutation in the *USP8* gene has been described (Reincke et al. 2014). The *USP8* gene encodes for a deubiquitinase, which removes ubiquitin from the activated epidermal growth factor receptor (EGFR), rescuing it from lysosomal degradation and preventing its downregulation. Mutation in *USP8* gene results in increased EGFR signaling activation, suggesting a role of this signaling in the pathogenesis of ACTH-secreting pituitary tumors (Lacroix et al. 2015; Perez-Rivas and Reincke 2016; Reincke et al. 2014). Additionally, a reduced sensitivity to the cortisol feedback, potentially due to an increased inactivation of cortisol by 11β hydroxysteroid dehydrogenase type 2 (11β-HSD2) and to an altered expression of the brg1 (ATPase subunit of the Swi/Snf complex) or

histone deacetylase-2 (HDAC2) proteins, which are involved in glucocorticoid receptor (GR) signaling, might play a role in the pathogenesis of ACTH-secreting pituitary tumors (Lacroix et al. 2015; Perez-Rivas and Reincke 2016; Dworakowska and Grossman 2012). Only rarely some different somatic mutations, such as mutation of *GR* gene, encoding for glucocorticoid receptor, which might also cause a reduced sensitivity to the cortisol feedback, and mutations in *TP53* (encoding for the tumor suppressor protein p53), have been described (Lacroix et al. 2015; Perez-Rivas and Reincke 2016). ACTH-dependent CS can also be caused by extrapituitary ACTH- (or CRH-) secreting tumors. This form of hypercortisolism is named ECS. Several tumor types, ranging from clearly benign to highly malignant tumors, can cause ECS: these include most frequently small cell lung carcinomas and neuroendocrine tumors of the lung, thymus, and pancreas, but also medullary thyroid carcinomas, and pheochromocytoma (Lacroix et al. 2015; Newell-Price et al. 2006; Raff et al. 2014; Alexandraki and Grossmann 2010). The reasons of this aberrant expression are unclear, but an abnormal methylation of the *POMC* promoter might be involved (Newell-Price et al. 2006; Ye et al. 2005; Newell-Price et al. 2001). Generally, ACTH-secreting ectopic tumors secrete large amounts of ACTH and unprocessed POMC (Ye et al. 2005; Newell-Price et al. 2001; Raffin-Sanson et al. 2003). The pathogenesis of ACTH-secreting ectopic tumors still needs to be fully clarified and depends on the tumor type. Most of these tumors are sporadic or their genetic background has not been described, but some cases associated with familial syndrome, particularly MEN1, multiple endocrine neoplasia type 2 (MEN2; a rare condition due to germline mutation of the oncogene *RET*), Von Hippel-Lindau (a rare condition due to germline mutation of the tumor suppressor gene *VHL*), and neurofibromatosis type 1 (NF1; a rare condition due to germline mutation of the tumor suppressor gene *NF1*) have been reported (Lacroix et al. 2015; Ilias et al. 2005; Takagi et al. 2006; Hatipoglu et al. 2013; Bano et al. 2013).

The *ACTH-independent CS* includes a heterogeneous group of diseases characterized by an increased adrenal cortisol production, independent from the ACTH stimulation. Hypercortisolism exerts a normal negative feedback on hypothalamus and pituitary, which results in low circulating ACTH levels. However, particularly in adrenal tumors, androgen levels might be elevated because of a possible autonomous androgen production (Lacroix et al. 2015; Newell-Price et al. 2006; Raff et al. 2014). The most common causes of endogenous ACTH-independent CS are the cortisol secreting adrenocortical tumors (adrenocortical adenomas and less frequently adrenocortical carcinomas). Additional rare causes include BMAH and BmAH (Lacroix et al. 2015; Newell-Price et al. 2006). The mechanism leading to an abnormal cortisol secretion in adrenocortical tumors remains to be clarified. However, an abnormal expression of specific steroidogenic enzymes is suggested by the frequent production of GC precursors, observed particularly in adrenocortical carcinomas, which are in more than half cases secreting tumors (up to 70%). In these malignant tumors of the adrenal cortex, the most frequent hormone produced is cortisol (50–80% with hormone excess), followed by androgens (40–60%, in most cases associated with cortisol secretion), and only rarely estrogens or aldosterone (Lacroix et al. 2015; Else et al. 2014). Adrenocortical carcinomas are rare whereas

adrenocortical adenomas are more frequent (up to 10%), although most of them (about 80%) are nonsecreting adenomas. The pathogenesis of benign and malignant adrenocortical tumors needs to be further investigated and, to date, the progression from benign to malignant tumors remains still debated (Lacroix et al. 2015; Else et al. 2014; Lerario et al. 2014). Generally, adrenocortical tumors are sporadic and a clear association between the genotype and the presence of hormonal secretion has not been described; however, rarely, adrenocortical tumors can also arise in the context of familial syndromes caused by germline gene alterations (Lerario et al. 2014). Among these syndromes, they can be part of MEN1 syndrome (bilateral adrenal hyperplasia and both benign and malignant adrenocortical tumors); Li–Fraumeni syndrome, which is caused by germline mutations in *TP53* gene (particularly in childhood and, less frequently, in adult adrenocortical carcinomas); Lynch syndrome, which is caused by germline mutations in DNA mismatch repair genes *MSH2*, *MSH6*, *MLH1*, and *PMS2*; Beckwith-Wiedemann syndrome, caused by alterations of the DNA methylation at the 11p15 locus, which leads to an overexpression of *IGF2* gene [encoding for the insulin like growth factor (IGF) type 2], and reduced expression of the tumor suppressor gene *CDKN1C* (encoding for the protein cyclin dependent kinase inhibitor 1C), and the untranslated RNA *H19* (both benign and malignant adrenocortical tumors); familial adenomatous polyposis, which is caused by germline mutations in *APC* gene encoding for a downstream regulator of the Wnt/beta-catenin pathway; and NF1 (Else et al. 2014). In adrenocortical adenomas, genes found altered at somatic levels are generally members of cAMP (activation of *GNAS* or *PRKAR1A* genes or inactivation of *PRKAR1A* gene) or Wnt/beta-catenin (oncogenes *CTNNB1* and *AXIN2*) pathways, suggesting a role of these pathways in the pathogenesis of this benign tumors (Lacroix et al. 2015; Else et al. 2014; Lerario et al. 2014). Adrenocortical carcinomas are very heterogeneous tumors in which numerous somatic genetic abnormalities have been described. The most common (up to 90% of cases) are somatic structural alterations of the 11p15 locus, which cause abnormal expression of *IGF2* and *CDKN1C* genes, and the untranslated RNA *H19*, as described in Beckwith-Wiedemann syndrome. The upregulation of *IGF2* has suggested a role of the IGF and mTOR (a mediator of IGFs) pathways in pathogenesis of adrenocortical carcinomas (Else et al. 2014; Lerario et al. 2014; De Martino et al. 2012). Additionally, adrenocortical carcinomas can harbor sporadic mutations or different somatic gene alterations in genes encoding for members of the Wnt/beta-catenin (oncogene *CTNNB1*, *APC*, *ZNFR3*), cell cycle (*TP53*, *CDK4*, *CDKN2A*, *CDKN2B*, *RB1*, *MDM2*), and chromatin remodeling/DNA repair (*MEN1*, *DAXX*, *ATM*, *ATRX*) pathways, supporting a role of these pathways in these malignancies (Else et al. 2014; Lerario et al. 2014; De Martino et al. 2013; Assié et al. 2014). Aside from genetic predisposition, no risk factors have been firmly established, although, considering the higher frequency of adrenocortical carcinomas in women, a role of estrogens might be suggested (Else et al. 2014). In BMAH hypercortisolism is frequently induced by the increased expression of receptors normally expressed [such as luteinizing hormone/choriogonadotropin receptor (LHCGR) for luteinizing hormone and choriogonadotropin;

arginine vasopressin receptor (AVPR1) for vasopressin; serotonin receptor type 7 (5HT7R) for serotonin] or ectopic receptors normally not expressed [such as arginine vasopressin receptor 2 (AVPR2) and 3 (AVPR3) for vasopressin], in normal adrenals. The mechanisms leading to this abnormal expression are still unknown (Lacroix et al. 2015; Newell-Price et al. 2006). The so-called food-dependent Cushing's syndrome also belongs to this group of diseases and is caused by the abnormal expression of receptors for the gastric inhibitory peptide (Newell-Price et al. 2006). In the majority of cases, BMAH is sporadic; however, it might arise in the context of familial syndromes such as MEN1 syndrome, familial adenomatous polyposis, and McCune-Albright syndrome (Lacroix et al. 2015). MEN1 syndrome and familial adenomatous polyposis have been already reported above. In McCune-Albright syndrome, activating mutations in the gene *GNAS*, encoding for Gs-alpha subunit and resulting in constitutive cAMP activation, have been reported (Lacroix et al. 2015). Recently, frequent mutations in ARMC5 gene have been described in large families with BMAH (Assié et al. 2013); however, the role of these abnormalities in the pathogenesis of these diseases needs to be better described. Additional mutations have also been very rarely described, but they require further investigation (Lacroix et al. 2015). BmAH includes the primary pigmented nodular adrenocortical disease (PPNAD) and its nonpigmented variant, the isolated micronodular adrenocortical disease (IMAD); these disorders can be isolated or part of Carney complex (an autosomal dominant multiple neoplasia syndrome) (Lacroix et al. 2015; Newell-Price et al. 2006). The most frequent germline mutations, both in Carney complex and isolated PPNAD, are in *PRKAR1A* gene, which encodes for the regulatory subunit 1A of the PKA. Inactivating mutations lead to the constitutional activation of the catalytic subunits, and, consequently, to increased cAMP levels (Newell-Price et al. 2006; Lerario et al. 2014). Less frequently, patients with PPNAD and IMAD can harbor inactivating mutations of *PDE11A* and *PDE8B* genes, encoding phosphodiesterases. These mutations compromise the cAMP degradation, leading to a prolonged activation of this pathway (Lerario et al. 2014). McCune-Albright syndrome, above mentioned, might also be associated with BmAH (Newell-Price et al. 2006).

Cortisol acts on peripheral tissues mainly binding to its specific receptor GR. The binding of cortisol to the mineralocorticoid receptor (MR) is protected by the action of 11β-HSD2 enzyme; this enzyme converts cortisol to cortisone, which is unable to activate the MR. This inactivation is insufficient in presence of increased cortisol levels, therefore, in CS, some of the cortisol effects can be mediated also by the activation of MR (Newell-Price et al. 2006). Cortisol regulates several physiological functions including glucose, lipid, and protein metabolism, blood pressure, calcium and electrolyte balance, coagulation, immune, endocrine, and reproductive functions, mood and cognition. These pleiotropic actions of GCs explain the metabolic, cardiovascular, musculoskeletal, and neuropsychiatric diseases and the immune, endocrine, reproductive, and sexual disorders observed in CS and are responsible for the increased morbidity and mortality observed in CS (Pivonello et al. 2016a; Newell-Price et al. 2006).

Diagnosis

The diagnosis of CS is challenging, considering the broad clinical spectrum, with a limited number of discriminatory features, which are typical of CS, and the relevant number of nondiscriminatory features, which are shared with diseases characterized by a large prevalence in the general population, such as obesity, hypertension, diabetes, polycystic ovary syndrome, and depression (Nieman et al. 2008; Arnaldi et al. 2003). In addition, the hyperactivation of the HPA axis may also occur in the absence of CS, causing an overlap between CS and physiological or pathological causes of hypercortisolism, known as pseudo-Cushing states (PCS). These PCS, mainly including alcoholism, obesity and metabolic syndrome and depression, can be associated with mild hypercortisolism and produce biochemical test results that are suggestive of CS (Nieman et al. 2008; Arnaldi et al. 2003). It should be emphasized that although several screening tests have been extensively used, none has been proven to be fully capable in distinguishing CS from PCS or even from a physiological condition, making CS diagnosis a real demanding process (Nieman et al. 2008; Arnaldi et al. 2003). The diagnostic algorithm of CS, according to guidelines (Nieman et al. 2008; Arnaldi et al. 2003), consists of two different steps: the first step consists in the diagnosis of CS, through a pool of screening tests, characterized by high sensitivity, aimed at confirming CS, and particularly excluding PCS (Fig. 1a); the second step consists of differential diagnosis of CS, through a pool of differential tests, characterized by high specificity, aimed at identifying the CS etiology (Fig. 1b).

Diagnosis of CS

CS should be suspected in four different conditions: (1) the presence of multiple clinical signs and symptoms suggestive and predictive of CS; (2) the presence of clinical features unusual for age, such as vertebral osteoporosis, hypertension, or diabetes mellitus at young age; (3) the presence of adrenal incidentalomas; (4) the presence of increased weight and reduced growth rate in children (Nieman et al. 2008; Arnaldi et al. 2003). The diagnosis of CS requires the performance of one of the following initial screening tests: (1) measurement of urinary cortisol (UC) levels; (2) measurement of late-night salivary cortisol (LNSC) levels; (3) 1 mg-overnight dexamethasone (DMX) suppression test (DST) or Nugent test; (4) low-dose DST (2 mg/day for 48 h) or low-dose Liddle test (Nieman et al. 2008; Arnaldi et al. 2003).

Measurement of Urinary Cortisol Levels

The measurement of UC levels is a noninvasive screening test, which assesses the cortisol secretion in daily urinary samples, expression of cortisol secretion over the entire 24-h daily period. Considering the high intra-individual and inter-individual variability, at least two or three separate measurements are required

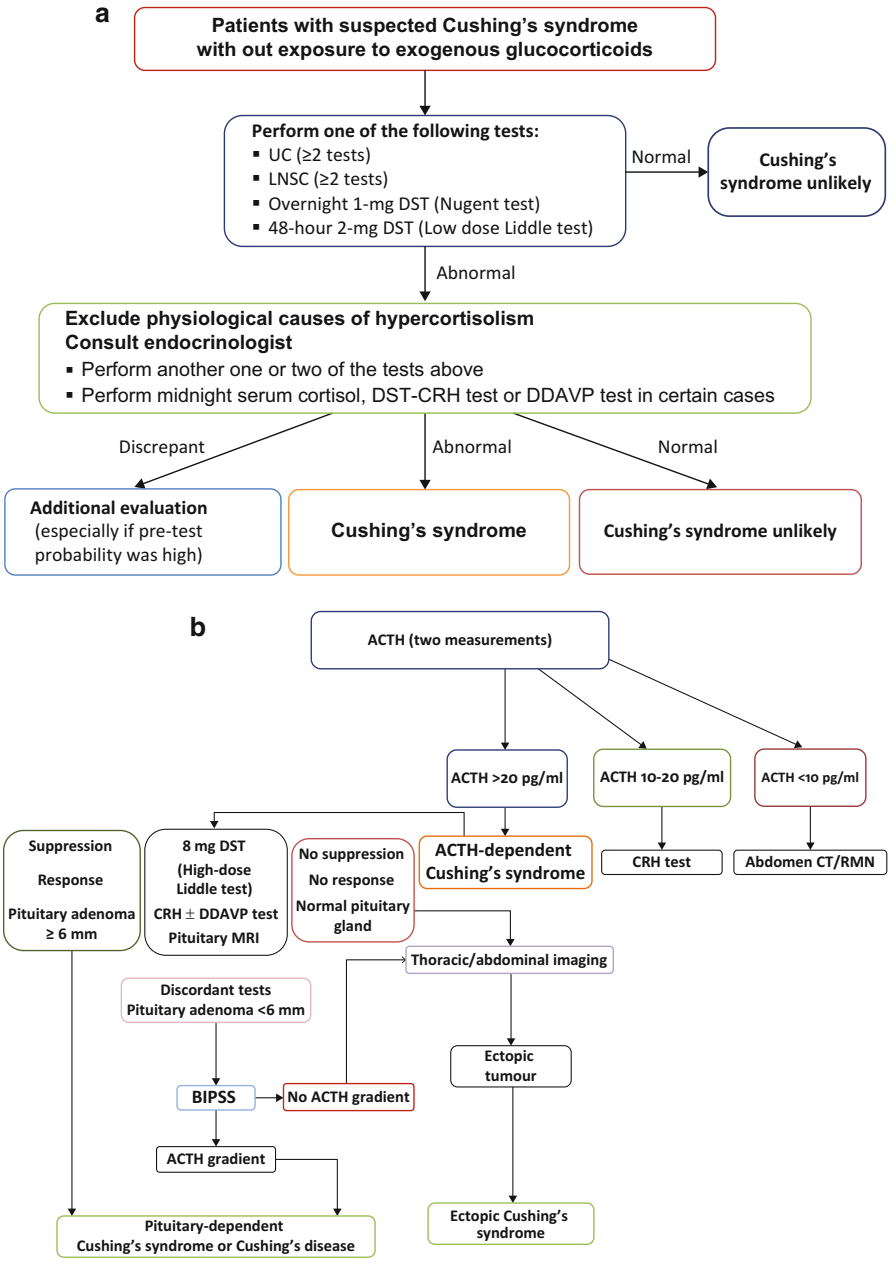


Fig. 1 (**a**) The diagnostic algorithm of CS: screening tests. (**b**) The diagnostic algorithm of CS: differential etiological tests

(Nieman et al. 2008; Arnaldi et al. 2003). To obtain a reliable 24-h cortisol secretion assessment, an appropriate 24-h urine collection is required. The first morning void should be discarded so that the collection begins with an empty bladder. All subsequent voids throughout the day and night should be included in the collection, as well as the first morning void on the second day; urine collection should be kept refrigerated. Once the bladder has been emptied into the collection on the second morning, the sample may be considered complete (Nieman et al. 2008). In case 24-h urinary collection is not possible, the presence of hypercortisolism might be also evaluated using the ratio between UC and urine creatinine, evaluated on the first morning void, indeed, an increased ratio is consistent with possible CS. The main assay method used to quantify UC levels is immunoassay (IA) (El-Farhan et al. 2017). However, limitations of IA techniques are represented by the low specificity rates and the cross-reactivity with cortisol metabolites and synthetic GCs (El-Farhan et al. 2017). More recently, new methodologies with higher sensitivity and specificity, such as the high performance liquid chromatography (HPLC) or liquid chromatography tandem mass spectrometry (LCMS/MS), have been developed (Nieman et al. 2008). These techniques are not affected by cross reactivity with either cortisol metabolites or synthetic GCs. Depending on assay methods, UC sensitivity and specificity range from 38% to 100% and from 44% to 100%, respectively (Elamin et al. 2008). The upper limit of normal (ULN) of the specific assay is the best criterion suggested to consider a test as positive because of the lack of a single value that can be considered a standardized diagnostic cut-off applicable to all the methodologies used to measure UC (Nieman et al. 2008). UC values fourfold higher than the ULN are highly suggestive of CS (Arnaldi et al. 2003). Considering that UC is a measure of cortisol unbound to cortisol binding globulin (CBG) or "free" cortisol, directly filtered by kidneys, the measurement of UC levels is not affected by conditions and drugs that alter CBG; however, false positive and false negative results may occur. False positive results might occur in different conditions: (1) fluid intake higher than 5 liters/day, resulting in filtered cortisol reabsorption decrease, with consequent UC excretion increase, in presence of unchanged urine creatinine excretion (Mericq and Cutler 1998); (2) use of substances inhibiting the 11β-HSD2, including carbenoxolone and liquorice, which contain the 11β-HSD2 inhibitor glycyrrhizic acid, resulting in increased UC levels, due to the inactivation of the physiological cortisol conversion in inactive cortisone (Nieman et al. 2008); (3) use of specific drugs, such as carbamazepine and fenofibrate, that can coelute with cortisol, producing false UC increase, if HPLC assay is used (Nieman et al. 2008; Arnaldi et al. 2003); (4) use of synthetic GCs, mainly including prednisolone and methylprednisolone, that cross-react with cortisol metabolites, if IA assay is used (Arnaldi et al. 2003). False negative results might occur in case of moderate to severe renal failure, with creatinine clearance <60 mL/min, resulting in reduced UC excretion (Nieman et al. 2008).

Measurement of Late-Night Salivary Cortisol Levels

The measurement of LNSC levels is a simple and non-invasive screening test to measure cortisol in late-night saliva samples. Salivary cortisol is in equilibrium with

biologically active free circulating cortisol, and there is a good correlation between salivary and simultaneous serum cortisol values. Additionally, salivary cortisol assessment remains stable at room or refrigerator temperature for several weeks, being suitable for outpatient assessment (Nieman et al. 2008). However, considering the high assay variability, at least two separate evening samples, collected between 23.00 and 24.00 h, are required (Nieman et al. 2008). To obtain a reliable salivary cortisol assessment, samples should be collected either by passive drooling into a plastic tube or by placing a salivette into the mouth and chewing for 1–2 min; considering that eating, blood (due to blood leakage, periodontal diseases, and vigorous tooth brushing), and the use of lotions or oral gels containing steroids could contaminate the sample, the patient should be adequately informed and salivary sample collection should be avoided in all these circumstances (Nieman et al. 2008). Various methods have been used resulting in different ranges, and yielding differences in sensitivity (92–100%) and specificity (93–100%) (Nieman et al. 2008). Normal ranges are assay-dependent, requiring local laboratory validations (Nieman et al. 2008; Arnaldi et al. 2003; El-Farhan et al. 2017). According to Endocrine Society Clinical Practice Guidelines (Nieman et al. 2008), the best validated assays are enzyme-linked immunosorbent assay (ELISA) and LCMS/MS; considering these two assay techniques, salivary cortisol levels at bedtime, or between 23.00 and 24.00 h, higher than 145 ng/dl (4 nmol/liter) are consistent with CS (Nieman et al. 2008). Several factors might interfere with the reliability of LNSC results, mainly causing false positive results. False positive results might occur in case of use of liquorice, as well as in stress conditions, shift working, variable bedtimes, crossing widely different time zones, and in depression or critically ill conditions, due to circadian rhythm disruption (Nieman et al. 2008). Cigarette smoke also may induce ACTH/cortisol secretion, causing false positive results, due to the activation of central nicotine receptors (Badrick et al. 2007). Lastly, although the influence of gender, age, and coexisting medical conditions has not been fully characterized, men aged 60 year or older, diabetic and hypertensive subjects may report a high percentage of false positive results, due to HPA axis hyperactivation and circadian rhythm disruption (Nieman et al. 2008; Liu et al. 2005).

Nugent Test

The Nugent test aims at evaluating whether the physiological negative feedback exerted by cortisol on ACTH and CRH secretion is maintained. It is an easily performed outpatient suppression test, although less simple than measurement of UC and LNSC levels. The current suppression test is based on the administration of 1 mg of DMX, between 23.00 and 24.00 h, and subsequent measurement of serum cortisol levels between 8.00 and 9.00 h on the following morning. This suppression test has shown a sensitivity rate of 98–100%, and specificity rate of 58–80% (Nieman et al. 2008; Arnaldi et al. 2003; Wood et al. 1997; Pecori Giraldi et al. 2007). Serum cortisol levels $\geq$1.8 μg/dl (50 nmol/l) are suggestive of CS (Nieman et al. 2008; Arnaldi et al. 2003). Variable absorption and metabolism of DMX may influence the Nugent test results. Particularly, drugs such as phenobarbital, phenytoin, carbamazepine, rifampicin, and alcohol, accelerate DMX metabolism by

induction of CYP 3A4, therefore reducing the plasma DMX concentrations, and causing false positive results. False positive results are recognized also in case of increased CBG levels, such as in patients treated with mitotane and in women taking contraceptive drugs (Nieman et al. 2008). Conversely, drugs such as itraconazole, ritonavir, fluoxetine, diltiazem, and cimetidine decrease DMX metabolism by inhibition of CYP 3A4, therefore increasing the plasma DMX concentrations, and causing false negative results. False negative results might also occur in case of liver and/or renal failure, due to decreased DMX metabolism (Nieman et al. 2008).

Low-Dose Liddle Test

Low-dose Liddle test, similarly to the previous DST, aims at evaluating if the physiological negative feedback exerted by cortisol on ACTH and CRH secretion is maintained. It is an outpatient suppression test, preferred by some authors, as initial screening test, because of its better specificity as compared with the Nugent test, although it is less easily performed compared with measurement of UC and LNSC levels, and Nugent test. The current suppression test is based on the administration of 0.5 mg DMX every 6 h, for 48 h (2 days), starting at 9.00 h or at 12.00 h of the first day. The measurement of serum cortisol levels should be assessed at 9.00 h or at 8.00 h, 6 or 2 h after the last DMX dose, accordingly to different protocols (Nieman et al. 2008). This suppression test has shown sensitivity ranging from 98 to 100% and specificity ranging from 70% to 88% (Nieman et al. 2008; Wood et al. 1997; Martin et al. 2006). As for Nugent test, serum cortisol levels $\geq$1.8 μg/dl (50 nmol/l) are suggestive of CS (Nieman et al. 2008; Arnaldi et al. 2003). Variable absorption and metabolism of DMX may influence also the low-dose Liddle test results, as reported above for the Nugent test.

In case of normal initial screening test results, CS is very unlikely; therefore, the patient can be reassured and no further tests are required. However, in case of symptoms or complications progress or occurrence of additional features, a new assessment should be performed (Nieman et al. 2008; Arnaldi et al. 2003). In case of abnormal initial screening test results, or in case of normal initial screening test results associated with the persistence of a high clinical suspicion, the diagnostic algorithm should continue by performing one or two screening tests among the abovementioned tests, or one of the following additional screening tests: (1) measurement of midnight serum cortisol levels; (2) DST-CRH test; (3) desmopressin (DDAVP) test (not officially recommended as routinely screening test, but useful in certain cases) (Nieman et al. 2008; Arnaldi et al. 2003). The screening test results must be carefully interpreted, excluding the possible interferences of several drugs (Nieman et al. 2008; Arnaldi et al. 2003).

Measurement of Midnight Serum Cortisol Levels

The measurement of midnight serum cortisol levels is a reliable additional screening test, aimed at evaluating if the physiological nocturnal cortisol nadir is maintained. However, it is a cumbersome test, requiring inpatient admission. It is evaluable in a sleeping or awake state. Particularly, in case of sleeping test, blood must be drowned through an in-welling line or within 5–10 min of awaking patients. The measurement

of sleeping midnight serum cortisol levels has shown a very high degree of sensitivity (98–100%) (Nieman et al. 2008; Pecori Giraldi et al. 2007) with poor specificity (18–26%) (Pecori Giraldi et al. 2007; Nieman et al. 2008; Vilar et al. 2007) compared with different screening tests. Values higher than 1.8 μg/dl (50 nmol/l) are suggestive of CS (Nieman et al. 2008). The measurement of awake midnight serum cortisol levels have shown a sensitivity of 56–100% (Friedman et al. 2007; Nieman et al. 2008; Papanicolau et al. 1998) and specificity of 83–100% (Friedman et al. 2007; Nieman et al. 2008; Vilar et al. 2007). Values higher than 7.5 μg/dl (207 nmol/l) are suggestive of CS (Nieman et al. 2008). False positive results may be due to increased CBG levels, such as in patients treated with mitotane and in women taking contraceptive drugs, and may occur in case of hospitalization stress (Nieman et al. 2008).

Dexamethasone-CRH Test

DST-CRH test is a combined suppression/stimulation additional screening test, aimed at evaluating whether the physiological HPA axis suppression, induced by DMX, is maintained after CRH stimulation. Although this additional screening test was developed in an effort to improve the sensitivity of low-dose Liddle test, its diagnostic accuracy and its advantages over the other screening tests remain to be estabilished (Arnaldi et al. 2003). This test is performed administering 48 h 2 mg-DMX followed by 1 μg/kg/i.v. CRH 2 h after the last DMX dose. The measurement of serum cortisol levels should be assessed 15 min after CRH injection. Serum cortisol levels higher than 1.4 μg/dl (38 nmol/l) have been proposed to be suggestive of CS, with sensitivity of 94–100% and specificity of 60–100% (Nieman et al. 2008; Martin et al. 2006; Yanovski et al. 1993; Pecori Giraldi et al. 2007; Reimondo et al. 2008). However, more recently, a different cut-off for serum cortisol, corresponding to 1.6μg/dl (44 nmol/l) has been proposed, being associated with a sensitivity of 93.7% and a specificity of 93.3%. (Reimondo et al. 2008). As discussed above, any DMX test may give either false positive or false negative results, in conditions that alter the metabolic clearance of this drug.

Desmopressin Test

DDAVP stimulation test, aimed at evaluating the ACTH secretion in response to the stimulation, is not recommended as a routine additional screening test in confirming CS, because of limited experience and poor validation (Nieman et al. 2008). Conversely, it seems to be promising in the differential diagnosis, particularly between CS and PCS, and between pituitary and ectopic ACTH-dependent CS forms (Nieman et al. 2008). This stimulation test consists in the administration of 10 μg i.v. DDAVP, resulting in the stimulation of ACTH secretion, which is increased in CD, likely due to upregulation of tumor vasopressin receptors. Plasma ACTH levels are mainly assessed at 30 or 15 min before, and immediately before, the injection of DDAVP, as well as 15, 30, 45, 60, 90, and 120 min after the injection. This stimulation test has shown a sensitivity of 75–87%, and a specificity of 90–91%, using Δ-ACTH increase $\geq$6 pmol/l (1.6 pg/ml) as criteria to suggest CS diagnosis (Findling and Raff 2017). More recently, this stimulation test has shown a slightly higher sensitivity (90.3%)

and specificity (91.5%), using the simultaneous presence of basal serum cortisol levels greater than 331 nmol/l (12 μg/dl) and Δ-ACTH increase >4 pmol/l (1.1 pg/ml) as criteria to suggest CS diagnosis (Tirabassi et al. 2010).

The initial and additional screening tests are detailed in Table 1. The list of drugs interfering with screening tests is summarized in Table 2.

Pseudo-Cushing States

The crucial aim of the process of confirmation of CS diagnosis is the differentiation between CS and PCS, which are clinical conditions characterized by the presence of a chronic and functional hyperactivation of HPA axis, associated with clinical features overlapping with CS. Particularly, many PCS, mainly including alcoholism, obesity and metabolic syndrome and depression, can be associated with mild cortisol excess and may produce screening test results suggestive of CS, including elevated UC and LNSC levels, as well as abnormal DST results (Nieman et al. 2008). Conversely, DST–CRH and DDAVP tests seem to be useful to discriminate the two states, reporting in this context a sensitivity of 88–100% and 75–90%, and a specificity of 50–100% and 90–92%, respectively. CS patients seem to typically respond to these two tests, compared to those with all forms of PCS (Findling and Raff 2017). In women, the CS diagnosis may present a further challenging difficulty, considering the broad possible overlap between CS and polycystic ovary syndrome characterized by the presence of hyperandrogenism, including acne, hirsutism, oligomenorrhea and amenorrhea, visceral obesity, and insulin resistance.

PCS are summarized in Table 3.

Alcoholism: The functional hyperactivation of HPA axis induces increased UC and LNSC levels, due to the increase in CRH and vasopressin secretion, as well as impairment of cortisol clearance, probably due to liver dysfunction (Findling and Raff 2017). False positive results are reported using UC and LNSC tests, as well as Nugent test, due to the accelerated DMX metabolism, related to alcohol intake (Nieman et al. 2008). Therefore, it seems that the most reliable screening tests, useful to differentiate alcoholism from CS, are DST-CRH and DDAVP tests (Findling and Raff 2017); nevertheless, active alcohol intake may cause false positive results in the DST-CRH test, by limiting its use in case of active alcoholism, and few evidences are available for the DDAVP test (Findling and Raff 2017).

Obesity and metabolic syndrome: The pathogenetic mechanism involved in the functional hyperactivation of HPA axis in obesity and metabolic syndrome is not well known, although it may be due to the chronic exposure to stress factors (Anagnostis et al. 2009). However, in obese patients, UC and LNSC are either normal or only slightly elevated, rarely causing false positive results (Findling and Raff 2017). DST-CRH and DDAVP tests are limited experienced but promising screening tests (Findling and Raff 2017). Conversely, considering that GCs negative feedback appears to be maintained, DST are reliable tests to discriminate obesity and metabolic syndrome from CS (Anagnostis et al. 2009).

Depression: The functional hyperactivation of HPA axis in depression induces increased UC and LNSC levels, due to the increase in CRH secretion (Holsboer and Barden 1996) and reduced GR sensitivity, with a consequent resistance to cortisol

Table 1 Tests used as screening tests

Test	Protocol	Response suggestive of CS	Sensitivity and specificity
UC	At least two UC 24 h urine collections	ULN for the specific assay	Sensitivity: 38–100% Specificity: 44–100%
LNSC	At least two samples between 23.00 and 24.00 h	LNSC: >145 ng/dl (4 nmol/liter)	Sensitivity: 92–100% Specificity: 93–100%
Nugent test	DMX dose: 1 mg between 23.00 and 24.00 h Serum cortisol: 8.00–9.00 h	Serum cortisol: ≥1.8 μg/dl (50 nmol/l)	Sensitivity: 98–100% Specificity: 58–80%
Low-dose Liddle test	DMX dose: 0.5 mg 6-hourly for 48 h Serum cortisol: 6 or 2 h after last DMX dose (9.00 h or 8.00 h)	Serum cortisol: ≥1.8 μg/dl (50 nmol/l)	Sensitivity: 98–100% Specificity: 70–88%
Midnight serum cortisol (sleeping)	Blood drown through an in-welling line or within 5–10 min of waking patients	Serum cortisol: >1.8 μg/dl (50 nmol/l)	Sensitivity: 98–100% Specificity: 18–26%
Midnight serum cortisol (awake)	Blood drown in awake patients	Serum cortisol: >7.5 μg/dl (207 nmol/l)	Sensitivity: 56–100% Specificity: 83–100%
DST-CRH test	DMX dose: 0.5 mg 6-hourly for 48 h CRH dose: 1 μg/kg 2 h after DMX last dose Serum cortisol: 15 min after CRH dose	Serum cortisol: >1.4 μg/dl (38 nmol/l)	Sensitivity: 94–100% Specificity: 60–100%
DDAVP test	DDAVP dose: 10 μg Plasma ACTH: −30/or-15, 0 min before DDAVP dose 15, 30, 45, 60, 90, 120 min after DDAVP dose	Plasma ACTH: Δ ACTH ≥6 pmol/l (1.6 pg/ml) **Or** Δ ACTH >4 pmol/l (1.1 pg/ml) + Basal serum cortisol >331 nmol/l (12 μg/dl)	Sensitivity: 75–87% Specificity: 90–91% **Or** Sensitivity: 90.3% Specificity: 91.5%

negative feedback (Holsboer and Barden 1996). Increased active intracellular cortisol concentrations, secondary to a reduced activity of the cortisol-deactivating enzymes 5α-reductase and 11β-HSD2, have also been reported to be involved in

Table 2 All drugs interfering with screening tests

Drugs that accelerate DMX metabolism by induction of CYP3A4	Drugs that impair DMX metabolism by inhibition of CYP3A4	Drugs that increase CBG and may falsely elevate cortisol results	Drugs that increase UFC results
Alcohol Carbamazepine Ethosuximide Phenobarbital Phenytoin Pioglitazone Pyrimidone Rifampin Rifapentine	Aprepitant Cimetidine Diltiazem Fluoxetine Fosaprepitant Itraconazole Ritonavir	Estrogens Mitotane	11-HSD2 inhibitors (e.g., liquorice, carbenoxolone) Carbamazepine and fenofibrate (HPLC) Some synthetic GCs mainly prednisolone and methylprednisolone (IA)
False positive	**False negative**	**False positive**	**False positive**

Table 3 Pseudo-Cushing states

Alcoholism
Obesity and metabolic syndrome
Depression
Uncontrolled diabetes mellitus
Glucocorticoids resistance
Renal failure
Malnutrition
Pregnancy
Physical stress (e.g., intense exercise, hospitalization, surgery)

the HPA axis hyperactivation (Römer et al. 2009). It seems that there is no single reliable test to discriminate depression from CS. False positive results are registered using the measurements of UC and LNSC levels, Nugent test, because of reduced cortisol suppression due to GCs negative feedback resistance, as well as using DST-CRH test, because of an exaggerated cortisol release after CRH injection (Holsboer and Barden 1996). However, the DDAVP test use is restricted by the limited experience (Findling and Raff 2017).

Particular Physiological and Pathological States

Some recommendations should be done for particular physiological and pathological states, mainly including pregnancy, epilepsy, renal failure, and cyclic CS.

Pregnancy: CS screening is more difficult during pregnancy, due to the physiological increase of UC levels during second and third trimesters, up to threefold the ULN (Nieman et al. 2008). Therefore, only UC values threefold higher than the ULN can be considered suggestive of CS (Nieman et al. 2008). Moreover, midnight serum

cortisol levels are reported to be higher, although specific cut-offs to predict CS in pregnant women are not available (Nieman et al. 2008). As concerns DST, suppression of cortisol by DMX is blunted during pregnancy, by increasing the risk of false positive results. Therefore, the use of these suppression tests is not suggested in the evaluation of pregnant women (Nieman et al. 2008).

Epilepsy: In the treatment of epilepsy some anticonvulsants, such as phenobarbital, phenytoin, and carbamazepine, by accelerating DMX metabolism and reducing its plasma concentrations, may cause DST false positive results (Nieman et al. 2008). However, wherever possible, these drugs should be withdrawn before testing, switching to non-enzyme inducing drugs, although no data are available to guide the needed time to washout (Nieman et al. 2008). Therefore, patients suffering from epilepsy should be screened using LNSC and midnight serum cortisol assays (Nieman et al. 2008).

Renal failure: Moderate to severe renal failure (creatinine clearance <60 mL/min) might induce UC false negative results. Diagnostic cut-offs for either LNSC or midnight serum cortisol are unknown in this peculiar patients category. Therefore, the Nugent test is the preferred biochemical test to be used in the initial screening of CS in these patients, although false negative results, due to decreased DMX metabolism, cannot be excluded (Nieman et al. 2008).

Cyclic CS: Cyclic CS, a clinical condition characterized by episodic cortisol excess secretion in a cyclical pattern, with exacerbations occurring at irregular intervals, ranging from few days to several months, requires a challenging diagnostic process (Nieman et al. 2008). First of all, it should be noted that patients suffering from cyclic CS might have normal screening test results in the "quiescent phase." Indeed, both UC and DST results may be normal if a patient has cyclic disease and collects urine when the disease is inactive. In this case, further evaluations and follow-up are the suggested approaches, mainly repeating measurement of UC or LNSC levels over time (Nieman et al. 2008).

Differential Diagnosis of CS

The algorithm of the differential diagnosis of CS, according to consensus statement (Arnaldi et al. 2003), consists of two different steps: the first step consists in the differential diagnosis between ACTH-dependent and ACTH-independent forms (Fig. 1b), through the measurement of ACTH levels; the second step consists of differential diagnosis among ACTH-dependent forms, differentiating between pituitary and ectopic ACTH sources.

In case of ACTH-dependent forms, the diagnostic algorithm should continue performing the following tests: (1) high-dose DST (HDDST) or high-dose Liddle test; (2) CRH or DDAVP stimulation test; (3) pituitary MRI; (4) bilateral inferior petrosal sinus sampling (BIPSS) (if needed). In case of ECS, diagnostic algorithm should continue performing body computed tomography (CT) or magnetic resonance imaging (MRI) scan, and as second step ^{111}In-pentetreotide scintigraphy (octreoscan), ^{18}fluoro-2-deoxyglucose-positron emission tomography (FDG-PET),

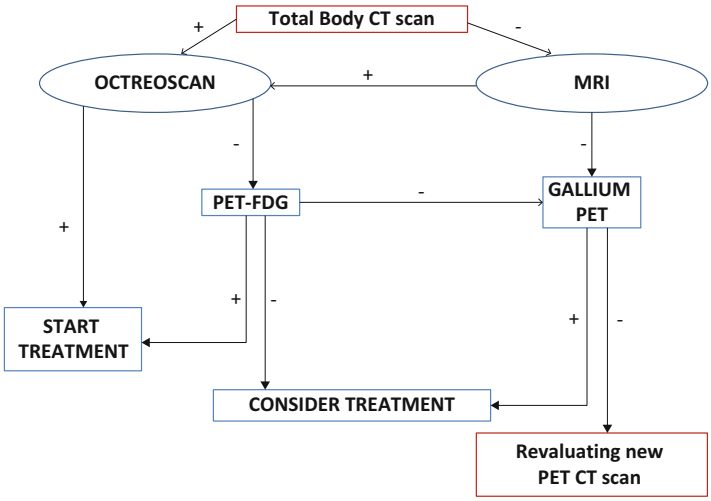


Fig. 2 The imaging diagnostic algorithm of ECS

^{68}Gallium-SSTR-PET/CT scan as described in Fig. 2. In case of ACTH-independent forms, diagnostic algorithm should continue performing adrenal CT or MRI.

The entire series of differential tests is detailed and summarized in Table 4.

Measurement of ACTH Levels

The measurement of ACTH levels is a differential test, aimed at distinguishing the ACTH-dependent and ACTH-independent forms of CS. Reliable ACTH assays are essential to distinguish between elevated and suppressed ACTH levels. ACTH assays have evolved considerably since the first radioimmunoassay (RIA), and currently available IA are mostly fully automated, requiring minimal plasma samples. However, a considerable variability has been reported, mainly related to assay variability, starting with the different standards used by individual assays, as not all ACTH (1–39) formulations are equally recognized by assay antibodies. It is also important to recall that ACTH adsorbs to unsiliconized glass tubes and degrades rapidly at room temperature, therefore, the use of non-hemolyzed EDTA-coated plastic tubes, rapid sample processing, and cool storage prior to assay is necessary (Arnaldi et al. 2003; Newell-Price et al. 1998). Non-suppression of morning plasma ACTH values, with levels higher than 4 pmol/l (20 pg/ml), suggests an ACTH-dependent CS form; frequently, ACTH levels tend to be higher in ECS than in CD. Conversely, suppression of morning plasma ACTH values, with levels less than 2 pmol/l (10 pg/ml), suggests an adrenal ACTH-independent CS form (Arnaldi et al. 2003). Lastly, in case of ACTH levels ranging from 2 to 4 pmol/l (10–20 pg/ml), a CRH stimulation test should be performed to discriminate between ACTH-dependent and ACTH-independent forms with an enphatic ACTH response in ACTH-dependent forms and a blunted ACTH response in ACTH-independent forms (Arnaldi et al. 2003).

Table 4 Tests used as differential diagnostic tests

Test	Protocol	Response suggestive	Sensitivity and specificity
ACTH	Nonhemolyzed 9.00 h plasma ACTH blood sample placed in ice and immediately treated with a refrigerated centrifugation	**ACTH < 2 pmol/l (10 pg/ml):** Adrenal ACTH-independent CS **ACTH 2–4 pmol/l (10–20 pg/ml):** CRH stimulation test **ACTH > 4 pmol/l (20 pg/ml):** ACTH-dependent CS	
High-dose Liddle test	DMX dose: 2 mg 6-hourly for 48 h Serum cortisol: 6 or 2 h after last DMX dose (9.00 h or 8.00 h)	Serum cortisol: Suppression >50% in CD	Sensitivity: 65–100% Specificity: 60–100%
	Or DMX 8 mg at 23.00 h Serum cortisol: 8.00–9.00 h	Serum cortisol: Suppression >50% in CD	
	Or 1 mg/h for 5–7 h Serum cortisol: Before at the end of infusion	Serum cortisol: Suppression >50% in CD after 5 mg Cortisol fall >190 nmol/l in CD after 7 mg	
CRH test	CRH dose: 1 μg/kg or 100 μg Plasma ACTH and serum cortisol: −30/or −15, 0, 15, 30, 45, 60, 90, and 120 min after CRH dose	Plasma ACTH after dose: Increase of >50% in CD Serum cortisol after dose: Increase of >20% in CD Plasma ACTH 15–30′ after dose: Increase of 35–50% in CD Serum cortisol 15–45′ after dose: Increase of 20% in CD	Sensitivity: 86–93% Specificity: 88–100%
DDAVP test	DDAVP dose: 10 μg Plasma ACTH and serum cortisol: −30/or −15, 0, 15, 30, 45, 60, 90, and 120 min after DDAVP dose	Plasma ACTH after dose: Increase of 35% in CD Serum cortisol after dose: Increase of 20% in CD Or Peak plasma cortisol >4 times the intra-assay coefficient of variation	Sensitivity: 77–84% Specificity: 73–83%
BIPSS	CRH dose: 1 μg/kg or 100 μg Central and peripheral plasma ACTH: 0, 3, 5, (10) minutes after CRH dose	ACTH ratio in basal condition: >2 in CD And/or ACTH ratio after stimulation: >3 in CD	Sensitivity: 95–99% Specificity: 95–99%

High-Dose Liddle Test

The high-dose Liddle test aims at distinguishing between pituitary and extra-pituitary ACTH source: the partial inhibitory feedback induced by high doses of DMX is maintained in pituitary ACTH source causing CD, compared with extra-pituitary ACTH source causing ECS, in which this inhibitory feedback is lost. This noninvasive, cumbersome suppression test consists in the administration of DMX mainly according to three different protocols: (1) oral administration of 2 mg DMX every 6 h, for 48 h, starting at 9.00 h or at 12.00 h of the first day and assessing serum cortisol levels at 9.00 h or at 8.00 h, 6 or 2 h after the last dose of DMX; (2) oral administration of 8 mg DMX in one single dose at 23.00 h and assessment of serum cortisol levels between 8.00 and 9.00 h on the following morning; (3) intravenous DMX infusion at a rate of 1 mg/h for 5–7 h (5–7 mg) and assessment of serum cortisol levels before and at the end of the infusion (Newell-Price et al. 1998). A reduction in serum cortisol levels greater than 50% after oral and 5 mg intravenous test, or a serum cortisol levels fall greater than 190 nmol/l after 7 mg intravenous test, suggests a pituitary ACTH source with a sensitivity and specificity within the ranges of 65–100% and 60–100%, respectively (Newell-Price et al. 1998). As reported above for Nugent and low-dose Liddle test, variable absorption and metabolism of DMX may also influence the high-dose Liddle test results.

CRH Stimulation Test

CRH stimulation test aims at distinguishing between pituitary and extra-pituitary ACTH source: ACTH and consequently cortisol secretion is typically stimulated in pituitary ACTH source causing CD, compared with extra-pituitary ACTH source causing ECS (Arnaldi et al. 2003; Newell-Price et al. 1998). This stimulation test is very reliable, but CRH is expensive and not easily available. CRH stimulation test consists of the administration of 1 μg/kg or 100 μg of CRH i.v. The measurement of plasma ACTH and serum cortisol levels should be assessed 15 or 30 min before and immediately before the CRH injection, as well as 15, 30, 45, 60, 90, and 120 min after the injection. A great variability exists in the interpretation of CRH test, mainly depending on the type of CRH used (human vs. ovine), the biochemical parameters considered (ACTH peak, 35–50%, or cortisol peak, 14–20%), and the evaluated time points (ACTH, 15–30 min, or cortisol, 15–45 min) (Arnaldi et al. 2003). It has been reported that, after CRH stimulation, considering a rise from basal in peak ACTH of $\geq$50%, or a rise in peak cortisol of $\geq$20%, the test has a sensitivity of 86% and a specificity of 95%, for ACTH response, whereas plasma cortisol responses provide an improved sensitivity of 91%, and a similar specificity of 95% (Newell-Price et al. 1998). However, as reported in a large serie from National Institutes of Health the best discriminatory criterion between pituitary and extra-pituitary ACTH source seems to be a rise of 35% or more in the mean ACTH concentrations at 15 and 30 min above the mean basal value at −5 and −1 min, which gives a sensitivity of 93% and a specificity of 100%, with cortisol response being less impressive, with a rise of 20% or more at the mean of the levels at 30 and 45 min, giving a sensitivity of 91% and a specificity of 88% (Newell-Price et al. 1998).

Desmopressin Stimulation Test

DDAVP stimulation tests aim at distinguishing between pituitary and extra-pituitary ACTH source. ACTH and consequently cortisol secretion is typically stimulated in pituitary ACTH source causing CD, because of tumor vasopressin receptors upregulation, even if a maintained response has been also reported in 20–50% of ECS expressing ectopic tumor vasopressin receptors (Arnaldi et al. 2003; Newell-Price et al. 1998). DDAVP is easily available and inexpensive, and it does not cause significant side effects, although it is less reliable than CRH test (Arnaldi et al. 2003; Newell-Price et al. 1998). This stimulation test consists of the administration of 10 μg/i.v (Arnaldi et al. 2003; Newell-Price et al. 1998). The measurement of plasma ACTH and serum cortisol levels should be assessed 15 or 30 min before and immediately before the DDAVP injection, as well as 15, 30, 45, 60, 90, and 120 min after the injection. A great variability and limited experience exists in the interpretation of DDAVP test. A rise in plasma cortisol of 20% or a rise from baseline in peak plasma cortisol of more than four times the intra-assay coefficient of variation or a rise in plasma ACTH of 35%, have been described as consistent with CD. Combining all published data, the desmopressin cortisol response has a sensitivity of 84% and specificity of 83%, whereas the ACTH has a sensitivity of 77% and specificity of 73% (Newell-Price et al. 1998).

Pituitary MRI

A pituitary MRI with gadolinium enhancement, a procedure able to detect the presence of pituitary tumors in up to 70% of cases (Arnaldi et al. 2003; Vitale et al. 2017), should be performed in all patients with an ACTH-dependent CS form (Arnaldi et al. 2003). In patients with differential test results suggestive of CD and a pituitary MRI showing a pituitary tumor $\geq$6 mm, the CD diagnosis can be confirmed, and no additional differential tests are required (Arnaldi et al. 2003). Conversely, in patients showing discordant differential test results and/or a negative pituitary MRI or the MRI evidence of a pituitary tumor $<$6 mm, an additional evaluation, by using BIPSS, should be performed (Arnaldi et al. 2003). False positive results may occur in case of incidental pituitary lesions, occurring in 10–20% of the general population, and consist of incidentally discovered pituitary tumors not responsible for CD, (Arnaldi et al. 2003; Scangas and Laws 2014).

Bilateral Inferior Petrosal Sinus Sampling (BIPSS)

BIPSS represents the best differential test able to identify pituitary ACTH source causing CD, directly measuring ACTH levels at inferior petrosal sinuses. However, this procedure should be performed only in specialized centers, due to the required experience of the radiologist, and to the possible occurrence of rare adverse events, such as deep vein thrombosis, pulmonary embolism, and brain stem vascular damages (Arnaldi et al. 2003; Pecori Giraldi et al. 2015). This procedure consists in the catheterization of both inferior petrosal sinuses (IPS), and in the measurement of plasma ACTH levels simultaneously at left and right IPS (central sites) and at a peripheral vein (peripheral site). These simultaneous central and peripheral ACTH

measurements should be evaluated in basal conditions, and at 3 and 5 min (and at 10 min in some centers) after the intravenous CRH infusion (1 μg/kg or 100 μg) (Arnaldi et al. 2003). An IPS to peripheral ACTH ratio (IPS/P) greater than 2.0 in basal conditions and/or greater than 3.0 after CRH stimulation confirms the pituitary ACTH source with high sensitivity and specificity rates (95–99%) (Arnaldi et al. 2003). The use of BIPSS for the localization of a pituitary tumor at right or left side of the pituitary gland remains controversial (Arnaldi et al. 2003). False negative results may be due to technical factors, such as the anomalous venous drainage.

Ectopic Imaging

In patients showing differential test results suggestive of ECS, the ACTH-secreting or CRH-secreting extra-pituitary tumor should be detected. Approximately half of ECS sources are readily diagnosed, whereas extensive investigations are needed to discover the riddle sources in up to 30% of cases, due to difficulties in localizing ectopic secreting tumors (Isidori et al. 2015a). However, despite intensive investigations, 18.6% of the tumors remains occult, indicating the need for further improvement in available imaging techniques, characterized by false positive and false negative results (Isidori et al. 2015a). Most tumors causing ECS are in the lungs, mediastinum, or neck. For this reason, a CT body scan is the most useful first line examination (Isidori et al. 2015a). If CT is successful in identifying the lesion, octreoscan is found to be the best confirmatory and the most requested second line examination (Isidori et al. 2015a). When CT and octreoscan findings are concordant, most clinicians start treatment, normally opting for surgery (Isidori et al. 2015a). In contrast, where the octreoscan is negative, FDG-PET is the best performing third line examination (Isidori et al. 2015a). With FDG-PET confirming a previous investigation, treatment is started. With only one positive CT scan, clinicians should be cautious in performing treatment: an alternative approach may be represented by highly sensitive ^{68}Gallium-SSTR-PET/CT scan (Isidori et al. 2015a). In case of CT fails to reveal any lesion, MRI is successful in 35.3% of cases when performed (Isidori et al. 2015a). After false negative CT or MRI scans, octreoscan appears able to detect the source in 66.7% of cases, and subsequent FDG-PET in 72.2%, suggesting that these exams could be performed as second-level and third-level investigations, after a negative CT or MRI (Isidori et al. 2015a). The ^{68}Gallium-SSTR-PET/CT has shown its possible superiority over other imaging techniques in localizing covert ECS (Isidori et al. 2015a). However, only a minority of studies report its use, indicating limited availability (Isidori et al. 2015a). The imaging diagnostic algorithm of ECS is detailed in Fig. 2.

Adrenal Imaging

Adrenal imaging plays an important role in the diagnostic algorithm of ACTH-independent forms and includes contrast enhanced CT or MRI (Newell-Price et al. 1998). In case of adrenal tumor, several features help in the distinction between adrenocortical adenoma and adrenocortical carcinoma. A lesion less than 4 cm, in absence of necrosis, hemorrhage, calcifications, with homogeneous CT density

(<10 hounsfield; HU) or homogeneous MRI signal drop and homogeneous MRI contrast enhancement is suspected to be an adrenocortical adenoma. Conversely, a lesion higher than 4 cm, in presence of necrosis, hemorrhage, calcifications, with heterogeneous CT density (>10HU) or heterogeneous MRI signal drop and heterogeneous MRI contrast enhancement is suspected to be an adrenocortical carcinoma (Else et al. 2014).

Treatment

The aims of CS treatment, according to guidelines (Nieman et al. 2015), include: (1) the normalization of cortisol secretion; (2) the reversal of clinical picture in terms of clinical signs and symptoms; (3) the prevention or improvement of concomitant comorbidities; and (4) the long-term control without recurrence (Pivonello et al. 2015a; Nieman et al. 2015). The main treatment approaches include surgery, radiotherapy, and medical treatment (Pivonello et al. 2015a; Nieman et al. 2015). Surgery represents the first line treatment for all CS forms (Pivonello et al. 2015a; Nieman et al. 2015). Second line treatment approaches are strictly dependent on CS etiology. CD approaches include repeat pituitary surgery, pituitary radiotherapy, bilateral adrenalectomy, and medical treatment (Pivonello et al. 2015a; Nieman et al. 2015). ECS approaches mainly include radiotherapy or chemotherapy, bilateral adrenalectomy, and medical treatment (Nieman et al. 2015). Lastly, adrenal CS approaches include chemotherapy, radiotherapy, and medical treatment (Nieman et al. 2015). A multimodal and individualized approach is suggested considering the high rates of CS persistence and recurrence, especially for CD and ECS (Pivonello et al. 2015a; Nieman et al. 2015). The treatment algorithm of CS is detailed in Fig. 3.

Cushing's Disease

CD treatment is based mainly on four different approaches: pituitary surgery, pituitary radiotherapy, adrenal surgery, and medical treatment.

Pituitary Surgery

Pituitary surgery is the first line treatment for CD, consisting in selective removal of the pituitary tumor, performed by a transsphenoidal approach with microscopic or endoscopic techniques (Pivonello et al. 2015a; Nieman et al. 2015). Pituitary tumors of large size or extra-sellar extension may require the historical transcranial approach, which is currently less used in the clinical routine (Pivonello et al. 2015a). The overall initial remission rates of pituitary surgery range from 25% to 100%, with a mean (m) remission rate of 77.8%, being lower for macrodenomas (30.8–100%, m = 62.3%) than microdenomas (48.7–100%, m = 82.1%). The recurrence rate when calculated as a percentage of the patients who obtained initial remission ranges from 0% to 65.6% (m = 13.2%), whereas when calculated as a

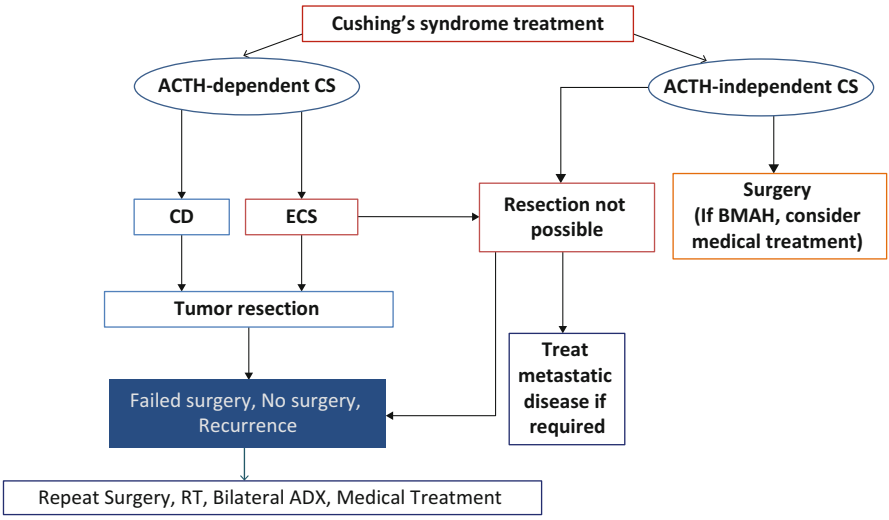


Fig. 3 Treatment algorithm of CS. *CS* Cushing's syndrome, *CD* Cushing's disease, *ECS* ectopic Cushing's syndrome, *BMAH* bilateral macronodular adrenal hyperplasia, *RT* radiotherapy, *ADX* adrenalectomy

percentage of the whole population of patients ranges from 0% to 51.2% (m = 9.6%). Therefore, pituitary surgery is associated with long-term failure, including persistence and recurrence, in 0–75% of cases (m = 31.7%). Particularly, recurrence rates are higher for macroadenomas (0–59%, m = 18.8%) than microadenomas (0–36.4%, m = 11.7%), with a recurrence risk persistence maintained for up to 30 years, mainly within the first 10 years (Mampalam et al. 1988; Hofmann et al. 2008; Lambert et al. 2013; Jagannathan et al. 2009; Invitti et al. 1999; Patil et al. 2008a; Cavagnini and Pecori Giraldi 2001). Several factors can negatively influence pituitary surgery outcome, and mainly including male gender, tumor size greater than 2 cm, tumors with supra-sellar extension or cavernous sinus invasion, undetected pituitary tumors at presurgical MRI or during surgical procedure, the absence of histological confirmation or peritumoral Crooke's cells, as well as unskilled surgeon (Pivonello et al. 2015a; Mampalam et al. 1988; Jagannathan et al. 2009; Invitti et al. 1999; Hammer et al. 2004; Cannavò et al. 2003; Wagenmakers et al. 2013; Ciric et al. 1997; Bigos et al. 1980). Pituitary surgery is a well tolerated and safe approach when performed by an experienced pituitary surgeon. Most common complications include hypopituitarism and diabetes insipidus, occurring in 0.9–93.3% (m = 29.6%) and in 0.9–32.5% (m = 12.3%) of cases, respectively (Pivonello et al. 2015a; Mampalam et al. 1988; Hofmann et al. 2008; Lambert et al. 2013; Jagannathan et al. 2009; Invitti et al. 1999; Patil et al. 2008a; Cavagnini and Pecori Giraldi 2001). Myocardial infarction, pneumonia infection, and meningitis are the main mortality causes, with a mortality rate of 0–7.1% and a mean mortality rate of 0.6% (Pivonello et al. 2015a).

Repeat Pituitary Surgery

A repeat pituitary surgery is a second line treatment option, particularly suggested in patients with a clear evidence of a residual tumor at pituitary MRI performed after the first surgical procedure (Pivonello et al. 2015a; Nieman et al. 2015). However, patients with large and/or invasive tumors appear not to benefit from a repeat surgical approach (Pivonello et al. 2015a). Indeed, remission rates appear lower than those of the first surgery, ranging from 30% to 87.5% (m = 58%), with higher recurrence rates from 0% to 60% (m = 16.1%) (Pivonello et al. 2015a; Hofmann et al. 2008; Benveniste et al. 2005; Patil et al. 2008b). Residual tumor size and localization seem to be the main negative predictive factors, by influencing surgery outcome (Pivonello et al. 2015a; Hofmann et al. 2008; Benveniste et al. 2005; Patil et al. 2008b). As far as safety profile was concerned, the most common complication is hypopituitarism occurring in 9.1–78.6% (m = 38%) of cases (Pivonello et al. 2015a; Hofmann et al. 2008; Benveniste et al. 2005; Patil et al. 2008b).

Pituitary Radiotherapy

Pituitary radiotherapy should be considered as a second line option in case of pituitary surgery failure, especially in cases of aggressive and/or invasive tumors (Pivonello et al. 2015a). However, radiotherapy may rarely represent a first line approach, in patients without indication for surgery, with contraindications to surgery, and in patients who refuse surgery (Pivonello et al. 2015a; Nieman et al. 2015). The recent development of medical treatment may limit its use to a third line option, in patients who are unresponsive or intolerant to medical treatment, or after a period of cortisol excess control by medical therapy (Pivonello et al. 2015a). Different radiotherapy techniques have been developed, including conventional radiotherapy (CRT) and stereotactic radiotherapy (SRT). CRT, historically used as the first radiation treatment, but presently less used, is a technique delivering ionizing radiation to the target tumor in small daily doses, over a period of 25–30 days. SRT, presently most commonly used, is a technique delivering ionizing radiation to the target tumor by stereotactic methods, which provides the precise identification of tumor localization, with three spatial coordinates. SRT can be delivered as a single treatment (stereotactic radiosurgery) by means of three different techniques, including a multiheaded cobalt unit (Gamma Knife), a linear accelerator system (LINAC), or a proton-beam system (Proton-beam), or as a fractionated treatment (stereotactic conformal radiotherapy) (Pivonello et al. 2015a). CRT remission rates, in terms of cortisol excess control, range from 19.6% to 100% (m = 63.8%), with recurrence rates from 0% to 62.5% (m = 15.9%) (Pivonello et al. 2015a; Estrada et al. 1997; Orth and Liddle 1971; Minniti et al. 2007; Tsang et al. 1994). Considering the efficacy in terms of tumor growth control, evaluated as growth arrest or tumor size reduction, remission rates range from 53% to 100% (m = 98.5%) (Pivonello et al. 2015a; Estrada et al. 1997; Orth and Liddle 1971; Minniti et al. 2007; Tsang et al. 1994). CRT seems to be less effective if used as exclusive treatment, and at dosage less than 40 Gy, although no studies have identified specific factors able to predict its outcome (Pivonello et al. 2015a). SRT remission rates, in terms of cortisol excess control, range from 10% to 100% (m = 60.8%), with

recurrence rates from 0% to 100% (m = 12.3%) (Pivonello et al. 2015a; Estrada et al. 1997; Orth and Liddle 1971; Minniti et al. 2007; Tsang et al. 1994). Considering the efficacy in terms of tumor growth control, remission rates range from 50% to 100% (m = 90.9%) (Pivonello et al. 2015a; Estrada et al. 1997; Orth and Liddle 1971; Minniti et al. 2007; Tsang et al. 1994). Treatment response rates appear similar in the different SRT methods (Pivonello et al. 2015a). Although further studies are needed to better identify the involved factors able to predict the SRT outcome, radiation dose seems to play a key role (Pivonello et al. 2015a). Indeed, patients with postsurgical residual tumors >1 cm and tumors with a distance from the optic chiasm <5 mm, which would require a radiation dose superior to those tolerated by the optic apparatus or the other structures, are potentially undertreated, and the lower doses used may negatively impact on radiotherapy outcome (Pivonello et al. 2015a). Similarly, the use of some medical treatments able to exert a radioprotective role results in a delayed achievement of remission (Pivonello et al. 2015a). The most frequent complication of radiotherapy is represented by the occurrence of hypopituitarism, which ranges from 0% to 100% (m = 39.3%) using CRT, from 0% to 66% (m = 22.9%) using Gamma Knife, from 0% to 40% (m = 17.%) using LINAC, and from 0% to 52% (m = 26%) using proton-beam method (Pivonello et al. 2015a; Estrada et al. 1997; Orth and Liddle 1971; Minniti et al. 2007; Tsang et al. 1994). The incidence and severity of hormonal deficiencies appear increased over time (Pivonello et al. 2015a; Estrada et al. 1997; Orth and Liddle 1971; Minniti et al. 2007; Tsang et al. 1994). Additional complications include cerebrovascular accidents, mainly reported after CRT, with an incidence of 4%, 11%, and 21% after 5, 10, and 20 years, respectively (Pivonello et al. 2015a; Estrada et al. 1997; Orth and Liddle 1971; Minniti et al. 2007; Tsang et al. 1994). Moreover, radiotherapy seems to be associated with a higher incidence of secondary brain tumors, especially in patients who receive high radiation doses and CRT, although the specific incidence is not clear yet (Pivonello et al. 2015a; Estrada et al. 1997; Orth and Liddle 1971; Minniti et al. 2007; Tsang et al. 1994). A specific complication of SRT is the optic neuropathy, occurring with an incidence of up to 11.1% of cases, showing a variable clinical picture, ranging from quadrantanopsia to the complete visual loss (Pivonello et al. 2015a; Estrada et al. 1997; Orth and Liddle 1971; Minniti et al. 2007; Tsang et al. 1994).

Bilateral Adrenalectomy

Nowadays, bilateral adrenalectomy, which is reserved to a limited number of patients with a very severe CD, is generally considered as a second or third line option, after failure of previous treatments. Rarely, it can be used as first line option, when an immediate hypercortisolism resolution is necessary, and results in an immediate reduction of cortisol levels and rapid improvement of the clinical picture (Nieman et al. 2015). The laparoscopic approach is the most commonly used, and represents the gold standard, whereas the laparotomic approach is currently used only in case of contraindications for laparoscopic surgery, primarily obesity, and scarring due to previous surgery (Pivonello et al. 2015a). Remission rates range from 78.9% to 100% (m = 96.8%), with persistence or recurrence rates from 0% to 12%

(m = 1.7%), mainly due to adrenal rests (Pivonello et al. 2015a; Ernest and Ekman 1972; Kelly et al. 1983; Welbourn 1985; Nagesser et al. 2000; Chow et al. 2008; Smith et al. 2009; Ding et al. 2010). Bilateral adrenalectomy results in permanent adrenal insufficiency in all patients, by determining the requirement of lifelong GC and mineralocorticoid (MC) replacement treatments (Pivonello et al. 2015a). Another important complication, occurring in 0–34.6% (m = 18.6%) of patients, is represented by the ACTH-secreting pituitary tumor progression due to the loss of the negative feedback exerted by GCs on ACTH-secreting cells (Pivonello et al. 2015a; Ernest and Ekman 1972; Kelly et al. 1983; Welbourn 1985; Nagesser et al. 2000; Chow et al. 2008; Smith et al. 2009; Ding et al. 2010). Cardiovascular events, mainly myocardial infarction and secondary postoperative hemorrhages, are the main mortality causes, with a mortality rate from 0% to 11.1% of cases (m = 1.8%) (Pivonello et al. 2015a).

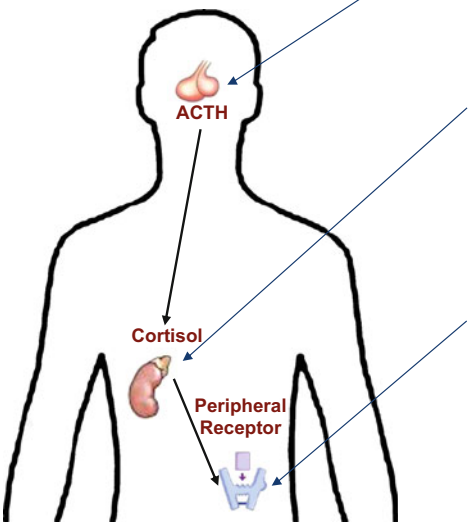
Medical Treatment

Medical treatment has historically played a minor role in the CD management; however, recently, thanks to the availability of novel compounds and to the employment of drugs previously used with different indications, it has been acquiring a more important role in different steps of the treatment schedule (Pivonello et al. 2015a). Particularly, medical treatment can be advocated before pituitary surgery, as preoperative treatment, especially in patients with severe disease, in order to control cortisol excess and improve the clinical picture, or after pituitary surgery, as adjuvant treatment, in patients with persistent or recurrent CD, before or after pituitary radiotherapy, or, lastly, as primary alternative treatment in case of contraindication to surgery, for instance, in patients with invisible tumors or with tumors with unfavorable location or extra-sellar expansion, or in case of refusal of surgery (Pivonello et al. 2015a). The spectrum of available drugs includes three main categories of compounds: (1) the pituitary-directed agents, which act at the pituitary level by inhibiting ACTH, and, only secondarily, cortisol secretion; (2) the adrenal-blocking agents or steroidogenesis inhibitors, which act at the adrenal level by blocking cortisol production, by inhibiting steroid hormone synthesis; (3) the glucocorticoid receptor antagonist, which blocks the activation of GC receptors (Ferone et al. 2014; Pivonello et al. 2015a). The spectrum of available drugs is detailed in Fig. 4.

Pituitary-Directed Drugs

Pituitary-directed drugs, acting at pituitary level and targeting the cause of CD, namely, the pituitary tumor, theoretically represents the ideal medical approach for CD management, although they are safely effective only in a group of patients with CD (Ferone et al. 2014). He two main pituitary-directed drugs are the dopamine agonist cabergoline and the somatostatin analogue pasireotide.

Cabergoline, a powerful agonist of dopamine receptor type 2 (D2), which are highly expressed in ACTH-secreting pituitary tumors, is currently an off-label treatment for CD (Pivonello et al. 2015a). Cabergoline, orally administered at dosages of 0.5–7 mg/week, has shown remission rates, in terms of cortisol excess



Pituitary-targeted drugs	
	Indication
Cabergoline	• Off-label
Pasireotide	• Cushing's disease

Adrenal-targeted drugs	
	Indication
Ketoconazole	• Cushing's syndrome
Metyrapone	• Cushing's syndrome
Mitotane	• Off-label

Glucocorticoid-receptor antagonist	
	Indication
Mifepristone	• Hyperglycemia in patients with Cushing's syndrome and diabetes mellitus or Impairment of glucose tolerance

Fig. 4 Classification of the drugs availbale for the treatment of CS: pituitary-directed agents, adrenal-blocking agents, glucocorticoid receptor antagonists

control, of 25–40% (m = 31.2%), associated with an improvement in the clinical picture, mainly hypertension and glucose intolerance, with an escape rate of 18.2–33.3% (m = 25%) in patients with a documented initial response (Pivonello et al. 2009; Vilar et al. 2010; Barbot et al. 2014; Lila et al. 2010; Godbout et al. 2010). Moreover, in 50% of patients, tumor shrinkage has been described (Pivonello et al. 2009). As far as safety profile was concerned, cabergoline is very well tolerated with rare adverse events reported, including hypotension and severe asthenia (10–20%, m = 15%), dizziness and nausea (5–25%, m = 13.3%) (Pivonello et al. 2009; Vilar et al. 2010; Godbout et al. 2010). The increased risk of cardiac valve diseases, which has been reported at the higher doses used for neurological disorders, is less important at the lower dosage used to treat CD (Pivonello et al. 2009; Vilar et al. 2010; Barbot et al. 2014; Lila et al. 2010; Godbout et al. 2010). Nevertheless, further controlled studies on larger populations of patients are needed to evaluate the clinical advantages, in terms of drug benefits and side effects.

Pasireotide, a new multiligand somatostatin analogue with high affinity for somatostatin receptor type 5 (SSTR5), which is highly expressed in ACTH-secreting pituitary tumors, is the first medical therapy officially approved for the treatment of adult CD patients, which experienced a failure of pituitary surgery, or are not candidates for pituitary surgery and require medical therapeutic intervention (Pivonello et al. 2015a). Pasireotide, subcutaneously administered twice daily, at a dosage of 300–900 μg, has shown remission rates, in terms of cortisol excess control,

ranging from 17% to 22.2% in the short-term follow-up (shorter or equal 12 months), reaching 34.5% (longer 24 months), 68.8% (up to 5 years), and 50% (up to 10 years) of cases in the long-term follow-up (Boscaro et al. 2009; Colao et al. 2012; Schopohl et al. 2015; Petersenn et al. 2017; Trementino et al. 2016). Notably, cortisol reduction appeared to be accompanied by an improvement in clinical picture and metabolic profile, including facial rubor, bruising, supraclavicular and dorsal fat pads, weight, body mass index, waist circumference, blood pressure, and total and LDL-cholesterol levels, as well as depression and QoL (Colao et al. 2012; Schopohl et al. 2015; Pivonello et al. 2014). Moreover, pasireotide has also been demonstrated to be able to reduce pituitary tumor volume in 46.3–100% (m = 73.15%) of patients at 12-months follow-up (Colao et al. 2012; Simeoli et al. 2015). As far as safety profile was concerned, pasireotide is generally well tolerated: the most frequently reported significant adverse events were hyperglycemia-related events (72.8%), diarrhea (58%), nausea (52%), cholelithiasis-related events (30%), mild transient elevations in liver enzyme levels (29%), headache (28%), abdominal pain (24%), fatigue (19%), asthenia (11%), hypocortisolism-related events (8%), and prolongation of the corrected QTc interval >480 msec (2%) (Colao et al. 2012). Compared with conventional somatostatin analogues, pasireotide is associated with a higher rate of hyperglycemia-related adverse events, due to the decrease in insulin and incretin secretion (Henry et al. 2013). During treatment, patients need to be carefully monitored, and an appropriate antidiabetic therapy should be initiated with metformin and staged treatment intensification with a dipeptidyl peptidase-4 inhibitor, with a switch to a GLP-1 receptor agonist and initiation of insulin, as required, to achieve and maintain glycemic control (Colao et al. 2014).

Pasireotide long acting release (LAR), a new formulation with monthly administration at a dosage of 10–30 mg, is currently under investigation for the treatment of CD.

Adrenal-Directed Drugs

Adrenal-directed drugs, acting at the adrenal level, block the cortisol production by inhibiting steroid hormone synthesis. He three main adrenal-directed drugs are ketoconazole, metyrapone, and mitotane. Additionally, new adrenal-directed drugs, represented by osilodrostat and levoketoconazole, are currently under investigation.

Ketoconazole, an imidazole derivative, firstly used as antifungal drug, acts by blocking different adrenal steroidogenesis enzymes (cholesterol side-chain cleavage complex, 17,20-lyase, 11β-hydroxylase, 17α-hydroxylase), and consequently reducing cortisol synthesis; the compound is officially approved for the treatment of patients older than 12 years, suffering from CS (Loose et al. 1983; Feldman 1986; Sonino 1987). Apart from adrenal blocking effects, ketoconazole may also have direct effects on corticotroph tumor cells in patients with CD. Surprisingly, evidences on mouse and human ACTH-secreting pituitary tumor cell lines have demonstrated its efficacy in inhibiting ACTH secretion and cell growth by the induction of apoptosis (Feelders et al. 2010a; Jimenez Reina et al. 1989). Ketoconazole, orally administered twice or thrice daily at a dosage of 200–1200 mg, has shown remission

rates, in terms of cortisol excess control, ranging from 44.7% to 92.9% (m = 64.3%), with escape rates of 7.1–22.7% (m = 14.5%) registered in initially responsive patients (Sonino et al. 1991; Moncet et al. 2007; Castinetti et al. 2008; van Bosch et al. 2014; Castinetti et al. 2014). However, it should be considered that these remission rates are extrapolated by studies analyzing patients suffering from all CS forms, including CD but also adrenal CS and ECS, resulting in overall CS remission rates of 84.6–94.1% (m = 89.3%) (Sonino et al. 1991; Moncet et al. 2007). Notably, cortisol reduction appeared accompanied by an improvement in signs and comorbidities of CD, including body weight, hirsutism, myopathy and muscle weakness, bone status, psychiatric symptoms, glucose metabolism, and blood pressure (Sonino et al. 1991; Moncet et al. 2007; Castinetti et al. 2008; van Bosch et al. 2014; Castinetti et al. 2014). As far as safety profile was concerned, the most severe and frequent adverse event related to ketoconazole is hepatotoxicity, occurring in 2.6–18.7% of cases, early after starting treatment or at dosage increase, requiring a strict monitoring of liver enzymes, particularly within the first month of treatment (Sonino et al. 1991; Moncet et al. 2007; Castinetti et al. 2008; van Bosch et al. 2014; Castinetti et al. 2014). Fatal hepatitis is rarely reported in patients using ketoconazole as antifungal drug (Duarte et al. 1984). Additional adverse events less frequently reported include gastrointestinal disturbances, skin rash, adrenal insufficiency, pruritus, fatigue, headache, and gynecomastia (Sonino et al. 1991; Moncet et al. 2007; Castinetti et al. 2008; van Bosch et al. 2014; Castinetti et al. 2014). Considering the impact of ketoconazole on gonadal testosterone synthesis, which results in hypogonadism, this drug should be preferred in women (Pivonello et al. 2015a).

Metyrapone, a pyridine derivative, acts by blocking different adrenal steroidogenesis enzymes (cholesterol side chain cleavage, 17α-hydroxylase, 18-hydroxylase, and particularly 11β-hydroxylase complex), and consequently reducing cortisol synthesis; the compound is officially approved for treatment of patients suffering from CS (Chart et al. 1958; Liddle et al. 1958; Carballeira et al. 1976; Gower 1974). Metyrapone, orally administered at a dosage of 500–6000 mg, four or six times daily, has shown remission rates, in terms of cortisol excess control, ranging from 45.4% to 100% (m = 71%), with escape rates of 0–18.7% (m = 7.8%) registered in initially responsive patients (van Bosch et al. 2014; Jeffcoate et al. 1977; Thorén et al. 1985; Verhelst et al. 1991; Valassi et al. 2012). However, it should be considered that, also for metyrapone, the mentioned remission rates are extrapolated by studies analyzing patients suffering from all CS forms, resulting in overall CS remission rates of 56.5–80% (m = 70.8%) (Thorén et al. 1985; Verhelst et al. 1991; Valassi et al. 2012). Notably, cortisol reduction appeared accompanied by an improvement in signs and symptoms of CD, including facial plethora, round face, muscle weakness, psychiatric symptoms, glucose metabolism, and blood pressure (Jeffcoate et al. 1977; Thorén et al. 1985; Verhelst et al. 1991; Valassi et al. 2012; Igaz et al. 2008). As far as safety profile was concerned, metyrapone is well tolerated, and the most frequent adverse events are hyperandrogenism in women, reported in 4.5–71.4%, hypertension in 48.4%, and hypokalemia in 6.7–13.6% of patients (van Bosch et al. 2014; Jeffcoate et al. 1977; Thorén et al. 1985; Verhelst et al. 1991; Valassi et al. 2012; Igaz et al. 2008). These adverse events are due to ACTH

increase, with consequent androgen and cortisol or aldosterone precursors overproduction. Therefore, considering the impact of metyrapone on androgen synthesis, this drug should be preferred in men (Feelders et al. 2010a; Igaz et al. 2008). Additional less frequently reported adverse events include dizziness, headache, arthralgia, myalgia, fatigue, gastrointestinal disturbances, adrenal insufficiency, and skin rash (van Bosch et al. 2014; Castinetti et al. 2014; Duarte et al. 1984; Chart et al. 1958; Liddle et al. 1958; Carballeira et al. 1976; Gower 1974; Jeffcoate et al. 1977; Thorén et al. 1985; Verhelst et al. 1991; Valassi et al. 2012).

Mitotane, a diphenylmethane derivative known as adrenolytic agent, officially approved for treatment of patients suffering from adrenocortical carcinoma, may be occasionally used in CS treatment. Mitotane acts by blocking different adrenal steroidogenesis enzymes (cholesterol side-chain cleavage, 11β-hydroxylase, 18-hydroxylase, and 3β-hydroxysteroid-dehydrogenase), and consequently reducing cortisol synthesis, and is an off-label drug used for the treatment of CS (Vilar and Tullner 1959; Young et al. 1973). Mitotane, orally administered at a dosage of 1–12 g/day, has shown remission rates, in terms of cortisol excess control, ranging from 71.6% to 100% (m = 86.9%), without escape phenomenon (Orth and Liddle 1971; Luton et al. 1979; Schteingart et al. 1980; Baudry et al. 2012). As far as safety profile was concerned, mitotane is associated with significant adverse events, mainly including gastrointestinal disturbances, lipid disorders, neurological manifestations, gynecomastia, liver enzymes increase, leukopenia, skin rash, hypersialorrhea, and chloasma (Orth and Liddle 1971; Luton et al. 1979; Schteingart et al. 1980; Baudry et al. 2012). In order to reduce the risk of developing adverse events, mitotane plasma concentrations should be monitored, and maintained between 8.5 mg/l and 18 mg/l; to avoid adrenal insufficiency, mitotane should be coadministered with exogenous GCs, mainly hydrocortisone (Luton et al. 1979; Baudry et al. 2012).

Osilodrostat is a new adrenal directed drug, which exerts a blocking action of 11β-hydroxylase enzyme, by consequently reducing cortisol synthesis, representing a novel and potential new treatment for CD, and theoretically for all forms of CS. Data from phase II studies in patients with CD have shown that osilodrostat, orally administered twice daily at a dosage of 2–50 mg, induced remission rates, in terms of cortisol excess control, ranging from 78.9% to 91.7% (m = 85.3%) (Fleseriu et al. 2016; Bertagna et al. 2014). Notably, cortisol reduction appeared accompanied by a modest improvement in blood pressure, glucose metabolism, and lipid profile (Fleseriu et al. 2016). This drug is well tolerated, showing as most common adverse events asthenia, gastrointestinal disturbances, adrenal insufficiency, headache, and hyperandrogenism (Fleseriu et al. 2016; Bertagna et al. 2014). Despite these promising results, a phase III study on a larger number of patients followed for a longer period of time is mandatory to evaluate the real potential efficacy and safety of this novel adrenal-directed drug, as well as to determine whether patients will experience a late escape from response.

Levoketoconazole, a 2S,4R enantiomer of ketoconazole, acts by blocking different steroidogenesis enzymes (CYP17A1, CYP21A2, and CYP11B1) and consequently reducing cortisol synthesis, likely with a higher potency than ketoconazole, probably

permitting to obtain a better cortisol levels control, using lower doses than ketoconazole. This drug is presently under investigation.

Glucocorticoid Receptor Antagonist

Glucocorticoid receptor antagonist antagonizes cortisol effects by blocking glucocorticoid receptors. The only available compound is mifepristone that acts by blocking central and peripheral type II GR, and consequently antagonizing cortisol receptor binding (Jung-Testas and Baulieu 1983; Bertagna et al. 1984). The compound is officially approved in the USA for the treatment of patients suffering from CS associated with diabetes mellitus or impairment of glucose tolerance. Due to its mechanism of action, plasma ACTH and serum cortisol levels cannot be used as efficacy parameters, by limiting the evaluation of drug efficacy to clinical parameters (Morgan and Laufgraben 2013). Mifepristone, orally administered at a dosage of 300–1200 mg once daily, has shown efficacy rates up to 87%, in terms of clinical picture improvement, including decrease in body weight, waist circumference, and body fat and increase in insulin sensitivity (Sitruk-Ware and Spitz 2003; Castinetti et al. 2009). Moreover, 60% of patients improved diabetes mellitus or impaired glucose metabolism, and 38.1% improved hypertension (Fleseriu et al. 2012). As far as safety profile was concerned, the most important adverse events are represented by endometrial thickening (38.5%), due to the antagonism of mifepristone on progesterone and androgen receptors, hypokalemia (25–34%) and hypertension (24–25%), both due to the "spill-over" effect exerted by cortisol excess on MC receptors. Additional common adverse events include gastrointestinal disturbances, fatigue, headache, arthralgia, peripheral edema, adrenal insufficiency, and dizziness (Castinetti et al. 2009, 2012; Carmichael and Fleseriu 2013; Fleseriu et al. 2012, 2013; van Uum et al. 2004).

Combined Medical Therapy

The rational of combined treatment is to improve the endogenous hypercortisolism control, compared with monotherapy, and to improve the safety profile, particularly by using lower doses compared to those classically used in monotherapy (Pivonello et al. 2015a). Available data, regarding combined therapy with the three different steroidogenesis inhibitors, ketoconazole, metyrapone, and mitotane, suggest a more rapid and efficacious cortisol excess control in all severe ACTH-dependent CS patients studied, associated with a rapid clinical improvement (Kamenicky et al. 2011). As far as safety profile was concerned, during this combined treatment, caution should be paid to side effects, mainly including hypokalemia (100%), nausea and vomiting (63.7%), acute adrenal insufficiency (36.4%), dizziness and confusion (9.1%), and increases in liver enzymes (18.2–81.8%), which have been reported in a significant percentage of patients (Kamenicky et al. 2011). Additional combined CD treatment schedules, associating drugs acting at adrenal and pituitary level, have shown interesting results (Vilar et al. 2010; Barbot et al. 2014; Feelders et al. 2010b). Combination of cabergoline and ketoconazole has shown remission rates, in terms of cortisol excess control, ranging from 75% to 79% of patients, associated with good tolerability (Vilar et al. 2010; Barbot et al. 2014). Another notable study has

evaluated the efficacy of a stepwise combination approach combining pasireotide, cabergoline, and ketoconazole and has reported remission rates, in terms of cortisol excess control, up to 88% of patients, with potential additional effects on tumor mass, associated with a better safety profile (Feelders et al. 2010b). Further studies on larger populations of patients are needed to evaluate clinical advantages, in terms of drug benefits and side effects (Pivonello et al. 2015a).

Ectopic Cushing's Syndrome

Treatment for ECS has the aim to prevent or inhibit tumor progression and control cortisol excess, and it mainly includes surgery, radiotherapy, chemotherapy, bilateral adrenalectomy, and medical treatment (Ilias et al. 2005; Nieman et al. 2015; Pavel et al. 2016; Garcia-Carbonero et al. 2016). Surgery is the first line treatment for ECS, consisting in resection of ACTH-secreting tumor after staging, with node dissection, as appropriate (Nieman et al. 2015). Ectopic ACTH-producing tumor resection is associated with remission rates ranging from 12% to 83%, depending on the causes of ECS (Ilias et al. 2005; Zeiger et al. 1992; Aniszewski et al. 2001; Isidori et al. 2006). In case of surgery failure, or when surgery is not possible due to a not removable, occult or metastatic tumor, different treatment approaches are required, mainly including bilateral adrenalectomy and medical treatment (Ilias et al. 2005; Verhelst et al. 1991; Castinetti et al. 2009; Kamenicky et al. 2011; Zeiger et al. 1992; Aniszewski et al. 2001; Isidori et al. 2006; van der Pas et al. 2012; Sharma and Nieman 2012; Pivonello et al. 2005a, 2007; von Werder et al. 1996). Bilateral adrenalectomy is suggested for occult or metastatic ECS or as a life-preserving emergency treatment, in patients with very severe ECS who cannot be promptly controlled by medical therapy (Nieman et al. 2015; Zeiger et al. 1992; Aniszewski et al. 2001). Medical therapies can be considered as first line treatment, with or without debulking surgery, radiotherapy, and or other loco-regional and ablative therapies, when surgery is not possible due to a not removable, occult or metastatic tumor. Medical therapies include drugs aiming at control of cortisol excess, such as steroidogenesis inhibitors, mifepristone and some targeted therapies, and systemic therapies aiming at inhibiting or preventing tumor progression, including targeted therapies and chemotherapy according with the different aetiology and the tumor grading (Ilias et al. 2005; Nieman et al. 2015; Pavel et al. 2016; Garcia-Carbonero et al. 2016).

Treatment with steroidogenesis inhibitors, including ketoconazole, metyrapone, and mitotane, administered alone or in combination, has shown remission rates ranging from 70% to 100%, even in the long-term follow-up, and has shown clinical and biochemical improvement, and sustained remission in some patients, even after medical discontinuation (Ilias et al. 2005; Verhelst et al. 1991; Kamenicky et al. 2011; Isidori et al. 2006; Sharma and Nieman 2012). Mild gastrointestinal disturbances, hypokalemia, and increases in cholesterol and liver enzyme levels are mainly reported as adverse events (Kamenicky et al. 2011; Sharma and Nieman 2012). Few available data regarding mifepristone have shown a clinical improvement in all treated patients, associated with a worsening of hypokalemia and

hypertension, which has been reported in 100% and 67.7% of cases, respectively (Castinetti et al. 2009).

Among targeted therapies somatostatin analogues (octreotide, lanreotide, and pasireotide), and cabergoline administered alone or in combination, have also been used to control cortisol excess, but data available mainly derived from small series or case reports (Ilias et al. 2005; Verhelst et al. 1991; Castinetti et al. 2009; Kamenicky et al. 2011; Isidori et al. 2006; van der Pas et al. 2012; Sharma and Nieman 2012; Pivonello et al. 2005a, 2007; von Werder et al. 1996; De Bruin et al. 2009). Octreotide and lanreotide are currently used in the management of patients with NETs to control tumor progression and some clinical syndromes associated with hormonal hypersecretion (Pavel et al. 2016; De Bruin et al. 2009). The role of somatostatin analogues in controlling ACTH secretion and CS has been scantly investigated, although case reports support the effectiveness of the somatostatin analogue octreotide, reporting remission rates up to 56% of patients (von Werder et al. 1996).

The use of cabergoline has been described in a small case series showing remission rates of 66.7% after 3 months of treatment, and of 33.3% after 6 months, due to treatment escape demonstrated in one patient; no significant adverse events have been reported (Pivonello et al. 2007). Cabergoline, in combination with the somatostatin analogue lanreotide, has demonstrated efficacy in a patient with a lung carcinoid that had developed resistance to either agents administered alone. This case supports the hypothesis that somatostatin analogues and dopamine agonists may reciprocally potentiate their actions in patients with ECS due to some types of NETs (Pivonello et al. 2005a). The role of cabergoline associated to pasireotide in the treatment of ECS remains to be determined and future studies should further explore their combined efficacy (Nieman et al. 2015; van der Pas et al. 2012).

Tyrosine kinase inhibitors (such as sunitinib, vandetanib, and cabozantinib) and mTOR inhibitors (everolimus) are currently used mainly to control tumor progression in patients with some types of NETs (Pavel et al. 2016; Valerio et al. 2017). The use of these drugs to control ECS has been scantly described. A rapid control of hypercortisolism using the tyrosine kinase inhibitors vandetanib and sorafenib in metastatic medullary thyroid carcinomas has been described in few case reports (Barroso-Sousa et al. 2014; Baudry et al. 2013; Nella et al. 2014).

The role that everolimus and other systemic and loco-regional therapies used to control tumor progression in patients with extra-pituitary ACTH-(or CRH-)secreting tumors, can play in controlling ECS remains to be established.

Adrenal Cushing's Syndrome

The first line treatment approach for adrenal CS due to adrenocortical adenomas, adrenocortical carcinomas, or bilateral adrenal hyperplasia is usually surgery, unless surgery is not possible or unlikely to significantly reduce the hypercortisolism (Nieman et al. 2015).

The first line treatment of adrenocortical adenomas is generally represented by adrenalectomy (Nieman et al. 2015). In referral centers, unilateral adrenalectomy for

all cases of unilateral benign diseases, generally performed by an experienced adrenal surgeon via either transperitoneal or retroperitoneal laparoscopy, is curative in nearly 100% of adults (Park et al. 2009). Laparoscopic adrenalectomy is considered as the gold standard treatment for adrenal lesions without suspicion of malignancy (Alemanno et al. 2017; Sautter et al. 2016). Complications are infrequent (15% in adrenal CS) and mainly include bleeding, injury to peritoneal and retroperitoneal organs, wound infection or hematoma, hernia, thromboembolic, urinary, gastrointestinal, pulmonary, and cardiovascular problems (Sautter et al. 2016). A higher complication rate may be associated to less experienced surgeons (Nieman et al. 2015; Sautter et al. 2016). Adrenal insufficiency can also occur and it requires preventive GCs administration. However, the requirement of long-term replacement treatment has to be confirmed with appropriate testing, 1–2 months after surgery (Terzolo et al. 2011).

In adrenocortical carcinomas, when possible, clinicians should attempt definitive treatment through a complete resection of the tumor, mainly by using laparotomic surgery, which is recommended to stage and remove the tumor (Else et al. 2014; Nieman et al. 2015; Miller et al. 2010). Unfortunately, even in patients that undergo to an apparent complete tumor resection the rate of recurrence ranges from 19% to 34%, giving the rational to use adjuvant treatments (Else et al. 2014). The reported rate of complications of adrenalectomy for adrenocortical carcinomas is 37.5%, with a significantly more pronounced risk in patients with CS (51.2% vs. nonfunctional, 32.0%) (Margonis et al. 2016). Surgery can also be considered in patients with tumor recurrence or advanced and metastatic disease in selected cases (Else et al. 2014).

Medical treatment of adrenocortical carcinomas includes drugs aiming at controlling cortisol excess and drugs aiming at preventing (adjuvant setting) and/or inhibiting (palliative setting) tumor progression (Else et al. 2014). Mitotane is effective in controlling CS in almost all patients, although the use of other steroidogenesis inhibitors either alone or in combination might be useful to obtain a more rapid resolution of hypercortisolism (Else et al. 2014). Mitotane as monotherapy (both in an adjuvant and palliative setting) or in combination with chemotherapy (in a palliative setting) is also used to prevent and/or inhibit adrenocortical carcinoma progression (Else et al. 2014). Adjuvant treatment with mitotane has been reported to improve the median tumor-free survival (Else et al. 2014). Palliative therapies include mitotane, chemotherapy, and radiotherapy (Else et al. 2014). Treatment with mitotane in patients with recurrent or persistent disease determines a normalization of cortisol levels in almost all the patients with associated CS and a stabilization or partial remission of disease in 30% of cases. Tumor response is generally observed when mitotane treatment is performed reaching and maintaining the therapeutic blood levels of mitotane between 14 and 20 mg/l (Else et al. 2014). The starting dose of mitotane is 2 g/day and is increased every 4–7 days by 0.5–1 g/day, until reaching a dose of 5–7 g/day (Else et al. 2014). Gastrointestinal disorders, as well as neurological disturbances, adrenal insufficiency, and hypercholesterolemia, represent the most frequent adverse events, which could be limited by controlling mitotane blood concentrations (Else et al. 2014). First line chemotherapy is represented by etoposide, doxorubicin, cisplatin, mitotane (EDPM) regimen for which response rates of 20–50%, (including

stable disease) have been reported. Lower response rates have been reported with second/third line treatments which mainly include mitotane plus streptozocin or gemcitabine-5 fluorouracil (Baudin et al. 2011; Else et al. 2014). Although traditionally considered ineffective for adrenocortical carcinomas, in recent series, radiotherapy has been shown to improve disease control, both in adjuvant and palliative settings. Nevertheless, further studies are required to define the role of radiotherapy in the management of adrenocortical carcinomas (Berruti et al. 2012; Else et al. 2014).

Bilateral adrenalectomy still remains the first line treatment of bilateral adrenal hyperplasia (Nieman et al. 2015). Medical therapy has been proposed only for BMAH, aimed at blocking the action of the aberrant hormone receptors, avoiding the life-threatening hypocortisolism (Nieman et al. 2015). In a case of ACTH-independent BMAH causing a GIP-related CS, somatostatin analogues, such as octreotide and pasireotide, have shown a short efficacy in terms of cortisol excess control, limited to postprandial phase, without hormonal control in the long-term period, and without effects on clinical and metabolic abnormalities associated with CS (Preumont et al. 2011). In a case of ACTH-independent BMAH induced by catecholamines through an aberrant expression of β-adrenergic receptors, propanolol has shown a long-term efficacy in terms of disease control (Lacroix et al. 1997). In a woman suffering from CS induced by luteinizing hormone and chorionic gonadotropin, a complete reversal has been obtained by using leuprolide acetate (Lacroix et al. 1999). Nevertheless, available data are based only on case reports (Preumont et al. 2011; Lacroix et al. 1997, 1999).

Complications and Mortality

CS is complicated by several comorbidities including metabolic syndrome, characterized by visceral obesity, impairment of glucose metabolism and dyslipidemia, strictly associated with systemic arterial hypertension and cardiovascular diseases, including vascular atherosclerosis and cardiac damage, which, together with thromboembolism, contribute to the increased cardiovascular risk. Additional clinical complications include musculoskeletal diseases, such as osteoporosis, skeletal fractures, and myopathy; neuropsychiatric diseases, such as impairment of cognitive function, depression, anxiety, and bipolar disorders; immune disorders with higher susceptibility to infections, possibly complicated by sepsis; impairment of reproductive and sexual function with consequent infertility or sexual disturbances (Feelders et al. 2012; Lacroix et al. 2015; Pivonello et al. 2008, 2015a, 2016a). These clinical complications negatively impact on QoL and increase the mortality, mainly due to cardiovascular events and sepsis (Lacroix et al. 2015; Pivonello et al. 2008, 2015a, 2016a).

Metabolic Syndrome

Cortisol excess is mainly associated with visceral obesity with preferential visceral fat disposition, rather than subcutaneous accumulation (Pivonello et al. 2005b,

2016a). The pathogenetic mechanisms underlying the typical fat distribution are still not completely understood. Weight excess, as documented by the BMI increase, is higher in females than in males, reported in 57–100% of patients (overweight in 33–48% and obesity in 25–100%), but without any significant differences considering the CS etiology (Colao et al. 1999; Faggiano et al. 2003; Mancini et al. 2004; Pivonello et al. 2005b, 2016a). Surgical remission can improve weight excess, although frequently without reaching weight normalization. Pharmacological treatments, mainly including ketoconazole, mitotane, mifepristone, cabergoline, and pasireotide, are demonstrated to be able in ameliorating weight excess and visceral fat deposition, both at short- and long-term follow-up (Colao et al. 1999; Faggiano et al. 2003; Giordano et al. 2011; Pivonello et al. 2016a). An impairment of glucose metabolism is described in 27–87% of CS patients: particularly, impaired fasting glucose in 6–14% of patients, impaired glucose metabolism in 7–64%, and diabetes in 11–47% (Mancini et al. 2004; Faggiano et al. 2003; Pivonello et al. 2010, 2016a). Although no differences have been demonstrated comparing different CS forms and between the genders of patients, the diabetes prevalence in ECS is reported higher (74%) than in CD (33%) and adrenal CS (34%) patients (Pivonello et al. 2016a). Surgical remission can improve but does not always normalize, glucose abnormalities, recovering faster in adrenal CS than in CD (Giordano et al. 2011; Faggiano et al. 2003; Colao et al. 1999; Pivonello et al. 2016a). Generally, pharmacological treatments, mainly including mifepristone and cabergoline, are associated with an improvement in glucose metabolism, with the exception of pasireotide, which showed a worsening in glycemic control in CD patients, particularly if they suffered from preexisting alterations of glucose metabolism (Pivonello et al. 2016a). Dyslipidemia is described in 12–72% of CS patients, mainly characterized by increase in total and LDL cholesterol, and triglyceride levels, and decrease in HDL cholesterol levels, without differences among CS forms (Mancini et al. 2004; Faggiano et al. 2003; Pivonello et al. 2016a). Dyslipidemia persists after surgical remission, probably due to the persistence of obesity. The effects of pharmacological treatments on lipid profile are variable: mitotane increased total, LDL, HDL, and triglycerides levels in CD patients, while pasireotide appeared able to reduce total and LDL levels (Pivonello et al. 2016a).

Cardiovascular Diseases

Cardiovascular diseases are commonly reported as the main causes of death in CS patients, mainly due to hypertension, vascular atherosclerosis, and cardiac remodeling and dysfunction (Pivonello et al. 2016a; Isidori et al. 2015b; De Leo et al. 2010). Hypertension is a very common clinical feature, occurring in 25–93% of CS patients with a similar prevalence in males and females, and different CS forms (Pivonello et al. 2016a). Surgical remission can improve, but it does not always normalize hypertension, which is reported to persist in 25–54% of patients (Isidori et al. 2015b; Mancini et al. 2004; Faggiano et al. 2003; Pivonello et al. 2016a). As far as medical treatment was concerned, adrenal-directed drugs, mainly 11β-hydroxylase

inhibitors, metyrapone, and osilodrostat, can worsen hypertension; mifepristone improved blood pressure in nearly 50% of treated patients, although, in some patients, hypertension was reported as worsened. Cabergoline and pasireotide improved hypertension. Nevertheless, pharmacological antihypertensive treatments are often required (Pivonello et al. 2015a, 2016a; Isidori et al. 2015b). CS is associated with an increased risk of developing myocardial infarction, cardiac failure, left ventricle hypertrophy, increased myocardial fibrosis, vascular atherosclerosis with endothelial dysfunction, and stroke; these cardiovascular damages are only partially reversible after successful CS treatment (Pivonello et al. 2016a; Pereira et al. 2010; Faggiano et al. 2003; Colao et al. 1999). Venous thromboembolism and thromboembolic events are reported in 6–20% of CS patients, representing another important mortality cause (Pivonello et al. 2016a). Alterations of coagulation and fibrinolysis with rise in factor VIII, fibrinogen, and von Willebrand factor levels, and a shortening of the activated partial thromboplastin time, are reported, accompanied by a rise in the number of platelets, thromboxane B2, and thrombin–antithrombin complexes, with a compensatory increase in the activity of endogenous coagulation inhibitors (Pivonello et al. 2016a). Hemostatic alterations seem to improve after CS remission, although they do not fully normalize. Conversely, successful pharmacological treatment does not seem to improve the hypercoagulable state (Pivonello et al. 2016a).

Musculoskeletal Diseases

An impairment of bone status is described in 64–100% of CS patients, particularly, osteopenia in 40–78%, osteoporosis in 22–57%, and skeletal fractures in 11–76% of patients (Pivonello et al. 2016a; Tauchmanovà et al. 2006, 2007; Minetto et al. 2004; Di Somma et al. 2002, 2003). Osteoporosis prevalence appeared higher in adrenal CS than in CD patients, as well as in males than in females; bone mineral density appeared lower and vertebral fractures prevalence higher, in ECS than in CD patients, as well as higher in males than in females (Pivonello et al. 2016a; Tauchmanovà et al. 2006). Surgical remission appeared able to improve bone density, generally more slowly in the femoral neck than in the lumbar spine, and more efficiently in males than in females, although the time to complete bone recovery is relatively long and variable (Pivonello et al. 2016a; Kristo et al. 2006; Di Somma et al. 2003; Kawamata et al. 2008). Few data regarding the effects of pharmacological treatments on bone disease, mainly focused on ketoconazole, are available (Pivonello et al. 2016a; Di Somma et al. 1998). Specific guidelines for treatment of osteoporosis induced by endogenous hypercortisolism are still needed (Pivonello et al. 2016a; Scillitani et al. 2014). Myopathy is frequently described in CS patients, with a prevalence ranging from 42% to 83%, higher in ECS than in adrenal CS, and in males than in females (Pivonello et al. 2016a). Musculoskeletal pain and acute bilateral carpal tunnel syndrome have been reported after surgical remission. However, the myopathy reversibility after surgical or medical CS remission and the use of anabolic factors or physical activity programs need further investigation (Pivonello et al. 2016a; Scillitani et al. 2015).

Neuropsychiatric Diseases

The most common psychiatric diseases in CS are depression, anxiety, and bipolar disorders, occurring in 50–81%, 66%, and 30% of patients, respectively (Pivonello et al. 2015b, 2016a). Improvements in neuropsychiatric disorders after CS remission, obtained by either surgery or pharmacological treatments, have been reported, but hypercortisolism resolution is not always followed by complete recovery, suggesting irreversible effects on central nervous system (Pivonello et al. 2016a). Beyond cortisol normalization, psychotherapeutic strategies including cognitive behavioral therapies and psychotropic drugs, such as antidepressant agents, can be useful, whereas benzodiazepines may be used to treat severe anxiety (Pivonello et al. 2016a).

Immune Disorders

Immunosuppression, observed in endogenous hypercortisolism and affecting both cellular and humoral components of immune system, is responsible for the increased risk of susceptibility to infections, with a reported prevalence of 21–51%, higher in ECS (23–51%) than in CD (21%), especially consisting in opportunistic infections (Pivonello et al. 2016a; Sarlis et al. 2000). Autoimmune disorders, mainly thyroid autoimmunity, are reported in 0–20% of patients during active hypercortisolism, and with higher prevalence, up to 60%, after hypercortisolism remission (Pivonello et al. 2016a; da Mota et al. 2011).

Reproductive and Sexual Function Disorders

Decreased libido (24–90%), hypogonadotropic hypogonadism in men (50–75%) causing erectile dysfunction and oligospermia, and menstrual irregularity in women (43–80%) are the most common reproductive and sexual disorders, the latter being more frequent in CD patients than in those suffering from adrenal CS (Pivonello et al. 2016a). These disorders, and several different factors, mainly including lower frequency of sexual activity and metabolic alterations, seem to impair fertility (Pivonello et al. 2014, 2016a). Ovarian status impairment appeared to be reversible, by improving after CS remission, and leading to successful pregnancies, conversely, in men, although testosterone levels are reported to spontaneously normalize after CS remission, no data are available on semen quality (Pivonello et al. 2016a).

Mortality

CS is associated with excessive mortality. In patients with CD, the overall standardized mortality ratio (SMR) ranges from 0.98 to 9.3 being similar or significantly

higher than the general population (Clayton et al. 2011; Graversen et al. 2012; Pivonello et al. 2016a). Cardiovascular disease is the major cause of death in these patients, whereas infectious diseases and sepsis represent frequent causes of death, and suicide associated with psychiatric disorders has also been described (Pivonello et al. 2016a). In patients with ECS, SMR ranges from 13.3 to 68.5, as expected for the frequently malignant origin or aggressive behavior of this disease (Pivonello et al. 2016a). Beyond neoplastic progression, causes of death are typically represented by infectious diseases or sepsis. In patients with adrenal CS, SMR ranges substantially from 1.35 to 7.5 in adrenocortical adenomas, from 1.14 to 12 in bilateral adrenal hyperplasia, and up to 48 in adrenocortical carcinomas (Pivonello et al. 2016a). The main causes of death are cardiovascular and cerebrovascular diseases, thromboembolism, infectious diseases or sepsis, neoplastic progression, and suicide (Pivonello et al. 2016a; Clayton et al. 2011; Graversen et al. 2012).

Summary

Endogenous CS is a severe endocrine disorder, caused by chronic hypercortisolism, consequence of an ACTH hypersecretion, or a direct consequence of autonomous cortisoloverproduction by the adrenal glands, and is associated with increased morbidity, mortality, and impaired QoL. Considering the strict association between cortisol excess exposure duration and mortality, a prompt screening, a confirmatory diagnosis, and an effective multidisciplinary therapeutic approach are mandatory in the attempt to improve clinical picture, morbidity, mortality, and QoL. The challenging diagnostic algorithm of CS includes sequential diagnostic initial and additional screening tests, aimed at confirming CS. Initial screening tests, mainly including UC, LNSC, Nugent test, and low-dose Liddle test, should be performed in patients displaying multiple clinical signs and symptoms, particularly suggestive and predictive of CS, aimed at confirming endogenous cortisol excess. In case of abnormal initial screening test results, or in case of normal initial screening test results associated with the persistence of a high clinical suspicion, the diagnostic algorithm should continue by performing one or two screening tests among the abovementioned, or by performing midnight serum cortisol or DST-CRH test, which are aimed at confirming CS and at excluding PCS. Additionally, DDAVP stimulation test could be also used to exclude PCS, because it seems to be promising in the differential diagnosis, especially between CD and PCS, even if it is not recommended as a routinely additional screening test. Once a clinical and biochemical CS diagnosis has been confirmed, the next step is to discriminate among the different causes of CS. Plasma ACTH firstly discriminate ACTH-dependent forms and ACTH-independent forms. In case of ACTH-independent forms, abdomen CT or MRI scanning should be performed to detect the adrenal tumor or hyperplasia. In case of ACTH-dependent forms, high-dose Liddle test, CRH, and DDAVP stimulation tests, as well as pituitary MRI and, eventually, BIPSS, should be performed to identify ACTH secreting sources. In case of ECS, conventional and functional imaging are needed to detect the ectopic tumor. CS treatment requires a

multidisciplinary and individualized approach. Surgery represents the first line treatment for all endogenous CS forms, including pituitary surgery, adrenal surgery, and ectopic tumors removal in order to eliminate the source of ACTH or cortisol excess. Second line treatment approaches are strictly dependent on CS etiology: CD approaches include repeat pituitary surgery, pituitary radiotherapy, bilateral adrenalectomy, and medical treatment; ECS approaches mainly include radiotherapy or chemotherapy, bilateral adrenalectomy, and medical treatment; adrenal CS approaches include chemotherapy, radiotherapy, and medical treatment. In recent years, the role of medical treatment has significantly increased, particularly in CD management, being a suitable choice as presurgical or postsurgical treatment, in case of unsuccessful pituitary surgery, as bridging treatment before and after the administration of radiotherapy while awaiting its definitive effects, and as primary treatment in case of severe disease, lack of indications, or contraindications, and in case of refusal of surgery and/or radiotherapy. Different categories of drugs have been used, including pituitary-directed drugs, mainly cabergoline and pasireotide, adrenal-directed drugs, mainly including ketoconazole, metyrapone, and mitotane, and the glucocorticoid receptor antagonist mifepristone. Currently, the availability of different drugs has raised the possibility of combined treatment aimed at improving the endogenous hypercortisolism control, compared with monotherapy, particularly by using different drugs acting at different levels, and to improve safety profile, particularly by using lower doses compared to those classically used in the monotherapy; nevertheless, further studies on larger populations of patients are needed to evaluate clinical advantages, in terms of drug benefits and side effects. Beyond the type of treatment, goals of CS management are the normalization of cortisol secretion, the reversal of clinical picture in terms of clinical signs and symptoms, the prevention or improvement of concomitant comorbidities, and the long-term control without recurrence.

References

Alemanno G, Bergamini C, Prosperi P, Valeri A. Adrenalectomy: indications and options for treatment. Updat Surg. 2017;69(2):119–25.

Alexandraki K, Grossmann A. The ectopic ACTH syndrome. Rev Endocr Metab Disord. 2010;11:117–26.

Anagnostis P, Athyros VG, Tziomalos K, Karagiannis A, et al. Clinical review: the pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. J Clin Endocrinol Metab. 2009;94(8):2692–701.

Aniszewski JP, Young WF Jr, Thompson GB, Grant CS, et al. Cushing syndrome due to ectopic adrenocorticotropic hormone secretion. World J Surg. 2001;25:934–40.

Arnaldi G, Angeli A, Atkinson AB, Bertagna X, et al. Diagnosis and complications of Cushing's syndrome: a consensus statement. J Clin Endocrinol Metab. 2003;88(12):5593–602.

Assié G, Libé R, Espiard S, Rizk-Rabin M, et al. ARMC5 mutations in macronodular adrenal hyperplasia with Cushing's syndrome. N Engl J Med. 2013;369:2105–14.

Assié G, Letouzé E, Fassnacht M, Jouinot A, et al. Integrated genomic characterization of adrenocortical carcinoma. Nat Genet. 2014;46(6):607–12.

Badrick E, Kirschbaum C, Kumari M. The relationship between smoking status and cortisol secretion. J Clin Endocrinol Metab. 2007;92(3):819–24.
Bano G, Mir F, Beharry N, et al. A novel medical treatment of Cushing's due to ectopic ACTH in a patient with Neurofibromatosis type 1. Int J Endocrinol Metab. 2013;11(1):52–6.
Barbot M, Albiger N, Ceccato F, Zilio M, et al. Combination therapy for Cushing's disease: effectiveness of two schedules of treatment: should we start with cabergoline or ketoconazole? Pituitary. 2014;17(2):109–17.
Barroso-Sousa R, Lerario AM, Evangelista J, Papadia C, et al. Complete resolution of hypercortisolism with sorafenib in a patient with advanced medullary thyroid carcinoma and ectopic ACTH (adrenocorticotropic hormone) syndrome. Thyroid. 2014;24(6):1062–6.
Baudin E, Leboulleux S, Al Ghuzlan A, Chougnet C, Young J, Deandreis D, Dumont F, Dechamps F, Caramella C, Chanson P, Lanoy E, Borget I, Schlumberger M. Therapeutic management of advanced adrenocortical carcinoma: what do we know in 2011? Horm Cancer. 2011;2(6):363–71. https://doi.org/10.1007/s12672-011-0094-2. Review.
Baudry C, Coste J, Bou Khalil R, Silvera S, et al. Efficiency and tolerance of mitotane in Cushing's disease in 76 patients from a single center. Eur J Endocrinol. 2012;167(4):473–81.
Baudry C, Paepegaey AC, Groussin L. Reversal of Cushing's syndrome by vandetanib in medullary thyroid carcinoma. N Engl J Med. 2013;369:584.
Benveniste RJ, King WA, Walsh J, Lee JS, et al. Repeated transsphenoidal surgery to treat recurrent or residual pituitary adenoma. J Neurosurg. 2005;102(6):1004–12.
Berruti A, Baudin E, Gelderblom H, Haak HR, Porpiglia F, Fassnacht M, Pentheroudakis G, ESMO Guidelines Working Group. Adrenal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012;23 Suppl 7:vii131–8.
Bertagna X, Bertagna C, Luton JP, Husson JM, et al. The new steroid analog RU 486 inhibits glucocorticoid action in man. J Clin Endocrinol Metab. 1984;59(1):25–8.
Bertagna X, Pivonello R, Fleseriu M, Zhang Y, et al. LCI699, a potent 11β-hydroxylase inhibitor, normalizes urinary cortisol in patients with Cushing's disease: results from a multicenter, proof-of-concept study. J Clin Endocrinol Metab. 2014;99:1375–83.
Bigos ST, Somma M, Rasio E, Eastman RC, et al. Cushing's disease: management by transsphenoidal pituitary microsurgery. J Clin Endocrinol Metab. 1980;50(2):348–54.
Biller BM, Alexander JM, Zervas NT, Hedley-Whyte ET, et al. Clonal origins of adrenocorticotropin-secreting pituitary tissue in Cushing's disease. J Clin Endocrinol Metab. 1992;75:1303–9.
Boscaro M, Ludlam WH, Atkinson B, Glusman JE, et al. Treatment of pituitary-dependent Cushing's disease with the multireceptor ligand somatostatin analog pasireotide (SOM230): a multicenter, phase II trial. J Clin Endocrinol Metab. 2009;94(1):115–22.
Cannavò S, Almoto B, Dall'Asta C, Corsello S, et al. Long-term results of treatment in patients with ACTH-secreting pituitary macroadenomas. Eur J Endocrinol. 2003;149(3):195–200.
Carballeira A, Fishman LM, Jacobi JD. Dual sites of inhibition by metyrapone of human adrenal steroidogenesis: correlation of in vivo and in vitro studies. J Clin Endocrinol Metab. 1976;42(4):687–95.
Carmichael JD, Fleseriu M. Mifepristone: is there a place in the treatment of Cushing's disease? Endocrine. 2013;44(1):20–32.
Castinetti F, Morange I, Jaquet P, Conte-Devolx B, et al. Ketoconazole revisited: a preoperative or postoperative treatment in Cushing's disease. Eur J Endocrinol. 2008;158(1):91–9.
Castinetti F, Fassnacht M, Johanssen S, Terzolo M, et al. Merits and pitfalls of mifepristone in Cushing's syndrome. Eur J Endocrinol. 2009;160(6):1003–10.
Castinetti F, Brue T, Conte-Devolx B. The use of the glucocorticoid receptor antagonist mifepristone in Cushing's syndrome. Curr Opin Endocrinol Diabetes Obes. 2012;19(4):295–9.
Castinetti F, Guignat L, Giraud P, Muller M, et al. Ketoconazole in Cushing's disease: is it worth a try? J Clin Endocrinol Metab. 2014;99(5):1623–30.
Cavagnini F, Pecori Giraldi F. Epidemiology and follow-up of Cushing's disease. Ann Endocrinol (Paris). 2001;62(2):168–72.

Chart JJ, Sheppard H, Allen MJ, Bencze WL, et al. New amphenone analogs as adrenocortical inhibitors. Experientia. 1958;14(4):151–2.

Chow JT, Thompson GB, Grant CS, Farley DR, et al. Bilateral laparoscopic adrenalectomy for corticotrophin-dependent Cushing's syndrome: a review of the Mayo Clinic experience. Clin Endocrinol. 2008;68(4):513–9.

Ciric I, Ragin A, Baumgartner C, Pierce D. Complications of transsphenoidal surgery: results of a national survey, review of the literature, and personal experience. Neurosurgery. 1997;40(2): 225–36; discussion 236–227.

Clayton RN, Raskauskiene D, Reulen RC, Jones PW. Mortality and morbidity in Cushing's disease over 50 years in Stoke-on-Trent, UK: audit and meta-analysis of literature. J Clin Endocrinol Metab. 2011;96:632–42.

Colao A, Pivonello R, Spiezia S, et al. Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. J Clin Endocrinol Metab. 1999;84:2664–72.

Colao A, Ochoa AS, Auriemma RS, Faggiano A, et al. Pituitary carcinomas. Front Horm Res. 2010;38:94–108.

Colao A, Petersenn S, Newell-Price J, Findling JW, et al. A 12-month phase 3 study of pasireotide in Cushing's disease. N Engl J Med. 2012;366(10):914–24.

Colao A, De Block C, Gaztambide MS, Kumar S, et al. Managing hyperglycemia in patients with Cushing's disease treated with pasireotide: medical expert recommendations. Pituitary. 2014;17(2):180–6.

da Mota F, Murray C, Ezzat S. Overt immune dysfunction after Cushing's syndrome remission: a consecutive case series and review of the literature. J Clin Endocrinol Metab 2011;96:E1670–74.

De Bruin C, Feelders RA, Lamberts SW, Hofland LJ. Somatostatin and dopamine receptors as targets for medical treatment of Cushing's syndrome. Rev Endocr Metab Disord. 2009;10(2): 91–102.

De Leo M, Pivonello R, Auriemma RS, Cozzolino A, et al. Cardiovascular disease in Cushing's syndrome: heart versus vasculature. Neuroendocrinology. 2010;92(Suppl 1):50–4.

De Martino MC, van Koetsveld PM, Feelders RA, Sprij-Mooij D, et al. The role of mTOR inhibitors in the inhibition of growth and cortisol secretion in human adrenocortical carcinoma cells. Endocr Relat Cancer. 2012;19(3):351–64.

De Martino MC, Al Ghuzlan A, Aubert S, Assie G, et al. Molecular screening for a personalized treatment approach in advanced adrenocortical cancer. J Clin Endocrinol Metab. 2013;98: 4080–8.

Di Somma C, Colao A, Pivonello R, et al. Effectiveness of chronic treatment with alendronate in the osteoporosis of Cushing's disease. Clin Endocrinol (Oxf). 1998;48:655–62.

Di Somma C, Pivonello R, Loche S, et al. Severe impairment of bone mass and turnover in Cushing's disease: comparison between childhood-onset and adulthood-onset disease. Clin Endocrinol (Oxf). 2002;56:153–58.

Di Somma C, Pivonello R, Loche S, et al. Effect of 2 years of cortisol normalization on the impaired bone mass and turnover in adolescent and adult patients with Cushing's disease: a prospective study. Clin Endocrinol (Oxf). 2003;58:302–08.

Ding XF, Li HZ, Yan WG, Gao Y, et al. Role of adrenalectomy in recurrent Cushing's disease. Chin Med J. 2010;123(13):1658–62.

Duarte PA, Chow CC, Simmons F, Ruskin J. Fatal hepatitis associated with ketoconazole therapy. Arch Intern Med. 1984;144(5):1069–70.

Dworakowska D, Grossman AB. The molecular pathogenesis of corticotroph tumours. Eur J Clin Investig. 2012;42(6):665–76.

Elamin MB, Murad MH, Mullan R, Erickson D, et al. Accuracy of diagnostic tests for Cushing's syndrome: a systematic review and metaanalyses. J Clin Endocrinol Metab. 2008;93(5): 1553–62.

El-Farhan N, Rees DA, Evans C. Measuring cortisol in serum, urine and saliva – are our assays good enough? Ann Clin Biochem. 2017;54(3):308–22.

Else T, Kim AC, Sabolch A, Raymon VM, et al. Adrenocortical carcinoma. Endocr Rev. 2014;35: 282–326.
Ernest I, Ekman H. Adrenalectomy in Cushing's disease. A long-term follow-up. Acta Endocrinol Suppl (Copenh). 1972;160:3–41.
Estrada J, Boronat M, Mielgo M, Magallón R, et al. The long-term outcome of pituitary irradiation after unsuccessful transsphenoidal surgery in Cushing's disease. N Engl J Med. 1997;336(3): 172–7.
Faggiano A, Pivonello R, Spiezia S, et al. Cardiovascular risk factors and common carotid artery caliber and stiff ness in patients with Cushing's disease during active disease and 1 year after disease remission. J Clin Endocrinol Metab. 2003;88:2527–33.
Feelders RA, Hofland LJ, de Herder WW. Medical treatment of Cushing's syndrome: adrenal-blocking drugs and ketaconazole. Neuroendocrinology. 2010a;92(suppl 1):111–5.
Feelders RA, de Bruin C, Pereira AM, Romijn JA, et al. Pasireotide alone or with cabergoline and ketoconazole in Cushing's disease. N Engl J Med. 2010b;362(19):1846–8.
Feelders RA, Pulgar SJ, Kempel A, Pereira AM. The burden of Cushing's disease: clinical and health-related quality of life aspects. Eur J Endocrinol. 2012;167:311–26.
Feldman D. Ketoconazole and other imidazole derivatives as inhibitors of steroidogenesis. Endocr Rev. 1986;7(4):409–20.
Ferone D, Pivonello C, Vitale G, Zatelli MC, Colao A, Pivonello R. Molecular basis of pharmacological therapy in Cushing's disease. Endocrine. 2014;46(2):181–98. https://doi.org/10.1007/s12020-013-0098-5. Epub 2013 Nov 23. Review.
Findling JW, Raff H. DIAGNOSIS OF ENDOCRINE DISEASE: differentiation of pathologic/neoplastic hypercortisolism (Cushing's syndrome) from physiologic/non-neoplastic hypercortisolism (formerly known as pseudo-Cushing's syndrome). Eur J Endocrinol. 2017;176(5): R205–16.
Fleseriu M, Biller BM, Findling JW, Molitch ME, et al. Mifepristone, a glucocorticoid receptor antagonist, produces clinical and metabolic benefits in patients with Cushing's syndrome. J Clin Endocrinol Metab. 2012;97(6):2039–49.
Fleseriu M, Molitch ME, Gross C, Schteingart DE, et al. A new therapeutic approach in the medical treatment of Cushing's syndrome: glucocorticoid receptor blockade with mifepristone. Endocr Pract. 2013;19(2):313–26.
Fleseriu M, Pivonello R, Young J, Hamrahian AH, et al. Osilodrostat, a potent oral 11beta-hydroxylase inhibitor: 22-week, prospective, Phase II study in Cushing's disease. Pituitary. 2016;19:138–48.
Friedman TC, Zuckerbraun E, Lee ML, Kabil MS, Shahinian H. Dynamic pituitary MRI has high sensitivity and specificity for the diagnosis of mild Cushing's syndrome and should be part of the initial workup. Horm Metab Res. 2007;39(6):451–6.
Garcia-Carbonero R, Sorbye H, Baudin E, Raymond E, et al. ENETS consensus guidelines for high-grade Gastroenteropancreatic neuroendocrine tumors and neuroendocrine carcinomas. Neuroendocrinology. 2016;103(2):186–94.
Giordano R, Picu A, Marinazzo E, et al. Metabolic and cardiovascular outcomes in patients with Cushing's syndrome of diff erent aetiologies during active disease and 1 year after remission. Clin Endocrinol (Oxf). 2011;75:354–60.
Godbout A, Manavela M, Danilowicz K, Beauregard H, et al. Cabergoline monotherapy in the long-term treatment of Cushing's disease. Eur J Endocrinol. 2010;163(5):709–16.
Gower DB. Modifiers of steroid-hormone metabolism: a review of their chemistry, biochemistry and clinical applications. J Steroid Biochem. 1974;5:501–23.
Graversen D, Vestergaard P, Stochholm K, Gravholt CH, Jørgensen JO. Mortality in Cushing's syndrome: a systematic review and meta-analysis. Eur J Intern Med. 2012;23: 278–82.
Hammer GD, Tyrrell JB, Lamborn KR, Applebury CB, et al. Transsphenoidal microsurgery for Cushing's disease: initial outcome and long-term results. J Clin Endocrinol Metab. 2004;89(12):6348–57.

Hatipoglu E, Kepicoglu H, Rusen E, et al. Von Hippel Lindau disease with metastatic pancreatic neuroendocrine tumor causing ectopic Cushing's syndrome. Neuro Endocrinol Lett. 2013;34(1):9–13.

Henry RR, Ciaraldi TP, Armstrong D, Burke P, et al. Hyperglycemia associated with pasireotide: results from a mechanistic study in healthy volunteers. J Clin Endocrinol Metab. 2013;98(8): 3446–53.

Hofmann BM, Hlavac M, Martinez R, Buchfelder M, et al. Long-term results after microsurgery for Cushing disease: experience with 426 primary operations over 35 years. J Neurosurg. 2008;108(1):9–18.

Holsboer F, Barden N. Antidepressants and hypothalamic-pituitary-adrenocortical regulation. Endocr Rev. 1996;17(2):187–205.

Holthouse DJ, Robbins PD, Kahler R, Knuckey N, et al. Corticotroph pituitary carcinoma: case report and literature review. Endocr Pathol. 2001;12:329–41.

Hopkins RL, Leinung MC. Exogenous Cushing's syndrome and glucocorticoid withdrawal. Endocrinol Metab Clin N Am. 2005;34(2):371–84.

Igaz P, Tömböl Z, Szabó PM, Likó I, et al. Steroid biosynthesis inhibitors in the therapy of hypercortisolism: theory and practice. Curr Med Chem. 2008;15(26):2734–47.

Ilias I, Torpy DJ, Pacak K, et al. Cushing's syndrome due to ectopic corticotropin secretion: twenty years' experience at the national institutes of health. J Clin Endocrinol Metab. 2005;90(8): 4955–62.

Invitti C, Pecori Giraldi F, de Martin M, Cavagnini F. Diagnosis and management of Cushing's syndrome: results of an Italian multicentre study. Study Group of the Italian Society of endocrinology on the pathophysiology of the hypothalamic-pituitary-adrenal Axis. J Clin Endocrinol Metab. 1999;84(2):440–8.

Isidori AM, Kaltsas GA, Pozza C, Frajese V, et al. The ectopic adrenocorticotropin syndrome: clinical features, diagnosis, management, and long-term follow-up. J Clin Endocrinol Metab. 2006;91:371–7.

Isidori AM, Sbardella E, Zatelli MC, Boschetti M, et al. Conventional and nuclear medicine imaging in ectopic Cushing's syndrome: a systematic review. J Clin Endocrinol Metab. 2015a;100(9):3231–44. Review.

Isidori AM, Graziadio C, Paragliola RM, Cozzolino A, Ambrogio AG, Colao A, Corsello SM, Pivonello R, ABC Study Group. The hypertension of Cushing's syndrome: controversies in the pathophysiology and focus on cardiovascular complications. J Hypertens. 2015b;33(1):44–60. https://doi.org/10.1097/HJH.0000000000000415. Review. PubMed PMID: 25415766; PubMed Central PMCID: PMC4342316.

Jacobson L. Hypothalamic-pituitary-adrenocortical axis regulation. Endocrinol Metab Clin N Am. 2005;34(2):271–92, vii.

Jagannathan J, Smith R, DeVroom HL, Vortmeyer AO, et al. Outcome of using the histological pseudocapsule as a surgical capsule in Cushing disease. J Neurosurg. 2009;111:531–9.

Jeffcoate WJ, Rees LH, Tomlin S, Jones AE, et al. Metyrapone in long-term management of Cushing's disease. Br Med J. 1977;2(6081):215–7.

Jimenez Reina L, Leal-Cerro A, Garcia J, Garcia-Luna PP, et al. In vitro effects of ketoconazole on corticotrope cell morphology and ACTH secretion of two pituitary adenomas removed from patients with Nelson's syndrome. Acta Endocrinol. 1989;121(2):185–90.

Jung-Testas I, Baulieu EE. Inhibition of glucocorticosteroid action in cultured L-929 mouse fibroblasts byRU486, a new anti-glucocorticosteroid of high affinity for the glucocorticosteroid receptor. Exp Cell Res. 1983;147(1):177–82.

Kamenicky P, Droumaquet C, Salenave S, Blanchard A, et al. Mitotane, metyrapone, and ketoconazole combination therapy as an alternative to rescue adrenalectomy for severe ACTH-dependent Cushing's syndrome. J Clin Endocrinol Metab. 2011;96:2796–804.

Kawamata A, Iihara M, Okamoto T, Obara T. Bone mineral density before and after surgical cure of Cushing's syndrome due to adrenocortical adenoma: prospective study. World J Sur. 2008; 32(5):890–6.

Kelly WF, MacFarlane IA, Longson D, Davies D, et al. Cushing's disease treated by total adrenalectomy: longterm observations of 43 patients. Q J Med. 1983;52(206):224–31.
Kristo C, Jemtland R, Ueland T, Godang K, Bollerslev J. Restoration of the coupling process and normalization of bone mass following successful treatment of endogenous Cushing's syndrome: a prospective, long-term study. Eur J Endocrinol. 2006;154:109–18.
Lacroix A, Tremblay J, Rousseau G, Bouvier M, et al. Propranolol therapy for ectopic beta-adrenergic receptors in adrenal Cushing's syndrome. N Engl J Med. 1997;337:1429–34.
Lacroix A, Hamet P, Boutin JM. Leuprolide acetate therapy in luteinizing hormone-dependent Cushing's syndrome. N Engl J Med. 1999;341:1577–81.
Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. Lancet. 2015;386(9996): 913–27.
Lambert JK, Goldberg L, Fayngold S, Kostadinov J, et al. Predictors of mortality and long-term outcomes in treated Cushing's disease: a study of 346 patients. J Clin Endocrinol Metab. 2013;98(3):1022–30.
Lerario AM, Moraitis A, Hammer GD. Genetics and epigenetics of adrenocortical tumors. Mol Cell Endocrinol. 2014;386(1–2):67–84.
Liddle GW, Island D, Lance EM, Harris AP. Alterations of adrenal steroid patterns in man resulting from treatment with a chemical inhibitor of 11 β hydroxylation. J Clin Endocrinol Metab. 1958;18(8):906–12.
Lila AR, Gopal RA, Acharya SV, George J, et al. Efficacy of cabergoline in uncured (persistent or recurrent) Cushing disease after pituitary surgical treatment with or without radiotherapy. Endocr Pract. 2010;16(6):968–76.
Liu H, Bravata DM, Cabaccan J, et al. Elevated late-night salivary cortisol levels in elderly male type 2 diabetic veterans. Clin Endocrinol. 2005;63(6):642–9.
Loose DS, Kan PB, Hirst MA, Marcus RA, et al. Ketoconazole blocks adrenal steroidogenesis by inhibiting cytochrome P450-dependent enzymes. J Clin Invest. 1983;71(5):1495–9.
Luton JP, Mahoudeau JA, Bouchard P, Thieblot P, et al. Treatment of Cushing's disease by O,p'DDD. Survey of 62 cases. N Engl J Med. 1979;300(9):459–64.
Mampalam TJ, Tyrrell JB, Wilson CB. Transsphenoidal microsurgery for Cushing disease. A report of 216 cases. Ann Intern Med. 1988;109(6):487–93.
Mancini T, Kola B, Mantero F, Boscaro M, Arnaldi G. High cardiovascular risk in patients with Cushing's syndrome according to 1999 WHO/ISH guidelines. Clin Endocrinol (Oxf). 2004;61:768–77.
Margonis GA, Kim Y, Tran TB, et al. Outcomes after resection of cortisol-secreting adrenocortical carcinoma. Am J Surg. 2016;211(6):1106–13.
Martin NM, Dhillo WS, Banerjee A, Abdulali A, Jayasena CN, Donaldson M, Todd JF, Meeran K. Comparison of the dexamethasone-suppressed corticotropin-releasing hormone test and low-dose dexamethasone suppression test in the diagnosis of Cushing's syndrome. J Clin Endocrinol Metab. 2006;1:2582–6.
Mericq MV, Cutler GB. High fluid intake increases urine free cortisol excretion in normal subjects. J Clin Endocrinol Metab. 1998;83(2):682–4.
Miller BS, Ammori JB, Gauger PG, Broome JT, et al. Laparoscopic resection is inappropriate in patients with known or suspected adrenocortical carcinoma. World J Surg. 2010;34:1380–5.
Minetto M, Reimondo G, Osella G, Ventura M, Angeli A, Terzolo M. Bone loss is more severe in primary adrenal than in pituitary-dependent Cushing's syndrome. Osteoporos Int. 2004;15:855–61.
Minniti G, Osti M, Jaffrain-Rea ML, Esposito V, et al. Long-term follow-up results of postoperative radiation therapy for Cushing's disease. J Neuro-Oncol. 2007;84(1):79–84.
Moncet D, Morando DJ, Pitoia F, Katz SB, et al. Ketoconazole therapy: an efficacious alternative to achieve eucortisolism in patients with Cushing's syndrome. Medicina (B Aires). 2007;67(1): 26–31.
Morgan FH, Laufgraben MJ. Mifepristone for management of Cushing's syndrome. Pharmacotherapy. 2013;33(3):319–29.

Nagesser SK, van Seters AP, Kievit J, Hermans J, et al. Long-term results of total adrenalectomy for Cushing's disease. World J Surg. 2000;24(1):108–13.
Nella AA, Lodish MB, Fox E, Balis FM, et al. Vandetanib successfully controls medullary thyroid cancer-related Cushing syndrome in an adolescent patient. J Clin Endocrinol Metab. 2014;99(9):3055–9.
Newell-Price J, Trainer P, Besser M, Grossman A. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. Endocr Rev. 1998;19(5):647–72.
Newell-Price J, King P, Clark AJ. The CpG island promoter of the human proopiomelanocortin gene is methylated in nonexpressing normal tissue and tumors and represses expression. Mol Endocrinol. 2001;15:338–48.
Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. Lancet. 2006;367:1605–17.
Nieman LK, Biller BM, Findling JW, Newell-Price J, et al. The diagnosis of Cushing's syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2008;93(5):1526–40.
Nieman LK, Biller BM, Findling JW, Murad MH, et al. Treatment of Cushing's syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2015;100(8):2807–31.
Orth DN, Liddle GW. Results of treatment in 108 patients with Cushing's syndrome. N Engl J Med. 1971;285(5):243–7.
Papadimitriou A, Priftis KN. Regulation of the hypothalamic-pituitary-adrenal axis. Neuroimmunomodulation. 2009;16(5):265–71.
Papanicolau DA, Yanovski JA, Cutler GB, et al. A single midnight serum cortisol measurement distinguishes Cushing's syndrome from pseudo-Cushing states. J Clin Endocrinol Metab. 1998;83(4):1163–7.
Park HS, Roman SA, Sosa JA. Outcomes from 3144 adrenalectomies in the United States: which matters more, surgeon volume or specialty? Arch Surg. 2009;144:1060–7.
Patil CG, Prevedello DM, Lad SP, Vance ML, et al. Late recurrences of Cushing's disease after initial successful transsphenoidal surgery. J Clin Endocrinol Metab. 2008a;93(2):358–62.
Patil CG, Veeravagu A, Prevedello DM, Katznelson L, et al. Outcomes after repeat transsphenoidal surgery for recurrent Cushing's disease. Neurosurgery. 2008b;63:266–70; discussion 270–271.
Pavel M, O'Toole D, Costa F, Capdevila J, et al. ENETS consensus guidelines update for the management of distant metastatic disease of intestinal, pancreatic, bronchial neuroendocrine neoplasms (NEN) and NEN of unknown primary site. Neuroendocrinology. 2016;103(2):172–85.
Pecori Giraldi F, Pivonello R, Ambrogio AG, De Martino MC, De Martin M, Scacchi M, Colao A, Toja PM, Lombardi G, Cavagnini F. The dexamethasone-suppressed corticotropin-releasing hormone stimulation test and the desmopressin test to distinguish Cushing's syndrome from pseudo-Cushing's states. Clin Endocrinol (Oxf). 2007;66(2):251–7. Erratum in: Clin Endocrinol (Oxf). 2007;67(3):477.
Pecori Giraldi F, Cavallo LM, Tortora F, Pivonello R, Colao A, Cappabianca P, Mantero F, Altogether to Beat Cushing's Syndrome Group. The role of inferior petrosal sinus sampling in ACTH-dependent Cushing's syndrome: review and joint opinion statement by members of the Italian Society for Endocrinology, Italian Society for Neurosurgery, and Italian Society for Neuroradiology. Neurosurg Focus. 2015;38(2):E5. https://doi.org/10.3171/2014.11.FOCUS14766. Review.
Pereira AM, Delgado V, Romijn JA, Smit JW, Bax JJ, Feelders RA. Cardiac dysfunction is reversed upon successful treatment of Cushing's syndrome. Eur J Endocrinol. 2010;162:331–40.
Perez-Rivas LG, Reincke M. Genetics of Cushing's disease: an update. J Endocrinol Investig. 2016;39(1):29–35.
Petersenn S, Salgado LR, Schopohl J, Portocarrero-Ortiz L, et al. Long-term treatment of Cushing's disease with pasireotide: 5-year results from an open-label extension study of a Phase III trial. Endocrine. 2017;57(1):156–65.
Pivonello R, Ferone D, Lamberts SW, Colao A. Cabergoline plus lanreotide for ectopic Cushing's syndrome. N Engl J Med. 2005a;352:2457–8.

Pivonello R, Faggiano A, Lombardi G, Colao A. The metabolic syndrome and cardiovascular risk in Cushing's syndrome. Endocrinol Metab Clin N Am. 2005b;34(2):327–39.
Pivonello R, Ferone D, de Herder WW, Faggiano A, et al. Dopamine receptor expression and function in corticotroph ectopic tumors. J Clin Endocrinol Metab. 2007;92:65–9.
Pivonello R, De Martino MC, De Leo M, Lombardi G, et al. Cushing's Syndrome. Endocrinol Metab Clin N Am. 2008;37(1):135–49.
Pivonello R, De Martino MC, Cappabianca P, De Leo M, et al. The medical treatment of Cushing's disease: effectiveness of chronic treatment with the dopamine agonist cabergoline in patients unsuccessfully treated by surgery. J Clin Endocrinol Metab. 2009;94(1):223–30.
Pivonello R, De Leo M, Vitale P, Cozzolino A, et al. Pathophysiology of diabetes mellitus in Cushing's syndrome. Neuroendocrinology. 2010;92(Suppl 1):77–81.
Pivonello R, Petersenn S, Newell-Price J, Findling JW, et al. Pasireotide treatment significantly improves clinical signs and symptoms in patients with Cushing's disease: results from a Phase III study. Clin Endocrinol. 2014;81(3):408–17.
Pivonello R, De Leo M, Cozzolino A, Colao A. The treatment of Cushing's disease. Endocr Rev. 2015a;36(4):385–486.
Pivonello R, Simeoli C, De Martino MC, Cozzolino A, et al. Neuropsychiatric disorders in Cushing's syndrome. Front Neurosci. 2015b;9:129.
Pivonello R, Isidori AM, De Martino MC, Newell-Price J, et al. Complications of Cushing's syndrome: state of the art. Lancet Diabetes Endocrinol. 2016a;4(7):611–29.
Pivonello R, De Martino MC, Iacuaniello D, Simeoli C, et al. Metabolic alterations and cardiovascular outcomes of cortisol excess. Front Horm Res. 2016b;46:54–65.
Pivonello R, De Martino MC, De Leo M, Simeoli C, et al. Cushing's disease: the burden of illness. Endocrine. 2017;56(1):10–8.
Preumont V, Mermejo LM, Damoiseaux P, Lacroix A, et al. Transient efficacy of octreotide and pasireotide (SOM230) treatment in GIP-dependent Cushing's syndrome. Horm Metab Res. 2011;43:287–91.
Raff H, Sharma ST, Nieman LK. Physiological basis for the etiology, diagnosis, and treatment of adrenal disorders: Cushing's syndrome, adrenal insufficiency, and congenital adrenal hyperplasia. Compr Physiol. 2014;4(2):739–69.
Raffin-Sanson ML, de Keyzer Y, Bertagna X. Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. Eur J Endocrinol. 2003;149:79–90.
Reimondo G, Bovio S, Allasino B, De Francia S, Zaggia B, Micossi I, Termine A, De Martino F, Paccotti P, Di Carlo F, Angeli A, Terzolo M. The combined low-dose dexamethasone suppression corticotropinreleasing hormone test as a tool to rule out Cushing's syndrome. Eur J Endocrinol. 2008;159(5):569–76. https://doi.org/10.1530/EJE-08-0402. Epub 2008 Aug 19.
Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, et al. Mutations in the deubiquitinase gene USP8 cause Cushing's disease. Nat Genet. 2014;47:31–8.
Römer B, Lewicka S, Kopf D, Lederbogen F, et al. Cortisol metabolism in depressed patients and healthy controls. Neuroendocrinology. 2009;90(3):301–6.
Sarlis NJ, Chanock SJ, Nieman LK. Cortisolemic indices predict severe infections in Cushing syndrome due to ectopic production of adrenocorticotropin. J Clin Endocrinol Metab. 2000;85:42–47.
Sautter AE, Cunningham SC, Kowdley GC. Laparoscopic adrenalectomy for adrenal cancer-a systematic review. Am Surg. 2016;82(5):420–6.
Scangas GA, Laws ER Jr. Pituitary incidentalomas. Pituitary. 2014;17(5):486–91.
Schopohl J, Gu F, Rubens R, Van Gaal L, et al. Pasireotide can induce sustained decreases in urinary cortisol and provide clinical benefit in patients with Cushing's disease: results from an open-ended, open-label extension trial. Pituitary. 2015;18(5):604–12.
Schteingart DE, Tsao HS, Taylor CI, McKenzie A, et al. Sustained remission of Cushing's disease with mitotane and pituitary irradiation. Ann Intern Med. 1980;92(5):613–9.

Scillitani A, Mazziotti G, Di Somma C, Moretti S, Stigliano A, Pivonello R, Giustina A, Colao A, ABC Group. Treatment of skeletal impairment in patients with endogenous hypercortisolism: when and how? Osteoporos Int. 2014;25(2):441–6. https://doi.org/10.1007/s00198-013-2588-y. Epub 2013 Dec 6.

Scillitani A, Mazziotti G, Di Somma C, Moretti S, Stigliano A, Pivonello R, Giustina A, Colao A, ABC 2011. Cortisol and the muscle-bone axis: response to comments by Molfino et al. Osteoporos Int. 2015;26(5):1661–2. https://doi.org/10.1007/s00198-014-2876-1. Epub 2015 Mar 6.

Sharma ST, Nieman LK. Prolonged remission after long-term treatment with steroidogenesis inhibitors in Cushing's syndrome caused by ectopic ACTH secretion. Eur J Endocrinol. 2012;166:531–6.

Simeoli C, Auriemma RS, Tortora F, De Leo M, et al. The treatment with pasireotide in Cushing's disease: effects of long-term treatment on tumor mass in the experience of a single center. Endocrine. 2015;50(3):725–40.

Sitruk-Ware R, Spitz IM. Pharmacological properties of mifepristone: toxicology and safety in animal and human studies. Contraception. 2003;68(6):409–20.

Smith PW, Turza KC, Carter CO, Vance ML, et al. Bilateral adrenalectomy for refractory Cushing disease: a safe and definitive therapy. J Am Coll Surg. 2009;208(6):1059–64.

Sonino N. The use of ketoconazole as an inhibitor of steroid production. N Engl J Med. 1987;317(13):812–8.

Sonino N, Boscaro M, Paoletta A, Mantero F, et al. Ketoconazole treatment in Cushing's syndrome: experience in 34 patients. Clin Endocrinol. 1991;35(4):347–52.

Takagi J, Otake K, Morishita M, et al. Multiple endocrine neoplasia type I and Cushing's syndrome due to an aggressive ACTH producing thymic carcinoid. Intern Med. 2006;45(2):81–6.

Tauchmanovà L, Pivonello R, Di Somma C, et al. Bone demineralization and vertebral fractures in endogenous cortisol excess: role of disease etiology and gonadal status. J Clin Endocrinol Metab. 2006;91:1779–84.

Tauchmanovà L, Pivonello R, De Martino MC, et al. Effects of sex steroids on bone in women with subclinical or overt endogenous hypercortisolism. Eur J Endocrinol. 2007;157:359–66.

Terzolo M, Stigliano A, Chiodini I, Loli P, et al. AME position statement on adrenal incidentaloma. Eur J Endocrinol. 2011;164(6):851–70.

Thorén M, Adamson U, Sjöberg HE. Aminoglutethimide and metyrapone in the management of Cushing's syndrome. Acta Endocrinol. 1985;109(4):451–7.

Tirabassi G, Faloia E, Papa R, Furlani G, et al. Use of the desmopressin test in the differential diagnosis of pseudo-Cushing state from Cushing's disease. J Clin Endocrinol Metab. 2010;95(3):1115–22.

Trementino L, Michetti G, Angeletti A, Marcelli G, et al. A single-center 10-year experience with Pasireotide in Cushing's disease: Patients' characteristics and outcome. Horm Metab Res. 2016;48(5):290–8.

Tsang RW, Brierley JD, Panzarella T, Gospodarowicz MK, et al. Radiation therapy for pituitary adenoma: treatment outcome and prognostic factors. Int J Radiat Oncol Biol Phys. 1994;30(3):557–65.

Valassi E, Crespo I, Gich I, Rodríguez J, et al. A reappraisal of the medical therapy with steroidogenesis inhibitors in Cushing's syndrome. Clin Endocrinol. 2012;77(5):735–42.

Valerio L, Pieruzzi L, Giani C, Agate L, et al. Targeted therapy in thyroid cancer: state of the art. Clin Oncol (R Coll Radiol). 2017;29(5):316–24.

van den Bosch OF, Stades AM, Zelissen PM. Increased long-term remission after adequate medical cortisol suppression therapy as presurgical treatment in Cushing's disease. Clin Endocrinol. 2014;80(2):184–90.

van der Pas R, de Herder WW, Hofl LJ, Feelders RA. New developments in the medical treatment of Cushing's syndrome. Endocr Relat Cancer. 2012;19:R205–23.

van Uum SH, Lenders JW, Hermus AR. Cortisol, 11β-hydroxysteroid dehydrogenases, and hypertension. Semin Vasc Med. 2004;4(2):121–8.

Verhelst JA, Trainer PJ, Howlett TA, Perry L, et al. Short and long-term responses to metyrapone in the medical management of 91 patients with Cushing's syndrome. Clin Endocrinol. 1991;35(2): 169–78.

Vilar O, Tullner WW. Effects of o,p' DDD on histology and 17-hydroxycorticosteroid output of the dog adrenal cortex. Endocrinology. 1959;65(1):80–6.

Vilar L, Freitas MC, Faria M, et al. Pitfalls in the diagnosis of Cushing's syndrome. Arq Bras Endocrinol Metabol. 2007;51(8):1207–16.

Vilar L, Naves LA, Azevedo MF, Arruda MJ, et al. Effectiveness of cabergoline in monotherapy and combined with ketoconazole in the management of Cushing's disease. Pituitary. 2010;13(2):123–9.

Vitale G, Tortora F, Baldelli R, Cocchiara F, et al. Pituitary magnetic resonance imaging in Cushing's disease. Endocrine. 2017;55(3):691–6.

von Werder K, Muller OA, Stalla GK. Somatostatin analogs in ectopic corticotropin production. Metabolism. 1996;45(Suppl 1):129–31.

Wagenmakers MA, Boogaarts HD, Roerink SH, Timmers HJ, et al. Endoscopic transsphenoidal pituitary surgery: a good and safe primary treatment option for Cushing's disease, even in case of macroadenomas or invasive adenomas. Eur J Endocrinol. 2013;169(3):329–37.

Welbourn RB. Survival and causes of death after adrenalectomy for Cushing's disease. Surgery. 1985;97(1):16–20.

Wood PJ, Barth JH, Freedman DB, Perry L, Sheridan B. Evidence for the low dose dexamethasone suppression test to screen for Cushing's syndrome – recommendations for a protocol for biochemistry laboratories. Ann Clin Biochem. 1997;34(Pt 3):222–9.

Yanovski JA, Cutler GB Jr, Chrousos GP, Nieman LK. Corticotropin-releasing hormone stimulation following low-dose dexamethasone administration. A new test to distinguish Cushing's syndrome from pseudo-Cushing's states. JAMA. 1993;269(17):2232–8.

Ye L, Li X, Kong X, Wang W, et al. Hypomethylation in the promoter region of POMC gene correlates with ectopic overexpression in thymic carcinoids. J Endocrinol. 2005;185:337–43.

Young RB, Bryson MJ, Sweat ML, Street JC. Complexing of DDT and o,p'DDD with adrenal cytochrome P-450 hydroxylating systems. J Steroid Biochem. 1973;4(6):585–91.

Young WF Jr, Scheithauer BW, Gharib H, Laws ER Jr, et al. Cushing's syndrome due to primary multinodular corticotrope hyperplasia. Mayo Clin Proc. 1988;63:256–62.

Zeiger MA, Pass HI, Doppman JD, Nieman LK, et al. Surgical strategy in the management of non-small cell ectopic adrenocorticotropic hormone syndrome. Surgery. 1992;112:994–1000; discussion 1000–1001.

Physiopathology, Diagnosis, and Treatment of Diabetes Insipidus

12

Ana M. Ramos-Leví and Mónica Marazuela

Contents

A. M. Ramos-Leví (✉) · M. Marazuela
Department of Endocrinology, Hospital Universitario La Princesa, Instituto de Investigación Princesa, Universidad Autónoma, Madrid, Spain
e-mail: ana_ramoslevi@hotmail.com; monicamarazuela@salud.madrid.org

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_13

Abstract

Diabetes insipidus (DI) is a clinical disorder characterized by an excessive hypotonic and diluted urine output. There are four main mechanisms involved in the pathophysiology of DI: (1) central DI (CDI), when the main alteration occurs in the hypothalamus or posterior pituitary and an insufficient amount of the antidiuretic hormone (ADH or AVP) is synthesized or released; (2) nephrogenic DI (NDI), when the kidney does not respond correctly to AVP; (3) transient DI of pregnancy, where an altered AVP metabolism occurs, presumably during a limited period of time; and (4) primary polydipsia, where the initial pathogenic mechanism is related to the intake of fluid, and not as much in its excretion. Consideration of the basic physiopathologic mechanisms involved in AVP regulation enables subsequent understanding of the potential diagnostic and treatment alternative tools for a patient with DI. In this chapter, we will thoroughly revise the different clinical settings of DI, and we will discuss the algorithms that need to be followed for a correct approach and management of patients with this syndrome.

Keywords

Diabetes insipidus · Central diabetes insipidus · Nephrogenic diabetes insipidus · Desmopressin, pituitary surgery · Aquaporin · Primary polydipsia · Arginine-vasopressin · Antidiuretic hormone

Introduction

Diabetes insipidus (DI) is a clinical disorder characterized by an excessive urine output (diabetes) in which, as opposed to what is observed in diabetes mellitus, urine is hypotonic (<250 mmol/kg), diluted, and insipid. There are several mechanisms involved in the pathogenesis of the different clinical forms of DI, all of which are related to an insufficient amount or action of the antidiuretic hormone or arginine-vasopressin (ADH or AVP).

Consideration of the basic physiopathologic mechanisms involved in AVP regulation enables subsequent understanding of the potential diagnostic and treatment alternative tools that may be considered in the approach of a patient with DI. In this chapter, we will thoroughly revise the different clinical settings of DI, and we will discuss the algorithms that need to be followed for a correct approach and management of patients with this syndrome.

Basics in Anatomy and Physiology of the Posterior Pituitary

Normal Anatomy

The posterior pituitary (neurohypophysis) comprises the distal axons from hypothalamic magnocellular neurons whose cellular bodies are located in the supraoptic and paraventricular nuclei (Fig. 1). Although the anterior pituitary receives the majority of its vascularization from the hypothalamic-pituitary portal system, the posterior pituitary is mainly irrigated directly from posterior pituitary arteries derived from the internal carotid artery and the posterior communicant artery. Venous drainage is mainly conducted through the cavernous sinus and the internal jugular vein.

Cerebral magnetic resonance imaging (MRI) has enabled the identification of the posterior pituitary as hyperintense (bright) in T1-weighted images. Furthermore, it allowed the documentation of some patients who exhibited this bright spot in ectopic locations, mostly in cases with associated anterior pituitary deficiencies and growth retardation. Abnormalities in the location of the posterior pituitary are probably due to genetic alterations in its migration down the pituitary stalk (Bichet 2011).

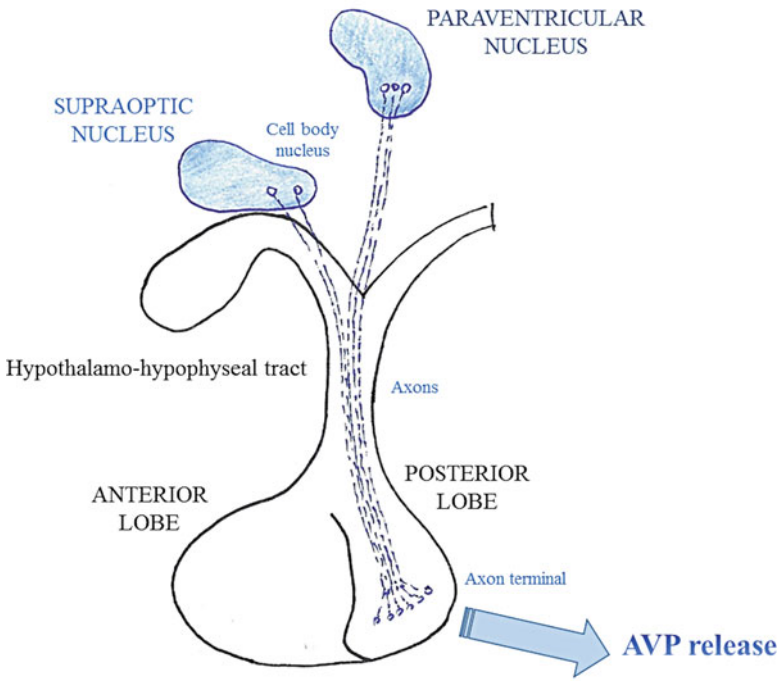


Fig. 1 Schematic representation of the hypothalamic-pituitary axis, and where AVP is secreted. The precursor of AVP is packaged into neurosecretory granules and transported axonally into the posterior pituitary, through the pituitary stalk, and transformed into the active hormone

Antidiuretic Hormone or Arginine-Vasopressin (ADH or AVP): A Complex Regulatory System

AVP is a nonapeptidic hormone that consists of a six-amino-acid ring with a cysteine-cysteine bonding and a three-amino-acid terminal region. It is synthesized as precursor forms by the magnocellular and parvocellular neurons in the supraoptic and paraventricular nuclei of the hypothalamus. These precursors of AVP are packaged into neurosecretory granules, follow an axonal transport into the posterior pituitary through the pituitary stalk, and are then transformed into the active hormone (Fig. 1). AVP will thereafter be stored in vesicles in the posterior pituitary and will be ready to be released in response to stimulus (Table 1).

Specifically, AVP is mainly regulated, thanks to the osmotic and the pressure-volume systems, which function as two independent, but related, mechanisms (Fig. 2). In fact, the roles of these two systems are so distinct that the existence of two different hormones, an antidiuretic hormone and a vasopressin hormone, was historically believed. This is why we currently name this hormone with either of the two terms.

Table 1 Stimulatory and inhibitory factors of arginine-vasopressin (AVP) secretion

	Stimulatory	Inhibitory
Mediating mechanism		
Osmorreceptors	Increased plasma osmolality - Extracellular fluid hyperosmolality (hypernatremia) - Saline infusion - Cellular dehydration (cell shrinking – hypovolemia) - Thirst	Decreased plasma osmolality: - Extracellular fluid hypoosmolality - Drinking water - Cellular hyperhydration
Barorreceptors	Reduced stimulation of barorreceptors (left atrium, carotid sinus, and aortic arch)	Increased stimulation of barorreceptors (left atrium, carotid sinus, and aortic arch)
Drugs	- Morphine - Nicotine - Beta-adrenergic - Angiotensin II - Barbiturics - Carbamacepine - Metoclopramide - Acetylcholine - Histamine - Ciclofosfamide, vincristine - Clofibrate - Prostaglandine E	- Atrial natriuretic peptide - Alcohol - Alpha-adrenergic, phenytoin
Other mediators	- Pain - Nausea, emesis - Hypoglycemia - Stress - Hypoxia, hypercapnia	Activation of cold-sensitive oropharyngeal receptors

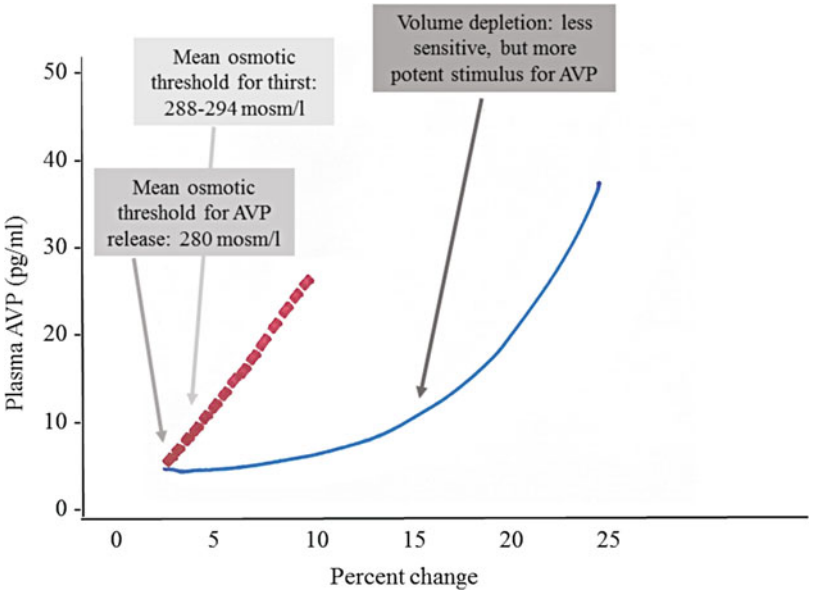


Fig. 2 Stimulation of AVP release by osmotic and volume changes. Schematic representation of the normal physiological relationships between plasma AVP levels and percentage increase in plasma osmolality (red thick dotted line) and plasma AVP levels and blood volume percentage depletion (blue continuous line). Note that AVP release is more sensitive to changes (increase) in osmolality (mediated by osmoreceptors), in comparison to changes (depletion) in volume (mediated by baroreceptors). However, when the non-osmotic stimulation of AVP occurs, the resultant plasma AVP concentrations are much higher than when the main trigger is the osmotic stimulation. Moreover, the non-osmotic stimulation of AVP can override the effect of hypoosmolality to suppress AVP

Concerning the osmotic regulation, AVP secretion is relatively simple and straightforward. In this regard, subtle increases in serum osmolality (hypernatremia) will precede evident increases in AVP synthesis and release. This response is mediated by specific neurons, termed osmoreceptors, which are capable of detecting subtle changes in extracellular fluid osmolality and are present in both the central nervous system and in the periphery. The specific osmotic threshold for osmoreceptor sensitivity and vasopressin release is considerably variable between individuals and is also probably genetically determined, but, in general, it lies around 280 mosm/kg (Fig. 2).

The regulatory influence of the pressure-volume system is a little more complicated. Firstly, more pronounced decreases in extracellular fluid (hypovolemia) are required to stimulate AVP synthesis and release, an action which is mediated by stretch receptors, termed baroreceptors, located in the left atrium, carotid sinus, and aortic arch (Fig. 2). In this case, stimulus arising from these receptors inhibits vasopressin secretion, while a reduced discharge rate enhances vasopressin release. However, other inhibitory phenomena and/or the role of other simultaneous

sympathetic impulses have to be also considered. For instance, the renin-angiotensin-aldosterone system plays an important role, and angiotensin II increases vasopressin secretion; opioid peptides that act through k-receptors inhibit it; cholecystokinin increases AVP release; and nitric oxide acts as an inhibitory modulator. The dopaminergic transmission seems to potently stimulate AVP release, for example, in situations like nausea and emesis; this effect is generally less significant but may acquire a relevant role in pathologic situations. In addition, vasopressin secretion is under the influence of a glucocorticoid-negative feedback system, and the usual AVP responses to a variety of stimuli (including hemorrhage, hypoxia, and hypertonic saline) may be attenuated, or even suppressed, by previously administering glucocorticoids.

In the majority of normal physiologic circumstances, AVP stimulation results from a coincident and synergistic combination of an increased osmolality and a decreased effective volume, such as in the setting of dehydration. In addition, there is evidence enough to suggest that a reduction in effective volume moves the curve of vasopressin response to osmolality to the left, thus reducing the osmotic threshold and leading to a greater AVP stimulation. In other words, the osmotic stimulus is much more sensitive to trigger AVP release than the stimulus of a reduced effective volume. However, when a greater decrease in effective volume is capable of triggering AVP release, its effect is much more potent than the osmotic stimulus, and the increase in AVP is significantly higher (Fig. 2). In a similar way, an excess in body fluids will reduce the effective osmolality and increase the effective volume, thus reducing AVP secretion. Therefore, the sensibility of AVP synthesis and release to osmoregulation allows plasma osmolality to be kept within a small range, mediated through kidney adaptation for water excretion regulation in response to subtle osmotic changes. Thirst may behave as an essential and additional protective mechanism to maintain an adequate plasma osmolality and volume, but, in normal circumstances, it does not really play a relevant role, since liquid ingestion is usually above real needs and excess water is normally excreted by the kidney. However, if water intake is not sufficiently enough to replace water losses, like in the setting of maximal antidiuresis, serum osmolality increases and stimulates thirst to an extent which parallels the existing osmolality increase.

Cellular Actions of Vasopressin Are Mediated by Specific Receptors

Vasopressin receptors (AVPR or V) differ concerning the target organ. In this regard, V1 receptors are mainly expressed in the vasculature (vascular smooth muscle), platelets, brain, liver, and anterior pituitary, although they can also be found in other locations; and V2 receptors are heavily expressed in cells of the renal collecting ducts. The third type of vasopressin receptor (V3) mediates stimulation of the adrenocorticotropin hormone (ACTH) in the anterior pituitary, which is a particular nonclassical action of AVP, and a V2 subtype receptor may stimulate the production of clot factor VIII.

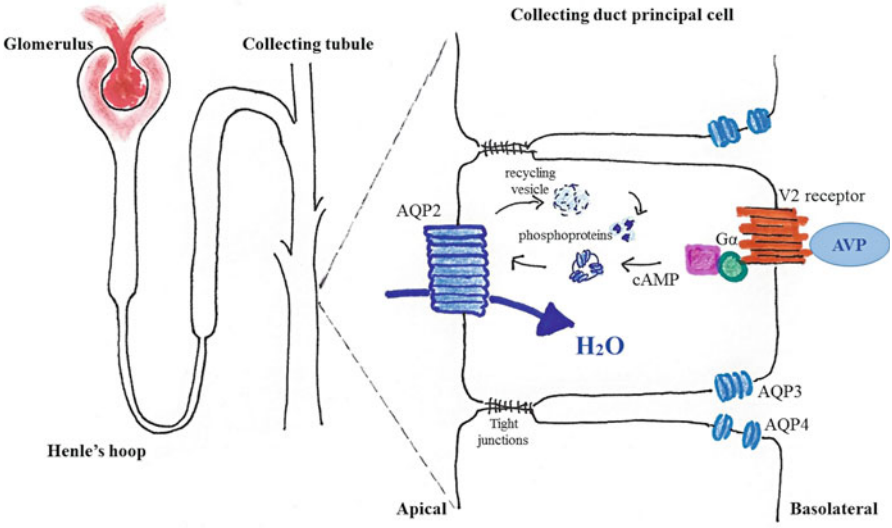


Fig. 3 Schematic representation of water transport in the renal cell. Binding of AVP to the vasopressin receptor 2 (V2) on the basolateral side of the renal principal cell stimulates the release of the G alpha (Gα) protein, which, in turn, activates the adenyl cyclase and increases cAMP production. As a result, several phosphoproteins are activated, enabling trafficking of the aquaporin 2 (AQP2) to the apical membrane and the formation of AQP2 water channels. AQP2 may be continuously regenerated in recycling vesicles. Therefore, ongoing water permeability depends on the availability of AQP2 on the apical membrane, either by new generation or by recycling vesicles. Water enters the renal cells through this AQP2 channel and diffuses across the concentration gradient, from the tubular lumen, into the principal cell, and exits into the interstitium through other aquaporin channels (AQP3 and AQP4)

Vasopressin actions are mediated through these vasopressin receptors, and include inhibition of diuresis, mainly through the protein aquaporin 2 (AQP2), which rapidly turns renal cells in the collecting ducts permeable to water, allowing reabsorption of water from urine into the blood and concentrating urine (Fig. 3), contraction of smooth muscle, aggregation of platelets, stimulation of liver glycogenolysis, modulation of ACTH release, central regulation of somatic functions such as thermoregulation and blood pressure, and modulation of social and reproductive behavior (for instance, stimulation of the thirst center in the cerebral cortex) (Fig. 4).

It is important to note that several situations, like pregnancy or stress, may produce alterations in the usual AVP regulation. Also, age may modify the effective circulating volume and renal function, which explains why the elderly may be predisposed to hydro-electrolytic imbalances. For instance, although their AVP secretion may be preserved, the elderly usually exhibit a reduced thirst reflex and a reduced ability to obtain the maximal or minimum urine concentrations, in order to retain water or excrete it, respectively.

A good understanding of these actions helps anticipate the clinical alterations which can be identified in cases with altered AVP secretion or action.

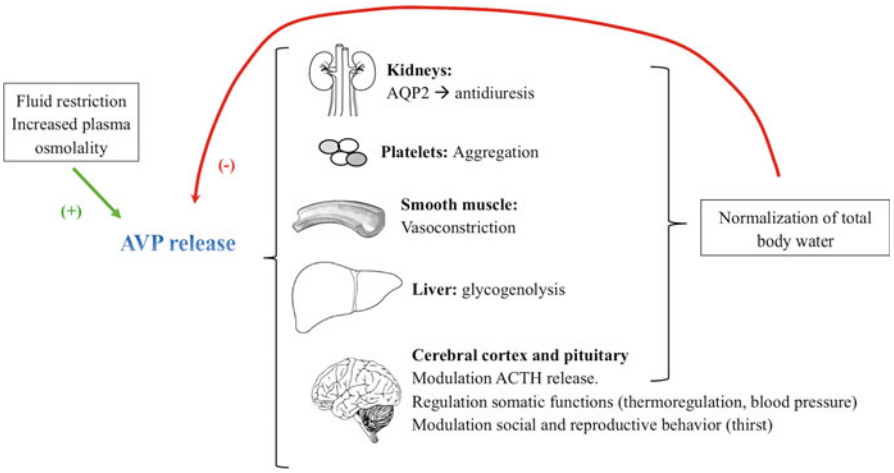


Fig. 4 Simplified summary of the mechanisms involved in hydro-electrolytic regulation, mediated by AVP. "(+)", stimulus; "(−)", inhibition. Fluid restriction and increased plasma osmolality stimulate the release of AVP. AVP, on its side, acts at different target organs in the body: it stimulates the activity of AQP2 in renal cells, to reabsorb water and contribute to the antidiuretic effect; stimulates aggregation of platelets; stimulates the smooth muscle in blood vessels, leading to vasoconstriction and contributing to the increase on effective intracellular volume; stimulates liver glycogenolysis; and modulates ACTH release, thermoregulation, blood pressure, and thirst in the pituitary gland and cerebral cortex. All these actions subsequently lead to normalization of total body water and will eventually lead to a negative feedback regulation for AVP release

Subtypes of Diabetes Insipidus: Different Etiologies to one Same Syndrome

There are four main mechanisms which can be distinguished in the pathophysiology of DI:

- Central DI (CDI): when the main alteration is found in the hypothalamus or posterior pituitary and an insufficient amount of AVP is synthesized or released. This type has been termed as hypothalamic, neurogenic, or pituitary DI.
- Nephrogenic DI (NDI): when the kidney does not respond correctly to AVP.
- Transient DI of pregnancy: where an altered AVP metabolism occurs, presumably during a limited period of time.
- Primary polydipsia: where the initial pathogenic mechanism is related to the intake of fluid, and not as much in its excretion.

Table 2 shows a list of the most relevant causes under these previous mechanisms.

Table 2 Etiology of diabetes insipidus

Central diabetes insipidus	Genetic/familial: - Autosomal dominant: mutations in the *AVP-neurophysin II* gene - Autosomal recessive - Inactivating mutations of the *AVP* gene - X-linked (Xq28) - Wolfram syndrome (*WFS* gene, 4p16) Congenital: - Septo-optic dysplasia - Pituitary hypogenesis - Midline malformations - Holoprosencephaly Acquired: - Traumatic brain injury - Neurosurgery - Tumors: Craneopharingioma, pituitary adenoma, pinealoma, dysgerminoma, meningioma Metastasis (lung in male; breast in female) Hematologic: Lymphoma, leukemia Granulomas: Histiocytosis, Wegener's granulomatosis, sarcoidosis - Infections: Chronic meningitis, tuberculosis, syphilis, viral encephalitis, toxoplasmosis - Vascular diseases: Sheehan's syndrome, carotid artery aneurysms, aorto-coronary bypass, hypoxic encephalopathy - Autoimmune: lymphocytic hypophysitis - Inflammatory: systemic lupus eritematosus, schlerodermia - Toxins: snake's venom, tetradotoxin - Drugs: ethanol, phenytoin, corticoids, alpha-agonists - Idiopathic
Nephrogenic diabetes insipidus	Genetic/familial - X-linked (mutations in AVPR2 gene, which encodes V2) - Autosomal recessive mutations in AQP2 gene Acquired: - Drugs: Lithium, cisplatin, anphotericin, demeclocyclin, rifampicin. . . - Metabolic alterations: Hypercalcemia, hypokaliemia - Renal diseases: Amiloydosis, pielonephritis, renal poliquistosis, obstructive uropathy, acute tubular necrosis, drepanocitic anemia - Granulomas: Sarcoidosis - Tumors: Sarcoma - Idiopathic - Excessive liquid intake and diuretics
Primary polidipsia	- Psychiatric - Dipsogenic diabetes insipidus - Iatrogenesis
Gestational diabetes insipidus	- Pregnancy

Neurogenic (Central) Diabetes Insipidus (CDI)

There are several clinical entities in which we can observe an absent or deficient release of AVP due to a defective neurohypophyseal function.

Hypothalamic Hereditary Diabetes Insipidus

This clinical syndrome is characterized by the development of early diabetes insipidus in infants, with the classical symptoms of thirst and increased fluid intake and urine output. As opposed to familial NDI, in familial CDI, the age of onset of symptoms may differ depending on the mutation. In fact, it may be asymptomatic during the first years, and, therefore, the diagnosis may be frequently overlooked, an observation which lies in accordance with the finding of a progressive decrease, or even disappearance, of the bright spot in MRI over time.

In the majority of cases, familial CDI is an autosomal dominant disorder caused by mutations in arginine vasopressin-neurophysin II (*AVP-NPII*) gene, which lead to aberrant preprohormone processing and gradual destruction of AVP-secreting cells,

Wolfram's syndrome, also known as DIDMOAD (diabetes insipidus + diabetes mellitus + optic atrophy + sensorineural deafness), is an autosomal recessive neurodegenerative disease in which patients may develop diabetes insipidus. In this case, AVP deficiency is usually partial and of gradual onset. It is important to be aware that polyuria may sometimes be wrongly attributed to poor glycemic control, and a severe hyperosmolar state can occur if an untreated diabetes mellitus is associated with an unrecognized posterior pituitary deficiency. The genetic defect in this syndrome is located in chromosome region 4p16.1, which encodes a putative 890-amino acid transmembrane protein termed wolframin (Miller et al. 1970; Turkkahraman et al. 2015).

Central Diabetes Insipidus in the Setting of Solid Tumors or Hematologic Diseases

Suprasellar tumors such as craniopharyngioma, pinealoma, and germinoma are characteristically found in the basal area of the hypothalamus and are frequently associated with the development of diabetes insipidus. In fact, polydipsia and polyuria may be one of the first symptoms appearing in a patient with these types of tumors and should alert clinicians for the suspicion of these entities, especially when concomitant hypopituitarism exists. MRI will characteristically evidence an enlargement of the pituitary stalk, together with the hypothalamic mass.

Pituitary metastases are not frequent, but, when they appear, locations in the posterior lobe are much more frequent than those in the anterior lobe, presumably as a result of the greater and direct vascularization that it receives.

There have also been cases of pituitary lymphomas with diabetes insipidus as one of its cardinal symptoms. In addition, there have been cases of diabetes insipidus in

patients with hepatic C virus (HCV) or human immunodeficiency virus (HIV), who develop lymphoproliferative diseases, and in patients with leukemia. The underlying pathophysiology may involve infiltration of the hypothalamus, thrombosis, or infection.

Diabetes Insipidus in the Neurosurgical Patient

Disorders of water homeostasis are frequent in neurosurgical patients, especially after subarachnoid hemorrhage; after traumatic brain injury, with intracranial tumors; and after pituitary surgery. In fact, diabetes insipidus is quite common in the acute phase after a neurosurgical intervention, although it is usually transient. Serum sodium levels are rarely significantly altered if the thirst reflex is maintained, but many neurosurgical patients have a diminished consciousness level because of brain injury, postoperative cerebral irritation, cerebral edema, sedation for airway management, or a combination of all these previous factors. Therefore, awareness of thirst and the ability to respond to plasma osmolality increases should be taken into account, and sodium levels should be frequently monitored.

Diabetes insipidus in subarachnoid hemorrhage (*SAH*). DI may occur acutely in around 15% of patients with SAH, and, although it is transient in the majority of patients, up to 8% may present persistent DI 3 months after discharge. Hemorrhage from anterior communicating artery aneurysms is associated with an increased risk of developing DI, presumably due to a higher risk of compromising the vascularization of the anterior hypothalamus. Fluid replacement will be especially important in these patients, since hypovolemia may predispose to cerebral vasospasm and worsen outcomes.

Diabetes insipidus in traumatic brain injury (*TBI*) (Scranton and Baskin 2015). Polyuria may be observed immediately after brain injury in around 20% of patients, nearly always within the first 2 or 3 days. However, as it occurred after SAH, the majority of cases will resolve spontaneously, although it is possible that some "subclinical" cases remain undiagnosed and maintain normal plasma osmolality through increased water intake. The severity of head trauma, assessed with the Glasgow Coma Scale, and the presence of cerebral edema on imaging studies are directly related to the risk of developing DI after TBI. MRI may be useful to complement the diagnosis of CDI in this clinical setting, where hypothalamic hemorrhage, neurohypophyseal hemorrhage or apoplexy, or pituitary stalk section may be the frequent findings.

Diabetes insipidus in the context of pituitary adenomas. As it was previously highlighted, pituitary adenomas themselves rarely produce DI, so if DI develops in a patient with a pituitary mass, it should prompt the suspicion of craniopharyngioma or other granulomatous diseases. However, DI is very frequent after surgical intervention of pituitary adenomas, especially after transsphenoidal surgery. In fact, up to 80% of cases have been reported to develop transient DI in the early phase after pituitary surgery, usually within 2 days, with an abrupt onset of hypotonic polyuria and accompanying thirst. But, once again, most cases are resolved by the third

postoperative day, and rates of persistent DI are usually not above 15%. Transection of the pituitary stalk, on the contrary, will almost inevitably lead to permanent DI.

It is important to exclude confounding factors of polyuria, including steroid-induced hyperglycemia, mannitol, and diuretics. If there is evidence of a plasma sodium >145 mmol/l in the presence of hypotonic (urine osmolality <300 mOsm/kg, or urine density <1.005) polyuria (>300 ml/h for 2 consecutive hours or >3 l/day), then it is highly suggestive of acute DI and warrants prompt management.

Some factors may be associated with the development of DI after pituitary surgery: for instance, young age, male sex, large tumors, intraoperative cerebral-spinal fluid fistula, Rathke cysts, craniopharyngiomas, ACTH-producing tumors, and the experience of the neurosurgical team. Also, patients receiving prophylactic high doses of hydrocortisone may have a higher risk of postoperative DI. This is probably because free water excretion is dependent, to some extent, on adequate adrenal function, and may be impaired in the presence of cortisol deficiency, so patients on an insufficient dose of parenteral hydrocortisone postoperatively may have their DI "masked" by the presence of relative cortisol deficiency. Interestingly, although radiotherapy has been associated with the development of anterior pituitary insufficiency in the long-term follow-up, DI does not seem to occur, presumably because of a different radiosensitivity for the neurohypophysis in comparison to the adenohypophysis.

A small group of patients will develop what has been known as "a triphasic response." In this case, after the initial occurrence of DI, there is a putative release of pre-stored AVP from damaged neurohypophyseal cells, leading to antidiuresis and hyponatremia (syndrome of inadequate secretion of antidiuretic hormone – SIADH), mostly around day 7 after surgery, and where exogenous AVP or excessive fluids may exacerbate the clinical picture. But then, these damaged neurons undergo gliosis and lose their functional capacity to synthesize or release AVP, and permanent DI establishes.

Diabetes insipidus in other less frequent neurosurgical settings. DI may also develop in the context of granulomatous diseases. In this case, clinical manifestations in other extrapituitary locations will guide clinicians to the diagnosis. In addition, DI may develop, although less commonly, after other neurosurgical conditions, such as intracerebral hemorrhage, subdural hematoma, and brain abscesses. Some cases, however, will still be considered "idiopathic," and an underlying autoimmune mechanism should be borne in mind, such as what is known as lymphocytic hypophysitis (Hannon et al. 2012; Loh and Verbalis 2007).

Adipsic Diabetes Insipidus: Essential Hypernatremia

Adipsic DI or adipsic hypernatremia is an infrequent complicated clinical variant, in which there is an absent responsiveness to osmotic stimuli because of an altered osmostat, but a normal response of baroreceptors is preserved. The neurosurgical setting in which this rare disorder has been most frequently described is in patients who have had clipping of an anterior communicating artery aneurysm, presumably reflecting an underlying lesion of hypothalamic osmoreceptors.

These patients do not exhibit thirst nor secrete AVP in response to hyperosmolality, leading to hypotonic polyuria, although they retain the ability to synthesize and release AVP in response to other non-osmotic stimuli such as hypotension. As a result, limited fluid intake and increased water excretion lead to hypernatremic dehydration. When dehydration is sufficiently significant so as to stimulate baroreceptors, some AVP is released, urine concentrates, and patients may achieve a hypernatremic equilibrium state with mild dehydration, and hypernatremia itself may subsequently lead to sodium excretion, which maintains this new equilibrium state. Fluid supplementation with variable infusion of hypotonic fluids to slowly reduce sodium concentrations and maintain them in the normal range and prophylaxis against thromboembolic complications deem necessary in the approach of these patients, as well as providing a fixed dose of desmopressin to limit fluid loss. Body weight can serve as a surrogate of adequate water and sodium balance. Further explanations of this complicated variant are beyond the scope of this chapter (Crowley et al. 2007).

Nephrogenic Diabetes Insipidus

In nephrogenic diabetes insipidus (NDI), the kidney is unable to concentrate urine despite normal or elevated AVP concentrations (Bockenhauer and Bichet 2015).

Congenital Nephrogenic Diabetes Insipidus

In congenital NDI, the classic and unequivocal symptoms of DI, polyuria, and polydipsia are present early during the first week after birth and are frequently also associated with vomiting, constipation, development alterations, and fever. Therefore, prompt recognition is vital to avoid the severe consequences derived from dehydration.

Two clinical subtypes can be distinguished: a “pure” NDI phenotype, which is characterized by loss of water exclusively and is caused by mutations in the *AVPR2* (which encodes the vasopressin V2 receptor) or *AQP2* genes, and a more “complex” type, characterized by loss of water and ions, caused by inactivating mutations in genes (*SLC12A1*, *KCNJ1*, *CLCNKB*, *CLCNKA*, *BSND*) that encode the membrane proteins of the thick ascending limb of the loop of Henle. A summary of the mutations identified in congenital NDI is shown in Table 3.

Acquired Nephrogenic Diabetes Insipidus

The acquired form of NDI is much more common than congenital forms. The ability to elaborate a hypertonic urine is usually preserved, although the nephron is unable to maximally concentrate urine. Therefore, DI symptoms are generally more moderate, and polyuria-polydipsia is rarely greater than 3 to 4 l/day.

Table 3 Genetic mutations causing nephrogenic diabetes insipidus

Gene	Location	Type of mutation	Phenotype
AVPR2	Xq28	Recessive X-linked Mostly missense mutations	Male patients Loss of function or dysregulation of V2: - Type 1 mutant V2 receptors: Reach cell surface, but display impaired ligand binding. Unable to induce cAMP production - Type 2 mutant V2 receptors (most frequent): Defective intracellular transport - Type 3 mutant V2 receptors: Ineffective transcription, unstable mRNA and rapid degradation
AQPR2	12q12.13	Autosomal recessive	Male and female Misfolded and misrouted mutant proteins, which are trapped in the endoplasmic reticulum
SLC12A1, KCNJ1, CLCNKB, CLCNKA, BSND	Various	Inactivating mutations	Consider additional symptoms such as polyhydramnios, salt wasting, hypokaliemia and nephrocalcinosis

Drugs are the most frequent cause of acquired NDI, with lithium therapy being at the top of the list. In fact, rates have been described to be as high as 85%, so if we take into account the relatively large number of patients who take lithium, it is not surprising to encounter this entity in everyday clinical practice. The precise underlying pathophysiology remains to be elucidated. However, it seems that there is a cytotoxic accumulation of lithium, which enters renal cells via the highly lithium-permeable epithelial sodium channels (ENaC) on the apical membrane and leads to the inhibition of signaling pathways that involve glycogen synthase kinase type 3 beta. Consequently, the normal function of AQP2 is dysregulated, and renal tubular cells suffer a constitutive damage due to the electrolyte imbalance caused (Fig. 5).

Lithium is frequently prescribed in psychiatric disorders, and normal therapeutic lithium levels are enough to produce this hypotonic polyuric effect. Although interruption of treatment will eventually restore normality, cessation of lithium therapy is rarely an option, because, in the majority of cases, the beneficial effects of the drug on the psychiatric disorder outweigh the negative impact of the polyuric complications on the quality of life (Behl et al. 2015).

If long-term treatment with lithium is required, administration of ENaC blockers such as amiloride may block the uptake of lithium in the collecting ducts, thus preventing the inhibitory effect of intracellular lithium on water transport, increase urine osmolality, and ameliorate polyuria.

Other acquired causes of typically transient NDI include hypercalcemia, hypercalciuria, and obstructive uropathy. In this latter clinical setting, NDI seems to occur because hydrostatic pressure directly suppresses AQP2 expression for up to 30 days, which explains why after obstruction release, an increased diuresis occurs.

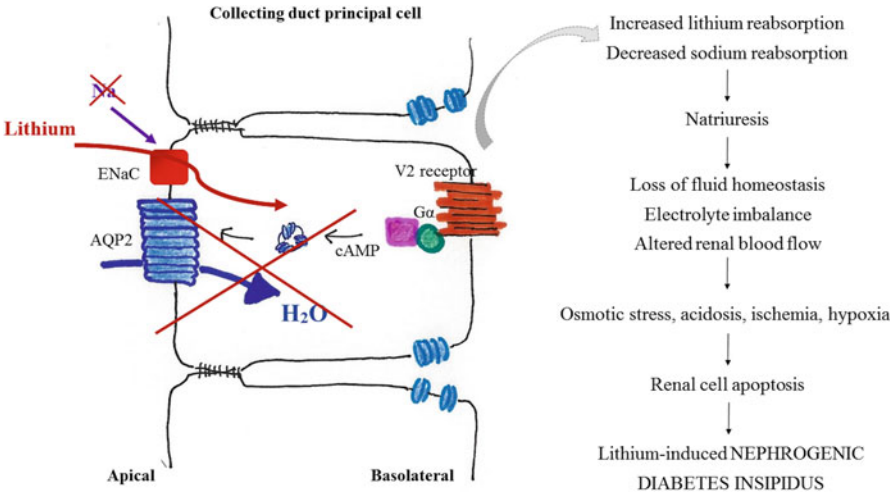


Fig. 5 Schematic representation of the effects of chronic lithium exposure in renal tubular cells. Lithium competes with sodium in its entry into the renal tubular cell through the epithelial sodium channel (ENaC) on the apical membrane. This leads to the inhibition of the normal signaling pathway of aquaporin 2 (AQP2) formation, so the normal function of AQP2 is dysregulated. In addition, lithium accumulation is responsible for the development of cytotoxicity due to the electrolyte imbalance caused, which constitutively damages the renal cells

In addition, chronic renal diseases in which the kidney structure is significantly altered, such as renal poliquistosis, renal infarctions, or infiltrative diseases, may also present with a clinical picture of DI.

Diabetes Insipidus in Pregnancy

Pregnancy is usually accompanied by a physiologic redistribution of body fluids, decreasing plasma osmolality and increasing intravascular volume, leading to an osmotic threshold reset. This normal effect is probably mediated by the hormone known as relaxin and is usually unremarkable.

Diabetes insipidus may occur transiently during pregnancy as a result of an increased release and activity of the enzyme cysteine-aminopeptidase (oxytocinase), which is synthesized by the placenta from week 20 of pregnancy onward and breaks down AVP. An extreme and abnormal oxytocinase activity would lead to the typical "resistant DI of pregnancy" and has sometimes been associated with concomitant preeclampsia, acute hepatic steatosis, and coagulopathies. Normal delivery and subsequent recovery of normal body fluids will usually resolve this situation. On the other hand, in a pregnant woman with an already insufficient AVP activity due to a preexisting DI of any other cause, an abnormally increased metabolism will lead to an increased clearance, which cannot be overcome by a corresponding increase in AVP secretion and release, and will cause a clinically significant DI.

Primary Polydipsia

Primary polydipsia (PP) is a state of hypotonic polyuria, and, in general, mean plasma osmolality is lower due to excessive fluid intake. Its complete pathophysiology has not been fully understood yet.

It is possible that a selective defect in osmoregulation of thirst exists, with normal AVP secretion, but an abnormally low osmotic threshold for thirst, leading to a "dipsogenic DI." In addition, PP may develop due to hypothalamic lesions (for instance, hypothalamic sarcoidosis), drugs which cause mucosal dryness, or any disease that causes an elevation of the renin-angiotensin-aldosterone axis. However, the majority of cases occur in patients with psychiatric disorders such as schizophrenia and mania. In these patients, osmolalities may be higher early in the morning because of a relative fluid restriction during the night sleep. AVP administration will be useless, since these patients have altered concentration renal mechanisms.

Differential Diagnosis: Investigating a Patient with Polyuria and Polydipsia, where Do we Start our Clinical Approach?

There are several variables that should be considered when approaching a patient with polyuria and polydipsia:

- Urine volume in 24 h. Patients in whom DI is suspected should record their urine output by collecting urine or quantifying the time and amount of each urination.
- Rule out the presence of any osmotic agents such as hyperglycemia.
- Rule out the existence of previous nephropathy.
- Serum sodium concentration, osmolality, and renal function.
- Urine sodium concentration and osmolality.
- Preserved thirst mechanism.

Certain clinical features should alert clinicians for the potential presence of DI. For instance, if a patient acknowledges an increased thirst, especially for cold fluids, and/or his urine output is excessive and hypotonic ("clear"), we should probably search for biochemical parameters to rule out potential causes of polyuria/polydipsia (Fenske and Allolio 2012).

Basal Measurements of Serum and Urine Sodium and Osmolality

A serum osmolality >295 mosm/kg, serum sodium >145 mEq/l, and urine osmolality inadequately diluted (<300 mosm/kg or urine density <1.005) in the setting of polyuria/polydipsia are highly suspicious of DI, and dynamic studies may not be necessary.

The Urinary Concentration Test as an Indirect Measure of AVP Activity

Diabetes insipidus is diagnosed if, after fluid restriction, AVP secretion is normal, but inadequately low or with insufficient action in relation to urine osmolality. As a result, the initial most frequent diagnostic approach to a patient with suspected DI consists of the evaluation of the capacity of urinary concentration in a supervised fluid privation test (dehydration test), followed by the evaluation of urinary response to the administration of a vasopressin analogue or vasopressin itself (Miller et al. 1970). Although the urinary concentration mechanism may be disturbed under certain circumstances, such as anterior pituitary insufficiency, overhydration, or reduced glomerular filtration rate, which could limit the test validity, in general, its evaluation after supervised dehydration and subsequent vasopressin administration is widely used, and results may provide sufficient information. If polyuria is mild, the test can begin late in the afternoon and continue the following morning, so that most of the dehydration occurs overnight. However, in more severe forms, it is safer to directly proceed to a supervised test starting early in the morning.

A possible way of carrying out the dehydration test in order to obtain a differential diagnosis in a patient whose clinical setting is highly suspicious, but biochemical parameters are equivocal, is as follows:

- The patient should have fasted for 8 h if possible, including restriction of any sort of food or liquid. Special attention should be paid to restriction of alcohol, coffee, tea, and tobacco during the previous days.
- The patient will empty his bladder and his weight will be recorded.
- Basal levels of plasma and urine osmolality and sodium are collected.
- The patient is asked to refrain from any fluid or food intake during the duration of the dynamic test, and supervision is mandatory to avoid deviations.
- Urine volume and urine osmolality and weight are repeated hourly.
- Serum osmolality and sodium levels are recorded every 2 h.
- The test is ended if:
  - Weight decreases >5%.
  - Plasma osmolality is >295 mOsm/kg.
  - Plasma sodium is >145 mEq/L.
  - Urine osmolality plateaus (less than 30 mOsm/kg increase in three consecutive samples).
- Urine osmolality >800 mOsm/kg is highly suggestive of mild primary polydipsia.
- If urine osmolality is <800 mOsm/kg, we will reevaluate plasma osmolality and sodium; then, we will administer 1 mcg of desmopressin (DDAVP) subcutaneously, and repeat urine osmolality after 30 and 60 min.

Interpretation of this test's results is usually relatively straightforward (Fig. 6, Table 4). A normal response is characterized by plasma sodium and osmolality within stable and normal reference values, with progressive urine volume decrease,

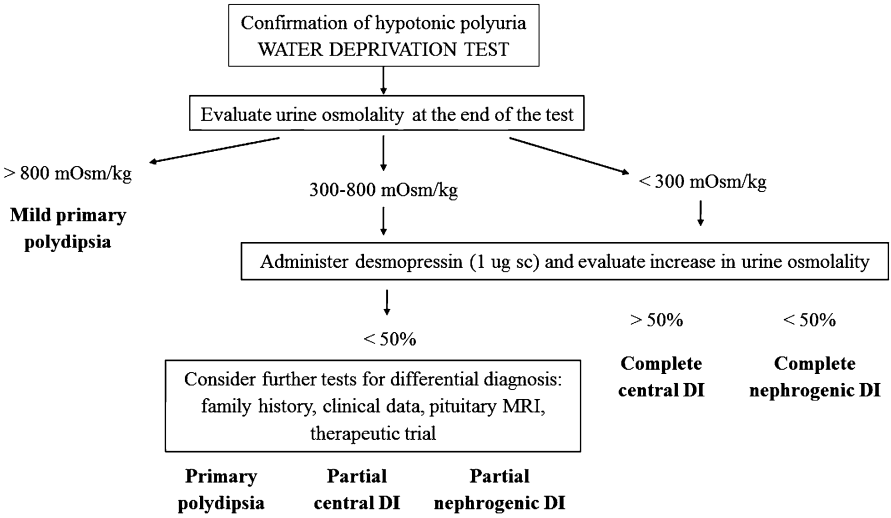


Fig. 6 Diagnostic approach to the patient with hypotonic polyuria. Differential diagnosis of diabetes insipidus

Table 4 Simple summary of the differential diagnosis in a patient with polyuria and polydipsia according to test results (see also Fig. 6). ↑: increased; ↓: decreased; AVP: arginine-vasopressin; DDAVP: pharmacological AVP analogue

	Normal	Central diabetes insipidus	Nephrogenic diabetes insipidus	Primary polydipsia
Plasma osmolality	Normal	↑	↑	↓
Urine osmolality	Normal	↓	↓	↓
Urine osmolality/plasma osmolality (ratio)	>2	<1 (complete) Variable (partial)	<1	>1
Urine osmolality after dehydration (mOsm/kg)	>750	<300	<300	300–750
Urine osmolality after DDAVP administration (mOsm/kg)	>750	>750	<300	<750
Urine osmolality after DDAVP administration (% related to previous)	<9%	>50% (complete) 10–50% (partial)	<50%	<9%
Serum AVP	Normal	↓	Normal – ↑	↓

an increase in urine osmolality >800 mOsm/kg, and an increase in urine osmolality of <9% after DDAVP administration. Central DI, however, will be mainly characterized by persistent hypotonic urine after fluid privation, but an evident increase of >50% after exogenous DDAVP. Nephrogenic DI, on its side, is characterized by an insufficient response to the administration of DDAVP.

Direct Measurement of Plasma AVP Activity

With the development of the first sensitive and specific AVP radioimmunoassay (RIA) during the 1970s, a new tool became available to help in the differential diagnosis of patients with suspected DI. In this regard, for instance, undetectable levels of AVP after a dehydration test would be highly suggestive of central DI. AVP results should be cautiously interpreted, however, and always in the setting of its corresponding serum and urine osmolality.

Comparison between the diagnostic efficacies and accuracies of AVP measurement and the classical urine osmolality evaluation described above for the assessment of DI is difficult, since there is no "gold standard" with which test results may be compared. In addition, the area of normality of AVP release in response to a specific serum osmolality is variable and has been a matter of debate in several studies. Furthermore, there are well-characterized technical difficulties of the AVP assay, which entail a high pre-analytical instability. Taken together, these limitations in AVP measurement jeopardize the possibility of it being considered as an essential tool in the differential diagnosis of DI, although they might be useful in specific ambiguous cases.

Direct Measurement of Plasma Copeptin (Pro-AVP)

Plasma concentrations of copeptin and AVP in relation to serum osmolality are highly correlated. In fact, they have an equimolar secretion and response to osmotic, hemodynamic, and stress-related stimuli. Copeptin corresponds to the C-terminal glycoprotein of the AVP prohormone and represents a stable surrogate for endogenous plasma AVP levels, as opposed to AVP, whose quantification may be difficult. Therefore, copeptin has been suggested as a promising diagnostic marker for the diagnosis of AVP-dependent fluid disorders and has been considered very useful for identification of patients with severe neurohypophyseal damage after transsphenoidal surgery.

The relevance of copeptin in the differential diagnosis of DI has been studied by using the urinary concentration tests, as a surrogate of AVP activity. In this regard, in the absence of prior fluid deprivation, baseline copeptin levels >20 pmol/l identify patients with NDI, while baseline copeptin levels with prior fluid deprivation <2.5 pmol/l are highly suggestive of complete CDI. Upon osmotic stimulation, copeptin levels are accurate for the challenging clinical setting of differentiating patients with partial CDI and primary polydipsia (Fig. 7); in this case, the ratio of copeptin increase during an 8-h dehydration period to the serum sodium concentration measured after 16 h of water deprivation has a high specificity and sensitivity (100 and 86%, respectively) and a diagnostic yield of 94% to distinguish between the two clinical entities.

Evaluation of copeptin levels may also be useful in the approach to patients with hyponatremia; in this setting, low levels of copeptin, together with low urine osmolality, would be suggestive of primary polydipsia, while the ratio of copeptin

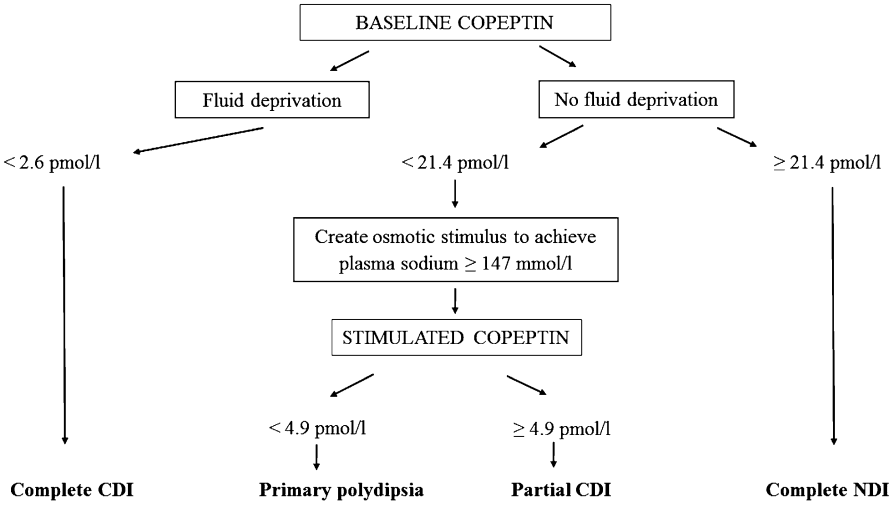


Fig. 7 Differential diagnosis of diabetes insipidus using copeptin levels. CDI, central diabetes insipidus; NDI, nephrogenic diabetes insipidus

to urinary sodium would be useful to distinguish the syndrome of inappropriate antidiuretic hormone secretion (SIADH) from other AVP-dependent forms of hyponatremia.

Again, the lack of a "gold standard" with which to compare the results obtained by the evaluation of copeptin levels, the absence of very large study populations, and the still pending validation studies for cutoff levels in post hoc analyses limit the diagnostic relevance of this parameter. In addition, the sandwich immunoluminometric copeptin assay (LUMItest CT-pro-AVP) is not yet commercially available for everyday clinical practice all over the world, so its clinical implementation is still limited (Christ-Crain 2016).

Therapeutic Trial of DDAVP

In selected patients in which the diagnosis is still uncertain despite the previous approaches, a closely monitored therapeutic trial of DDAVP may be attempted. In this case, we would administer 10 ug of DDAVP intranasally twice a day for 2–3 days. If this procedure causes a significant antidiuretic effect, nephrogenic DI is effectively excluded. If polydipsia and polyuria are abolished and plasma sodium does not decrease below the normal range, the most probable diagnosis will be central (neurohypophyseal) DI. If DDAVP administration causes a reduction of urine output, without reduction in water intake, and hyponatremia appears, the patient will probably have primary polydipsia. Water intoxication is not frequent, but monitoring of this trial is recommended to avoid it.

AQP2 Measurements

Urinary AQP2 excretion could be measured by radioimmunoassay or quantitative Western analysis and could serve as an additional indicator of the response to AVP of the renal collecting duct in cases were nephrogenic DI is suspected. However, this is still an infrequent approach used in everyday clinical practice, since it is not readily available in most centers.

Carrier Detection and Perinatal Testing

In families with known autosomal CDI, or X-linked or autosomal NDI, mutation analysis is recommended before the birth of an infant, in order to allow early diagnosis and establish prompt treatment, avoiding the physical and mental retardation associated with dehydration episodes. Gene analysis is also important for the identification of nonobligatory female carriers in families with X-linked NDI.

Imaging Techniques in Diabetes Insipidus: Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) allows visualization of the anterior and posterior pituitary, as well as the pituitary stalk. Because of the direct vascularization of the neurohypophysis, this latter lobe can be more rapidly visualized in a dynamic mode after gadolinium administration during an MRI. In fact, it is easily identified, as it was briefly mentioned previously, as a "bright spot" in the posterior part of the sella turcica on T1-weighted images. This hyperintensity may be seen in 80% of normal individuals but is absent in the majority of patients with CDI. It represents the normal AVP storage in the posterior pituitary lobe and has been seen to be correlated with AVP levels (Fig. 8).

In a patient who is being evaluated for the suspicion of diabetes insipidus, if this pituitary hyperintense spot is not seen in a pituitary MRI, it will probably reflect the loss of functional integrity of the neurohypophysis, serving as a non-specific indicator in the diagnosis of CDI. On the contrary, in patients with primary polydipsia, the bright spot is usually seen, while findings in patients with NDI may be variable.

Pituitary MRI has been reported to be the best technique with which to evaluate the sella turcica area, not only for identification of this bright spot but also to rule out other pituitary, pituitary stalk, and infundibulum alterations. Given the fact that more than 90% of AVP-producing neurons have to be destroyed for the development of clinical diabetes insipidus, it is easy to understand how a pituitary lesion needs to be considerably big in order to destroy a significant amount of hypothalamic cells, or the lesion should be located specifically where hypothalamic axons come together in the pituitary stalk. Therefore, small pituitary lesions rarely produce neurogenic DI. Conversely, pituitary stalk enlargement, which, by the way, is frequently

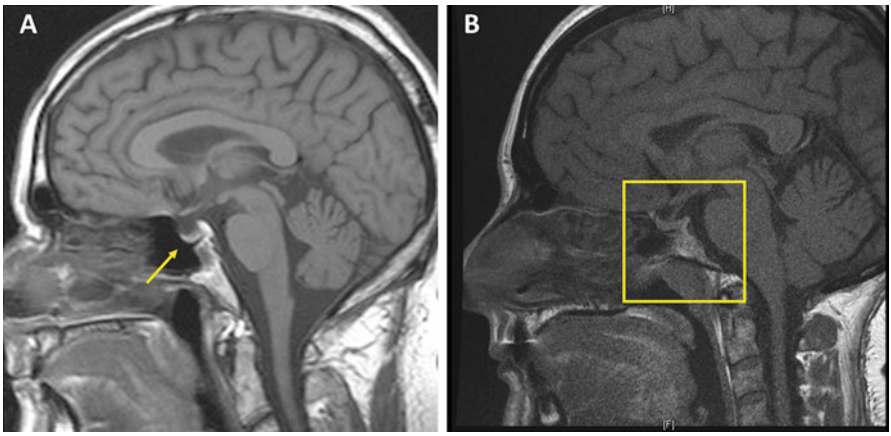


Fig. 8 Magnetic resonance imaging (MRI) showing the bright spot in a normal patient (**a**, pointing arrow) and its absence in a patient with central diabetes insipidus (**b**, square)

associated with the absence of the bright spot, could be suggestive of infiltrative lesions or systemic diseases, as previously explained.

Follow-up MRI may be useful to evaluate the outcome of reversible diseases, for example, in cases with enlargement of the pituitary stalk. However, if no other pituitary lesion is observed, and the only finding is the absence of the hyperintense spot reflecting the absence of AVP storage, serial MRI may not be necessary.

Practical Clues for the Differential Diagnosis of Diabetes Insipidus in Ambiguous Cases

In ambiguous cases, clinical clues can guide us through the diagnosis. In this regard, findings suggestive of primary polydipsia include history of psychiatric disease or neurotic personality, fluctuating symptoms, and gradual onset of polydipsia, posterior pituitary bright spot and normal thickness of the pituitary stalk, plasma sodium in the low range of normal, and persistent polydipsia, or even development of hyponatremia after a therapeutic trial with desmopressin. Findings suggestive for partial central DI include previous head trauma or pituitary surgery, family history of DI, recent and distinct onset of symptoms, and consistent need for fluid intake during the night, preference for cold fluids, missing pituitary bright spot and/or enlargement of the pituitary stalk beyond 2–3 mm, serum sodium in the upper range of normal, and abolishment of thirst, polydipsia, and polyuria without development of hyponatremia after a standard dose of desmopressin. Finally, findings suggestive for partial nephrogenic diabetes insipidus include history of lithium or other drug therapies (e.g., cisplatin) interfering with urine concentration and presence of electrolyte disorders (like hypercalcemia or hypokalemia), posterior pituitary bright spot and normal pituitary stalk, and no effect of desmopressin on polyuria or polydipsia.

In addition, although ureic nitrogen (BUN) may not be useful for a differential diagnosis, serum uric acid levels may, in fact, differ: in central DI, uric acid levels may be high because of a coexistent moderate volume contraction and because AVP acts on V1 kidney receptors and increase urate clearance. Therefore, in the absence of AVP, uric acid levels will rise.

Treatment of Diabetes Insipidus

Treatment goals for patients with diabetes insipidus should seek the maintenance of sodium serum levels within normality; a comfortable urine output, around 2–4 l/day; and an acceptable and optimal quality of life. Patients and caregivers' education regarding this disease is especially important; they should be trained on how to monitor liquid intake and urine output and how to promptly identify treatment overdose or insufficiency.

Current alternatives to emulate AVP's action include formulations of the native antidiuretic hormone, vasopressin (Pitressin), and its analogue, desmopressin (DDAVP). Both produce antidiuresis by stimulating renal V2 receptors. High concentrations of vasopressin also stimulate contraction of smooth muscle in the gastrointestinal tract and blood vessels via its action at V1 receptors. However, DDAVP does not have this effect because it is relatively inactive at V1 receptors, thus avoiding a vasopressor effect.

The mainstay of treatment for patients with CDI involves the direct administration of desmopressin. However, standard doses of this drug will rarely be useful for the management of patients with NDI, who are insensitive to AVP. Although supranormal doses may be effective in some cases, the only practical approach for NDI concerns symptomatic control, i.e., reducing polyuria, thirst, and polydipsia, by reducing sodium intake, and using thiazide diuretics or prostaglandin synthetase inhibitors, as it will be now explained (Lamas et al. 2014).

Central Diabetes Insipidus

The first option of treatment for patients with CDI (i.e., AVP deficiency) is the administration of desmopressin (DDAVP). The formulation suitable for intravenous or subcutaneous administration is supplied in single-dose 1 mL ampoules and multidose 10 mL vials containing 4 ug/mL of the compound. This formulation has been the most extensively used in multiple reports and clinical trials. Nowadays, oral and intranasal formulations are also available, so patients requiring long-term treatment with DDAVP may choose between oral, subcutaneous, or intranasal routes; the intravenous route, although less frequently required, may be useful in the acute and emergency clinical setting. Urine output is rapidly decreased 15 or 60 min after its subcutaneous or oral administration, respectively.

Doses required may be highly variable depending on the patient and the route of administration. For instance, there are interindividual variations in the volume of

distribution; clearance of DDAVP; differences in V2 sensitivity, or in the kinetics of the biochemical mechanisms that mediate the antidiuretic effect; and even because of adsorption of DDAVP to the plastic syringes used for its administration. Furthermore, the oral formulation has a 10–20% of the potency of the nasal formulation, since only 5% is absorbed in the intestine; and oral absorption may be up to 40–50% less if taken with food, so, in general, a 2–3-h fasting interval is advised in cases of oral formulations.

Increasing doses do not seem to achieve higher urine concentration capacity; however, the magnitude and duration of the antidiuretic effect have a direct relationship with the dosage administered. The fact that the kidney's concentrating capacity decreases in the absence of AVP, requiring more than 8 h of continued stimulation to fully recover, may explain this issue. Table 5 shows the usual formulations and equivalent doses of DDAVP in everyday clinical practice. It can be observed how a 0.1 mg tablet is equivalent to 2.5–5 ug in intranasal spray.

Hyponatremia is a logical adverse effect of DDAVP use, mainly because of high fluid retention in the presence of high doses of AVP, so minimal doses will be always recommended. To avoid overdosing, patients are recommended to monitor their urine output for a minimum of, for example, 2 l/day. If urine output is significantly decreased, the DDAVP dose may be withheld, and reevaluation for persistence of DI may be necessary in some cases in which DI remission may be evidenced.

As it was explained before, in the acute setting of patients who underwent neurosurgery, development of DI is mainly mild and transient, and a preserved thirst mechanism will compensate for temporally increased liquid intake requirements. If esteemed necessary because of excessive and uncomfortable hypotonic polyuria, especially at night, or development of hypernatremia, one single parenteral dose of desmopressin (1–2 ug subcutaneous or intramuscular), which is active for 6–12 h, is usually enough. Further injections are seldom needed, only in cases with persistent or recurrent polyuria for more than 48 h. Associated hypokalemia must be ruled out, and, if present, it should be treated, since this may be a cause of renal resistance to desmopressin therapy. It is typical to withdraw desmopressin treatment at least once before discharge of patients from the hospital, to help identify those who have or have not resumed endogenous AVP secretion. In any case, patients and caregivers should be advised to monitor their balance between liquid intake and urine output during the first days after hospital discharge, and explain the potential symptoms of hyponatremia, in an attempt to promptly identify disorders of water homeostasis in these patients. This is especially relevant in today's everyday clinical practice, where there is a trend for rapid hospital discharge after pituitary surgery. If diabetes insipidus persists, the usual forms of administering desmopressin may be used, according to patients' tolerability and preference and in a proportional dose to the degree of AVP deficiency. For instance, a single nocturnal dose may be sufficient for patients with only partial CDI. Regular monitoring of serum electrolytes is mandatory in patients using DDAVP.

If the thirst mechanism is compromised and/or the patient is unable to compensate his urinary losses with liquid intake, maintenance of hydro-electrolytic homeostasis is complicated but may be preserved with administration of intravenous fluids and

Table 5 Comparison between different formulations of desmopressin

Formulation	Melt	Tablets	Spray	Drops	Solution for injection
Route of administration	Oral (sublingual)	Oral	Intranasal	Intranasal	Intravenous or subcutaneous
Bioavailability	0.25 (0.21–0.31)%	0.16 ± 0.17%	6.00 ± 2.29%	Similar to spray	N/A
Dose comparison	60 ug 120 ug 240 ug	100 ug 200 ug 400 ug	2.5 ug 5 ug 10 ug	2.5 ug 5 ug 10 ug	N/A <0.5 ug <1 ug
Usual prescription	100–400 ug 2–3 times/day	100–400 ug 2–3 times/day	10–20 ug 2–3 times/day	0.05–0.40 μg 2–3 times/day	1–2 ug 1–2 times/day
Commercial products	Minurin Flas® (60, 120, 240 ug)	Wetirin® (tablets 0.1, 0.2 mg) Desmopressin® (tablets 0.2 mg)	Minurin® spray (10 ug/pulse) Octostim ® (150 ug/pulse) Desmopressin® (10 ug/pulse)	Minurin® drops (100 ug/mL – 2.5 mL susp)	Minurin® sol (4ug/mL)
Comments	Better bioavailability than tablets.	Stable antidiuretic effect and dose-response relationship, despite low bioavailability Easy to use	Variable absorption and efficacy Individualized dose (metered dose spray)	Variable absorption and efficacy Individualized dose (rhinyl tube)	Hospitalized patients

strict monitoring of body weight, urine output, and general physical examination. Water deficiency may be calculated with the following formula, 0.6 × body weight (kg) × ([serum Na/140] − 1), and serum electrolytes should be monitored every 6–8 h.

Vasopressin (Pitressin), as an injectable aqueous solution (20 U/ml in a 1 mL vial), has been available for decades, and some authors have advocated its intravenous infusion to achieve short-term control of antidiuresis. In this regard, the rate of infusion usually starts at 0.25–1 uU/kg/h and is increased every 30 min thereafter until the urine specific gravity (density) reaches 1.010–1.020 or the rate of urine output falls to around 100 mL/h (approximate average infusion rate of 0–5-3 uU/kg/h). Accidental overdoses, usually due to inadequate dilution of the concentrated formulation, may result in severe abdominal cramping, diarrhea, vomiting, and pallor due to stimulation of V1 receptors. However, Pitressin is, in general, less frequently used in daily clinical practice.

There are other several drugs for whom an antidiuretic effect and subsequent utility in CDI have been described. For instance, carbamazepine, frequently used in neurological disorders, reduces urine output by 30–90%, with an accompanying proportional increase in urine osmolality, when used at conventional doses of 200–800 mg. It seems that this effect may be related to plasma AVP reduction, but the exact underlying mechanism is not yet fully understood. The sulfonylurea chlorpropamide has also a significant antidiuretic effect in CDI; in this case, at conventional doses of 250–500 mg/day, it reduces urine volume by 30–90%, reaching a maximum within 3–10 days of treatment, and with an associated proportionate rise in urine osmolality, but no change in solute excretion or glomerular filtration rate. Apparently, the antidiuretic effect is achieved by potentiating the very low levels of plasma AVP that persist even in patients with severe CDI or by a direct effect on V2 receptors. Combination with chlorothiazide may enhance chlorpropamide's effects. Clofibrate, on its side, at doses of 2 g per day, demonstrated to decrease urine volume by 50%, with a proportional urine osmolality increase. However, the underlying pathophysiology was even more uncertain, and the increased mortality observed in patients with long-term treatment led to its discontinuation in 2002.

Thiazide diuretics are mainly useful for the management of NDI, as it will be further explained below, but they may also play a relevant role in patients with CDI, especially with an accompanying restriction of sodium intake. Moreover, they potentiate the antidiuretic effect of other agents, so they may be frequently used in combination regimes. However, as with all antidiuretic treatments, an accurate diagnosis of the specific type of DI is essential for a safe and effective management (Oiso et al. 2013).

Nephrogenic Diabetes Insipidus

The main objective in the treatment of congenital NDI is to ameliorate symptoms and avoid the consequences of dehydration. Conversely, for acquired NDI, treatment

should target the underlying cause. If this is not possible, treatment approaches will be similar, targeting the control of symptoms.

For pediatric NDI, correct dietetic counseling is essential to enable patients' normal growth and development. Specifically, the diet's osmotic load, i.e., the osmotically active substances in diet (mainly proteins and salt), should be minimized to avoid an increased urine output.

In the setting of acute decompensations of patients with NDI, they may develop hypernatremic dehydration states, exhibiting ongoing losses of essentially pure water. In this context, infusion of 0.9% saline may be excessive and worsen hypernatremia, leaving isotonic fluids only for acute intravascular volume expansion in the rare cases of hypovolemic shock. Therefore, patients with NDI should be generally treated with hypotonic fluids. If possible, prompt oral administration is preferred, for example, with water or milk, to enable the thirst physiology to properly regulate fluid intake. If necessary, 5% dextrose in water for intravenous supplementation may be used, which will not provide an osmotic load, and may help urine output to decrease substantially. However, it is important to remember that hypotonic fluids must not be administered as intravenous bolus. Instead, they should be given at an infusion rate only slightly greater than the urine output, providing just enough water to safely normalize plasma sodium levels at a rate <0.5 mmol/l/h (<10–12 mmol/l/day), but avoiding the risk of developing cerebral edema. Careful and close management of fluid balance in specialized centers warrants a favorable outcome of these patients.

The use of diuretics in polyuric syndromes may seem rather paradoxical, but they are relatively effective for long-term management of patients with NDI. In fact, treatment with hydrochlorothiazide reduces sodium excretion, reduces urine volume, and increases urine osmolality, together with a concomitant reduction of plasma volume and body weight. This occurs due to a decreased salt reabsorption through the thiazide-sensitive co-transporter SLC12A3 in the distal tubule, which results in a decreased dilution of urine and a reduction in extracellular fluid volume, which stimulates a compensatory increase in proximal tubular reabsorption of sodium and water, and thereby diminishes delivery of filtrate to the distal nephron and collecting tubules, where the defect in urinary concentration exists. Another complementary mechanism by which thiazides are useful for the management of NDI is inhibition of carbonic anhydrase in the proximal tubule, resulting in a reduced proximal sodium uptake and subsequent decreased glomerular filtration. Furthermore, thiazides may increase the expression of AQP2 in the apical membrane, favoring water reabsorption, although this effect in vivo still needs to be demonstrated.

Another family of drugs that have become a key component in the management of NDI, especially during the first years, are inhibitors of prostaglandins synthesis. Specifically, indomethacin effectively reduces water diuresis by a synergistic and independent effect of AVP, presumably by enhancing the proximal reabsorption of salt and water, but without a significant change in urine osmolality.

Novel approaches to NDI focus on restoring AVP signaling upstream of AQP2, provided there are no AQP2 mutations. Because in the majority of congenital NDI cases there is a misfolding and mistransporting of AVPR2, pharmacological

chaperones may be a possible therapeutic approach. These chaperones are cell-permeable AVPR2 antagonists that fit into the AVP binding pocket of AVPR2 and allow a correct escape from the endoplasmic reticulum, subsequent transport to the cell membrane, and, thus, normal AVP signaling. An important issue to consider with this strategy is the AVPR2 antagonist's affinity. In this regard, chaperones with high affinities will fit better into the binding pocket and allow a more efficient surface expression; however, they will less likely diffuse off the receptor and jeopardize normal AVP signaling. On the other hand, compounds with low affinities will be less efficient for the promotion of surface expression, but more likely to diffuse off the receptors. Therefore, presumably, the most optimal chaperon will be one with an intermediate affinity. However, the only orally active non-peptide AVPR1A antagonist, known as SR49059, did not demonstrate enough effectiveness in reducing urine output and was even associated with idiosyncratic increases in liver enzymes, which led to discontinuation of its investigation and commercialization. Cell-permeable agonists that stimulate AVPR2 independent of AVP could be another alternative for NDI patients, but there is still much to investigate in this field.

Enhancing cAMP production independent of AVPR2 could also be an interesting target in patients with AVPR2 mutations, for example, by stimulating prostaglandin E2 receptors coupled to adenylyl cyclase. This strategy would not be effective in patients with autosomal AQP2 mutations, since these abnormalities result in a defect downstream of cAMP production. However, there are still no clinical data on the use of these prostaglandin-receptor agonists in patients with NDI, and, because prostaglandin synthesis inhibitors have proven efficient in the management of NDI, it is not likely that receptor agonists prosper as an alternative treatment option.

Other less common, and still preliminary, attempts for short-term therapy in NDI include a combination of secretin and fluvastatin for patients with X-linked forms; cGMP phosphodiesterase inhibitors, such as sildenafil, to increase cGMP levels, enhance trafficking of AQP2 to the liminal membrane, and increase urine osmolality; and statins, which may increase the expression levels of AQP2 channels, independent of cholesterol homeostasis. Genome editing of somatic tissue or embryos to correct mutant genes could be a future line of investigation, although with considerable ethics limitations, especially in patients who carry AQP2 mutations, in whom other target-directed approaches deem more difficult (Bonfrate et al. 2015).

Special Populations (Children, Pregnant Women, and the Elderly)

Given the fact that infants and children usually take diets with proportionally larger quantities of water, it may be necessary to allow a variable higher urine output to prevent hyponatremia, depending on age, diet, and if the child takes liquids ad libitum. Management may be easier if a steady amount of liquid intake is established, and treatment is tailored accordingly, with the complimentary help of monitoring plasma sodium; urine volume, which is virtually impossible to quantify in children, will rarely be useful.

DDAVP may be administered using different formulations, according to the child's age and caregivers' preference. For instance, in infants under 12 months, rhinyl preparation of nasal spray can be diluted 1:10 with physiological saline and administered orally one to two times per day (1–5 ug). Subcutaneous formulations (0.02 ug/day – 0.08 ug × 2 times a day) may also achieve stable urine volumes and sodium levels. In general, starting treatment at low doses is recommended, with subsequent upward titration according to the effect. Usual doses in children will range from 10 to 20 ug intranasally, 60–240 ug of melt, or 100–400 ug of the oral tablet. It is particularly important to educate children with DI and/or their caregivers about their disease and its complications, to avoid the dangers of excessive fluid intake during treatment, dehydration if doses are omitted, or signs and symptoms of water intoxication.

Women with preexisting CDI who become pregnant and women who develop temporary gestational DI can usually be treated successfully with oral DDAVP, which is resistant to degradation in the placenta by leucine aminopeptidase. Doses required may be similar or slightly greater than usual, and, in general, in cases of gestational DI, DDAVP may be withdrawn 4–6 weeks after delivery. Dose monitoring is normally based on plasma sodium levels, but it should be noted that it might be 5 mmol/l lower that in the non-gravid state. DDAVP can be safely given to nursing mothers, since very little appears in breast milk.

Management of DI in the elderly is similar to that in young adults, but careful monitoring should be performed, since there is a higher risk of developing hyponatremia, especially with intranasal formulations. Presumably, the underlying explanation involves increased renal sensitivity to AVP and abnormalities in the osmoregulation of thirst and fluid intake.

Summary and Conclusions

Recent investigation has provided a substantial increase in the knowledge of the molecular physiology involved in urine concentration. Diabetes insipidus is characterized by an excessive hypotonic and diluted urine output, due to an insufficient amount or action of AVP.

Several circumstances can cause CDI, being the neurosurgical setting the most frequently observed in everyday clinical practice. The treatment of choice for long-term management of CDI is desmopressin tablet or melt, which should be individually titrated to achieve an optimal control of polyuria, thirst, and polydipsia.

Inherited forms of NDI are mainly due to mutations in the processing of two key proteins in the regulation of water permeability in the collecting duct, AVPR2 and AQP2; but the majority of cases of NDI are acquired, especially due to chronic lithium treatment. Management of NDI includes dietary modifications, thiazides, and inhibitors of prostaglandin synthesis, leaving other novel therapies, such as molecular chaperones or gene therapy approaches, for future investigation.

Patient and caregivers' education on the pathophysiology of the disease deems necessary for a safe and effective management.

References

Behl T, Kotwani A, Kaur I, Goel H. Mechanisms of prolonged lithium therapy-induced nephrogenic diabetes insipidus. Eur J Pharmacol. 2015;755:27–33.

Bichet DG. The Posterior Pituitary. In: Melmed S, editor. The Pituitary. 3rd ed; 2011. p. 261–99.

Bockenhauer D, Bichet DG. Pathophysiology, diagnosis and management of nephrogenic diabetes insipidus. Nat Rev. Nephrol. 2015;11:576–88.

Bonfrate L, Procino G, Wang DQH, Svelto M, Portincasa P. A novel therapeutic effect of statins on nephrogenic diabetes insipidus. J Cell Mol Med. 2015;19:265–82.

Christ-Crain M, Fenske W. Copeptin in the diagnosis of vasopressin-dependent disorders of fluid homeostasis. Nat Rev Endocrinol. 2016;12:168–76.

Christ-Crain M, Morgenthaler NG, Fenske W. Copeptin as a biomarker and a diagnostic tool in the evaluation of patients with polyuria-polydipsia and hyponatremia. Best Pract Res Clin Endocrinol Metab. 2016;30:235–47.

Crowley RK, Sherlock M, Agha A, Smith D, Thompson CJ. Clinical insights into adipsic diabetes insipidus: A large case series. Clin Endocrinol (Oxf). 2007;66:475–86.

Fenske W, Allolio B. Current state and future perspectives in the diagnosis of diabetes insipidus: a clinical review. J Clin Endocrinol Metab. 2012;97:3426–37.

Hannon MJ, Finucane FM, Sherlock M, Agha A, Thompson CJ. Clinical review: Disorders of water homeostasis in neurosurgical patients. J Clin Endocrinol Metab. 2012;97:1423–33.

Lamas C, Del Pozo C, Villabona C. Clinical guidelines for management of diabetes insipidus and syndrome of inappropriate antidiuretic hormone secretion after pituitary surgery. Endocrinol Nutr. 2014;61:e15–24.

Loh JA, Verbalis JG. Diabetes insipidus as a complication after pituitary surgery. Nat Clin Pract Endocrinol Metab. 2007;6:489–94.

Miller M, Dalakos T, Moses AM, Fellerman H, Streeten DH. Recognition of partial defects in antidiuretic hormone secretion. Ann Intern Med. 1970;73:721–9.

Oiso Y, Robertson GL, Nørgaard JP, Vinter Juul K. Treatment of Neurohypophyseal Diabetes Insipidus. J Clin Endocrinol Metab. 2013;98:3958–67.

Scranton RA, Baskin DS. Impaired Pituitary Axes Following Traumatic Brain Injury. J Clin Med. 2015;4:1463–79.

Turkkahraman D, Saglar E, Karaduman T, Mergen H. AVP-NPII gene mutations and clinical characteristics of the patients with autosomal dominant familial central diabetes insipidus. Pituitary. 2015;18:898–904.

Physiopathology, Diagnosis, and Treatment of Inappropriate ADH Secretion and Cerebral Salt Wasting Syndrome

13

Aoife Garrahy, Martin Cuesta, and Christopher J. Thompson

Contents

A. Garrahy · M. Cuesta · C. J. Thompson (✉)
Academic Department of Endocrinology, Beaumont Hospital/RCSI Medical School, Dublin, Ireland
e-mail: cuestamartintutor@gmail.com; christhompson@beaumont.ie

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_14

Abstract

The syndrome of inappropriate antidiuresis (SIAD) is the most frequent cause of hyponatremia in clinical practice. There is a well-recognized relationship between hyponatremia and increased morbidity and mortality in the medical literature, though whether SIAD confers the same mortality as other causes of hyponatremia remains unknown. The causes of SIAD comprise a wide variety of diseases, including malignancy, drugs, respiratory and central nervous system disorders among others. There have been significant advances in the treatment of SIAD in recent years, in particular since the introduction of the vasopressin-2 receptor antagonists, which provide a disease-specific tool to target the underlying pathophysiology of SIAD. The evidence base recommendations and the utility of the available therapies for SIAD are discussed in this chapter. Fluid restriction is the first line therapy for chronic SIAD recommended by all current guidelines, despite the lack of good evidence base to support its use in the medical literature. A number of key points relevant to the use of fluid restriction are presented in the manuscript which may be helpful for the physician. In addition, the evidence base to use other therapies such as demeclocycline, oral urea, furosemide, or sodium chloride tablets is very limited. Conversely, the clinical efficacy and safety of tolvaptan is supported by well-designed, randomized, placebo controlled, clinical trials. However, the cost of the therapy and the need for long term safety data may limit its use in clinical practice. Finally, we also review the management of acute hyponatremia, with a focus on the use of bolus therapy with 3% hypertonic sodium chloride for the treatment of patients with cerebral edema.

Keywords

Hyponatremia · SIAD · Mortality · Tolvaptan · Fluid restriction

Introduction

The Syndrome of Inappropriate antidiuresis (SIAD) is a common clinical and biochemical syndrome characterized by hyponatremia which occurs in a patient who is clinically euvolemic, and who displays evidence of inappropriate urinary concentration and reduced free water excretion. It is invariably due to elevated plasma concentrations of the antidiuretic hormone, arginine vasopressin (AVP) (Robertson et al. 1982). The first description of SIAD was published by Schwartz and Bartter in 1957. The two Boston researchers carefully documented the clinical and biochemical characteristics of two patients with bronchogenic carcinoma and hyponatremia (Schwartz et al. 1957). Clinical investigations in these two patients, alternating water restriction and fluid loading, demonstrated an inability to excrete free water. This prompted the authors to postulate that an excess of circulating "antidiuretic hormone" reduced renal free water clearance, resulting in dilutional hyponatremia. Their hypothesis was proven when the later development of a radioimmunoassay for AVP enabled researchers to demonstrate high plasma

concentrations of AVP, in patients diagnosed with SIAD (Zerbe et al. 1980). Since then, SIAD has been recognized to occur as a complication of a wide variety of conditions, particularly intracerebral, pulmonary, and malignant diseases, and to be shown to be a side effect of a large number of medications.

For many years, SIAD was regarded as clinically unimportant, but recent research has demonstrated a strong association between hyponatremia and poor clinical outcomes, including excess mortality, and new treatments are now available which specifically target the causation of SIAD. As a result, interest in SIAD has grown exponentially, and the volume of publications on SIAD has risen dramatically. However, there are still significant areas where current knowledge and research are deficient. Most published papers have concentrated their attentions on all-cause hyponatremia, rather than SIAD, so the natural history, mortality, and morbidity of SIAD have been poorly described. In addition, the measurement of biochemical criteria essential for the diagnosis of SIAD have been inadequate for accurate classification of hyponatremia, in audits of clinical practice (Tzoulis et al. 2014b) and in SIAD registries (Verbalis et al. 2015); in a recent Italian survey, less than half of clinicians utilized recommended biochemical parameters to diagnose SIAD (Peri and Giuliani 2014). Guidelines for management of hyponatremia (Verbalis et al. 2013; Spasovski et al. 2014) have emphasized that treatment of hyponatremia should be etiology-specific, so there is a great need for good quality prospective data in SIAD, which can provide the basis for accurate recommendations on management.

In this review, we will cover the etiology, diagnosis, and management of SIAD. We will separate out the scientific data referring directly to SIAD from that which refers to all-cause hyponatremia.

Pathophysiology of SIAD

Epidemiology

Hyponatremia is the commonest electrolyte disturbance found in clinical practice. SIAD has been shown to be the commonest cause of hyponatremia (Peri and Giuliani 2014). In our recent prospective study of 1323 patients admitted to our hospital with hyponatremia $<$130 mmol/l, 43% were classified as having SIAD, based on good ascertainment of the basic diagnostic clinical and biochemical parameters (Cuesta et al. 2016). This compared with 33% with hypovolemic and 21% with hypervolemic hyponatremia. To put this in clinical perspective, 58 patients per month were admitted to our 600 bed acute hospital with SIAD – close to two patients a day. The clinical impact of hyponatremia means that these patients occupy hospital beds for longer than their eunatremic counterparts (Sterns and Silver 2016) and contribute disproportionately to medical costs (Corona et al. 2016). As the causation of SIAD is so varied, SIAD enters the clinical practice of almost every speciality. Although management of SIAD falls mainly into the specialist interest of endocrinologists and nephrologists, it is important that all clinicians are aware of the basic principles of diagnosis and investigation of SIAD.

Etiology

SIAD is seen as a complication of many conditions including malignancy, CNS disorders, pulmonary disease, and medication. The etiology of SIAD in a large European and US cohort, published as the multicenter Hyponatremia Registry (Verbalis et al. 2015) was malignancy (24% – the commonest cause), medication (18%), pulmonary disease (11%), and intracerebral disease (9%). The results of a recent single-center retrospective study of 555 patients with SIAD showed broadly similar results, in that malignancy and medication were the commonest underlying causes (Shepshelovich et al. 2015). The results of the series published from our hospital were slightly different; central nervous system disease was the commonest cause of SIAD in our series (Table 1), which reflects the status of our hospital as the site of the Irish National Neurosurgical service.

In most published series, malignancy is the commonest cause of SIAD. A recent review of 2048 patients attending a Dubai oncology service demonstrated the presence of hyponatremia (<130 mmol/l) in 57% of prostate cancer patients. Hyponatremia was also found frequently in patients with pancreatic cancer (50%), liver cancer (49%), lung cancer (40%), and breast cancer (24%). All-cause mortality was increased fourfold in the entire oncology cohort, in those patients with plasma sodium concentration <130 mmol/l, though a direct causative role for hyponatremia could not be inferred. Poorer prognosis for patients with hyponatremia has also been reported in patients with small cell lung cancer (Tiseo et al. 2014), hepatocellular carcinoma (Biolato et al. 2014a), mesothelioma (Berardi et al. 2015a), and metastatic renal cell carcinoma (Bellmunt and Leow 2014). However, the increased mortality in these studies was associated with all-cause hyponatremia, not specifically SIAD, and this is a recurrent difficulty in analyzing the independent effects and prognostic implications of SIAD in the literature. The original report of SIAD by Schwartz and Bartter (Schwartz et al. 1957) described two patients with small cell lung cancer and approximately 15–18% of cases of small cell lung cancer develop SIAD (Tiseo et al. 2014), with a lower incidence in non-small cell carcinoma of the lung of 0.4–2% (Crowley and Thompson 2008). SIAD in lung cancer is often due to true ectopic secretion of AVP from tumor tissue, and immunostaining for AVP has been demonstrated in malignant cells derived from a bronchogenic tumor (George et al. 1972). As AVP is derived directly from the tumor, successful tumor treatment may be associated with a decrease in AVP secretion and normalization of plasma sodium concentration (Crowley and Thompson 2008).

We have demonstrated that hyponatremia occurs in 3% of intracranial tumors, almost all of which is due to SIAD; the incidence of hyponatremia rises to 15% after neurosurgery (Sherlock et al. 2009). SIAD also occurs frequently in head and neck tumors (Zohar et al. 1991; Ferlito et al. 1997), hematological malignancies, urological tumors and gastroenterological cancers (Crowley and Thompson 2008). Although SIAD in malignancy is nearly always due to the underlying cancer, a recent review showed that 27% of SIAD in malignant disease is due to other etiologies (Goldvaser et al. 2016), which serves as a reminder to consider other diagnoses in patients with malignancy.

Table 1 Description of the etiology in patients with SIAD in a prospective cohort of 573 patients (Reproduced with permission from Cuesta et al. 2016)

	Etiology of SIAD	n	%
CNS ($n = 148$, 26%)	Subarachnoid hemorrhage	30	20
	Ischemic stroke	22	15
	Intracranial tumor	28	19
	Traumatic brain injury	9	6
	Hemorrhagic	19	13
	Hydrocephalus	9	6
	Miscellaneous	31	21
Pulmonary diseases ($n = 111$, 19%)	Lobar pneumonia	50	45
	Other respiratory tract infections	41	37
	Bronchiectasis	9	8
	Miscellaneous: pneumothorax, pulmonary fibrosis, pulmonary embolism, etc	11	10
Malignancy ($n = 105$, 18%)	Lung cancer	35	33
	SCLCA $n = 14$		
	NSCLCA $n = 21$		
	Gastrointestinal malignancy	33	32
	Urological malignancy	12	12
	Hematological malignancies	10	9
	Other malignancies	15	14
Drug-related ($n = 47$, 8%)	Carbamazepine	20	43
	Oxcarbazepine	7	15
	SSRI	12	25
	Other	8	17
Postoperative ($n = 61$, 11%)	Orthopedic surgery	36	59
	Hip fracture	25	41
	Other	11	18
	Other surgeries	25	41
	Intraabdominal surgery	14	23
	Lower limb amputation	5	8
	Other	7	11

CNS central nervous system, *SSRI* selective serotonin reuptake inhibitor, *CA* carcinoma, *NSCLCA* non-small cell lung carcinoma, *SCLCA* small cell lung carcinoma

SIAD occurs as a complication of most pulmonary and intracerebral diseases, as well as a wide variety of drugs and infective processes.

Pathophysiology of SIAD

SIAD is characterized by inability to excrete free water, due to inappropriately elevated plasma AVP concentrations. The hyponatremia associated with SIAD is therefore dilutional, with minor expansion of blood volume, suppression of plasma renin, and elevation of plasma natriuretic peptide concentrations. However, as this does not produce clinical evidence of volume expansion, such as peripheral or sacral edema, SIAD is classified clinically as euvolemic hyponatremia. Urine is

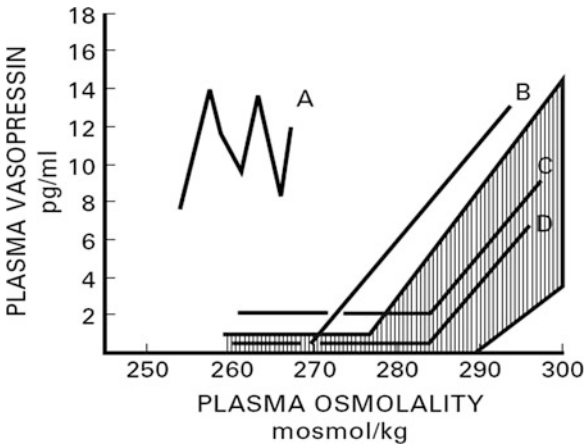


Fig. 1 Patterns of abnormal AVP secretion in SIAD (A–D) (Adapted with permission Smith et al. 2000)

inappropriately concentrated due to the antidiuretic activity of increased plasma vasopressin concentrations. The linear relationship between osmolality and AVP is very reproducible in man (Thompson et al. 1991) but clinical studies in SIAD have demonstrated that this relationship is profoundly disturbed, with four distinct categories of abnormal AVP secretion (Robertson et al. 1982) (Fig. 1).

Type A: Complete loss of the physiological relationship between plasma osmolality and plasma AVP, as a result of unregulated AVP secretion, is the most common pattern of abnormal AVP secretion, occurring in 40% of patients. Type A SIAD has classically been described in association with small cell lung tumors; these tumors synthesize and secrete bioactive AVP; tumor tissue specimens stain positively for mRNA for AVP. Plasma AVP secretion is not suppressed by lowering plasma sodium concentration (Smith et al. 2004), and as thirst is not suppressed in this variant of SIAD, drinking occurs even at plasma osmolalities which are below the physiological osmotic threshold of 284 mOsm/kg (Thompson et al. 1986), leading to persistent hyponatremia which is difficult to manage with fluid restriction.

Type B: This variant of abnormal AVP is characterized by resetting of the osmotic threshold for AVP secretion to a lower level than would be physiologically normal. Above the lower osmotic threshold, the linear relationship between plasma osmolality and plasma AVP concentration is maintained. Severe hyponatremia is less common in this variant, as lowering the plasma osmolality to below the osmotic threshold suppresses AVP and allows an aquaresis to occur. A lowered osmotic threshold for thirst has also been demonstrated in Type B SIAD (Smith et al. 2004).

Type C: This form of SIAD has been rarely reported. When plasma osmolality is above the physiological osmotic threshold for AVP secretion of approximately 284 mOsm/kg, the linear relationship between plasma AVP and plasma osmolality is preserved. However, if plasma osmolality drops below the physiological threshold, low grade AVP secretion continues, leading to hyponatremia. Although this type has been documented in the literature, we have never seen a case with this type of SIAD.

Type D: This is a very rare variant of SIAD, confined to occasional case reports. Patients develop hyponatremia which satisfies the clinical criteria for the diagnosis of SIAD, but plasma AVP concentrations are undetectable. Type D SIAD may be explained by several potential mechanisms. In some children an X-linked activating mutation of the V2 receptor (missense mutations in codon 137 from arginine to cysteine or leucine) has been described, leading to persistent water reabsorption in the kidney tubules (Feldman et al. 2005; Decaux et al. 2007). In other patients it might be due to an abnormal regulation of aquaporin-2 water channels in the renal collecting tubules or due to the secretion of an unidentified antidiuretic substance by tumor cells, which is undetectable in assays for vasopressin (Robertson 2006). The evidence base to recommend specific therapies in type D SIAD is very limited. However, a group from Belgium have reported their clinical experience in the long-term treatment with fluid restriction and low doses of urea in a subgroup of patients with good compliance, efficacy, and safety (Vandergheynst et al. 2012).

Inappropriate elevation of plasma AVP in SIAD leads to increased expression of AQP2 and AQP2 mRNA in the renal tubule. Sustained stimulation of aquaporins causes re-absorption of water from the renal tubules. The resultant increase in the ratio of total body water to sodium causes hyponatremia. Vasopressin antagonists can reverse the expression of AQP2 proteins and mRNA, demonstrating that such AQP2 activation is dependent on AVP or on a possible AVP analogue with action at the V2 receptor.

Prognostic Implications

Hyponatremia has been shown to be associated with increased mortality in almost every published series in the literature. Unfortunately, almost every published series has documented mortality associated with all cause hyponatremia, rather than SIAD per se. Unpublished data from our own unit has demonstrated that in the first month of hospital admission, mortality is increased in SIAD patients compared with eunatremic patients, but it is substantially less than the mortality for hypovolemic or hypervolemic patients (Fig. 2) (Cuesta et al. 2017a). This shows that published data on mortality in hyponatremic patients cannot be extrapolated to patients with SIAD. This may reflect the difference in underlying disease spectrum in the three types of hyponatremia or different comorbidities.

Diagnosis of SIAD

Essential Diagnostic Criteria

It is important to distinguish SIAD from other causes of hyponatremia, as the investigation and treatment of SIAD are quite different than the strategies employed in the management of hypovolemic or hypervolemic hyponatremia (Verbalis et al. 2013; Spasovski et al. 2014; Grant et al. 2015). There are clear diagnostic criteria for

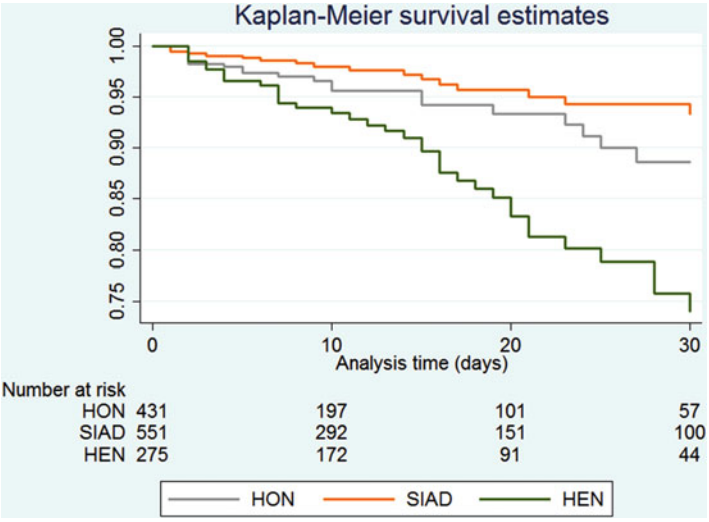


Fig. 2 Mortality risk in patients with hypovolemic hyponatremia (HON), hypervolemic hyponatremia (HEN), and SIAD during the 30 days following hospital admission (Cuesta et al. 2017a)

Table 2 Essential diagnostic criteria for SIAD (Janicic and Verbalis 2003)

Plasma osmolality <270 mOsm/kg
Evidence of inappropriate urinary concentration (UOsm >100 mOsm/kg)
Clinical euvolemia
Elevated urine sodium (>30 mmol/l) with normal salt intake
Hypothyroidism and hypoadrenalism excluded

SIAD (Table 2). It is essential to obtain a urine specimen as early as possible after presentation, and preferably before the institution of treatment such as intravenous fluids. Urine samples should be assayed for urine osmolality, to confirm inappropriate antidiuresis, and to exclude water intoxication, in which AVP concentrations are usually suppressed, leading to dilute urine (osmolality <100 mOsm/kg). In addition, urine sodium concentration should be >30 mmol/l; finding of a low urine sodium concentration (<20 mmol/l) would strongly suggest hyperaldosteronism and the presence of volume depletion. The other key diagnostic criteria are confirmation of euvolemia and exclusion of adrenal and thyroid failure, which are dealt with separately below.

Confirmation of Euvolemic Hyponatremia

The correct categorization of the blood volume status of the patient is important, as the treatment pathway differs according to whether the patient is hypovolemic,

euvolemic, or hypervolemic. Erroneous treatment for hyponatremia may be harmful, in that fluid restriction in a hypovolemic patient will not only fail to restore normonatremia but will also worsen hypovolemia. The clinical signs of hypervolemic are usually straightforward to recognize. The diagnostic challenge is the differentiation between mild dehydration and euvolemia. The clinical signs of mild dehydration may be subtle, and a number of additional measurements have been recommended to improve the recognition of mild dehydration. Measurement of the plasma copeptin to urine sodium ratio has been suggested to be useful in the differentiation between hypovolemic hyponatremia and SIAD (Fenske et al. 2009). Thiazide diuretics may cause SIAD or hypovolemic hyponatremia, and it has been suggested that the measurement of fractional uric acid excretion in patients on thiazides predicts SIAD (Fenske et al. 2008). Although measurement of these parameters is not common in clinical practice, they may be diagnostically useful in difficult clinical cases. Measurement of plasma AVP concentrations is rarely helpful in the diagnosis of SIAD; plasma AVP concentrations are elevated in almost all cases of hyponatremia, irrespective of blood volume status. In addition, reliable and accurate radioimmunoassay are available in very few, very specialized centers, and the results often become available too late to be diagnostically useful. Plasma copeptin concentrations are much easier to measure, with quicker delivery of results. A recent analysis of a large series of almost 300 hyponatremic patients did not, however, find that measurement of plasma copeptin was helpful diagnostically (Nigro et al. 2016). Although plasma concentrations were low in patients whose hyponatremia was derived from over drinking, due to primary polydipsia, it is unlikely that measurement of copeptin would offer a clinical advantage over the demonstration of dilute urine, which is a useful bioassay of suppressed plasma AVP concentrations.

A number of algorithms have been published as diagnostic aids to the approach to hyponatremia (Smith et al. 2000; Verbalis et al. 2013; Spasovski et al. 2014; Grant et al. 2015). All offer a guide to the difficult approach to complex patients but are not a substitute for careful history-taking and clinical examination, and the application of experience and acumen. It is important to recognize that clinical algorithms need to be used carefully when patients have multiple co-morbidities which may be responsible for hyponatremia. The expertise of the hospital specialist, with experience in managing hyponatremia, is invaluable. In situations where it is difficult to distinguish between mild hypovolemia and euvolemia, the empirical use of careful intravenous infusion of 0.9% sodium chloride is recommended. Intravenous saline is beneficial in hypovolemia and is unlikely to be harmful to those with SIAD. Indeed, the observational results of the Hyponatremia Registry study suggested that the rise in plasma sodium concentration in patients with SIAD treated with isotonic saline was greater than in those treated with fluid restriction, though the treatments were not randomized (Verbalis et al. 2015).

Exclusion of Other Causes of Euvolemia Hyponatremia

There are relatively few conditions that present as euvolemic hyponatremia. The essential diagnostic criteria for SIAD stress that cortisol and thyroid hormone

deficiency must be excluded (Table 2). In addition, excess water intake can present as euvolemic hyponatremia.

Primary polydipsia rarely causes hyponatremia, as healthy kidneys have such a capacity for free water excretion. However, if fluid intake exceeds the capacity for free water clearance, hyponatremia may develop. This is a relatively uncommon presentation; a recent Swiss series have shown that primary polydipsia accounts for 24/134 (18%) of cases of euvolemic hyponatremia (Nigro et al. 2016), though in our recent series we found much lower figures of 3/576 (0.5%), though we had a higher cutoff for definition of hyponatremia (130 vs. 125 mmol/l) (Cuesta et al. 2016). If hyponatremia is due to primary polydipsia, urine osmolality will be <100 mOsm/kg, indicating suppression of AVP secretio; whereas in SIAD, where the pathophysiology is inappropriate antidiuresis, urine osmolality will exceed 100 mOsm/kg.

Clinical guidelines stress that hypothyroidism should be excluded prior to the diagnosis of SIAD (Verbalis et al. 2013), as myxoedema is occasionally complicated by hyponatremia. Low plasma sodium concentrations have been reported following radioiodine therapy (Nozu et al. 2011). A recent study of 101 patients with thyroid cancer undergoing radioiodine treatment showed that only 4% of patients became hyponatremic (Vannucci et al. 2016), but hypothyroidism is so rarely the cause of significant hyponatremia that the continued inclusion of thyroid function tests in routine investigation of SIAD may be unnecessary (Berndt et al. 2015). Our view is that although measurement of thyroid function remains as part of the essential investigations of SIAD, the discovery of hypothyroidism should not stop the search for an underlying cause of hyponatremia; an alternative cause is likely.

The exclusion of glucocorticoid deficiency, which presents with a biochemical picture similar to SIAD is, however, essential. Primary adrenal failure presents with hypovolemic hyponatremia, accompanied by hypotension and hyperkalemia due to mineralocorticoid deficiency and is easily differentiated from SIAD. However, secondary adrenal failure presents in a similar way to SIAD. Glucocorticoid deficiency, which is severe enough to cause hyponatremia, is severe enough to cause a potentially life threatening adrenal crisis, so it is a key differential diagnosis for SIAD. A recent Swiss study found that 4/134 patients (3%) presenting with euvolemic hyponatremia were cortisol deficient (Nigro et al. 2016). Our own data was similar; in a large, prospective, carefully investigated series of almost 600 patients with euvolemic hyponatremia, we found that 3.8% of patients had steroid deficiency (Cuesta et al. 2016). Almost half had de novo pituitary disease. The other patients had steroid deficiency secondary to previous corticosteroid treatment of asthma or other inflammatory conditions; it is particularly important to consider previous inhaled steroids, which can have significant suppressive effects on the HPA axis (Woods et al. 2015). In our series one patient presented to the emergency room in our hospital every 2 weeks with euvolemic hyponatremia due to steroid deficiency, so this is not a small print, rare condition. The concern is that many of these patients are missed in routine clinical practice. In a recent English audit of routine clinical practice, only 40% of patients with all-cause hyponatremia had basal measurements of plasma cortisol, and only 2% had synacthen testing (Tzoulis et al. 2014b). Even in the prospective hyponatremia registry study, where patients were

carefully selected for inclusion, only 27% of SIAD patients had documented measurements of HPA axis function (Verbalis et al. 2015). The figure of 3% seems consistent, as a recent prospective, observational study of 298 patients with all-cause hyponatremia <125 mmol/l found that, of the 36% of patients who had SIAD, 3% had evidence of central adrenal insufficiency, although the criteria for making this diagnosis was unclear (Nigro et al. 2015). The consistency of the figures from these three recent studies emphasizes the importance of including formal assessment of the HPA axis essential in the approach to the patient with SIAD.

Establishing the Etiology of SIAD

Once the diagnosis of SIAD is confirmed, the extent of biochemical and radiological investigations to determine the cause of SIAD depends on the individual patient and the judgement of the investigating medical team. If the diagnosis is obvious, such as a history of candidate drugs such as serotonin reuptake inhibitors or carbamazepine, which can be discontinued, or acute lobar pneumonia, no further investigations may be required. Where the etiology is uncertain, we recommend CT scans of brain, thorax, abdomen, and pelvis and more specialized investigations at the discretion of the clinician. It is important to know that a normal chest X-ray does not exclude lung cancer, and CT thorax should be ordered if the chest X-ray is normal.

Differential Diagnosis of Neurosurgical Hyponatremia

Hyponatremia is exceptionally common in neurosurgical conditions. Retrospective data derived from a large cohort of over 3000 patients managed in our own establishment revealed that significant hyponatremia (<130 mmol/l) occurred in 10% of patients with traumatic brain injury (TBI), 6% of patients with pituitary tumors, 16% of patients with intracranial tumors, and 50% of patients with subarachnoid hemorrhage (Sherlock et al. 2009). The majority of neurosurgical hyponatremia is secondary to SIAD, but the differential diagnosis includes inappropriate IV fluids, drugs, acute ACTH deficiency (Hannon et al. 2011) and cerebral salt wasting (Hannon et al. 2012). Prospective evaluation of over 100 patients with subarachnoid hemorrhage (SAH), 50% of whom developed hyponatremia, indicated that 90% had SIAD (Hannon et al. 2014); 10% of those presenting with SIAD had underlying acute ACTH deficiency. Similarly, in over 100 patients with TBI, prospectively followed up, SIAD was the commonest cause of hyponatremia; however, over 80% of patients had evidence of transient inappropriately low ACTH/cortisol responses for a patient in intensive care (Hannon et al. 2013). These studies emphasize that in patients with neurosurgical hyponatremia, it is particularly important to consider the possibility of acute steroid deficiency as a cause of hyponatremia.

The biggest controversy in neurosurgical hyponatremia is whether or not the contribution of cerebral salt wasting (CSWS) is clinically significant. CSWS was first described by Peters et al. (1950) in three patients who presented with

hypovolemic hyponatremia, with clinical evidence of diuresis and natriuresis. CSWS was since been reported in TBI (Agha et al. 2004), SAH (Nelson et al. 1981), and clipping of intracranial aneurysms (Citerio et al. 2007), with characteristic evidence of hypovolemia, and hyponatremia secondary to increased renal sodium excretion. Supporting evidence came from retrospective data in 10 patients who presented with hyponatremia, increased natriuresis, inappropriate urine concentration, and hypovolemia (Nelson et al. 1981). Two subsequent studies provided evidence for a syndrome which was distinct from SIAD (Sivakumar 1994; Wijdicks 1985). In its most florid manifestation, CSWS is very dramatic (Hannon et al. 2012), with profound diuresis and natriuresis, requiring large volumes of intravenous saline to maintain blood pressure and to prevent serious hypovolemic hyponatremia. What is less certain is how often it is seen.

Some researchers have suggested that CSWS is the major cause of hyponatremia following SAH. A small prospective study of hyponatremia in ventilated patients with SAH concluded that CSWS was the predominant pathology contributing to hyponatremia after SAH (Audibert et al. 2009), whilst a large retrospective chart review in California estimated that a quarter of all cases of post-SAH hyponatremia was due to CSWS (Kao et al. 2009). Our own prospective studies in TBI and SAH (Hannon et al. 2013, 2014) were unable to identify a single patient who fitted the criteria for CSWS. Sequential measurement of plasma AVP, plasma BNP, cortisol, and renin, in patients recovering from SAH, showed that the usual pattern of hormonal abnormality was an initial rise in plasma AVP concentrations, followed later by a rise in plasma BNP concentrations. In fact plasma BNP concentrations rose in all patients after SAH, irrespective of the development of hyponatremia; plasma AVP concentrations were a far better predictor of the development of hyponatremia. Our conclusions, which were endorsed in an associated editorial (Verbalis 2014), was that hyponatremia following SAH was rarely due to CSWS. SIAD and acute ACTH/cortisol deficiency are far more important causes (Fig. 3).

Management of SIAD

In 1976, Arieff published a retrospective series, of patients who had been reviewed on an endocrine consult service, with severe hyponatremia. By dividing patients into acute onset hyponatremia (<12 h) and chronic (>3 days) hyponatremia, he was able to demonstrate a marked difference in outcome between the two groups. The acute hyponatremia were more likely to have neurological symptoms, including seizures and coma, and more likely to die, both from hyponatremia or underlying disease (Arieff et al. 1976). Although the series was highly selected, the two patient groups highlighted the difference in presentation and outcome between acute and chronic hyponatremia. A series of elegant studies in a rat model of SIAD showed that adaptation, by a process of loss of brain electrolytes, protected against cerebral edema in chronic hyponatremia (Verbalis and Drutarosky 1988). In contrast, rapid

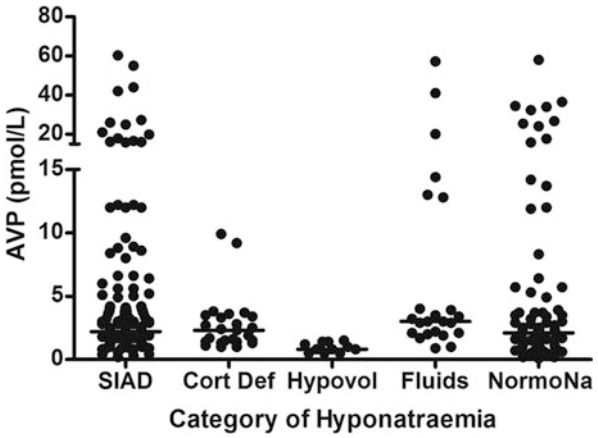


Fig. 3 Comparison of AVP levels between different patient groups in patients with hyponatremia following SAH, each point represents an individual AVP measurement (Adapted from Hannon et al. (2014) with permission). *SIAD* hyponatremia due to SIAD, *Cort Def* hyponatremia due to acute glucocorticoid insufficiency, *Hypovol* hypovolemic hyponatremia, *Fluids* hyponatremia due to inappropriate hypotonic intravenous fluid administration, *NormNa* normal plasma sodium concentration following SAH

onset of hyponatremia leaves no opportunity for osmotic equilibration, with the development of cerebral edema and high mortality (Sterns and Silver 2006).

The basis of the approach to the management of hyponatremia has been a recognition of the different effects of acute and chronic hyponatremia and the relative importance of the presence of neurological symptoms in deciding how aggressively to correct hyponatremia (Verbalis et al. 2013). Therefore in considering the management of SIAD, we shall approach chronic and acute hyponatremia separately.

Chronic Hyponatremia Due to SIAD

For the purposes of prognosis and approach to management, chronic hyponatremia is defined as hyponatremia that has taken more than 48 h to develop. Most cases of SIAD have hyponatremia which has been present for longer periods than this, though in the emergency room information about previous plasma sodium concentrations are not always available. The decision to treat hyponatremia in chronic SIAD needs to be carefully considered. Patients may be relatively asymptomatic because of adaptive changes in the brain and there may be little indication for aggressive correction of hyponatremia. On the other hand, if symptoms of cerebral irritation, particularly seizures or coma, are present, rapid correction of hyponatremia, in order to reverse cerebral edema and prevent coning, may be indicated. The decisions on how, and how fast to treat, are complex, and number of parameters are crucial to sensible decision-making:

1. **Chronicity of hyponatremia.** The quicker the onset of hyponatremia, the more the need for rapid correction, and the safer that correction is likely to be. The risk of osmotic demyelination secondary to over-correction is low in acute hyponatremia.
2. **The natural history of SIAD.** Some cases of SIAD are temporary and resolve with treatment of the underlying condition, and therefore may need no specific therapy. For instance, mild hyponatremia associated with SAH will resolve quickly without treatment (Hannon et al. 2011). The SIAD associated with pneumonia resolves after a mean of 5 days, simply with antibiotic treatment of the infection, and if asymptomatic, may need no treatment. Drug-induced SIAD may simply need withdrawal of the causative medication. Although not every case of SIAD necessarily needs specific therapy, chronic hyponatremia, due to permanent diseases such as bronchiectasis (van der Lubbe et al. 2009), will not correct and may need chronic, even lifelong, therapy.
3. **Side effects of treatment**. The first line treatment of SIAD is fluid restriction according to all published guidelines (Verbalis et al. 2013; Spasovski et al. 2014; Grant et al. 2015), but this may not be appropriate in hyponatremia associated with sepsis, particularly if intravenous antibiotics and intravenous fluids for circulatory support are needed. In addition, neurosurgeons are traditionally reluctant to permit fluid restriction to be implemented in patients under their care with SAH, because of concern that it will precipitate cerebral vasospasm. In both of these circumstances, fluid restriction is undesirable. The decision to treat, with pharmacological agents, must be taken on an individual basis, according to symptoms of cerebral irritation and clinical risk.
4. **Symptoms of hyponatremia**. It is now recognized that even mild hyponatremia may predispose to gait instability and falls (Renneboog et al. 2006), increased fracture rate after falls (Kinsella et al. 2010), and the development of osteoporosis (Verbalis et al. 2010); all of these symptoms are pertinent to the older population, who are at greater risk of SIAD and hyponatremia (Giordano et al. 2016; Miller et al. 2016). Reversal of hyponatremia in SIAD has the potential to reduce the likelihood of developing these complications. Certainly, Renneboog's study showed reversal of gait instability after correction of plasma sodium concentration in SIAD patients (Renneboog et al. 2006). However, the evidence base for symptomatic treatment of SIAD is sparse. The SALT studies, which included a composite group of SIAD and heart failure patients, did show an improvement in the SF-12 questionnaires in the cohort actively managed with tolvaptan, results which were confirmed in the subsequent subgroup analysis in the SIAD cohort (Schrier et al. 2006; Verbalis et al. 2011). Although the evidence base for outcome-based therapy for SIAD is poor, treatment of hyponatremia-associated symptoms is justified on clinical grounds.
5. **Prevention of complications**. There is no evidence to suggest that treatment of hyponatremia will prevent complications such as osteoporosis or fracture, and further work in this area is needed.
6. **Reduction in mortality**. Although mortality is increased in hyponatremia in almost every cohort studied, there are few mortality studies in SIAD patients

specifically, so prospective randomized trials on the effect of treatment of hyponatremia on mortality in SIAD are needed.

In the absence of the type of evidence usually required to treat, management of SIAD should be targeted at the causative condition; specific treatment of hyponatremia per se requires the clinical judgement of the treating physician. There are a number of non-pharmacological and pharmacological options, and the relatives merits have been reviewed in recommendations produced from USA/Ireland (Verbalis et al. 2013), and clinical guidelines from Europe (Spasovski et al. 2014), Britain (Grant et al. 2015), and Spain (Runkle et al. 2013). Although there are areas of common agreement between the various guidelines, including the prioritization of fluid restriction as first line therapy for chronic hyponatremia, there are fundamental differences in opinion in some areas. The European guidelines, for instance, were unable to recommend the use of vaptans in any circumstance, prioritizing the use of urea, whereas the other three clinical recommendations all agreed on the judicious use of vaptans in certain circumstances.

Fluid Restriction

Fluid restriction, to a daily oral intake of 500 mls of fluid less then urine output, was recommended as first line therapy for mild-to-moderate hyponatremia secondary to SIAD in all of the recent guidelines. It is cheap, free of side effects, and available to be used in all hospitals, including nonspecialized units. However, there is a lack of randomized controlled trial evidence to prove the clinical effectiveness, or the safety, of fluid restriction. It is also difficult for patients to implement as long-term therapy, as the downward resetting of the osmotic thirst threshold in SIAD (Smith et al. 2004) means that patients remain thirsty at plasma osmolalities where thirst should be physiologically suppressed. In addition, there is doubt regarding the acute impact of fluid restriction. The Hyponatremia Registry reported management of hyponatremia in a large cohort of selected patients with SIAD; the results of observed clinical practice showed that 48% of patients were treated first line with fluid restriction, but that this treatment was no better than no treatment at all, with a mean increase in plasma sodium concentration of only 1 mmol/l/day (Verbalis et al. 2015). Fluid restriction is often difficult to implement in patients who are acutely unwell, as intravenous fluids, antibiotics, and nutritional support take the fluid intake above that necessary to elevate plasma sodium concentration.

The US guidelines have suggested that a number of parameters may assist the clinician in predicting patients who will not respond to fluid restriction (Verbalis et al. 2013). Urine osmolality, which is a bioassay for the action of AVP, gives an indication of the level of circulating antidiuretic hormone. Urine osmolality >500 mOsm/kg has been suggested to be strongly predictive of poor response to fluid restriction. Both the US (Verbalis et al. 2013) and the British guidelines (Grant et al. 2015) recommend the application of the Furst formula (urine Na + urine K/serum sodium) (Furst et al. 2000), with a ratio >1 strongly predictive of failure to respond to fluid restriction. In addition,

fluid intake >1.5 L/day and low urine volume (<1 L/24 h) also predict failure of fluid restriction. Data from prospective studies conducted in our unit shows that over 50% of SIAD patients have biochemical or clinical data at diagnosis which predicts poor response to fluid deprivation (Cuesta et al. 2017b). It is probably fair to say that these recommendations are more likely to be useful in predicting the response to fluid restriction in chronic hyponatremia; new onset SIAD will often show normalization of plasma sodium with treatment of the underlying condition. There is a clear need for these putative predictors of response to fluid restriction to be tested experimentally. However as fluid restriction is unproven, and potentially ineffective, there is a growing move to consider other treatments to be first line in patients with SIAD.

Demeclocycline

Demeclocycline is a tetracycline derivative which acts on the collecting tubules in the nephron, causing nephrogenic diabetes insipidus, and an increase in free water clearance (Dousa and Wilson 1974). It has been used as second-line therapy for SIAD for many years, despite the fact that in many countries it has no license for use in the management of hyponatremia. The increase in renal water excretion leads to an increase in plasma sodium concentration, but the rate of rise is variable and unpredictable. Elevation in plasma sodium concentration is delayed, and disappointing in some patients, whereas in others, hypernatremia from over-vigorous aquaresis can occur. Plasma sodium therefore needs very careful monitoring. Adverse reactions include azotemia, nephrotoxicity, hepatic dysfunction, and photosensitive skin rashes (Miller et al. 1980). In a recent systematic analysis, there was no evidence to support the safety or efficacy of demeclocycline in the literature (Miell et al. 2015). In the Hyponatremia Registry only 3% clinicians, from many different countries, had selected demeclocycline as first line therapy for SIAD (Verbalis et al. 2015). It is still included as second line therapy in most clinical guidelines, though the US recommendations were that it should no longer be considered (Verbalis et al. 2013).

Urea

Urea increases free water clearance and decreases natriuresis, leading to an elevation in plasma sodium concentration. It is used to treat hyponatremia in SIAD in some European countries. In a systematic review of therapies for SIAD, no randomized controlled trials were found which supported the use of urea (de Solà-Morales and Riera 2014); six retrospective trials of the use of urea were considered to have significant bias or methodological problems. However, the data from these retrospective, nonrandomized studies do suggest that urea may be effective in treating SIAD following subarachnoid hemorrhage (Pierrakos et al. 2012) and in the management of hyponatremia in acutely unwell patients (Decaux et al. 2010; Coussement et al. 2012). In one small, nonrandomized study, the effect of urea treatment on hyponatremia due to SIAD was found to be similar to that of vaptans

(Soupart et al. 2012), though the study design meant that the findings were of limited impact. Urea is unlicensed for the treatment of hyponatremia, and there is no commercially available preparation for clinical use. Hospital pharmacies must make up sachets for treatment; patients may find the taste unpalatable. Urea has been recommended as "first choice" second-line therapy for SIAD in the European Guidelines (ECE) and as a potential second-line therapy in the US recommendations. Prospective, randomized studies are necessary to clearly define the value of urea therapy (Decaux et al. 2014).

Furosemide Plus Sodium Chloride Supplementation

Loop diuretics cause natriuresis and aquaresis, but the addition of oral sodium chloride will theoretically offset renal sodium loss, leaving a net loss of renal water, and a rise in plasma sodium concentration. The combination of loop diuretic and oral sodium chloride offers a physiological treatment of SIAD, where there is an excess of body water. However, there are no data from randomized controlled trials to support this form of treatment. Anecdotal evidence suggests that this treatment can induce an equivalent aquaresis to vaptans without the financial burden, but good evidence to support these suggestions is absent.

Vasopressin Receptor Antagonists

In the vast majority of cases of SIAD, hyponatremia is a direct response to elevation in plasma AVP concentration (Robertson et al. 1982), so the development of vasopressin receptor antagonist drugs (vaptans) means that there is now a treatment which specifically targets the pathophysiology of the condition. Vaptans competitively bind to the V2 receptor, preventing AVP-mediated antidiuresis, and causing nephrogenic diabetes insipidus, with an increase in free water clearance. Plasma sodium concentration rises steadily and predictably in most patients, though clinical experience, and the results of clinical trials, show that over-correction of hyponatremia may occur, particularly if the initial plasma sodium is <120 mmol/l (Schrier et al. 2006). There is a need therefore for frequent monitoring of plasma sodium concentrations, particularly in the first 24 h of treatment. Tolvaptan is the only vaptan licensed as an oral agent, for the treatment of SIAD, in Europe.

There is evidence from randomized controlled trials to support the clinical efficacy of tolvaptan. The SALT studies showed that tolvaptan was effective in causing an elevation of plasma sodium concentration in patients with chronic hyponatremia due to SIAD or heart failure (Schrier et al. 2006). Tolvaptan was more effective than placebo in causing an increase in plasma sodium concentration, with an elevation in excess of the maximum recommended correction rate in $<2\%$ of cases. The greatest rise in plasma sodium occurred when baseline plasma sodium concentration was <120 mmol/l, but no case of osmotic demyelination was documented. A subsequent subgroup analysis of the patient cohort who had

hyponatremia due to SIAD showed that tolvaptan was efficacious in this subgroup (Verbalis et al. 2011). The rise in plasma sodium concentration was not due to a reversal of the underlying cause of SIAD, as discontinuation of tolvaptan after 30 days was associated with a drop in plasma sodium concentration. Although the SALT studies were of short duration, the longer SALTWATER study indicated that tolvaptan continued to be effective for up to 4 years of therapy (Verbalis et al. 2011).

The use of vaptans was the major difference between the European guidelines and the other published guidelines and recommendations. The results of the carefully conducted, prospective, placebo-controlled randomized trials of tolvaptan therapy were downgraded by the authors of the European guidelines on the basis of the industry sponsorship (Spasovski et al. 2014). However, while recognizing the industry support of the SALT studies, the strength of the data in the trials was sufficient for the USA, UK, and Spanish guidelines (Verbalis et al. 2013; Runkle et al. 2013; Grant et al. 2015) to recommend the use of vaptans as second-line therapy in SIAD. There is no doubt that further studies comparing the use of vaptans with existing first-line therapies such as fluid restriction would be valuable however.

There are a number of safety considerations attached to the use of vaptans, which are derived from the US recommendations (Verbalis et al. 2013) (Table 3). Although there were relatively small numbers of patients with plasma sodium concentrations $<$120 mmol/l in the SALT studies, the data from these studies showed that the greatest elevations in plasma sodium occurred in those with the lowest baseline plasma sodium concentrations. The implication of this is that the potential for overcorrection, with the risk of osmotic demyelination, is greatest in those with the most severe hyponatremia. It is essential, therefore, to maintain frequent monitoring of electrolytes, if vaptans are used to treat severe hyponatremia.

There is no strong outcome for the use of vaptans in patients with mild/moderate hyponatremia. The SALT studies did show that improvements in plasma sodium concentration were associated with an improvement in the SF-12® Mental Component Score readings in the tolvaptan-treated patients, indicating improvement in quality of life (Schrier et al. 2006). A recent meta-analysis has also suggested that treatment of hyponatremia is associated with a reduction in overall mortality (Corona et al. 2015). As with so much of the literature, hyponatremia was not SIAD specific in this meta-analysis. However, improvements in parameters such as gait, falls, and cognitive function remain to be proven. The availability of this data is particularly important given the high unit cost of vaptans. It has been suggestion that the cost of vaptan therapy could be justified by reduction in duration of hospital admission (Imamura et al. 2016) and reduction of hospital costs (Miell et al. 2015) and associated morbidity (Dasta et al. 2012); a recent meta-analysis calculated that hyponatremia increased the cost of hospital admission by $3000 in US studies (Corona et al. 2015), so there are grounds to test the hypothesis that vaptan therapy can reduce the cost of hospital treatment by reducing length of stay. However there is a clear requirement for better outcome studies, specific to SIAD, and head to head comparisons with other interventions are also badly needed (Peri 2013).

A summary of the key aspects of the main therapies for SIAD is shown in Table 3.

Table 3 Summary of key aspects of therapies for SIAD. *pNa* plasma sodium, *RCT* randomized controlled trials, *LFTs* liver function tests

	Evidence	Practicality	Capacity for over-correction of pNa	Side effects
Fluid restriction	Nil	Cheap Poor patient tolerability Close supervision needed Ill patients may need fluid support	Unlikely	Dry mouth Patient tolerability
Demeclocycline	Nil	Available. Careful monitoring for side effects	No data, but clinical experience indicates a risk	Thirst Diarrhea Photosensitivity Liver disease Renal disease
Urea	Nonrandomized observational studies	No license or available preparation. Needs on-site preparation by pharmacy; cost dependent on pharmacy	Reported in literature Monitoring essential	Unpleasant taste Headache
Furosemide and oral salt	Anecdotal	Readily available Cheap	No data available	Thirst Hypokalemia Uremia
Vaptans	Good RCT data	Full license for SIAD Expensive and not universally reimbursable	Reported in literature Monitoring essential	Thirst Polyuria Raised LFTs

Acute Hyponatremia

Most patients with SIAD present with chronic hyponatremia, but in some circumstances, they can develop an acute drop in plasma sodium concentration due to supervening illness. Acute hyponatremia is defined as a drop in plasma sodium which occurs over less than 48 h. As there is no time for cerebral adaptation, cerebral edema is more likely to occur, and neurological symptoms, such as seizures and coma, are much more likely to develop; progression to death may occur (Sterns 1987; Sjøblom et al. 1997). In a patient who presents with hyponatremia complicated by neurological symptoms, an acute fall in plasma sodium concentration should be suspected, and cerebral herniation, with associated high mortality, should be considered (Arieff 1986; Adrogué and Madias 2000; Sterns et al. 2009, 2010).

Patients with chronic, stable, asymptomatic SIAD may develop acute hyponatremia in several circumstances. Acute infections, such as pneumonia, may add

a sudden drop in plasma sodium concentration to chronic hyponatremia, with the development of neurological symptoms. Gastroenteritis, with increased gut salt and water losses, may cause acute hypovolemic hyponatremia to be superimposed upon stable SIAD, whereas excess intravenous fluids administered post-operatively can also make stable hyponatremia rapidly symptomatic. Acute SIAD can develop in normonatremic patients who undergo intracerebral events such as subarachnoid hemorrhage or who undergo neurosurgery.

In any of these circumstances, symptomatic acute hyponatremia needs more urgent treatment than chronic hyponatremia. The US recommendations emphasize that the urgency of treatment is determined by the presence of neurological symptoms more than the absolute level of plasma sodium concentration. The traditional recommendation is to gradually elevate plasma sodium with low-dose infusion of hypertonic (3%) sodium chloride; the biochemical target of treatment was recommended to be a steady increase in plasma sodium concentration, of up to12 mmol/l, over 24 h. The theory was that a carefully monitored increase in plasma sodium, without exceeding set limits, would reduce the mortality associated with symptomatic hyponatremia, without exposing the patient to the risk of osmotic demyelination, due to overcorrection of plasma sodium. However, in the last few years, a number of experts have recommended an alteration in the targeted approach; in order to quickly decompress a brain compromised by cerebral edema, it has been suggested that an increase in plasma sodium concentration of 4–6 mmol/l over 4 h may be more effective in reducing mortality in acute hyponatremia (Sterns et al. 2009). Data in normonatremic cerebral edema has shown that hypertonic saline infusion, to cause an increase of 5 mmol/l in plasma sodium concentration, can reduce intracranial pressure and reverse clinical signs of cerebral herniation (Koenig et al. 2008).

The US recommendations have adopted this advice and have suggested that early management of acute symptomatic hyponatremia should be targeted at reduction of cerebral edema by using intravenous boluses of 3% sodium chloride, repeated until an increase in plasma sodium of 4–6 mmol/l over 4 h is achieved (Verbalis et al. 2013). This rapid early increase in plasma sodium is anecdotally sufficient to reduce symptoms of hyponatremia. A steady infusion of 3% sodium chloride can gradually increase plasma sodium concentration over the rest of the first 24 h, with a targeted rise of 8 mmol/l over that time. The use of bolus hypertonic saline is not evidence based but makes physiological sense, and we have found it effective in clinical practice. The evidence base for any form of treatment of acute hyponatremia is small.

Rapid reversal of hyponatremia runs the risk of osmotic demyelination, but in genuine acute hyponatremia, this dreaded complication is very rare. Caution should be exercise in high risk patient groups, which include alcoholics, the malnourished, and those with hepatic disease. Careful monitoring of plasma sodium, with measurements every 2–4 h, is recommended to identify over-rapid correction. The targets for avoidance of osmotic demyelination are summarized in Table 4.

Once the initial rise in plasma sodium has been achieved, the risk of death due to cerebral herniation has been significantly reduced, and the urgency of reversal of hyponatremia has abated. Plasma sodium can be increased slowly, using a traditional low-dose hypertonic saline infusion, over the subsequent 18–20 h, keeping plasma

Table 4 Targets for elevation in plasma sodium in hyponatremic patients, with lowered targets in patients at high risk of ODS (*ODS* osmotic demyelination syndrome) (Verbalis et al. 2013)

	Ideal rise of plasma sodium in first 24 h (mmol/l)	Maximum rise in plasma sodium in first 24 h (mmol/l)
Normal risk patient	8	12
High risk of ODS Plasma sodium <105 mmol/l Hypokalemia Alcoholism Malnutrition Advanced liver disease	6	8

sodium increases within the recommended parameters in Table 4. Over correction is less likely to cause osmotic demyelination in patients with acute hyponatremia, but if there is doubt over the duration of hyponatremia, plasma sodium can be re-lowered to safe limits with either intravenous dextrose, desmopressin, or both, according to clinician preference and experience (Verbalis et al. 2013).

Prognosis in SIAD

There is clear evidence from many studies that hyponatremia is associated with increased mortality (Hannon and Thompson 2010), and evidence from meta-analysis that treatment of hyponatremia may reduce mortality (Corona et al. 2015). However, the mortality data in the literature almost always refers to all-cause hyponatremia, rather than SIAD alone. This is important as it is not possible to extrapolate data from all cause hyponatremia to SIAD; the underlying diseases causing SIAD are too different from those causing hypo- or hypervolemic hyponatremia. The data from early mortality due to hyponatremia from our unit demonstrates this (Fig. 2), as it shows that early mortality in patients with hyponatremia secondary to SIAD is less than it is for other causes of hyponatremia (Cuesta et al. 2017a). Hopefully prospective data from the Hyponatremia registry (Verbalis et al. 2015) and other prospective studies will give a clearer indication of SIAD-specific mortality. The other great unknown is how much mortality and morbidity is related to hyponatremia per se, rather than the underlying cause of hyponatremia. Many of the conditions which predispose to SIAD, such as subarachnoid hemorrhage, lung cancer, and other malignancies have excess mortality associated with the condition itself, and the separate contribution of hyponatremia remains to be elucidated.

There is some evidence to suggest that the mortality and morbidity associated with hyponatremia is attributable to the low plasma sodium, as well as the etiological

condition. A case-controlled study of 139 patients with all-cause hyponatremia, showed that hyponatremic patients were three times more likely to die than matched controls (Tzoulis et al. 2014a). As the authors were careful to match patients with controls with the same disease profile, they felt confident in attributing excess mortality in part to hyponatremia. Similar studies, focusing on SIAD alone, have not been published; the SALT studies did not show a decrease in mortality in the vaptan-treated group (Schrier et al. 2006). However, as the mortality rate in chronic hyponatremia is relatively low, the studies may have been insufficiently powered to prove this association, which was not a primary endpoint of the studies.

An interesting retrospective review of SIAD patients showed that the mortality was lower in SIAD than in a control group of patients with hyponatremia and acute kidney injury (Basu and Ryder 2014). The 5 year mortality in the SIAD cohort was surprisingly high at 81%. Most of the early mortality in SIAD patients was within the subgroups with malignancy or sepsis, with lower rates in drug-induced SIAD. This data underlines the significance of the underlying disease in predicting mortality, with higher rates in more serious conditions. A single-center retrospective review of a large cohort of 555 patients with SIAD showed similar data, with higher mortality in patients with SIAD secondary to malignancy (Shepshelovich et al. 2015). The importance of prospective trials to distinguish between the effects of electrolyte imbalance from those of the underlying condition has been emphasized (Schrier et al. 2013). A number of disease-specific studies do suggest, however, that hyponatremia may worsen prognosis, independently of the underlying condition. Studies in a number of oncological conditions have demonstrated worse prognosis in hyponatremic patients with small cell lung cancer (Tiseo et al. 2014), hepatocellular cancer (Biolato et al. 2014b), malignant pleural mesothelioma (Berardi et al. 2015b), metastatic renal carcinoma (Bellmunt and Leow 2014) and a wide variety of other tumors (Abu Zeinah et al. 2015). However, prospective studies, in which the effect specifically of SIAD is studied, are needed before a confident conclusion about the prognosis in SIAD can be reached.

Summary

There is now treatment for SIAD, the vasopressin receptor antagonists, which specifically targets the underlying cause of the disease process. However, as data on the morbidity and mortality in hyponatremia has not separated SIAD from other causes of hyponatremia, our knowledge of the natural history and prognosis in SIAD is limited. It is very difficult to put a value on interventions in SIAD and to judge the clinical worth and cost-effectiveness of new therapies like the vaptans. Intervention trials will have to clearly differentiate chronic from acute hyponatremia – as the SALT studies did – as the prognosis of each is clearly different. The time has long passed when researchers should be focusing on disease-specific hyponatremia in order to separate SIAD from hyper- and hypovolemic hyponatremia, in order to provide an evidence base which accurately informs about the prognosis in SIAD, and the value of intervention.

References

Abu Zeinah GF, Al-Kindi SG, Hassan AA, Allam A. Hyponatraemia in cancer: association with type of cancer and mortality. Eur J Cancer Care (Engl). 2015;24:224–31. https://doi.org/10.1111/ecc.12187.

Adrogué HJ, Madias NE. Hyponatremia. N Engl J Med. 2000;342:1581–9. https://doi.org/10.1056/NEJM200005253422107.

Agha A, Thornton E, O'Kelly P, et al. Posterior pituitary dysfunction after traumatic brain injury. J Clin Endocrinol Metab. 2004;89:5987–92. https://doi.org/10.1210/jc.2004-1058.

Arieff AI. Hyponatremia, convulsions, respiratory arrest, and permanent brain damage after elective surgery in healthy women. N Engl J Med. 1986;314:1529–35. https://doi.org/10.1056/NEJM198606123142401.

Arieff AI, Llach F, Massry SG. Neurological manifestations and morbidity of hyponatremia: correlation with brain water and electrolytes. Medicine (Baltimore). 1976;55:121–9.

Audibert G, Steinmann G, de Talancé N, et al. Endocrine response after severe subarachnoid hemorrhage related to sodium and blood volume regulation. Anesth Analg. 2009;108:1922–8. https://doi.org/10.1213/ane.0b013e31819a85ae.

Basu A, Ryder REJ. The syndrome of inappropriate antidiuresis is associated with excess long-term mortality: a retrospective cohort analyses. J Clin Pathol. 2014;67:802–6. https://doi.org/10.1136/jclinpath-2014-202243.

Bellmunt J, Leow JJ. Hyponatremia associated with worse outcomes in metastatic renal cell cancer: a potential target for intervention? Eur Urol. 2014;65:731–2. https://doi.org/10.1016/j.eururo.2013.10.057.

Berardi R, Caramanti M, Fiordoliva I, et al. Hyponatraemia is a predictor of clinical outcome for malignant pleural mesothelioma. Support Care Cancer. 2015a;23:621–6. https://doi.org/10.1007/s00520-014-2398-6.

Berardi R, Caramanti M, Fiordoliva I, et al. Hyponatraemia is a predictor of clinical outcome for malignant pleural mesothelioma. Support Care Cancer. 2015b;23:621–6. https://doi.org/10.1007/s00520-014-2398-6.

Berndt M, Harbeck B, Lindner U, et al. Hyponatremia due to thyrotropin deficiency: a fairy tale? Mayo Clin Proc. 2015;90:1305–7. https://doi.org/10.1016/j.mayocp.2015.05.019.

Biolato M, Miele L, Vero V, et al. Hepatocellular carcinoma treated by conventional transarterial chemoembolization in field-practice: serum sodium predicts survival. World J Gastroenterol. 2014a;20:8158–65. https://doi.org/10.3748/wjg.v20.i25.8158.

Biolato M, Miele L, Vero V, et al. Hepatocellular carcinoma treated by conventional transarterial chemoembolization in field-practice: serum sodium predicts survival. World J Gastroenterol. 2014b;20:8158–65. https://doi.org/10.3748/wjg.v20.i25.8158.

Citerio G, Gaini SM, Tomei G, Stocchetti N. Management of 350 aneurysmal subarachnoid hemorrhages in 22 Italian neurosurgical centers. Intensive Care Med. 2007;33:1580–6. https://doi.org/10.1007/s00134-007-0700-5.

Corona G, Giuliani C, Verbalis JG, et al. Hyponatremia improvement is associated with a reduced risk of mortality: evidence from a meta-analysis. PLoS One. 2015;10:e0124105. https://doi.org/10.1371/journal.pone.0124105.

Corona G, Giuliani C, Parenti G, et al. The economic burden of hyponatremia: systematic review and meta-analysis. Am J Med. 2016;129:823–835.e4. https://doi.org/10.1016/j.amjmed.2016.03.007.

Coussement J, Danguy C, Zouaoui-Boudjeltia K, et al. Treatment of the syndrome of inappropriate secretion of antidiuretic hormone with urea in critically ill patients. Am J Nephrol. 2012;35:265–70. https://doi.org/10.1159/000336716.

Crowley RK, Thompson CJ. SIADH. In: Hay I, editor. Clinical endocrine oncology. Oxford: Blackwell; 2008.

Cuesta M, Garrahy A, Slattery D, et al. The contribution of undiagnosed adrenal insufficiency to euvolaemic hyponatraemia: results of a large prospective single-centre study. Clin Endocrinol. 2016;85:836–44. https://doi.org/10.1111/cen.13128.

Cuesta M, Garrahy A, Slattery A, Gupta S, Hannon AM, McGurren K, Sherlock M, Tormey W, Thompson CJ. Mortality rates are lower in SIAD, than in hypervolaemic or hypovolaemic hyponatraemia: Results of a prospective observational study. Clinical Endocrinology 2017a; 87 (4):400–406

Cuesta M, Ortolá A, Garrahy A, Calle Pascual A.L., Runkle I, Thompson CJ. Predictors of failure to respond to fluid restriction in SIAD in clinical practice; time to re-evaluate clinical guidelines?. QJM: An International Journal of Medicine 2017b; 110 (8):489–492

Dasta JF, Chiong JR, Christian R, et al. Update on tolvaptan for the treatment of hyponatremia. Expert Rev Pharmacoecon Outcomes Res. 2012;12:399–410. https://doi.org/10.1586/erp.12.30.

de Solà-Morales O, Riera M. Urea for management of the syndrome of inappropriate secretion of ADH: a systematic review. Endocrinol Nutr. 2014;61:486–92. https://doi.org/10.1016/j.endonu.2014.04.006.

Decaux G, Vandergheynst F, Bouko Y, et al. Nephrogenic syndrome of inappropriate antidiuresis in adults: high phenotypic variability in men and women from a large pedigree. J Am Soc Nephrol. 2007;18:606–12. https://doi.org/10.1681/ASN.2006090987.

Decaux G, Andres C, Gankam Kengne F, Soupart A. Treatment of euvolemic hyponatremia in the intensive care unit by urea. Crit Care. 2010;14:R184. https://doi.org/10.1186/cc9292.

Decaux G, Gankam Kengne F, Couturier B, et al. Actual therapeutic indication of an old drug: urea for treatment of severely symptomatic and mild chronic hyponatremia related to SIADH. J Clin Med. 2014;3:1043–9. https://doi.org/10.3390/jcm3031043.

Dousa TP, Wilson DM. Effects of demethylchlortetracycline on cellular action of antidiuretic hormone in vitro. Kidney Int. 1974;5:279–84.

Feldman BJ, Rosenthal SM, Vargas GA, et al. Nephrogenic syndrome of inappropriate antidiuresis. N Engl J Med. 2005;352:1884–90. https://doi.org/10.1056/NEJMoa042743.

Fenske W, Störk S, Koschker A-C, et al. Value of fractional uric acid excretion in differential diagnosis of hyponatremic patients on diuretics. J Clin Endocrinol Metab. 2008;93:2991–7. https://doi.org/10.1210/jc.2008-0330.

Fenske W, Störk S, Blechschmidt A, et al. Copeptin in the differential diagnosis of hyponatremia. J Clin Endocrinol Metab. 2009;94:123–9. https://doi.org/10.1210/jc.2008-1426.

Ferlito A, Rinaldo A, Devaney KO. Syndrome of inappropriate antidiuretic hormone secretion associated with head neck cancers: review of the literature. Ann Otol Rhinol Laryngol. 1997;106:878–83.

Furst H, Hallows KR, Post J, et al. The urine/plasma electrolyte ratio: a predictive guide to water restriction. Am J Med Sci. 2000;319(4):240.

George JM, Capen CC, Phillips AS. Biosynthesis of vasopressin in vitro and ultrastructure of a bronchogenic carcinoma. Patient with the syndrome of inappropriate secretion of antidiuretic hormone. J Clin Invest. 1972;51:141–8. https://doi.org/10.1172/JCI106784.

Giordano M, Ciarambino T, Castellino P, et al. Diseases associated with electrolyte imbalance in the ED: age-related differences. Am J Emerg Med. 2016;34:1923–6. https://doi.org/10.1016/j.ajem.2016.05.056.

Goldvaser H, Rozen-Zvi B, Yerushalmi R, et al. Malignancy associated SIADH: characterization and clinical implications. Acta Oncol. 2016;55:1190–5. https://doi.org/10.3109/0284186X.2016.1170198.

Grant P, Ayuk J, Bouloux P-M, et al. The diagnosis and management of inpatient hyponatraemia and SIADH. Eur J Clin Investig. 2015;45:888–94. https://doi.org/10.1111/eci.12465.

Hannon MJ, Thompson CJ. The syndrome of inappropriate antidiuretic hormone: prevalence, causes and consequences. Eur J Endocrinol. 2010;162(Suppl):S5–12. https://doi.org/10.1530/EJE-09-1063.

Hannon MJ, Sherlock M, Thompson CJ. Pituitary dysfunction following traumatic brain injury or subarachnoid haemorrhage – in "Endocrine Management in the Intensive Care Unit". Best Pract Res Clin Endocrinol Metab. 2011;25:783–98. https://doi.org/10.1016/j.beem.2011.06.001.

Hannon MJ, Finucane FM, Sherlock M, et al. Clinical review: disorders of water homeostasis in neurosurgical patients. J Clin Endocrinol Metab. 2012;97:1423–33. https://doi.org/10.1210/jc.2011-3201.

Hannon MJ, Crowley RK, Behan LA, et al. Acute glucocorticoid deficiency and diabetes insipidus are common after acute traumatic brain injury and predict mortality. J Clin Endocrinol Metab. 2013;98:3229–37. https://doi.org/10.1210/jc.2013-1555.

Hannon MJ, Behan LA, O'Brien MMC, et al. Hyponatremia following mild/moderate subarachnoid hemorrhage is due to SIAD and glucocorticoid deficiency and not cerebral salt wasting. J Clin Endocrinol Metab. 2014;99:291–8. https://doi.org/10.1210/jc.2013-3032.

Imamura T, Kinugawa K, Nitta D, Komuro I. Tolvaptan reduces long-term total medical expenses and length of stay in aquaporin-defined responders. Int Heart J. 2016;57:593–9. https://doi.org/10.1536/ihj.16-007.

Janicic N, Verbalis JG. Evaluation and management of hypo-osmolality in hospitalized patients. Endocrinol Metab Clin N Am. 2003;32:459–81. vii

Kao L, Al-Lawati Z, Vavao J, et al. Prevalence and clinical demographics of cerebral salt wasting in patients with aneurysmal subarachnoid hemorrhage. Pituitary. 2009;12:347–51. https://doi.org/10.1007/s11102-009-0188-9.

Kinsella S, Moran S, Sullivan MO, et al. Hyponatremia independent of osteoporosis is associated with fracture occurrence. Clin J Am Soc Nephrol. 2010;5:275–80. https://doi.org/10.2215/CJN.06120809.

Koenig MA, Bryan M, Lewin JL, Mirski MA, Geocadin RG, Stevens RD. Reversal of transtentorial herniation with hypertonic saline. Neurology. 2008;70:1023–9. https://doi.org/10.1212/01.wnl.0000304042.05557.60.

Miell J, Dhanjal P, Jamookeeah C. Evidence for the use of demeclocycline in the treatment of hyponatraemia secondary to SIADH: a systematic review. Int J Clin Pract. 2015;69:1396–417. https://doi.org/10.1111/ijcp.12713.

Miller PD, Linas SL, Schrier RW. Plasma demeclocycline levels and nephrotoxicity. Correlation in hyponatremic cirrhotic patients. JAMA. 1980;243:2513–5.

Miller AJ, Theou O, McMillan M, et al. Dysnatremia in relation to frailty and age in community-dwelling adults in the National Health and Nutrition Examination Survey. J Gerontol A Biol Sci Med Sci. 2016;72:376–81. https://doi.org/10.1093/gerona/glw114.

Nelson PB, Seif SM, Maroon JC, Robinson AG. Hyponatremia in intracranial disease: perhaps not the syndrome of inappropriate secretion of antidiuretic hormone (SIADH). J Neurosurg. 1981;55:938–41. https://doi.org/10.3171/jns.1981.55.6.0938.

Nigro N, Winzeler B, Suter-Widmer I, et al. Symptoms and characteristics of individuals with profound hyponatremia: a prospective multicenter observational study. J Am Geriatr Soc. 2015;63:470–5. https://doi.org/10.1111/jgs.13325.

Nigro N, Winzeler B, Suter-Widmer I, et al. Evaluation of copeptin and commonly used laboratory parameters for the differential diagnosis of profound hyponatraemia in hospitalized patients: "The Co-MED Study". Clin Endocrinol. 2016;86:456–62. https://doi.org/10.1111/cen.13243.

Nozu T, Yoshida Y, Ohira M, Okumura T. Severe hyponatremia in association with I(131) therapy in a patient with metastatic thyroid cancer. Intern Med. 2011;50:2169–74.

Peri A. Clinical review: the use of vaptans in clinical endocrinology. J Clin Endocrinol Metab. 2013;98:1321–32. https://doi.org/10.1210/jc.2012-4082.

Peri A, Giuliani C. Management of euvolemic hyponatremia attributed to SIADH in the hospital setting. Minerva Endocrinol. 2014;39:33–41.

Peters JP, Welt LG, Sims EAH, et al. A salt-wasting syndrome associated with cerebral disease. Trans Assoc Am Phys. 1950;63:57–64.

Pierrakos C, Taccone FS, Decaux G, et al. Urea for treatment of acute SIADH in patients with subarachnoid hemorrhage: a single-center experience. Ann Intensive Care. 2012;2:13. https://doi.org/10.1186/2110-5820-2-13.

Renneboog B, Musch W, Vandemergel X, et al. Mild chronic hyponatremia is associated with falls, unsteadiness, and attention deficits. Am J Med. 2006;119:71.e1–8. https://doi.org/10.1016/j.amjmed.2005.09.026.

Robertson GL. Regulation of arginine vasopressin in the syndrome of inappropriate antidiuresis. Am J Med. 2006;119:S36–42. https://doi.org/10.1016/j.amjmed.2006.05.006.

Robertson GL, Aycinena P, Zerbe RL. Neurogenic disorders of osmoregulation. Am J Med. 1982;72:339–53.
Runkle I, Villabona C, Navarro A, et al. The treament of hyponatremia secundary to the syndrome of inappropriate antidiuretic hormone secretion. Med Clin (Barc). 2013;141:507.e1–507.e10. https://doi.org/10.1016/j.medcli.2013.09.002.
Schrier RW, Gross P, Gheorghiade M, et al. Tolvaptan, a selective oral vasopressin V2-receptor antagonist, for hyponatremia. N Engl J Med. 2006;355:2099–112. https://doi.org/10.1056/NEJMoa065181.
Schrier RW, Sharma S, Shchekochikhin D. Hyponatraemia: more than just a marker of disease severity? Nat Rev Nephrol. 2013;9:37–50. https://doi.org/10.1038/nrneph.2012.246.
Schwartz WB, Bennett W, Curelop S, Bartter FC. A syndrome of renal sodium loss and hyponatremia probably resulting from inappropriate secretion of antidiuretic hormone. Am J Med. 1957;23:529–42.
Shepshelovich D, Leibovitch C, Klein A, et al. The syndrome of inappropriate antidiuretic hormone secretion: distribution and characterization according to etiologies. Eur J Intern Med. 2015;26:6–11. https://doi.org/10.1016/j.ejim.2015.10.020.
Sherlock M, O'Sullivan E, Agha A, et al. Incidence and pathophysiology of severe hyponatraemia in neurosurgical patients. Postgrad Med J. 2009;85:171–5. https://doi.org/10.1136/pgmj.2008.072819.
Sivakumar V, Rajshekhar V, Chandy MJ. Management of neurosurgical patients with hyponatremia and natriuresis. Neurosurgery. 1994;34(2):269–74; discussion 74.
Sjøblom E, Højer J, Ludwigs U, Pirskanen R. Fatal hyponatraemic brain oedema due to common gastroenteritis with accidental water intoxication. Intensive Care Med. 1997;23:348–50.
Smith DM, McKenna K, Thompson CJ. Hyponatraemia. Clin Endocrinol. 2000;52:667–78.
Smith D, Moore K, Tormey W, et al. Downward resetting of the osmotic threshold for thirst in patients with SIADH. Am J Physiol Endocrinol Metab. 2004;287:E1019–23. https://doi.org/10.1152/ajpendo.00033.2004.
Soupart A, Coffernils M, Couturier B, et al. Efficacy and tolerance of urea compared with vaptans for long-term treatment of patients with SIADH. Clin J Am Soc Nephrol. 2012;7:742–7. https://doi.org/10.2215/CJN.06990711.
Spasovski G, Vanholder R, Allolio B, et al. Clinical practice guideline on diagnosis and treatment of hyponatraemia. Eur J Endocrinol. 2014;170:G1–47. https://doi.org/10.1530/EJE-13-1020.
Sterns RH. Severe symptomatic hyponatremia: treatment and outcome. A study of 64 cases. Ann Intern Med. 1987;107:656–64.
Sterns RH, Silver SM. Brain volume regulation in response to hypo-osmolality and its correction. Am J Med. 2006;119:S12–6. https://doi.org/10.1016/j.amjmed.2006.05.003.
Sterns RH, Silver SM. Complications and management of hyponatremia. Curr Opin Nephrol Hypertens. 2016;25:114–9. https://doi.org/10.1097/MNH.0000000000000200.
Sterns RH, Nigwekar SU, Hix JK. The treatment of hyponatremia. Semin Nephrol. 2009;29:282–99. https://doi.org/10.1016/j.semnephrol.2009.03.002.
Sterns RH, Hix JK, Silver S. Treatment of hyponatremia. Curr Opin Nephrol Hypertens. 2010;19:493–8. https://doi.org/10.1097/MNH.0b013e32833bfa64.
Thompson CJ, Bland J, Burd J, Baylis PH. The osmotic thresholds for thirst and vasopressin release are similar in healthy man. Clin Sci (Lond). 1986;71:651–6.
Thompson CJ, Selby P, Baylis PH. Reproducibility of osmotic and nonosmotic tests of vasopressin secretion in men. Am J Phys. 1991;260:R533–9.
Tiseo M, Buti S, Boni L, et al. Prognostic role of hyponatremia in 564 small cell lung cancer patients treated with topotecan. Lung Cancer. 2014;86:91–5. https://doi.org/10.1016/j.lungcan.2014.07.022.
Tzoulis P, Bagkeris E, Bouloux P-M. A case-control study of hyponatraemia as an independent risk factor for inpatient mortality. Clin Endocrinol. 2014a;81:401–7. https://doi.org/10.1111/cen.12429.
Tzoulis P, Evans R, Falinska A, et al. Multicentre study of investigation and management of inpatient hyponatraemia in the UK. Postgrad Med J. 2014b;90:694–8. https://doi.org/10.1136/postgradmedj-2014-132885.

van der Lubbe N, Thompson CJ, Zietse R, Hoorn EJ. The clinical challenge of SIADH-three cases. NDT Plus. 2009;2:iii20–4. https://doi.org/10.1093/ndtplus/sfp155.

Vandergheynst F, Brachet C, Heinrichs C, Decaux G. Long-term treatment of hyponatremic patients with nephrogenic syndrome of inappropriate antidiuresis: personal experience and review of published case reports. Nephron Clin Pract. 2012;120:c168–72. https://doi.org/10.1159/000338539.

Vannucci L, Parenti G, Simontacchi G, et al. Hypothyroidism and hyponatremia: data from a series of patients with iatrogenic acute hypothyroidism undergoing radioactive iodine therapy after total thyroidectomy for thyroid cancer. J Endocrinol Investig. 2016;40:49–54. https://doi.org/10.1007/s40618-016-0525-6.

Verbalis JG. Hyponatremia with intracranial disease: not often cerebral salt wasting. J Clin Endocrinol Metab. 2014;99:59–62. https://doi.org/10.1210/jc.2013-4289.

Verbalis JG, Drutarosky MD. Adaptation to chronic hypoosmolality in rats. Kidney Int. 1988;34:351–60.

Verbalis JG, Barsony J, Sugimura Y, et al. Hyponatremia-induced osteoporosis. J Bone Miner Res. 2010;25:554–63. https://doi.org/10.1359/jbmr.090827.

Verbalis JG, Adler S, Schrier RW, et al. Efficacy and safety of oral tolvaptan therapy in patients with the syndrome of inappropriate antidiuretic hormone secretion. Eur J Endocrinol. 2011;164:725–32. https://doi.org/10.1530/EJE-10-1078.

Verbalis JG, Goldsmith SR, Greenberg A, et al. Diagnosis, evaluation, and treatment of hyponatremia: expert panel recommendations. Am J Med. 2013;126:S1–S42. https://doi.org/10.1016/j.amjmed.2013.07.006.

Verbalis J, Greenberg A, Burst V, et al. Diagnosing and treating the syndrome of inappropriate antidiuretic hormone secretion. Am J Med. 2015;129:537.e9–537.e23. https://doi.org/10.1016/j.amjmed.2015.11.005.

Wijdicks EFM, Vermeulen M, Hijdra A, van Gijn j, (1985) Hyponatremia and cerebral infarction in patients with ruptured intracranial aneurysms: Is fluid restriction harmful?. Annals of Neurology 17(2):137–140

Woods CP, Argese N, Chapman M, et al. Adrenal suppression in patients taking inhaled glucocorticoids is highly prevalent and management can be guided by morning cortisol. Eur J Endocrinol. 2015;173:633–42. https://doi.org/10.1530/EJE-15-0608.

Zerbe R, Stropes L, Robertson G. Vasopressin function in the syndrome of inappropriate antidiuresis. Annu Rev Med. 1980;31:315–27. https://doi.org/10.1146/annurev.me.31.020180.001531.

Zohar Y, Talmi YP, Finkelstein Y, et al. Syndrome of inappropriate antidiuretic hormone secretion in cancer of the head and neck. Ann Otol Rhinol Laryngol. 1991;100(4):341.

Physiopathology, Diagnosis, and Treatment of Hyperprolactinemia

14

Valentina Gasco and Silvia Grottoli

Contents

V. Gasco · S. Grottoli (✉)
Department of Medical Science, Division of Endocrinology, Diabetes and Metabolism, Turin, Italy
e-mail: valentina.gasco@unito.it; ninagro@yahoo.it

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_15

Abstract

Prolactin is a pituitary hormone that plays a pivotal role in a variety of reproductive functions. Hyperprolactinemia is a common condition that can result from a number of causes, including medication use and hypothyroidism as well as pituitary disorders. Depending on the cause and consequences of the hyperprolactinemia, selected patients require treatment. The underlying cause, sex, age, and reproductive status must be considered.

Keywords

Prolactin · Hyperprolactinemia · Treatment · Dopamine agonist · Pregnancy · Resistance · Cure

Introduction

Hyperprolactinemia (HPRL) is a common condition that can result from a number of causes, including medication use and hypothyroidism as well as pituitary disorders.

Indeed, HPRL is the more frequent pituitary dysfunction in clinical practice, and it is an important marker of hypothalamic-pituitary disease.

Regardless of etiology, HPRL may result in hypogonadism, infertility, and galactorrhea, or it may remain asymptomatic. Bone loss occurs secondary to HPRL-mediated sex steroid attenuation.

This chapter, after a brief recall on regulation of prolactin (PRL) secretion and its physiology, reviews the causes of HPRL, the diagnostic workup, and the treatment options and, finally, analyzes some treatment pitfalls.

Regulation of Prolactin Secretion

Human PRL is a 199-amino acid polypeptide of 23 kDa of molecular weight, similar in structure to growth hormone (GH) (Melmed and Kleinberg 2003). Its gene is located on chromosome 6. PRL, GH, and placental lactogen share a similar structure because they derive from a common ancestral gene.

PRL is secreted by the anterior pituitary gland by two populations of cells, lactotroph cells, which represent 15–25% of the anterior pituitary cells and secrete only PRL, and mammosomatotroph cells, a minor cell population, which co-secrete PRL and GH. Like most anterior pituitary hormones, PRL is under dual regulation

by hypothalamic hormones delivered through the hypothalamic-pituitary portal circulation: the balance between the two types of signals determines the amount of PRL released from the anterior pituitary gland. Under most conditions the predominant signal is inhibitory, preventing PRL release, and is mediated by the neurotransmitter dopamine (DA). The tuberoinfundibular dopamine (TIDA) neurons of the arcuate nucleus in the hypothalamus release DA into the portal pituitary circulation; DA is delivered via the pituitary stalk to the lactotroph cells, and here, acting via D2 receptors, DA inhibits PRL gene expression and secretion, increasing potassium conductance and thereby hyperpolarizing the cell membrane. Any mechanism that might interfere with the delivery or action of DA will increase serum PRL levels. However, DA is not the only PRL inhibitory factor. In fact, several molecules, including endothelin-1, transforming growth factor-β1, and calcitonin, which act in an endocrine or paracrine fashion, have been identified.

PRL is also regulated by stimulating agents. Among them, serotonin, thyrotropin-releasing hormone (TRH), basic fibroblast growth factor, epidermal growth factor, vasoactive intestinal peptide (VIP) (Kato et al. 1984), oxytocin, and opiates should be mentioned. Estrogens are important regulators of PRL production, enhancing growth of PRL-producing cells and stimulating PRL production directly, as well as inhibiting DA (Cookson 1981).

Beside the pituitary, 20% of PRL concentration relies on extrapituitary production. Extrapituitary PRL is produced by several tissues, such as the brain, mammary epithelial cells and tumors, endometrium, myometrium, lacrimal and sweat glands, skin fibroblasts, and lymphoid organs and cells (Ben-Jonathan et al. 1996).

Prolactin Physiology

Normal PRL levels are considered to be lower than 25 ng/ml for females and 20 ng/ml for males (1 ng/ml is equivalent to 21.2 mUI/l).

PRL is secreted in a pulsatile manner, following a circadian rhythm (Freeman et al. 2000; Rickenlund et al. 2004). PRL levels peak during REM sleep and in the early morning: maximum PRL level is reached 4 h after sleep onset, while the minimum level occurs 6 h after waking up (Frantz 1978).

The PRL receptor is a member of the type 1 cytokine receptor family (Cosman et al. 1990). PRL receptor is a single-pass transmembrane chain that is encoded by its gene on chromosome 5. The PRL receptor gene is comprised of at least ten coding exons. Multiple transcripts, reflecting alternative splicing variants and transcription start sites, account for some of the variability in PRL receptor structure and tissue distribution (Bole-Feysot et al. 1998). This mechanism provides three different isoforms that differ in the length of the cytoplasmic domain (short, intermediate, and long PRL receptor) (Goffin and Kelly 1997; Kelly et al. 1991). In addition, a soluble form, identical to the extracellular domain of the transmembrane PRL receptor, has been identified (Amit et al. 1997; Fuh and Wells 1995; Postel-Vinay et al. 1991).

PRL binds to its receptor through a binding site, forming an inactive complex, which allows for the interaction of a second PRL binding site to another PRL receptor (Goffin

and Kelly 1997; Goffin et al. 1996). The formation of 1:2 complexes of the hormone with its receptor is the essential first step in the transmission of the biologic signal within target cells. Several signaling pathways are activated by the PRL-PRL receptor interaction, but the most important is the phosphorylation of JAK/STAT molecules that leads to gene transcription. Upon ligand-induced dimerization of PRL receptors, JAK2 phosphorylates specific tyrosine residues on the receptor intracellular domain and autophosphorylates residues within the kinase. These phosphotyrosines serve as docking sites for the signal transducer and activator of transcription (STAT) protein, which then translocates to the cell nucleus and activates target genes.

The actions of the kinase are counteracted by multiple tyrosine phosphatases, which rapidly dephosphorylate specific proteins, and maintain the steady-state level of tyrosine phosphorylation at a very low level in the absence of hormonal stimulation (Berchtold et al. 1998).

In addition to the STAT-dependent events triggered by JAK2 activation, there are other STAT-independent signaling pathways that can be activated when PRL binds to its receptor. Src-family kinases may be involved in PRL signaling by virtue of their ability to couple to multiple signaling intermediates. Phosphatidylinositol-3'-kinase, mitogen-activated protein kinases (MAPKs), and protein kinase C have each been observed to be activated by PRL in some systems (Bole-Feysot et al. 1998). STAT-independent pathways have been proposed for PRL signaling, but the physiologic relevance of such mechanisms is not yet clear. It has been suggested that STAT-independent signaling mediates the mitogenic actions of PRL (Das and Vonderhaar 1997).

The best known role of PRL in humans is the development and stimulation of mammary glands during pregnancy to produce milk (lactogenesis). Plasma levels are raised after childbirth and during breastfeeding, and PRL suppresses gonadotrophins in the postpartum period, a contraceptive effect. Although in the pituitary PRL is present in equal amounts in males and females, its physiological role, well established for female reproduction, is still obscure for the male counterpart.

In addition, PRL plays a role in other functions tightly linked to maternity, as suggested by the observation that in mice with altered expression of PRL receptor, ovulation is impaired (Bole-Feysot et al. 1998) and induction of maternal behavior is compromised (Feldman et al. 1993). Recently, this role of PRL in parental behavior has been suggested also in men (Gettler et al. 2012).

Moreover, PRL receptor expression has been demonstrated not only in the breast but also in different cells and tissues, such as the brain, endometrium, ovary, testicle, prostate, pancreas, liver, kidney, intestine, skin, lung, myocardium, lymphoid cells, adipocytes, and endothelial cells (Ignacak et al. 2012; Melmed et al. 2011). Likewise, paracrine-type local PRL secretion (not DA dependent) is known to exist in some of these tissues.

Apart from the effects on other hormones of pituitary- gonadal axis, numerous other various biological functions of PRL have been identified in many aspects of physiological and metabolic processes. In mammals, it stimulates phospholipid synthesis in the alveolar cells of the fetal lung (Hamosh and Hamosh 1977) and also stimulates lipoprotein lipase activity in hepatocytes (Machida et al. 1990). It increases bile secretion as well (Lin et al. 1992). The direct action of PRL on the pancreas results in augmented insulin secretion (Sorenson et al. 1987). PRL is also reported to have a direct effect on

adrenal steroidogenesis. It increases androgens, dehydroepiandrosterone (DHEA), and also cortisol and aldosterone secretion by the adrenal cortex cells (Bole-Feysot et al. 1998; Freeman et al. 2000). In mammals PRL is involved in osmoregulation: it reduces renal Na+ and K+ excretion and stimulates Na+-K+ adenosine triphosphatase activity in the outer medulla of the rat kidney (Pippard and Baylis 1986; Richardsson 1973). Newer investigations show that PRL has an inhibitory effect on Na+-K+-ATPase of rat proximal tubular cells via interaction with renal dopaminergic system (Crambert et al. 2010; Ibarra et al. 2005). Furthermore, PRL increases sodium and chloride ion excretion with sweat and increases water and salt absorption in all segments of the intestine. Ultimately, it causes a reduction of water transport in the human amniotic membrane (Bole-Feysot et al. 1998; Freeman et al. 2000).

The autocrine and the paracrine actions of PRL locally synthesized by lymphocytes seem to have functional significance (Chavez-Rueda et al. 2005). In vitro investigations of human mononuclear cell cultures have shown that PRL alone is unable to induce proliferation of lymphocytes. However PRL acts as a co-stimulating factor for T lymphocytes, activated by an unspecific mitogen (concanavalin A) or by antigen presentations (Chavez-Rueda et al. 2005). The addition of neutralizing antibodies against PRL to the peripheral mononuclear cell cultures significantly decreases the activation and proliferation of T lymphocytes (Chavez-Rueda et al. 2005). It indicates that PRL is secreted locally by activated and proliferating T-cells and that it affects the proliferation on the basis of a positive reciprocal circuit (Chavez-Rueda et al. 2005).

PRL has over 300 described functions, but the clinical relevance is unclear as there is still no clearly recognized deficiency syndrome (Harvey et al. 2008). However, recent and emerging evidence shows that low PRL levels are associated with different pathological correlates and, for this reason, hypoprolactinemia may deserve a specific chapter in clinical manuals, as a new clinical syndrome.

Causes of Hyperprolactinemia

A number of physiological states including pregnancy, breastfeeding, stress, exercise, meals, sleep, and sexual intercourse can cause PRL elevation, as can medication and some pathological conditions (Table 1).

Pathophysiologic causes of HPRL include lactotroph adenomas, stalk disruption, primary hypothyroidism, and renal failure. However, the most frequent non-physiological HPRL cause is drug exposure.

Although the levels can hint at the etiology of the HPRL, there is significant overlap in the figures among different etiologies; this is especially true among those that are normally seen in microprolactinomas and in those secondary to drugs (Fig. 1).

Lactotroph Adenomas

PRL-secreting adenomas (lactotroph adenomas or prolactinomas) are pituitary adenomas that express and secrete PRL to variable degrees, are almost invariably

Table 1 Etiology of hyperprolactinemia

Physiological
Pregnancy and lactation
Sleep
Stress
Coitus
Exercise
Pharmacological
Anesthetics
Anticonvulsant
Anti-H2 histaminergic antagonists
Antihypertensive
Catecholamine depletors
Cholinergic agonists
Dopamine receptor blockers
Dopamine synthesis inhibitors
Estrogens
Neuroleptics/antipsychotics
Neuropeptides
Opiates and opiate antagonists
Tricyclic antidepressants
Pathological
Prolactinoma
Mixed adenoma
Pituitary or hypothalamic diseases with damage/interruption of the stalk
Pituitary mass
Inflammatory or granulomatous diseases
Trauma
Surgery
Radiotherapy
Empty sella
Primary hypothyroidism
Chronic renal failure
Polycystic ovarian disease
Liver cirrhosis
Neurogenic chest wall trauma, surgery, herpes zoster
Paraneoplastic syndrome
Idiopathic hyperprolactinemia

benign, but are nevertheless frequently clinically significant and may be challenging to manage. Prolactinomas are generally classified according to size as micro-adenomas (less than 10 mm in diameter) or macroadenomas (more than 10 mm in diameter). Over 90% of prolactinomas are small, intrasellar tumors that rarely increase in size (Molitch 1995). Occasionally, these adenomas can be aggressive

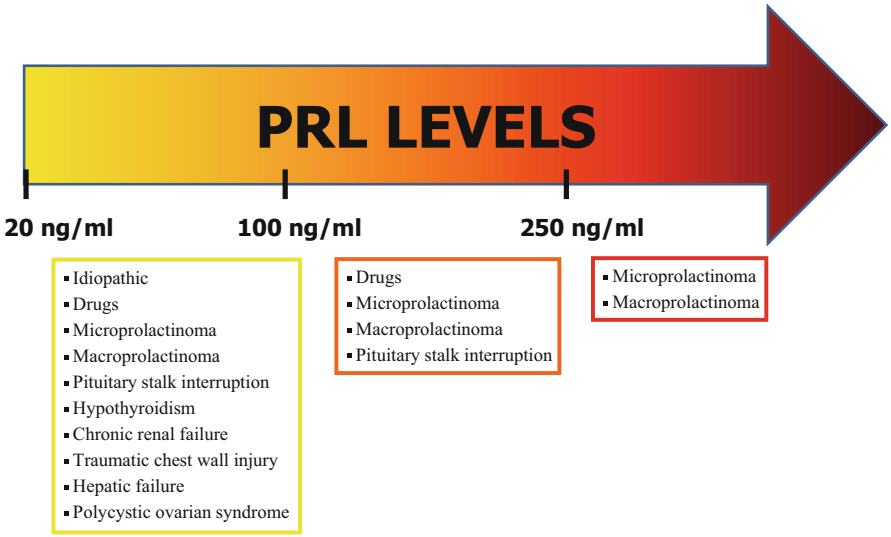


Fig. 1 Levels of PRL vary in function of the different aetiologies of the hyperprolactinaemia

or locally invasive and cause compression of vital structures. Malignant prolactinomas that are resistant to treatment and disseminate inside and outside the central nervous system (CNS) are very rare.

PRL levels typically correlate with tumor size. Individuals with large adenomas can have PRL levels on the order of 10^4 ng/ml (Delgrange et al. 1997), yet with poorly differentiated or cystic lesions, PRL levels will be lower than expected based on size.

It is not known how prolactinomas develop, but the process may involve an early genome mutation that results in a mutated pituitary stem cell. Various permissive factors may then stimulate the proliferation of these mutated cells. Familial prolactinomas have also been described (Sobrinho 1995) suggesting that a genetic component might contribute to the pathogenesis.

Prolactinomas are the most common subtype of secretory pituitary adenoma, accounting for approximately 40% of all pituitary tumors. The reported population prevalence of clinically apparent prolactinomas ranges from 6–10 per 100,000 to approximately 50 per 100,000 (Daly et al. 2006; Fernandez et al. 2010). In an analysis of 1607 patients with medically treated HPRL, the calculated mean prevalence was approximately 10 per 100,000 in men and approximately 30 per 100,000 in women, with a peak prevalence for women aged 25–34 years (Kars et al. 2009).

Stalk Disruption

Because PRL secretion is tonically inhibited by hypothalamic DA, disruption or compression of the pituitary stalk by traumatic injuries or a non-PRL-secreting

pituitary tumor or other parasellar mass will lead to HPRL: the so-called stalk effect. Usually serum PRL does not exceed 100 ng/ml in this setting (Karavitaki et al. 2006), but exceptions do occur.

Patients with large pituitary tumors, craniopharyngiomas, or granulomatous infiltration of the hypothalamus can develop HPRL because of pituitary stalk compression or dopaminergic neuronal damage. In 226 patients with histologically confirmed nonfunctioning pituitary macroadenomas, a PRL level greater than 94 ng/ml reliably distinguished between prolactinomas and nonfunctioning adenomas (Karavitaki et al. 2006).

It has been shown that successful surgical resection of nonfunctioning pituitary adenomas where DA delivery is restored results in rapid normalization of PRL levels (Arafah et al. 1995).

It is important to determine in the presence of a pituitary adenoma whether patients with HPRL also have acromegaly (Bonert and Melmed 2006) because PRL is elevated in up to 50% of patients with GH-secreting tumors (Kleinberg et al. 1977). This may be related to both the "stalk effect" due to a GH-secreting adenoma and the presence of a mixed adenoma secreting both GH and PRL.

Primary Hypothyroidism

Some patients with primary hypothyroidism may have moderate HPRL (Honbo et al. 1978; Molitch 1992). It has been proposed that this is due to the increased synthesis of, or sensitivity to, hypothalamic TRH, which is able to stimulate pituitary lactotroph cells, but the true cause is still unknown. Long-lasting or inadequately treated primary hypothyroidism can cause pituitary hyperplasia that may mimic a pituitary tumor. In this case, particular care should be taken to distinguish pituitary enlargement from prolactinoma. Treatment with L-thyroxine can reverse HPRL and enlargement of the pituitary gland due to thyroid failure (Ahmed et al. 1989; Keye et al. 1976).

Chronic Renal Failure

Patients with renal insufficiency may have moderate HPRL caused by impaired renal degradation of PRL and altered central PRL regulation (Hou et al. 1985; Lim et al. 1979). The latter may be the result of reduced lactotroph responsiveness to DA suppression (Sievertsen et al. 1980).

Drug-Induced Hyperprolactinemia

The most frequent nonphysiological HPRL cause is drug exposure, and, among all of them, the antipsychotics are the main culprits by far with respect to all the others (Kinon et al. 2003).

With drug-induced HPRL, PRL levels increase slowly after oral administration, and it usually takes 3 days for levels to return to normal after drug discontinuation

(Pollock and McLaren 1998; Spitzer et al. 1998). Medication-induced HPRL is usually associated with PRL levels ranging from 25 to 100 ng/ml, but metoclopramide, risperidone, and phenothiazines can lead to PRL levels exceeding 200 ng/ml (Smith et al. 2002a; Meltzer and Fang 1976) (Fig. 1).

The mechanism of antipsychotic-induced HPRL relates to the DA antagonist effect of these drugs on the D2 receptors of the TIDA system and lactotrophs (Molitch 2005a; Green and Brown 1988). By blocking the interaction between DA and the D2 receptors, the tonic inhibition of PRL secretion is negated, and increased PRL secretion results. This explains why antipsychotics with a greater D2 occupation index produce higher and more frequent PRL elevations. This is the case of risperidone and its 9-hydroxymetabolite paliperidone (Bellantuono and Santone 2012; Chwieduk and Keating 2010; Janicak and Winans 2007) considered the second-generation antipsychotics that cause HPRL most often, with levels even higher than those of haloperidol (Kinon et al. 2003). Another action mechanism involved is their ability to cross the blood-brain barrier: risperidone and paliperidone remain the longest outside of the barrier due to their low liposolubility; consequently, they act for a longer period in the TIDA pathway, provoking HPRL (Besnard et al. 2014). The stable link between HPRL and the use of risperidone, paliperidone, amisulpride, and the majority of the first-generation antipsychotics has led to this group being called "PRL-raising" or hyperprolactinemic antipsychotics in the literature. A study on 158 treatment-resistant patients that compared the PRL levels of various antipsychotics estimated that 60–100% of the women and 40–80% of the men treated with hyperprolactinemic antipsychotics presented HPRL (Volavka et al. 2004). In contrast, other atypical antipsychotics, called "PRL-sparing" antipsychotics in the literature, such as aripiprazole, asenapine, clozapine, quetiapine, and ziprasidone present a better profile with respect to limited PRL increase (Byerly et al. 2007; Cruz and Vieta 2011; Montejo et al. 2008; Smith 2008; Svestka et al. 2007).

In a recent meta-analysis on efficacy and tolerability of 15 antipsychotics, paliperidone and risperidone have been shown to be the antipsychotics most related to HPRL. Aripiprazole and quetiapine have the best hyperprolactinemic profile (Leucht et al. 2013).

In addition to antipsychotic medications, many other drugs can cause HPRL; among these verapamil causes HPRL in 8.5% of patients (Molitch 2005a), presumably by blocking hypothalamic DA. Opiates and cocaine act through the μ-receptor (Bart et al. 2003; Tolis et al. 1975; Zis et al. 1984) to cause mild HPRL (Mendelson et al. 1989). The role of estrogen in causing HPRL is controversial (Molitch 2005a). Twelve to 30% of women taking higher estrogen-containing oral contraceptives may have a small increase in serum PRL, but this finding is rarely an indication for therapy (Luciano et al. 1985).

Other Pathological Conditions Characterized by Hyperprolactinemia

Traumatic chest wall injury is a rare potential cause of HPRL (Morley et al. 1977). In a patient with HPRL after a severe burn to the chest wall, intercostal nerve blockade

resulted in normalization of her serum PRL level (Morley et al. 1977), suggesting that a neurogenic stimulus at the site of injury was responsible.

Hepatic failure is a cause of HPRL usually due to decreased clearance of PRL. However recent reports suggest that it is a very rare cause of HPRL (Ress et al. 2014).

The polycystic ovarian syndrome is commonly associated with elevated PRL levels as well (Yazigi et al. 1997).

Finally, the serum PRL level usually rises following an epileptic seizure (including after electroconvulsive therapy).

Idiopathic Hyperprolactinemia

Finally, idiopathic HPRL needs to be considered as diagnosis (Casanueva et al. 2006; Melmed et al. 2011).

Fewer than 10% of patients with idiopathic HPRL ultimately are found to harbor a microadenoma, and progression from a microadenoma to a macroadenoma is rare (Sluijmer and Lappöhn 1992). Spontaneous normalization of PRL levels occurs in approximately 30% of patients with idiopathic HPRL (Schlechte et al. 1989).

Diagnosis of Hyperprolactinemia

Updated guidelines recommend a single measurement of serum PRL to establish the diagnosis of HPRL: a level above the upper limit of normal confirms the diagnosis as long as the serum sample was obtained without excessive venipuncture stress (Casanueva et al. 2006; Melmed et al. 2011). The guidelines advise against performing dynamic tests for the diagnosis of HPRL (Casanueva et al. 2006; Melmed et al. 2011); indeed dynamic tests using TRH, L-dopa, nomifensine, domperidone, and insulin-induced hypoglycemia are not superior to measuring a single serum PRL sample for the diagnosis of HPRL (Casanueva et al. 2006; Melmed et al. 2011).

When initial PRL values are not diagnostic (e.g., above the normal laboratory range, but not high enough to clearly indicate a prolactinoma), sampling should be repeated on another day. In this case, to avoid the effect of pulsatile secretion, two to three samples separated by at least 15–20 min should be obtained.

However this recommendation has been recently criticized by Szosland et al. who suggested that daily profile of PRL in any patient with clinical suspicion of HPRL was the best mode for estimating mean circadian PRL concentration, while the attempts to diagnose HPRL based on a single PRL assay failed due to a high percentage of false-negative and false-positive results (Szosland et al. 2015).

Diagnosis of the Cause of Hyperprolactinemia

When evaluating a patient with symptoms consistent with HPRL and persistently elevated serum PRL, secondary causes should first be ruled out by a careful clinical history, physical examination, pregnancy test, routine biochemical analysis (to evaluate kidney and liver function), and TSH determination (Fig. 2).

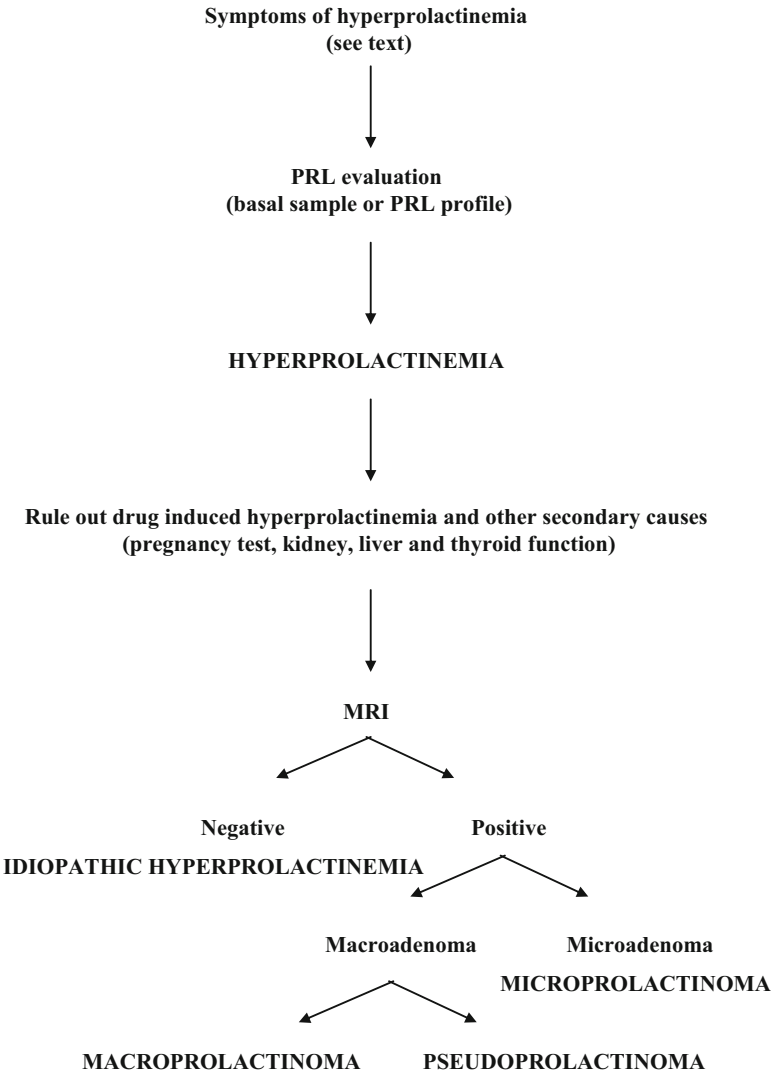


Fig. 2 Recommended diagnostic algorithm for hyperprolactinemia

If the patient is taking a drug known to cause HPRL, it is important to determine if the drug is indeed the cause by withdrawing the drug for at least 72 h, if this can be done safely. If feasible, the patient could be switched to an alternative drug that does not cause HPRL. However it is essential to discuss the issue with the prescribing physician and to obtain a psychiatric evaluation before stopping any psychiatric drugs suspected of causing HPRL. When the drug cannot be stopped, particularly in a patient with neurological symptoms, the evaluation should include magnetic resonance imaging (MRI) of the sella to exclude a mass lesion.

Although a PRL level greater than 250 ng/ml usually indicates the presence of a prolactinoma, selected drugs, including risperidone and metoclopramide, may cause PRL elevations above 200 ng/ml in patients without evidence of adenoma (Casanueva et al. 2006; Kearns et al. 2000; Melmed et al. 2011). Increases in PRL levels due to interference with DA action are usually modest, with levels rarely exceeding 150 ng/ml (Casanueva et al. 2006). However, such values are not absolute, and prolactinomas can present with variable elevations in PRL levels. Taking into account that there may be dissociation between tumor mass and hormonal secretion (Casanueva et al. 2006; Melmed et al. 2011; Mancini et al. 2008), even minimal PRL elevations may be consistent with the presence of a prolactinoma, but a non-PRL-secreting mass (pseudoprolactinoma) should first be considered (Fig. 1).

Diagnostic Pitfalls

Macroprolactin

Two high molecular mass forms of PRL have been identified by gel filtration chromatography in human serum: macroprolactin (macroPRL) (big-big PRL, >100 kDa) and big PRL (40–60 kDa). MacroPRL has a variable composition and structure but is most frequently a complex of PRL and IgG, with a molecular mass of 150–170 kDa. It is formed in the circulation following pituitary secretion of monomeric PRL but has a longer half-life, and the PRL in the complex remains reactive to a variable extent in immunoassays (Smith et al. 2002b). In the majority of subjects, little or no macroPRL can be detected in serum, but in some individuals it may be the predominant immunoreactive component of circulating PRL and the cause of apparent HPRL (Fig. 3a).

MacroPRL is an under-recognized cause of elevated PRL and is present in approximately 4% to 40% of hyperprolactinemic patients depending on the referral population (Samson et al. 2015).

Because of its high molecular weight, it is believed that macroPRL is confined to the intravascular compartment, and much evidence indicates that it has minimal bioactivity in vivo and is not of pathological significance. Therefore, a lack of recognition of the presence of macroPRL can lead to unnecessary laboratory investigations, imaging, and pharmacologic or surgical treatment.

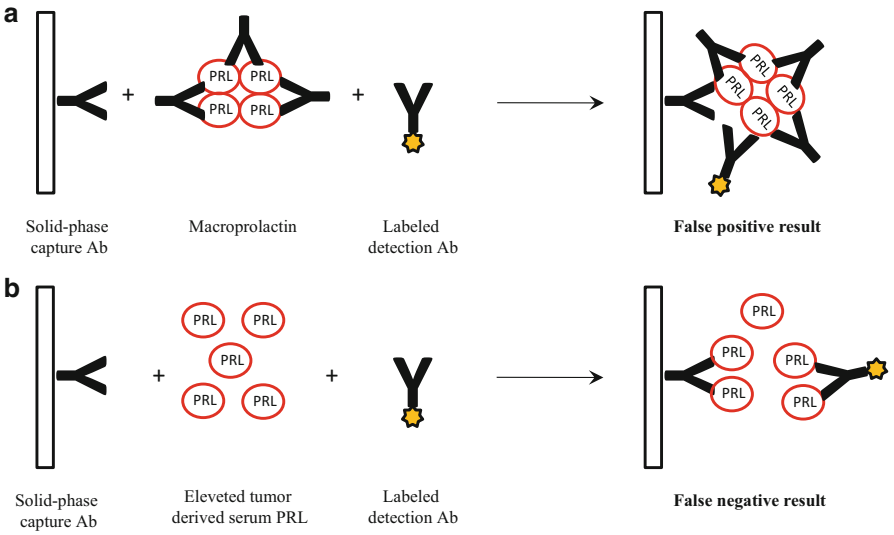


Fig. 3 Diagnostic pitfalls in hyperprolactinemic states: (**a**) **the macroprolactin problem:** if patients have circulating serum macroprolactin (preformed complexes of IgG and prolactin), with most immunoassays the sandwich formation occurs and the macroprolactin complex is therefore detected; however, because macroprolactin is bioinactive, the result is clinically misleading and constitutes a false-positive result. (**b**) **the "hook-effect" problem:** the "hook-effect" is caused by grossly elevated serum prolactin levels simultaneously saturating both the capture and detection antibodies, preventing immunoassay sandwich formation and quantitative detection of prolactin

MacroPRL is detected by most but not all PRL assays; therefore each center must know the specific characteristics of the PRL immunoassay they use. For confirmation of macroPRL, polyethylene glycol precipitation is the most practical method. Alternatively, size exclusion chromatography can be used, but is time-consuming and not suitable for routine use (Gibney et al. 2005).

In comparison with macroPRL, little is known about big PRL. It is a more consistent component of total serum PRL but rarely, if ever, the cause of HPRL.

The "Hook Effect"

Large amounts of antigen may produce falsely low values in immunoradiometric assays due to the so-called high-dose "hook effect" (Fig. 3b). Therefore, the "hook effect" may be observed when the PRL level is extremely high, as in some cases of giant prolactinomas. In this condition, extremely high levels of PRL can interfere with the assay and produce low readings. This high-dose "hook effect" may occur because there is not enough antibody to bind to both ends of PRL peptides; therefore most of the PRL is complexed to a single antibody. Only a little amount of PRL peptides are "sandwiched" and detectable. This results in a falsely low PRL value (St-Jean et al. 1996; Unnikrishnan et al. 2001; Yener et al. 2008). Hence, as the

antigen concentrations increase, there is a proportional increase in assay titers up to a certain level. Antigen concentrations above this threshold level would "hook" down the assay values resulting in very low measurements (Unnikrishnan et al. 2001; Yener et al. 2008). In addition, high-antigen titers can directly dissolve the antigen-antibody complex (Unnikrishnan et al. 2001). To overcome the "hook effect," an immunoradiometric PRL assay should be performed at a serum dilution at 1:100 or alternatively should include a washout between the binding to the first antigen and the second step in order to eliminate excess unbound PRL (Casanueva et al. 2006).

The immunoradiometric PRL assay must be performed with serum dilution in order to overcome the high-dose PRL "hook effect" in all new patients with large pituitary macroadenomas who have normal or mildly elevated PRL levels (Casanueva et al. 2006). Though repeatedly demonstrated in other immunoassays, the high-dose "hook effect" has only occasionally been observed in chemiluminescence assay systems for PRL estimation (Unnikrishnan et al. 2001).

Clinic of Hyperprolactinemia

The clinical consequences of pathological increased PRL levels can reveal themselves in the short, medium, and long term. The most immediate effects occur on sexual and gonadal function and on the breast, in both females and males. The long-term consequences are to a great extent conditioned by the permanence of these effects over time. However, they are also likely to be a consequence of other direct pleiotropic effects of PRL on different organs and tissues.

Hypogonadism

Pathological HPRL, whether or not it arises from a structural pituitary or hypothalamic lesion, is an important cause of reproductive dysfunction in both genders (Walsh and Pullan 1997).

In general, the degree of hypogonadism is proportional to the degree of PRL increase.

The exact mechanism by which HPRL causes hypogonadotropic hypogonadism is not known, but hyperprolactinemic women have reduced luteinizing hormone (LH)-pulse frequency and reduced LH responsiveness to estrogen (Matsuzaki et al. 1994; Sauder et al. 1984; Winters and Troen 1984), suggesting that GnRH suppression may be a key factor. CRH (Kooy et al. 1990) and kisspeptin, a protein made by neurons in the arcuate and periventricular nuclei of the hypothalamus, which stimulates GnRH release (Brown et al. 2014), may be important mediators of PRL-induced GnRH suppression. In rodent models, PRL receptor mRNA has been localized to kisspeptin neurons in the hypothalamus (Kokay et al. 2011), and kisspeptin administration to hyperprolactinemic female mice increases circulating gonadotropin levels and restores ovulation (Sonigo et al. 2012). HPRL has been associated with loss of the positive estrogen feedback on gonadotropin secretion at

mid-cycle (Glass et al. 1975), but whether this effect is mediated through kisspeptin is not known. Moreover, PRL also directly suppresses progesterone and estrogen secretion from human ovaries (Demura et al. 1982). PRL can decrease estrogen levels through direct effects on ovarian aromatase activity and by blocking the stimulatory effects of follicle-stimulating hormone (FSH) (Dorrington and Gore-Langton 1982; Krasnow et al. 1990). Although at low levels (<20 ng/ml) PRL is necessary for progesterone production in granulosa cell, at hyperprolactinemic levels it inhibits progesterone production (McNatty 1979). Direct PRL effects on ovarian granulosa cells include stimulation of the expression of type 2 3β-hydroxysteroid dehydrogenase (HSD), the enzyme responsible for catalyzing the final step in progesterone biosynthesis and the secretion of insulin-like growth factor 2 (IGF-2) (Feltus et al. 1999; Ramasharma and Li 1987).

Typically in women, menstrual disturbance occurs which may range in severity from luteal phase insufficiency with otherwise regular menses (Seppala et al. 1976) to irregular or infrequent periods (oligomenorrhea) and to amenorrhea. The oligo-amenorrhea in women is associated with infertility which is generally reversible upon restoration of normal PRL levels. In a compilation of three series of infertile women (total 367), approximately one-third had HPRL (Molitch and Reichlin 1982). That PRL excess may be important in this type of patient is suggested by the finding that treatment of similar patients with bromocriptine, a DA agonist, restored fertility (Skrabanek et al. 1980). Transient HPRL lasting for 1–2 days during the cycle has been shown in some infertile women, and such women may respond to DA agonists with increased progesterone during the luteal phase and improved fertility (Huang et al. 1991). Galactorrhea may also occur, alone or in combination with menstrual disturbance (Glass et al. 1975). Finally, in females due to hypogonadism there can be dyspareunia secondary to vaginal dryness.

In men, HPRL usually results in loss of libido and erectile dysfunction as a result of testosterone deficiency (Pinzone et al. 2000; Walsh and Pullan 1997). The combination of lowered libido with erectile dysfunction is the most frequent pattern in males with HPRL. Delay in or lack of orgasm can be associated with this pattern or, more rarely, present alone. The sexual dysfunction observed in hyperprolactinemic patients would be greatly conditioned by the situation of hypogonadism secondary to HPRL, although there are also data in favor of a direct HPRL effect on the libido and on erectile dysfunction (Buvat et al. 2006; Corona et al. 2007), probably mediated by the dopaminergic system (Drago et al. 1981). At local level, the erectile dysfunction would be related to endothelial dysfunction secondary to decreased nitric oxide (NO) production from inhibition of endothelial NO synthetase (Montes de Oca et al. 2005; Yu-Lee 2002) and vasoconstriction from β2-adrenergic effect (Molinari et al. 2007).

The role of PRL in male reproduction and fertility has been studied in several animal models, leading, however, to conflicting results. PRL receptor is expressed in the testis of rodents and mammals (Bole-Feysot et al. 1998), including men (Hair et al. 2002). In particular, PRL receptor expression has been demonstrated in Leydig, Sertoli, and germ cells (Ishida et al. 2010; Jabbour and Lincoln 1999). PRL can affect steroidogenesis by modulating the expression of LH receptors

(Dombrowicz et al. 1992; Takase et al. 1990) or by regulating the activity of steroidogenetic enzymes (Chandrashekar and Bartke 1988; Rubin et al. 1976), such as 5α-reductase, 3β-HSD, and 17β-HSD (Gunasekar et al. 1988; Takeyama et al. 1986). In animal models, PRL regulates spermatogenesis as well. PRL induces the expression of FSH on Sertoli cells and stimulates the progression of germ cells from spermatocyte to spermatide morphology (Gunasekar et al. 1991).

In men, studies on the effects of PRL on fertility are scanty, and, since mutations in PRL gene have not been described and mutations in PRL receptor were found only in women (Newey et al. 2013), no model of spontaneous defective PRL action is available. A study conducted in human sperms suggested that PRL is involved in the survival of these cells, since, after incubation with PRL, their motility was preserved for a longer time and spontaneous DNA strand fragmentation was decreased (Pujianto et al. 2010). However, a recent study conducted on 269 male partners of infertile couples failed to demonstrate any significant association between semen parameters and serum PRL levels (Lotti et al. 2013).

PRL has also a trophic effect on male seminal accessory glands. PRL and its receptor are expressed in the prostate, and studies in vitro and in vivo demonstrated that their regulation is androgen dependent (Nevalainen et al. 1997a). Moreover, PRL seems to be involved also in the secretory activity of male accessory glands; in fact the increase or suppression of PRL levels has been associated with a change in seminal vesicle and prostate secretion composition in different animal models (Arunakaran et al. 1988; Nicoll 1980; Ravault et al. 1977). PRL receptor expression has been demonstrated in human seminal accessory glands (Hair et al. 2002; Nevalainen et al. 1997b). However, the clinical implication of PRL activity on the male genital tract is still unclear.

Effects of Hyperprolactinemia on Calcium Metabolism and Bone

In both genders, HPRL and the resulting deficiency of sex steroid may lead to bone loss and osteoporosis (Greenspan et al. 1986; Klibanski et al. 1980). While correction of the elevated PRL improves bone density (Klibanski and Greenspan 1986), it seems that the bone loss is not directly mediated by the elevated PRL and restoration of sex steroid concentrations is required for subsequent improvement in bone density, even in the presence of ongoing HPRL (Greenspan et al. 1989). However, some experimental studies point to a possible direct adverse effect of PRL on osteoblastic function (Seriwatanachai et al. 2009). This would explain the finding in some clinical studies of a greater bone mineral density (BMD) deterioration in amenorrheic females with HPRL compared to normoprolactinemic females with similar estradiol levels and duration of amenorrhea (Schlechte et al. 1987), as well as the greater risk of vertebral fractures in males with HPRL, regardless of testosterone values (Mazziotti et al. 2011). Genetic evidence has shown that the PRL receptor is essential to normal bone formation and calcium homeostasis (Klibanski and Greenspan 1986). PRL receptor-deficient mouse displayed reductions in BMD and bone mineral content, as well as a deceleration in the apposition rate for new bone. Plasma

total calcium and parathyroid hormone were each higher in the receptor-deficient mice. The phenotypic characteristics of bone growth and calcium homeostasis in PRL receptor-deficient mice argue that there must be multiple sites of PRL action that influence calcium metabolism, including both direct effects on bone cells and systemic actions on other hormones or carriers.

Effects of Hyperprolactinemia on Cancer Risk

It seems that certain basically hormone-dependent tumors, such as those of the breast, may be related to the molecular signaling pathway of the PRL receptors.

Despite the limitations and the impossibility of having type I levels of evidence or clinical trials on the matter, there are an important number of studies, both experimental and epidemiological, that indicate that the increased PRL plasma levels seem to be related to increased risk of cancer, fundamentally breast cancer, especially in postmenopausal women (Clevenger et al. 2003; Faupel-Badger et al. 2014; Goffin et al. 2005; Hankinson et al. 1999; Harvey et al. 2006; Manjer et al. 2003).

In vitro studies have shown that PRL is mitogenic (Harvey et al. 2006), but a recent Dutch study showed no increase in breast cancer rate in women with idiopathic HPRL or prolactinomas (Dekkers et al. 2010a).

Furthermore, surgical series of breast cancer patients noted HPRL in a similar number of women with benign breast conditions as breast cancer (Nicol et al. 2002).

No increased risk of breast or prostate cancer was observed in hyperprolactinemic patients with prolactinomas (Berinder et al. 2011).

Therefore, although some epidemiologic studies suggest that HPRL is a risk for breast and prostate cancer, there is inadequate evidence to conclude a significant causal association between HPRL and cancer.

Effects of Hyperprolactinemia on Cardiovascular Diseases

HPRL has been associated with long-term cardiovascular effects, mainly mediated by sexual steroid deficit and by direct PRL action at cardiovascular level.

In vitro studies have shown that PRL per se is capable of modulating inflammatory response (Erem et al. 2010; Friedrich et al. 2011; Shibli-Rahhal and Schlechte 2009) and producing endothelial dysfunction by reducing NO production (Molinari et al. 2007; Montes de Oca et al. 2005; Yavuz et al. 2003; Yu-Lee 2002). PRL could also stimulate angiogenesis indirectly, as it encourages the synthesis of substances such as endothelial growth factor and fibroblast growth factor (Goldhar et al. 2005; Malaguarnera et al. 2002) stimulating the proliferation of smooth muscle cells of the vascular wall and the adhesion of mononuclear cells to vascular endothelium. In addition, PRL has been related to an increase in platelet aggregation (Wallaschofski et al. 2001), as well as to increased intima-media thickness at carotid level, compatible with preclinical atherosclerosis

(Arslan et al. 2014). Lastly, PRL receptor expression has been demonstrated in macrophages within atheroma plaque (Reuwer et al. 2009, 2011).

In women with early menopause, a correlation between PRL levels, blood pressure, and artery wall rigidity has been shown; this could indicate an acceleration of the atherosclerotic process with increased calculated risk of cardiovascular mortality at 10 years (Georgiopoulos et al. 2009). Recently, in a cohort study that included 3929 males and females followed for 10 years, a positive correlation between PRL levels and cardiovascular mortality was found in both sexes (Montejo 2008).

Effects of Hyperprolactinemia on Metabolism

There is also limited evidence that HPRL directly influences glucose and lipid metabolism (Ben-Jonathan et al. 2006).

Some clinical trials (Serri et al. 2006; Yavuz et al. 2003) carried out in a limited number of patients indicate the existence of insulin resistance in patients with HPRL that improves following treatment with bromocriptine. In vitro studies have also demonstrated the potential influence of PRL in the development of the pancreatic β-cells and in the secretion of insulin (Brelje et al. 2004; Freemark et al. 2002).

The association between HPRL and dyslipidemia has also been described (Erem et al. 2010). The PRL receptors are expressed in human adipose tissue (Brandebourg et al. 2007) where PRL reduces lipoprotein lipase activity and inhibits adiponectin secretion, leading to insulin resistance (Ling et al. 2003; Mingrone et al. 2008).

However, some authors find no correlation between PRL levels and different metabolic syndrome parameters (Ernst et al. 2009) or obtain insufficient evidence that the levels of PRL play a causal role as a risk factor for the development of metabolic syndrome or type 2 diabetes (Balbach et al. 2013).

HPRL has also been associated with ponderal increase, not always reversible, when PRL levels are normalized through drug treatment (Creemers et al. 1991; Delgrange et al. 1999; Doknic et al. 2002). Factors such as reduced dopaminergic tone, leptin resistance, or reduced adiponectin levels have been suggested for its pathogenesis (Nilsson et al. 2005).

Effects of Hyperprolactinemia on the Immune System

PRL receptors are found on a majority of immune precursor and effector cells in each of the major hematopoietic organs (bone marrow, spleen, thymus). PRL can potentiate the growth and effector function of lymphoid and myeloid cells, and hematopoietic cytokine receptors and signal transducers are closely related to those used by PRL.

In a rat model of immunodepression following acute hemorrhagic shock, PRL stimulated immune effector cell functions, as well as normal cytokine secretion (Zellweger et al. 1996). Whereas PRL can act as a positive stimulus for immune

cells when given to animals by injection, or to cells in culture, PRL deficiency does not significantly impair immune function or hematopoiesis (Horseman et al. 1997).

Several authors have described an association between HPRL and different autoimmune diseases, such as diabetes mellitus type 1, rheumatoid arthritis, and systemic erythematous lupus, among others (Atasoy et al. 2006; De Bellis et al. 2005; Leanos-Miranda and Cardenas-Mondragon 2006; Poyraz et al. 2008; Vera-Lastra et al. 2002). The physiopathological mechanism could be mediated by the antiapoptotic effect of PRL in the B lymphocytes (Orbach and Shoenfeld 2007; Shelly et al. 2012) and the stimulation of interferon-γ and interleukin-2 production by the T lymphocytes (De Bellis et al. 2005).

Effects of Hyperprolactinemia on the CNS

A recent study in a female population indicates that HPRL could have direct negative effects on cognitive function (Henry and Sherwin 2012), which could originate in low levels of gonad steroids (Craig et al. 2007, 2008; Grigorova et al. 2006; Phillips and Sherwin 1992). It has also been observed that in males low levels of testosterone are related to deterioration of the memory and of the visual-spatial abilities (Beer et al. 2006; Moffat et al. 2002; Pinsky and Hellstrom 2010) and with a greater risk of dementia (Moffat et al. 2004).

HPRL has also been associated with some psychiatric alterations, above all in females (Fava et al. 1983). Greater rates of hostility, anxiety, depression, and dysthymia have been reported in patients with HPRL (Oliveira et al. 2000; Reavley et al. 1997). The mechanism by which they would originate is unclear and is probably related to hypogonadism (Prabhakar and Davis 2008; Sobrinho 1998).

Treatment

The objective of HPRL treatment is to correct the clinical consequences of the hormonal excess. The primary goal of therapy in patients with HPRL is to restore gonadal and sexual function by normalizing PRL levels, but in patients with macroadenomas, control and reduction of tumor size are also important.

Indications for treatment include infertility, a pituitary tumor with neurological effects (particularly visual defects), bothersome galactorrhea, long-standing hypogonadism, alterations in pubertal development, and prevention of bone loss because of hypogonadism. Occasionally, patients with mild HPRL with regular menses who wish to become pregnant may also require treatment. HPRL will prove self-limiting in up to one-third of women, and in others pregnancy may induce a return to normal PRL function (Jeffcoate et al. 1996; Schlechte et al. 1986).

Women with HPRL who pass through menopause may normalize their PRL levels, and therefore in such women, reassessment of the need for continuing treatment of HPRL is indicated (Karunakaran et al. 2001).

Premenopausal women with normal menstrual cycles and tolerable galactorrhea, and postmenopausal women with tolerable galactorrhea who have idiopathic HPRL or microprolactinoma, should be reassured and not actively treated (Casanueva et al. 2006; Melmed et al. 2011).

Pharmacological Treatment

DA agonist is the therapy of choice to lower PRL levels, decrease tumor size, and restore gonadal function for patients harboring PRL-secreting macroadenomas. Asymptomatic patients harboring microprolactinomas should not be treated with DA agonists. Treatment with a DA agonist or oral contraceptive should be considered in patients with amenorrhea caused by a PRL-secreting microadenoma.

All DA agonists are efficacious, but pergolide and quinagolide are less commonly used. Cabergoline is recommended in preference to other DA agonists because it has higher efficacy in normalizing PRL levels, as well as a higher frequency of pituitary tumor shrinkage.

It is unclear why cabergoline is more effective than bromocriptine, but the greater efficacy may be explained by the fact that cabergoline has a higher affinity for DA receptor binding sites. Moreover, because the incidence of unpleasant side effects is lower with cabergoline, drug compliance may be superior for this medication (Verhelst et al. 1999). No clinical trials have directly compared the mass-reducing effects of different DA agonists. Nevertheless, results of various studies (Molitch et al. 1985) indicate that bromocriptine decreases pituitary tumor size by approximately 50% in two-thirds of patients, compared with a 90% decrease with cabergoline.

Nevertheless, bromocriptine has been used satisfactorily for years (Molitch et al. 1985), and since it is less expensive, it should be considered in medical settings with limited budgets.

Therapy with bromocriptine (tablet of 2.5 mg) is initiated with a dose of 0.625–1.25 mg daily and increased by 1.25 mg at weekly intervals until a dose generally of 2.5 mg twice or thrice daily is reached. Side effects such as upper gastrointestinal disturbances and postural hypotension can be reduced by using an incremental dosage schedule and taking tablets with a snack before retiring. Cabergoline (tablet of 0.5 mg) therapy is begun at a dose of 0.25–0.5 mg administered once or twice weekly, and the dose is increased monthly until PRL secretion normalizes (Schlechte 2003; Webster et al. 1994). Doses over 3 mg per week are rarely necessary.

Patients who are resistant to, or who cannot tolerate, a particular DA agonist should be switched to an alternative DA agonist (Colao et al. 1997). Cabergoline is effective in most patients, including those who did not previously respond to bromocriptine (Colao et al. 1997).

For patients with medication-induced HPRL, the primary treatment is to stop the drug or to switch to an alternative drug. For antipsychotic-induced HPRL, alternative medications include antipsychotic agents with lower DA antagonist potency

(Kinon et al. 2003; Volavka et al. 2004) or aripiprazole, an atypical antipsychotic with both DA agonist and DA antagonist activity (Lu et al. 2008) that can lower PRL and reverse HPRL-related side effects (Saitis et al. 2008). If the drug cannot be discontinued or substituted and the patient has hypogonadal symptoms or low bone mass, estrogen or testosterone therapy should be considered (Bhasin et al. 2010; Casanueva et al. 2006; Melmed et al. 2011).

Whether to treat a patient who has antipsychotic-induced HPRL with a DA agonist remains controversial. Some studies suggest that DA agonist therapy will normalize PRL levels in only up to 75% of such patients but may lead to exacerbation of the underlying psychosis (Cavallaro et al. 2004; Cohen and Biederman 2001; Smith 1992; Tollin 2000); therefore in such situations treatment with DA agonists should be considered only after careful psychiatric counselling (Casanueva et al. 2006; Melmed et al. 2011).

In an asymptomatic patient with medication-induced HPRL, no treatment is necessary.

Surgical Treatment

It is widely accepted that pharmacological treatment is the first choice for PRL-secreting pituitary adenoma, but surgery remains a good therapeutic option in patients intolerant or resistant to DA agonists or in cerebrospinal fluid leaks post tumor shrinkage (Kreutzer et al. 2008; Nomikos et al. 2001). With the advancement of surgical techniques, surgical intervention may be a reasonable option in microprolactinoma when the patient opts for surgical resection rather than medical management (Babey et al. 2011; Couldwell and Weiss 2004).

Surgery is also indicated for individuals with cystic tumors which do not respond to DA agonist therapy, tumors causing mass effect (cranial nerve palsies, visual impairment) which is not relieved by medical treatment, in case of intratumoral hemorrhage with mass effect or apoplexy requiring emergent/urgent decompression of the optic chiasm to preserve visual function on DA therapy.

Pituitary transsphenoidal surgery is associated with very low mortality (<1%) and very low morbidity rates. Both the expertise and experience of the neurosurgeon and the volume of the surgical center also play a role in these low rates of complications (Ikeda et al. 2013). Side effects of surgery include hypopituitarism, diabetes insipidus, cerebrospinal fluid leak, and local infection (Melmed et al. 2011).

Clinical results and economic costs of surgical resection are comparable to those of pharmacological management over a 10-year period. This suggests that surgery may be a more cost-effective option than lifelong medical therapy, especially in young patients (Amar et al. 2002).

Transsphenoidal surgery is an effective modality in the management of prolactinomas for patients with indications as described above (Table 2). Long-term chemical cure rates for microprolactinomas exceed 90% when performed by experienced pituitary surgeons (Amar et al. 2002; Buchfelder and Schlaffer 2009).

Table 2 Indication for neurosurgery in PRL-secreting pituitary adenomas

Intolerance to dopamine agonists
Resistance to dopamine agonists
Cerebrospinal fluid leaks post tumor shrinkage
Patient's preference (microprolactinoma)
Cystic tumors which do not respond to dopamine agonist therapy
Tumors causing mass effect (cranial nerve palsies, visual impairment) which is not relieved by medical treatment
Intratumoral hemorrhage with mass effect on dopamine therapy
Apoplexy requiring emergent/urgent decompression of the optic chiasm to preserve visual function

Postoperative hormonal levels are predictive for long-term cure; in fact, PRL levels lower than 10 ng/ml on postoperative day 1 demonstrated cure rates of up to 100% in microprolactinoma patients (Amar et al. 2002).

Surgical resections for macroprolactinomas are not as successful as for microprolactinomas in terms of biochemical cure rate. Cure rates drop to <50% for macroprolactinomas (Buchfelder and Schlaffer 2009). For macroprolactinomas, both the size and baseline PRL level serve as a predictor of surgical success. In a large microscopic transsphenoidal surgical series, the rate of recurrence was over 70% when preoperative PRL levels were over 250 ng/ml (Amar et al. 2002). The objective of surgical intervention for macroprolactinomas is mainly to debulk the tumor to relieve symptoms due to mass effect and as a cytoreductive strategy, rather than to cure them. Surgically decreasing the size of a large tumor may also help increase its responsiveness to DA agonist treatment. In patients with macroprolactinomas resistant to cabergoline, surgical debulking resulted in significantly lower postoperative PRL levels at significantly lower doses of cabergoline (Vroonen et al. 2012).

Radiotherapy

External radiation is rarely required to treat prolactinomas because it is associated with significant precious and delayed side effects as impairment of pituitary secretion, risk of optic chiasma damage, cognitive and neurological dysfunction, and increased incidence of stroke and secondary brain neoplasms.

Radiotherapy is therefore suggested as a third-line treatment for prolactinomas that are refractory to medical therapy or surgery, mainly for tumor growth control.

In Western countries radiotherapy means stereotactic technique, while conventional approach is completely abandoned and should be avoided.

With the advances in high-resolution imaging, image guidance, and dosage planning, stereotactic radiotherapy has been demonstrated to be relatively well tolerated with excellent accuracy and safety profiles (Wong et al. 2015).

Stereotactic Radiosurgery (SRS): Gamma Knife

During the past two decades, SRS has become a frequently used radiation technique because of its convenience, more rapid correction of hormone oversecretion (Kong et al. 2007), and a lower risk of radiation-induced neoplasms and carotid stenosis (Cohen-Inbar et al. 2015). SRS provides a highly conformal and selective therapeutic intervention in a single procedure performed with image guidance, achieving maximal precision. Such an approach reduces the long-term risk of larger-field radiotherapy.

There are only a few studies showing the effect of SRS on prolactinomas as a first-line treatment (Pan et al. 2000). A limit to Gamma Knife use as a primary treatment is that a number of studies have shown that radiosurgery is less effective in achieving endocrine remission for prolactinomas than for other types of pituitary adenoma patients. Clinical control was reported in 17–47% (Jezkova et al. 2009; Cohen-Inbar et al. 2015) though it has been reported even better control (52%) (Castinetti et al. 2009) in tumor mass effect; control rate has been reported higher than 95% if the follow-up time has been long enough (60–120 months).

Treatment Pitfalls

Pregnancy

Women with HPRL who wish to become pregnant or who are pregnant should be guided through the process by an endocrinologist. In particular there are four main issues with respect to gestation and HPRL: HPRL and fertility, safety of DA agonists, tumor growth, and lactation.

Hyperprolactinemia and Fertility

When starting DA treatment, women must be warned that restoration of ovulation and fertility may be immediate and even before their first normal menstruation (Casanueva et al. 2006; Melmed et al. 2011). Indeed, treatment with DA agonists in high percentage of cases normalizes PRL levels and consequently controls symptoms PRL related, i.e., oligo-amenorrhea and infertility. For this reason, when starting DA agonist treatment, mechanical contraception should be advised, and menses may serve as a guide. This is in order to discontinue DA agonist therapy as soon as patients discover that they are pregnant and to avoid an unnecessary and excessive exposure of the fetus to the effect of DA drugs.

When a female patient with a macroprolactinoma wishes to become pregnant, it is necessary to plan conception to occur after serum PRL is normalized and the tumor volume significantly reduced in order to avoid or reduce the risk of compression of the optic chiasm during pregnancy.

Women with macroprolactinomas who are intolerant or resistant to DA agonist therapy and want to reproduce can consider surgical debulking or radiotherapy

before attempting a pregnancy (Casanueva et al. 2006; Melmed et al. 2011). However it must be considered that surgery and radiotherapy can cause hypopituitarism, which may lead to the need for advanced reproductive technologies (e.g., ovulation induction with gonadotropins) to achieve pregnancy, as well as lifelong hormone replacement therapy (Melmed et al. 2011).

Safety of DA Agonists

Both bromocriptine and cabergoline are reported to be safely administered in premenopausal women (Casanueva et al. 2006; Melmed et al. 2011; Molitch 2015). Both drugs are not associated with increasing of abortions, ectopic/multiple pregnancies, and congenital malformations even if bromocriptine data are the most (Molitch 2015). On the other hand, guidelines recommended to discontinue DA agonist therapy once pregnancy is confirmed, usually 1–2 weeks after a missed period (Casanueva et al. 2006; Melmed et al. 2011). In selected patients with macroadenomas who become pregnant on dopaminergic therapy, it may be prudent to continue dopaminergic therapy throughout the pregnancy, especially if the tumor is invasive or is abutting the optic chiasm (Casanueva et al. 2006; Melmed et al. 2011)

The experience with pergolide and quinagolide in preparation for pregnancy is much more limited; for that reason these two drugs should not be used in this setting (Casanueva et al. 2006; Melmed et al. 2011).

Tumor Growth

During pregnancy in patients with prolactinoma, the size of pituitary adenoma may increase. The risk of increase is different in micro- or macroadenoma patients; in case of microadenoma, the risk of symptomatic growth is 3% while in macroadenoma is significantly higher being 30%; previous surgery or radiotherapy reduces this risk (2.8%) (Molitch 2015).

Therefore, DA agonists can be safely stopped in patients with microprolactinoma as soon as pregnancy has been confirmed (Casanueva et al. 2006; Melmed et al. 2011). The patients should be advised to report for urgent assessment in the event of a severe headache or visual disturbance (Casanueva et al. 2006; Melmed et al. 2011).

Options for patients with macroprolactinoma include stopping the DA agonist when pregnancy is confirmed with close surveillance thereafter or continuing the DA agonist through the pregnancy (Casanueva et al. 2006; Melmed et al. 2011).

It is not recommended to measure serum PRL levels because of their physiological increase during pregnancy and their expected increase upon discontinuation of DA agonists and to avoid uninterpretable results from laboratory tests and unnecessary testing.

If pregnancy is physiological, conducted spontaneous delivery is not contraindicated.

If visual field defects or progressive headaches develop, an MRI without gadolinium should be performed to assess changes in tumor size, and a DA agonist should be restarted if the tumor has grown significantly (Casanueva et al. 2006; Melmed et al. 2011). If the enlarged tumor does not respond to reinstitution of DA agonist

therapy, alternatives include delivery if the pregnancy is far enough advanced or transsphenoidal surgery (Casanueva et al. 2006; Melmed et al. 2011).

Lactation

There are no data to suggest that breastfeeding leads to an increase in tumor size; therefore, lactation is not contraindicated in patients harboring PRL-secreting adenomas. However, women wishing to breast-feed their infants should not be given DA agonists because the resulting decrease in serum PRL levels will impair lactation (Casanueva et al. 2006). After 3 months the end of lactation serum PRL levels should be assessed, and an MRI with contrast is indicated.

D2 Resistance

The majority of patients with prolactinomas treated with standard doses of DA agonists respond with normalization of PRL levels and a reduction in tumor size.

However, some patients do not respond satisfactorily (Molitch 2003), and some patients may have discordant responses, i.e., reduction in tumor size without normalization of PRL levels and vice versa, and others may be partially resistant and require higher than typical doses of DA agonists to achieve a response.

Although the literature is not totally consistent in defining the problem as different authors employ various definitions depending on the primary endpoints of their studies (Colao 2009; Molitch 2003), resistance to DA can be most properly described as inability to lower PRL within normal limits and to achieve a 50% reduction in tumor mass by the maximum tolerated dose of DA agonist (Gillam et al. 2006). To note that DA agonist resistance differs from intolerance, where side effects of the DA agonists preclude their use.

Microadenomas are less resistant to DA agonists than are macroadenomas, and men are more likely than women to be DA agonist resistant (Delgrange et al. 2009). The level of responsiveness depends also on the type of the DA used, and many prolactinomas that are resistant to bromocriptine respond to cabergoline therapy (Colao et al. 1997).

The mechanism of DA agonist resistance is not completely understood, and it is likely that different mechanisms underlie DA agonist resistance in prolactinomas: a decreased number of D2 receptors have been reported on resistant prolactinomas (Kukstas et al. 1991; Pellegrini et al. 1989), but this finding is not invariable (Kovacs et al. 1995). However DA receptor binding has been reported to be normal, and no DA receptor mutation has been identified in prolactinomas to date. D2 receptor isoform ratios may differ; it has been reported that resistant prolactinomas had a lower proportion of the short isoform of the D2 receptor mRNA compared to sensitive ones, while no significant difference was found between responsive adenomas and normal pituitary lactotroph cells (Caccavelli et al. 1994). Finally molecular alterations may occur downstream of the D2 receptor.

A number of therapeutic approaches may be employed when managing the resistant prolactinoma patients. These include substitution with another DA, gradual

dose augmentation to maximal efficient and tolerated levels, transsphenoidal surgery, and radiotherapy (Melmed et al. 2011). Some experimental treatments are also available, but their true clinical benefit still remains to be clearly demonstrated.

Since resistance is most prevalent among patients on bromocriptine, it is usually appropriate to switch them to another more potent DA. Quinagolide reduces PRL to normal ranges in 39–44% of bromocriptine-resistant patients and induces tumor shrinkage in about 30% of cases (Morange et al. 1996; Rohmer et al. 2000), but still a large proportion fail to respond.

Much better outcomes, however, are achieved with cabergoline. Accumulating evidence suggests that cabergoline is efficient in normalizing PRL in approximately 70–85% of patients resistant to bromocriptine and quinagolide and in 84% of patients intolerant of bromocriptine (Colao et al. 1997; Molitch 2005b; Verhelst et al. 1999). Another approach especially for cabergoline-resistant patients is the gradual dose increase, provided that the patient responds with some reduction of PRL levels to every dose adjustment (Melmed et al. 2011).

However, caution must be exhibited with protracted use of high-dose cabergoline because of the potential risk of cardiac valvular regurgitation. Even if patients with Parkinson's disease receiving at least 3 mg of cabergoline daily are at risk for moderate to severe cardiac valve regurgitation (Schade et al. 2007; Zanettini et al. 2007), six of seven studies analyzing cardiac valves in over 500 patients with prolactinomas receiving standard doses of cabergoline have shown no evidence of clinically significant valvular disease (Bogazzi et al. 2008; Herring et al. 2009; Kars et al. 2008; Lancellotti et al. 2008; Vallette et al. 2009; Wakil et al. 2008), and the only study that reported a 57% incidence of tricuspid regurgitation in patients treated with cabergoline also noted significant tricuspid regurgitation in the control group (Colao et al. 2008). However, in patients who require very high doses of cabergoline for prolonged periods, echocardiography may be necessary to assess for valvular abnormalities. Although the precise dose and duration cannot be identified at this time, patients receiving more than 3 mg of cabergoline weekly likely will require regular echocardiographic screening (Melmed et al. 2011).

Finally, in a very small number of cases, patients who initially respond to DA therapy may later become resistant. One study reported five patients who developed late DA resistance: two after bromocriptine and three following cabergoline treatment (Behan et al. 2011). Even more rarely such secondary resistance may be caused by malignant transformation (Hurel et al. 1997). A report by Lania et al. describes a case of aggressive prolactinoma successfully treated with DA for 15 years that surprisingly evolved in clinically and biochemically active acromegaly (Lania et al. 2010). The mechanisms underlying loss of responsiveness and transformation are not yet known.

Cure

There is an ongoing debate regarding the safety of the discontinuation and the optimal duration of therapy with DA agonist in prolactinomas (Casanueva et al. 2006;

Melmed et al. 2011). It has been suggested that DA agonist withdrawal may be safely undertaken after 2–3 years in patients who have achieved normoprolactinemia and significant tumor volume reduction (Casanueva et al. 2006; Melmed et al. 2011).

A landmark study by Colao et al. had demonstrated persistent normoprolactinemia post withdrawal in the majority of the study population (66.1% of microprolactinomas, 46.8% of macroprolactinomas) (Colao et al. 2003b). In general the risk of recurrence after withdrawal has been estimated to range from 26 to 69% (Biswas et al. 2005; Kharlip et al. 2009), and all studies have shown that recurrence is predicted by PRL levels at diagnosis and by tumor size. Recurrences are most likely to occur in the year after withdrawal, and in one study the risk of recurrence was 18% per millimeter of tumor mass (Kharlip et al. 2009). However, a meta-analysis of 19 studies and 743 patients by Dekker et al. demonstrated stable normoprolactinemia after DA agonist withdrawal in 32% of patients with idiopathic HPRL, 21% with microprolactinomas, and 16% with macroprolactinomas, and when the study by Colao et al. was excluded from the meta-analysis, these values decreased to 17%, 19%, and 12%, respectively (Dekkers et al. 2010b).

Some studies have shown that a longer treatment duration and the use of cabergoline were associated with a greater remission rate (Dekkers et al. 2010b; Oh and Aghi 2011), while some studies have not shown this (Anagnostis et al. 2012; Barber et al. 2011). The dosage of the DA agonist should be gradually decreased, while maintaining normal PRL levels, until the drug is completely discontinued (Melmed et al. 2011). HPRL recurrence is most commonly observed during the first 6 months to 1 year following cessation (Anagnostis et al. 2012; Barber et al. 2011; Colao et al. 2003b; Kharlip et al. 2009), and regular follow-ups are necessary. In particular, guidelines (Melmed et al. 2011) suggest that in patients for whom DA agonists have been tapered or discontinued, follow-up includes (1) measurement of serum PRL levels every 3 months for the first year and then annually thereafter and (2) MRI if PRL increases above normal levels. In women with microprolactinomas, it may be possible to discontinue dopaminergic therapy when menopause occurs. Surveillance for increasing size of the pituitary tumor should continue on a periodic basis.

Conclusions

PRL is secreted by pituitary lactotroph cells. The predominant signal is inhibitory, preventing PRL release, and is mediated by DA.

Pathological HPRL may develop due to lactotroph adenomas (prolactinomas), which account for approximately 40% of all pituitary tumors, or due to pharmacological or pathological interruption of hypothalamic-pituitary dopaminergic pathways. Finally, idiopathic HPRL needs to be considered as diagnosis.

Regardless of etiology, HPRL may result in hypogonadism, infertility, and galactorrhea, or it may remain asymptomatic. Bone loss occurs secondary to HPRL-mediated sex steroid attenuation.

Testing for HPRL is straightforward, owing to the case of ordering a serum PRL measurement. Accordingly, an evidence-based, cost-effective approach to management of thus relatively common endocrine disorder is required.

References

Ahmed M, Banna M, Sakati N, Woodhouse N. Pituitary gland enlargement in primary hypothyroidism: a report of 5 cases with follow-up data. Horm Res. 1989;32:188–92.

Amar AP, Couldwell WT, Chen JC, Weiss MH. Predictive value of serum prolactin levels measured immediately after transsphenoidal surgery. J Neurosurg. 2002;97:307–14.

Amit T, Dibner C, Barkey RJ. Characterization of prolactin and growth hormone-binding proteins in milk and their diversity among species. Mol Cell Endocrinol. 1997;130:167–80.

Anagnostis P, Adamidou F, Polyzos SA, Efstathiadou Z, Karathanassi E, Kita M. Long term follow-up of patients with prolactinomas and outcome of dopamine agonist withdrawal: a single center experience. Pituitary. 2012;15:25–9.

Arafah BM, Nekl KE, Gold RS, Selman WR. Dynamics of prolactin secretion in patients with hypopituitarism and pituitary macroadenomas. J Clin Endocrinol Metab. 1995;80:3507–12.

Arslan MS, Topaloglu O, Sahin M, Tutal E, Gungunes A, Cakir E, Ozturk IU, Karbek B, Ucan B, Ginis Z, Cakal E, Ozbek M, Delibasi T. Preclinical atherosclerosis in patients with prolactinoma. Endocr Pract. 2014;20:447–51.

Arunakaran J, Balasubramanian K, Srinivasan N, Aruldhas MM, Govindarajulu P. Effects of androgens, prolactin and bromocriptine on seminal vesicular enzymes of the pyruvate malate cycle involved in lipogenesis in castrated mature monkeys, macaca radiata. Int J Androl. 1988;11:133–9.

Atasoy M, Karatay S, Yildirim K, Kadi M, Erdem T, Senel K. The relationship between serum prolactin levels and disease activity in patients with Behcet's disease. Cell Biochem Funct. 2006;24:353–6.

Babey M, Sahli R, Vajtai I, Andres RH, Seiler RW. Pituitary surgery for small prolactinomas as an alternative to treatment with dopamine agonists. Pituitary. 2011;14:222–30.

Balbach L, Wallaschofski H, Völzke H, Nauck M, Dörr M, Haring R. Serum prolactin concentrations as risk factor of metabolic syndrome or type 2 diabetes? BMC Endocr Disord. 2013;13:12–9.

Barber TM, Kenkre J, Garnett C, Scott RV, Byrne JV, Wass JA. Recurrence of hyperprolactinaemia following discontinuation of dopamine agonist therapy in patients with prolactinoma occurs commonly especially in macroprolactinoma. Clin Endocrinol. 2011;75:819–24.

Bart G, Borg L, Schluger JH, Green M, Ho A, Kreek MJ. Suppressed prolactin response to dynorphin A1-13 in methadone-maintained versus control subjects. J Pharmacol Exp Ther. 2003;306:581–7.

Beer TM, Bland LB, Bussiere JR, Neiss MB, Wersinger EM, Garzotto M, Ryan CW, Janowsky JS. Testosterone loss and estradiol administration modify memory in men. J Urol. 2006;175:130–5.

Behan LA, Draman MS, Moran C, King T, Crowley RK, O'Sullivan EP, Smith D, Thompson CJ, Agha A. Secondary resistance to cabergoline therapy in a macroprolactinoma: a case report and literature review. Pituitary. 2011;14:362–6.

Bellantuono C, Santone G. Efficacy, tolerability and safety of paliperidone extended-release in the treatment of schizophrenia and schizoaffective disorder. Riv Psichiatr. 2012;47:5–20.

Ben-Jonathan N, Mershon JL, Allen DL, Steinmetz RW. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. Endocr Rev. 1996;17:639–69.

Ben-Jonathan N, Hugo ER, Brandebourg TD, LaPensee CR. Focus on prolactin as a metabolic hormone. Trends Endocrinol Metab. 2006;17:110–6.

Berchtold S, Volarevic S, Moriggl R, Mercep M, Groner B. Dominant negative variants of the SHP-2 tyrosine phosphatase inhibit prolactin activation of Jak2 (janus kinase 2) and induction of

Stat5 (signal transducer and activator of transcription 5)-dependent transcription. Mol Endocrinol. 1998;12:556–67.

Berinder K, Akre O, Granath F, Hulting AL. Cancer risk in hyperprolactinemia patients: a population-based cohort study. Eur J Endocrinol. 2011;165:209–15.

Besnard I, Auclair V, Callery G, Gabriel-Bordenave C, Roberge C. Antipsychotic-drug-induced hyperprolactinemia: physiopathology, clinical features and guidance. Encéphale. 2014;40:86–94.

Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM, Task Force, Endocrine Society. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010;95:2536–59.

Biswas M, Smith J, Jadon D, McEwan P, Rees DA, Evans LM, Scanlon MF, Davies JS. Long-term remission following withdrawal of dopamine agonist therapy in subjects with microprolactinomas. Clin Endocrinol. 2005;63:26–31.

Bogazzi F, Buralli S, Manetti L, Raffaelli V, Cigni T, Lombardi M, Boresi F, Taddei S, Salvetti A, Martino E. Treatment with low doses of cabergoline is not associated with increased prevalence of cardiac valve regurgitation in patients with hyperprolactinaemia. Int J Clin Pract. 2008;62:1864–9.

Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev. 1998;19:225–68.

Bonert VS, Melmed S (2006) Acromegaly with moderate hyperprolactinemia caused by an intrasellar macroadenoma. Nat Clin Pract Endocrinol Metab 2: 408-12

Brandebourg T, Hugo E, Ben-Jonathan N. Adipocyte prolactin: regulation of release and putative functions. Diabetes Obes Metab. 2007;9:464–76.

Brelje TC, Stout LE, Bhagroo NV, Sorenson RL. Distinctive roles for prolactin and growth hormone in the activation of signal transducer and activator of transcription 5 in pancreatic islets of langerhans. Endocrinology. 2004;145:4162–75.

Brown RS, Herbison AE, Grattan DR. Prolactin regulation of kisspeptin neurones in the mouse brain and its role in the lactation-induced suppression of kisspeptin expression. J Neuroendocrinol. 2014;26:898–908.

Buchfelder M, Schlaffer S. Surgical treatment of pituitary tumours. Best Pract Res Clin Endocrinol Metab. 2009;23:677–92.

Buvat J, Shabsigh R, Guay A, Gooren L, Torres LO, Meuleman E. Hormones, metabolism, aging, and men's health. In: Montorsi F, Basson R, Adaikan G, Becher E, Clayton A, Giuliano F, et al., editors. Standard practice in sexual medicine. Oxford: Blackwell Publishing; 2006. p. 225–86.

Byerly M, Suppes T, Tran QV, Baker RA. Clinical implications of antipsychotic-induced hyperprolactinemia in patients with schizophrenia spectrum or bipolar spectrum disorders: recent developments and current perspectives. J Clin Psychopharmacol. 2007;27:639–61.

Caccavelli L, Feron F, Morange I, Rouer E, Benarous R, Dewailly D, Jaquet P, Kordon C, Enjalbert A. Decreased expression of the two D2 dopamine receptor isoforms in bromocriptine-resistant prolactinomas. Neuroendocrinology. 1994;60:314–22.

Casanueva FF, Molitch ME, Schlechte JA, Abs R, Bonert V, Bronstein MD, Brue T, Cappabianca P, Colao A, Fahlbusch R, Fideleff H, Hadani M, Kelly P, Kleinberg D, Law E, Marek J, Scanlon M, Sobrinho LG, Wass JAH. Guidelines of the Pituitary Society for the diagnosis and management of prolactinomas. Clin Endocrinol (Oxf). 2006;65:265–73.

Castinetti F, Nagai M, Morange I, Dufour H, Caron P, Chanson P, Cortet-Rudelli C, Kuhn JM, Conte-Devolx B, Regis J, Brue T. Long-term results of stereotactic radiosurgery in secretory pituitary adenomas. J Clin Endocrinol Metab. 2009;94:3400–7.

Cavallaro R, Cocchi F, Angelone SM, Lattuada E, Smeraldi E. Cabergoline treatment of risperidone-induced hyperprolactinemia: a pilot study. J Clin Psychiatry. 2004;65:187–90.

Chandrashekar V, Bartke A. Influence of endogenous prolactin on the luteinizing hormone stimulation of testicular steroidogenesis and the role of prolactin in adult male rats. Steroids. 1988;51:559–76.

Chavez-Rueda K, Hernandez J, Zenteno E, Leanos-Miranda A, Legorreta-Haquet MV, Blanco-Favela F. Identification of prolactin as a novel immunomodulator on the expression of co-stimulatory molecules and cytokine secretions on T and B human lymphocytes. Clin Immunol. 2005;116:182–91.

Chwieduk CM, Keating GM. Paliperidone extended release: a review of its use in the management of schizophrenia. Drugs. 2010;70:1295–317.

Clevenger CV, Furth PA, Hankinson SE, Schuler LA. The role of prolactin in mammary carcinoma. Endocr Rev. 2003;24:1–27.

Cohen LG, Biederman J. Treatment of risperidone-induced hyperprolactinemia with a dopamine agonist in children. J Child Adolesc Psychopharmacol. 2001;11:435–40.

Cohen-Inbar O, Xu Z, Schlesinger D, Vance ML, Sheehan JP. Gamma knife radiosurgery for medically and surgically refractory prolactinomas: long-term results. Pituitary. 2015;18:820–30.

Colao A. Pituitary tumours: the prolactinoma. Best Pract Res Clin Endocrinol Metab. 2009;23:575–96.

Colao A, Di Sarno A, Sarnacchiaro F, Ferone D, Di Renzo G, Merola B, Annunziato L, Lombardi G. Prolactinomas resistant to standard dopamine agonists respond to chronic cabergoline treatment. J Clin Endocrinol Metab. 1997;82:876–83.

Colao A, Di Sarno A, Cappabianca P, Di Somma C, Pivonello R, Lombardi G. Withdrawal of long-term cabergoline therapy for tumoral and nontumoral hyperprolactinemia. N Engl J Med. 2003;349:2023–33.

Colao A, Galderisi M, Di Sarno A, Pardo M, Gaccione M, D'Andrea M, Guerra E, Pivonello R, Lerro G, Lombardi G. Increased prevalence of tricuspid regurgitation in patients with prolactinomas chronically treated with cabergoline. J Clin Endocrinol Metab. 2008;93:3777–84.

Cookson J. Oestrogens, dopamine and mood. Br J Psychiatry. 1981;139:365–6.

Corona G, Mannucci E, Fisher AD, Lotti F, Ricca V, Balercia G, Petrone L, Forti G, Maggi M. Effect of hyperprolactinemia in male patients consulting for sexual dysfunction. J Sex Med. 2007;4:1485–93.

Cosman D, Lyman SD, Idzerda RL, Beckmann MP, Park LS, Goodwin RG, March CJ. A new cytokine receptor superfamily. Trends Biochem Sci. 1990;15:265–70.

Couldwell WT, Weiss MH. Medical and surgical management of microprolactinoma. Pituitary. 2004;7:31–2.

Craig MC, Fletcher PC, Daly EM, Rymer J, Cutter WJ, Brammer M, Giampietro V, Wickham H, Maki PM, Murphy DG. Gonadotropin hormone releasing hormone agonists alter prefrontal function during verbal encoding in young women. Psychoneuroendocrinology. 2007;32:1116–27.

Craig MC, Fletcher PC, Daly EM, Picchioni MM, Brammer M, Giampietro V, Rymer J, McGuire PK, Maki PM, Murphy DG. A study of visuospatial working memory pre- and post-Gonadotropin Hormone Releasing Hormone agonists (GnRHa) in young women. Horm Behav. 2008;54:47–59.

Crambert S, Sjoberg A, Eklof AC, Ibarra F, Holtback U. Prolactin and dopamine 1-like receptor interaction in renal proximal tubular cells. Am J Phys. 2010;299:F49–54.

Creemers LB, Zelissen PM, van't Verlaat JW, Koppeschaar HP. Prolactinoma and body weight: a retrospective study. Acta Endocrinol. 1991;125:392–6.

Cruz N, Vieta E. Asenapine: a new focus on the treatment of mania. Rev Psiquiatr Salud Ment. 2011;4:101–8.

Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A. High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. J Clin Endocrinol Metab. 2006;91:4769–75.

Das R, Vonderhaar BK. Prolactin as a mitogen in mammary cells. J Mammary Gland Biol Neoplasia. 1997;2:29–39.

De Bellis A, Bizzarro A, Pivonello R, Lombardi G, Bellastella A. Prolactin and autoimmunity. Pituitary. 2005;8:25–30.

Dekkers OM, Lagro J, BurmanP JJO, Romijn JA, Pereira AM. Recurrence of hyperprolactinemia after withdrawal of dopamine agonists: systematic review and meta-analysis. J Clin Endocrinol Metab. 2010a;95:43–51.

Dekkers OM, Romijn JA, de Boer A, Vandenbroucke JP. The risk for breast cancer is not evidently increased in women with hyperprolactinemia. Pituitary. 2010b;13:195–8.

Delgrange E, Trouillas J, Maiter D, Donckier J, Tourniaire J. Sex-related difference in the growth of prolactinomas: a clinical and proliferation marker study. J Clin Endocrinol Metab. 1997;82:2102–7.

Delgrange E, Donckier J, Maiter D. Hyperprolactinaemia as a reversible cause of weight gain in male patients? Clin Endocrinol. 1999;50:271.

Delgrange E, Daems T, Verhelst J, Abs R, Maiter D. Characterization of resistance to the prolactin-lowering effects of cabergoline in macroprolactinomas: a study in 122 patients. Eur J Endocrinol. 2009;160:747–52.

Demura R, Ono M, Demura H, Shizume K, Oouchi H. Prolactin directly inhibits basal as well as gonadotropin-stimulated secretion of progesterone and 17b-estradiol in the human ovary. J Clin Endocrinol Metab. 1982;54:1246–50.

Doknic M, Pekic S, Zarkovic M, Medic-Stojanoska M, Dieguez C, Casanueva F, Popovic V. Dopaminergic tone and obesity: an insight from prolactinomas treated with bromocriptine. Eur J Endocrinol. 2002;147:77–84.

Dombrowicz D, Sente B, Closset J, Hennen G. Dose-dependent effects of human prolactin on the immature hypophysectomized rat testis. Endocrinology. 1992;130:695–700.

Dorrington JH, Gore-Langton RE. Antigonadal action of prolactin: further studies on the mechanism of inhibition of follicle-stimulating hormone-induced aromatase activity in rat granulosa cell cultures. Endocrinology. 1982;110:1701–7.

Drago F, Pellegrini-Quarantotti B, Scapagnini U, Gessa GL. Short-term endogenous hyperprolactinemia and sexual behaviour of me rats. Physiol Behav. 1981;26:277–9.

Erem C, Kocak M, Nuhoglu I, Yilmaz M, Ucuncu O. Blood coagulation, fibrinolysis and lipid profile in patients with prolactinoma. Clin Endocrinol. 2010;73:502–27.

Ernst B, Thurnheer M, Schultes B. Basal serum prolactin levels in obesity-unrelated to parameters of the metabolic syndrome and unchanged after massive weight loss. Obes Surg. 2009;19:1159–62.

Faupel-Badger JM, Duggan MA, Sherman ME, Garcia-Closas M, Yang XR, Lissowska J, Brinton LA, Peplonska B, Vonderhaar BK, Figueroa JD. Prolactin receptor expression and breast cancer: relationships with tumor characteristics among pre- and post-menopausal women in a population-based case-control study from Poland. Horm Cancer. 2014;5:42–50.

Fava M, Fava GA, Kellner R, Buckman MT, Lisansky J, Serafini E, DeBesi L, Mastrogiacomo I. Psychosomatic aspects of hyperprolactinemia. Psychother Psychosom. 1983;40:257–62.

Feldman M, Ruan W, Cunningham BC, Wells JA, Kleinberg DL. Evidence that the growth hormone receptor mediates differentiation and development of the mammary gland. Endocrinology. 1993;133:1602–8.

Feltus FA, Groner B, Melner MH. Stat5-mediated regulation of the human type II 3b-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene: activation by prolactin. Mol Endocrinol. 1999;13:1084–93.

Fernandez A, Karavitaki N, Wass JA. Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). Clin Endocrinol. 2010;72:377–82.

Frantz AG. Prolactin. N Engl J Med. 1978;298:201–7.

Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function and regulation of secretion. Physiol Rev. 2000;80:1523–631.

Freemark M, Avril I, Fleenor D, Driscoll P, Petro A, Opara E, Kendall W, Oden J, Bridges S, Binart N, Breant B, Kelly PA. Targeted deletion of the PRL receptor: effects on islet development, insulin production, and glucose tolerance. Endocrinology. 2002;143:1378–85.

Friedrich N, Schneider HJ, Spielhagen C, Markus MR, Haring R, Grabe HJ, Buchfelder M, Wallaschofski H, Nauck M. The association of serum prolactin concentration with inflammatory

biomarkers – cross-sectional findings from the population-based Study of Health in Pomerania. Clin Endocrinol. 2011;75:561–6.
Fuh G, Wells JA. Prolactin receptor antagonists that inhibit the growth of breast cancer cell lines. J Biol Chem. 1995;270:13133–7.
Georgiopoulos GA, Stamatelopoulos KS, Lambrinoudaki I, Lykka M, Kyrkou K, Rizos D, Creatsa M, Christodoulakos G, Alevizaki M, Sfikakis PP, Papamichael C. Prolactin and preclinical atherosclerosis in menopausal women with cardiovascular risk factors. Hypertension. 2009;54:98–105.
Gettler LT, McDade TW, Feranil AB, Kuzawa CW. Prolactin, fatherhood, and reproductive behavior in human males. Am J Phys Anthropol. 2012;148:362–70.
Gibney J, Smith TP, McKenna TJ. The impact on clinical practice of routine screening for macroprolactin. J Clin Endocrinol Metab. 2005;90:3927–32.
Gillam MP, Molitch ME, Lombardi G, Colao A. Advances in the treatment of prolactinomas. Endocr Rev. 2006;27:485–534.
Glass MR, Shaw RW, Butt WR, Edwards RL, London DR. An abnormality of oestrogen feedback in amenorrhoea–galactorrhoea. Br Med J. 1975;3:274–5.
Goffin V, Kelly PA. The prolactin/growth hormone receptor family: structure/function relationships. J Mammary Gland Biol Neoplasia. 1997;2:7–17.
Goffin V, Shiverick KT, Kelly PA, Martial JA. Sequence function relationships within the expanding family of prolactin, growth hormone, placental lactogen and related proteins in mammals. Endocr Rev. 1996;17:385–410.
Goffin V, Bernichtein S, Touraine P, Kelly P. Development and potential clinical uses of human prolactin receptor antagonists. Endocr Rev. 2005;26:400–22.
Goldhar AS, Vonderhaar BK, Trott JF, Hovey RC. Prolactin induced expression of vascular endothelial growth factor via Egr-1. Mol Cell Endocrinol. 2005;232:9–19.
Green AI, Brown WA. Prolactin and neuroleptic drugs. Endocrinol Metab Clin N Am. 1988;17:213–23.
Greenspan SL, Neer RM, Ridgway EC, Klibanski A. Osteoporosis in men with hyperprolactinemic hypogonadism. Ann Intern Med. 1986;104:777–82.
Greenspan SL, Oppenheim DS, Klibanski A. Importance of gonadal steroids to bone mass in men with hyperprolactinemic hypogonadism. Ann Intern Med. 1989;110:526–31.
Grigorova M, Sherwin BB, Tulandi T. Effects of treatment with leuprolide acetate depot on working memory and executive functions in young premenopausal women. Psychoneuroendocrinology. 2006;31:935–47.
Gunasekar PG, Kumaran B, Govindarajulu P. Prolactin and leydig cell steroidogenic enzymes in the bonnet monkey (macaca radiata). Int J Androl. 1988;11:53–9.
Gunasekar PG, Kumaran B, Govindarajulu P. Role of prolactin on leydig, sertoli and germ cellular neutral lipids in bonnet monkeys, macaca radiata. Endocrinologia Japonica. 1991;38:1–8.
Hair WM, Gubbay O, Jabbour HN, Lincoln GA. Prolactin receptor expression in human testis and accessory tissues: localization and function. Mol Hum Reprod. 2002;8:606–11.
Hamosh M, Hamosh P. The effect of prolactin on the lecithin content of fetal rabbit lung. J Clin Invest. 1977;59:1002–5.
Hankinson SE, Willett WC, Michaud DS, Manson JE, Colditz GA, Longcope C, Rosner B, Speizer FE. Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst. 1999;91:629–34.
Harvey PW, Everett DJ, Springall CJ. Hyperprolactinaemia as an adverse effect in regulatory and clinical toxicology: role in breast and prostate cancer. Hum Exp Toxicol. 2006;25:395–404.
Harvey PW, Everett DJ, Springall CJ. Adverse effects of prolactin in rodents and humans: breast and prostate cancer. J Psychopharmacol. 2008;22:20–7.
Henry JF, Sherwin BB. Hormones and cognitive functioning during late pregnancy and postpartum: a longitudinal study. Behav Neurosci. 2012;126:73–85.
Herring N, Szmigielski C, Becher H, Karavitaki N, Wass JA. Valvular heart disease and the use of cabergoline for the treatment of prolactinoma. Clin Endocrinol. 2009;70:104–8.

Honbo KS, van Herle AJ, Kellett KA. Serum prolactin levels in untreated primary hypothyroidism. Am J Med. 1978;64:782–7.

Horseman ND, Zhao W, Montecino-Rodriguez E, Tanaka M, Nakashima K, Engle SJ, Smith F, Markoff E, Dorshkind K. Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. EMBO J. 1997;16:6926–35.

Hou SH, Grossman S, Molitch ME. Hyperprolactinemia in patients with renal insufficiency and chronic renal failure requiring hemodialysis or chronic ambulatory peritoneal dialysis. Am J Kidney Dis. 1985;6:245–9.

Huang KE, Bonfiglio TA, Muechler EK. Transient hyperprolactinemia in infertile women with luteal phase deficiency. Obstet Gynecol. 1991;78:651–5.

Hurel SJ, Harris PE, McNicol AM, Foster S, Kelly WF, Baylis PH. Metastatic prolactinoma: effect of octreotide, cabergoline, carbo-platin and etoposide; immunocytochemical analysis of proto-oncogene expression. J Clin Endocrinol Metab. 1997;82:2962–5.

Ibarra F, Crambert S, Eklof AC, Lundquist A, Hansell P, Holtback U. Prolactin, a natriuretic hormone, interacting with renal dopamine system. Kidney Int. 2005;68:1700–7.

Ignacak A, Kasztelnik M, Sliwa T, Korbut R, Rajda K, Guzik T. Prolactin not only lactotrophin. A "new" view of the "old" hormone. J Physiol Pharmacol. 2012;63:435–43.

Ikeda H, Watanabe K, Tominaga T, Yoshimoto T. Transsphenoidal microsurgical results of female patients with prolactinomas. Clin Neurol Neurosurg. 2013;115:1621–5.

Ishida M, Yoshida M, Fukuta S, Uemura K, Iijima M, Horiguchi K, Harigaya T. Analysis of prolactin gene expression and cleaved prolactin variants in the mouse testis and spermatozoa. J Reprod Dev. 2010;56:567–74.

Jabbour HN, Lincoln GA. Prolactin receptor expression in the testis of the ram: localisation, functional activation and the influence of gonadotrophins. Mol Cell Endocrinol. 1999;148:151–61.

Janicak PG, Winans EA. Paliperidone ER: a review of the clinical trial data. Neuropsychiatr Dis Treat. 2007;3:869–97.

Jeffcoate WJ, Pound N, Sturrock ND, Lambourne J. Long-term follow-up of patients with hyperprolactinaemia. Clin Endocrinol. 1996;45:299–303.

Jezkova J, Hana V, Krsek M, Weiss V, Vladyka V, Liscak R, Vymazal J, Pecen L, Marek J. Use of the Leksell gamma knife in the treatment of prolactinoma patients. Clin Endocrinol. 2009;70:732–41.

Karavitaki N, Thanabalasingham G, Shore HC, Trifanescu R, Ansorge O, Meston N, Turner HE, Wass JA. Do the limits of serum prolactin in disconnection hyperprolactinaemia need re-definition? A study of 226 patients with histologically verified non-functioning pituitary macroadenoma. Clin Endocrinol. 2006;65:524–9.

Kars M, Delgado V, Holman ER, Feelders RA, Smit JW, Romijn JA, Bax JJ, Pereira AM. Aortic valve calcification and mild tricuspid regurgitation but no clinical heart disease after 8 years of dopamine agonist therapy for prolactinoma. J Clin Endocrinol Metab. 2008;93:3348–56.

Kars M, Souverein PC, Herings RM, Romijn JA, Vandenbroucke JP, de Boer A, Dekkers OM. Estimated age- and sex-specific incidence and prevalence of dopamine agonist-treated hyperprolactinemia. J Clin Endocrinol Metab. 2009;94:2729–34.

Karunakaran S, Page RC, Wass JA. The effect of the menopause on prolactin levels in patients with hyperprolactinaemia. Clin Endocrinol. 2001;54:295–300.

Kato Y, Shimatsu A, Matsushita N, Ohta H, Imura H. Role of vasoactive intestinal polypeptide (VIP) in regulating the pituitary function in man. Peptides. 1984;5:389–94.

Kearns AE, Goff DC, Hayden DL, Daniels GH. Risperidone associated hyperprolactinemia. Endocr Pract. 2000;6:425–9.

Kelly PA, Djiane J, Postel-Vinay MC, Edery M. The prolactin/growth hormone receptor family. Endocr Rev. 1991;12:235–51.

Keye WR, Yuen BH, Knopf RF, Jaffe RB. Amenorrhea, hyperprolactinemia and pituitary enlargement secondary to primary hypothyroidism. Successful treatment with thyroid replacement. Obstet Gynecol. 1976;48:697–702.

Kharlip J, Salvatori R, Yenokyan G, Wand GS. Recurrence of hyperprolactinemia after withdrawal of long-term cabergoline therapy. J Clin Endocrinol Metab. 2009;94:2428–36.

Kinon BJ, Gilmore JA, Liu H, Halbreich UM. Prevalence of hyperprolactinaemia in schizophrenic patients treated with conventional antipsychotic medications or risperidone. Psychoneuroendocrinology. 2003;28:55–68.

Kleinberg DL, Noel GL, Frantz AG. Galactorrhea: a study of 235 cases, including 48 with pituitary tumors. N Engl J Med Mar. 1977;296:589–600.

Klibanski A, Greenspan SL. Increase in bone mass after treatment of hyperprolactinemic amenorrhea. N Engl J Med. 1986;315:542–6.

Klibanski A, Neer RM, Beitins IZ, Ridgway EC, Zervas NT, McArthur JW. Decreased bone density in hyperprolactinemic women. N Engl J Med. 1980;303:1511–4.

Kokay IC, Petersen SL, Grattan DR. Identification of prolactin-sensitive GABA and kisspeptin neurons in regions of the rat hypothalamus involved in the control of fertility. Endocrinology. 2011;152:526–35.

Kong DS, Lee JI, Lim DH, Kim KW, Shin HJ, Nam DH, Park K, Kim JH. The efficacy of fractionated radiotherapy and stereotactic radiosurgery for pituitary adenomas: long-term results of 125 consecutive patients treated in a single institution. Cancer. 2007;110:854–60.

Kooy A, de Greef WJ, Vreeburg JT, Hackeng WH, Ooms MP, Lamberts SW, Weber RF. Evidence for the involvement of corticotropin-releasing factor in the inhibition of gonadotropin release induced by hyperprolactinemia. Neuroendocrinology. 1990;51:261–6.

Kovacs K, Stefaneanu L, Horvath E, Buchfelder M, Fahlbusch R, Becker W. Prolactin-producing pituitary tumor: resistance to dopamine agonist therapy. Case report. J Neurosurg. 1995;82:886–90.

Krasnow JS, Hickey GJ, Richards JS. Regulation of aromatase mRNA and estradiol biosynthesis in rat ovarian granulosa and luteal cells by prolactin. Mol Endocrinol. 1990;4:13–22.

Kreutzer J, Buslei R, Wallaschofski H, Hofmann B, Nimsky C, Fahlbusch R, Buchfelder M. Operative treatment of prolactinomas: indications and results in a current consecutive series of 212 patients. Eur J Endocrinol. 2008;158:11–8.

Kukstas LA, Domec C, Bascles L, Bonnet J, Verrier D, Israel JM, Vincent JD. Different expression of the two dopaminergic D2 receptors, D2415 and D2444, in two types of lactotroph each characterized by their response to dopamine, and modification of expression by sex steroids. Endocrinology. 1991;129:1101–3.

Lancellotti P, Livadariu E, Markov M, Daly AF, Burlacu MC, Betea D, Pierard L, Beckers A. Cabergoline and the risk of valvular lesions in endocrine disease. Eur J Endocrinol. 2008;159:1–5.

Lania AG, Ferrero S, Pivonello R, Mantovani G, Peverelli E, Di Sarno A, Beck-Peccoz P, Spada A, Colao A. Evolution of an aggressive prolactinoma into a growth hormone secreting pituitary tumor coincident with GNAS gene mutation. J Clin Endocrinol Metab. 2010;95:13–7.

Leanos-Miranda A, Cardenas-Mondragon G. Serum free prolactin concentrations in patients with systemic lupus erythematosus are associated with lupus activity. Rheumatology. 2006;45:97–101.

Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F, Samara M, Barbui C, Engel RR, Geddes JR, Kissling W, Stapf MP, Lässig B, Salanti G, Davis JM. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. Lancet. 2013;382:951–62.

Lim VS, Kathpalia SC, Frohman LA. Hyperprolactinemia and impaired pituitary response to suppression and stimulation in chronic renal failure: reversal after transplantation. J Clin Endocrinol Metab. 1979;48:101–7.

Lin Y, Hyde JF, Vore M. Prolactin regulates maternal bile secretory function post partum. J Pharmacol Exp Ther. 1992;261:560–6.

Ling C, Svensson L, Odén B, Weijdegård B, Edén B, Edén S, Billig H. Identification of functional prolactin (PRL) receptor gene expression: PRL inhibits lipoprotein lipase activity in human white adipose tissue. J Clin Endocrinol Metab. 2003;88:1804–8.

Lotti F, Corona G, Maseroli E, Rossi M, Silverii A, Degl'innocenti S, Rastrelli G, Forti G, Maggi M. Clinical implications of measuring prolactin levels in males of infertile couples. Andrology. 2013;1:764–71.

Lu ML, Shen WW, Chen CH. Time course of the changes in antipsychotic-induced hyperprolactinemia following the switch to aripiprazole. Prog Neuro-Psychopharmacol Biol Psychiatry. 2008;32:1978–81.
Luciano AA, Sherman BM, Chapler FK, Hauser KS, Wallace RB. Hyperprolactinemia and contraception: a prospective study. Obstet Gynecol. 1985;65:506–10.
Machida T, Taga M, Minaguchi H. Effect of prolactin (PRL) on lipoprotein lipase (LPL) activity in the rat fetal liver. Asia Oceania J Obstet Gynaecol. 1990;16:261–5.
Malaguarnera L, Pilastro MR, Quan S, Ghattas MH, Yang L, Mezentsev AV, Kushida T, Abraham NG, Kappas A. Significance of heme oxygenase in prolactin-mediated cell proliferation and angiogenesis in human endothelial cells. Int J Mol Med. 2002;10:433–40.
Mancini T, Casanueva FF, Giustina A. Hyperprolactinemia and prolactinomas. Endocrinol Metab Clin N Am. 2008;37:67–99.
Manjer J, Johansson R, Berglund G, Janzon L, Kaaks R, Agren A, Lenner P. Postmenopausal breast cancer risk in relation to sex steroid hormones, prolactin and SHBG (Sweden). Cancer Causes Control. 2003;14:599–607.
Matsuzaki T, Azuma K, Irahara M, Yasui T, Aono T. Mechanism of anovulation in hyperprolactinemic amenorrhea determined by pulsatile gonadotropin-releasing hormone injection combined with human chorionic gonadotropin. Fertil Steril. 1994;62:1143–9.
Mazziotti G, Porcelli T, Mormando M, De Menis E, Bianchi A, Mejia C, Mancini T, De Marinis L, Giustina A. Vertebral fractures in males with prolactinoma. Endocrine. 2011;39:288–93.
McNatty KP. Relationship between plasma prolactin and the endocrine microenvironment of the developing human antral follicle. Fertil Steril. 1979;32:433–8.
Melmed S, Kleinberg D. Anterior pituitary. In: Larsen P, Kronenberg H, Melmed S, Polonsky K, editors. Williams textbook of endocrinology. 10th ed. Philadelphia: Saunders; 2003. p. 177–279.
Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, Wass JA, Endocrine Society. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:273–88.
Meltzer HY, Fang VS. Serum prolactin levels in schizophrenia-effect of antipsychotic drugs: a preliminary report. In: Sachar EJ, editor. Hormones, behavior, and psychopathology. New York: Raven Press; 1976.
Mendelson JH, Mello NK, Teoh SK, Ellingboe J, Cochin J. Cocaine effects on pulsatile secretion of anterior pituitary, gonadal, and adrenal hormones. J Clin Endocrinol Metab. 1989;69:1256–60.
Mingrone G, Manco M, Iaconelli A, Gniuli D, Bracaglia R, Leccesi L, Calvani M, Nolfe G, Basu S, Berria R. Prolactin and insulin ultradian secretion and adipose tissue lipoprotein lipase expression in severely obese women after bariatric surgery. Obesity (Silver Spring). 2008;16:1831–7.
Moffat SD, Zonderman AB, Metter EJ, Blackman MR, Harman SM, Resnick SM. Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. J Clin Endocrinol Metab. 2002;87:5001–7.
Moffat SD, Zonderman AB, Metter EJ, Kawas C, Blackman MR, Harman SM, Resnick SM. Free testosterone and risk for Alzheimer disease in older men. Neurology. 2004;27(62):188–93.
Molinari C, Grossini E, Mary DA, Uberti F, Ghigo E, Ribichini F, Surico N, Vacca G. Prolactin induces regional vasoconstriction through the beta2-adrenergic and nitric oxide mechanisms. Endocrinology. 2007;148:4080–90.
Molitch ME. Pathologic hyperprolactinemia. Endocrinol Metab Clin N Am. 1992;21:877–901.
Molitch ME. Prolactinoma. In: Melmed S, editor. Pituitary. Cambridge: Blackwell Science; 1995. p. 433–77.
Molitch ME. Dopamine resistance of prolactinomas. Pituitary. 2003;6:19–27.
Molitch ME. Medication-induced hyperprolactinemia. Mayo Clin Proc. 2005a;80:1050–7.
Molitch ME. Pharmacologic resistance in prolactinoma patients. Pituitary. 2005b;8:43–52.
Molitch ME. Endocrinology in pregnancy: management of the pregnant patient with a prolactinoma. Eur J Endocrinol. 2015;172:R205–13.
Molitch ME, Reichlin S. Hyperprolactinemic disorders. Dis Mon. 1982;28:1–58.

Molitch ME, Elton RL, Blackwell RE, Caldwell B, Chang RJ, Jaffe R, Joplin G, Robbins RJ, Tyson J, Thorner MO. Bromocriptine as primary therapy for prolactin-secreting macroadenomas: results of a prospective multicenter study. J Clin Endocrinol Metab. 1985;60:698–705.

Montejo AL. Prolactin awareness: an essential consideration for physical health in schizophrenia. Eur Neuropsychopharmacol. 2008;18:101–30.

Montejo AL, Rico-Villademoros F, Spanish Working Group for the Study of Psychotropic-Related Sexual Dysfunction. Changes in sexual function for outpatients with schizophrenia or other psychotic disorders treated with ziprasidone in clinical practice settings: a 3-month prospective, observational study. J Clin Psychopharmacol. 2008;28:568–70.

Montes de Oca P, Macotela Y, Nava G, López-Barrera F, de la Escalera GM, Clapp C. Prolactin stimulates integrin-mediated adhesion of circulating mononuclear cells to endothelial cells. Lab Investig. 2005;85:633–42.

Morange I, Barlier A, Pellegrini I, Brue T, Enjalbert A, Jaquet P. Prolactinomas resistant to bromocriptine: long-term efficacy of quinagolide and outcome of pregnancy. Eur J Endocrinol. 1996;135:413–20.

Morley JE, Dawson M, Hodgkinson H, Kalk WJ. Galactorrhea and hyperprolactinemia associated with chest wall injury. J Clin Endocrinol Metab. 1977;45:931–5.

Nevalainen MT, Valve EM, Ahonen T, Yagi A, Paranko J, Härkönen PL. Androgen-dependent expression of prolactin in rat prostate epithelium in vivo and in organ culture. FASEB J. 1997a;11:1297–307.

Nevalainen MT, Valve EM, Ingleton PM, Nurmi M, Martikainen PM, Harkonen PL. Prolactin and prolactin receptors are expressed and functioning in human prostate. J Clin Invest. 1997b;99:618–27.

Newey PJ, Gorvin CM, Cleland SJ, Willberg CB, Bridge M, Azharuddin M, Drummond RS, van der Merwe PA, Klenerman P, Bountra C, Thakker RV. Mutant prolactin receptor and familial hyperprolactinemia. N Engl J Med. 2013;369:2012–20.

Nicol M, Willis C, Yiangou C, Sinnett D, Shousha S. Relationship between serum prolactin levels and histology of benign and malignant breast lesions: a detailed study of 153 consecutive cases. Breast J. 2002;8:281–5.

Nicoll C. Ontogeny and evolution of prolactins' function. Fed Proc. 1980;39:2563–6.

Nilsson L, Binart N, Bohlooly-Y M, Bramnert M, Egecioglu E, Kindblom J, Kelly PA, Kopchick JJ, Ormandy CJ, Ling C, Billig H (2005) Prolactin and growth hormone regulate adiponectin secretion and receptor expression in adipose tissue. Biochem Biophys Res Commun 331: 1120-1126

Nomikos P, Buchfelder M, Fahlbusch R. Current management of prolactinomas. J Neuro-Oncol. 2001;54:139–50.

Oh MC, Aghi MK. Dopamine agonist-resistant prolactinomas. J Neurosurg. 2011;114:1369–79.

Oliveira MC, Pizarro CB, Golbert L, Micheletto C. Hyperprolactinemia and psychological disturbance. Arq Neuropsiquiatr. 2000;58:671–6.

Orbach H, Shoenfeld Y. Hyperprolactinemia and autoimmune diseases. Autoimmun Rev. 2007;6:537–42.

Pan L, Zhang N, Wang EM, Wang BJ, Dai JZ, Cai PW. Gamma knife radiosurgery as a primary treatment for prolactinomas. J Neurosurg. 2000;93:10–3.

Pellegrini I, Rasolonjanahary R, Gunz G, Bertrand P, Delivet S, Jedynak CP, Kordon C, Peillon F, Jaquet P, Enjalbert A. Resistance to bromocriptine in prolactinomas. J Clin Endocrinol Metab. 1989;69:500–9.

Phillips SM, Sherwin BB. Effects of estrogen on memory function in surgically menopausal women. Psychoneuroendocrinology. 1992;17:485–95.

Pinsky M, Hellstrom W. Hypogonadism, ADAM, and hormone replacement. Ther Adv Urol. 2010;2:99–104.

Pinzone JJ, Katznelson L, Danila DC, Pauler DK, Miller CS, Klibanski A. Primary medical therapy of micro- and macroprolactinomas in men. J Clin Endocrinol Metab. 2000;85:3053–7.

Pippard C, Baylis PH. Prolactin stimulates Na+-K+-ATPase activity located in the outer renal medulla of the rat. J Endocrinol. 1986;108:95–9.

Pollock A, McLaren EH. Serum prolactin concentration in patients taking neuroleptic drugs. Clin Endocrinol. 1998;49:513–6.

Postel-Vinay MC, Belair L, Kayser C, Kelly PA, Djiane J. Identification of prolactin and growth hormone binding proteins in milk. Proc Natl Acad Sci U S A. 1991;188:6687–90.

Poyraz BC, Aksoy C, Balcioglu I. Increased incidence of autoimmune thyroiditis in patients with antipsychotic-induced hyperprolactinemia. Eur Neuropsychopharmacol. 2008;18:667–72.

Prabhakar VK, Davis JR. Hyperprolactinemia. Best Pract Res Clin Obstet Gynaecol. 2008;22:341–53.

Pujianto DA, Curry BJ, Aitken RJ. Prolactin exerts a prosurvival effect on human spermatozoa via mechanisms that involve the stimulation of Akt phosphorylation and suppression of caspase activation and capacitation. Endocrinology. 2010;151:1269–79.

Ramasharma K, Li CH. Human pituitary and placental hormones control human insulin-like growth factor II secretion in human granulosa cells. Proc Natl Acad Sci U S A. 1987;84:2643–7.

Ravault JP, Courot M, Garnier D, Pelletier J, Terqui M. Effect of 2-bromo-α-ergocryptine (CB154) on plasma prolactin, LH and testosterone levels, accessory reproductive glands and spermatogenesis in lambs during puberty. Biol Reprod. 1977;17:192–7.

Reavley A, Fisher AD, Owen D, Creed FH, Davis JR. Psychological distress in patients with hyperprolactinaemia. Clin Endocrinol. 1997;47:343–8.

Ress C, Maeser PA, Tschoner A, Loacker L, Salzmann K, Staudacher G, Melmer A, Zoller H, Vogel W, Griesmacher A, Tilg H, Graziadei I, Kaser S. Serum prolactin in advanced chronic liver disease. Horm Metab Res. 2014;46:800–3.

Reuwer AQ, Twickler MT, Hutten BA, Molema FW, Wareham NJ, Dallinga-Thie GM, Bogorad RL, Goffin V, Smink-Bol M, Kastelein JJ, Boekholdt SM, Khaw KT. Prolactin levels and the risk of future coronary artery disease in apparently healthy men and women. Circ Cardiovasc Genet. 2009;2:389–95.

Reuwer AQ, van Eijk M, Houttuijn-Bloemendaal FM, van der Loos CM, Claessen N, Teeling P, Kastelein JJ, Hamann J, Goffin V, von der Thüsen JH, Twickler MT, Aten J. The prolactin receptor is expressed in macrophages within human carotid atherosclerotic plaques: a role for prolactin in atherogenesis? J Endocrinol. 2011;208:107–17.

Richardsson BP. Evidence for a physiological role of prolactin in osmoregulation in the rat after its inhibition by 2-bromo-α-ergokryptine. Br J Pharmacol. 1973;47:623–4.

Rickenlund A, Thoren M, Carlstrom K, von Schoultz B, Hirschberg AL. Diurnal profiles of testosterone and pituitary hormones suggest different mechanisms for menstrual disturbances in endurance athletes. J Clin Endocrinol Metab. 2004;89:702–7.

Rohmer V, Freneau E, Morange I, Simonetta C. Efficacy of quinagolide in resistance to dopamine agonists: results of a multi-center study. Club de l'Hypophyse. Ann Endocrinol (Paris). 2000;61:411–7.

Rubin RT, Poland RE, Tower BB. Prolactin-related testosterone secretion in normal adult men. J Clin Endocrinol Metab. 1976;42:112–6.

Saitis M, Papazisis G, Katsigiannopoulos K, Kouvelas D. Aripiprazole resolves amisulpride and ziprasidone-induced hyperprolactinemia. Psychiatry Clin Neurosci. 2008;62:624.

Samson SL, Hamrahian AH, Ezzat S, AACE Neuroendocrine and Pituitary Scientific Committee, American College of Endocrinology (ACE). American association of clinical endocrinologists, American college of endocrinology disease state clinical review: clinical relevance of macroprolactin in the absence or presence of true hyperprolactinemia. Endocr Pract. 2015;21:1427–35.

Sauder SE, Frager M, Case GD, Kelch RP, Marshall JC. Abnormal patterns of pulsatile luteinizing hormone secretion in women with hyperprolactinemia and amenorrhea: responses to bromocriptine. J Clin Endocrinol Metab. 1984;59:941–8.

Schade R, Andersohn F, Suissa S, Haverkamp W, Garbe E. Dopamine agonists and the risk of cardiac-valve regurgitation. N Engl J Med. 2007;356:29–38.

Schlechte JA. Clinical practice. Prolactinoma. N Engl J Med. 2003;349:2035–41.

Schlechte JA, Sherman BM, Chapler FK, VanGilder J. Long-term follow-up of women with surgically treated prolactin-secreting pituitary tumours. J Clin Endocrinol Metab. 1986;62:1296–301.

Schlechte J, El-Khoury G, Kathol M, Walkner L. Forearm and vertebral bone mineral in treated and untreated hyperprolactinemic amenorrhea. J Clin Endocrinol Metab. 1987;64:1021–6.
Schlechte J, Dolan K, Sherman B, Chapler F, Luciano A. The natural history of untreated hyperprolactinemia: a prospective analysis. J Clin Endocrinol Metab. 1989;68:412–8.
Seppala M, Ranta T, Hirvonen E. Hyperprolactinaemia and luteal insufficiency. Lancet. 1976;1:229–30.
Seriwatanachai D, Krishnamra N, van Leeuwen JP. Evidence for direct effects of prolactin on human osteoblasts: inhibition of cell growth and mineralization. J Cell Biochem. 2009;107:677–85.
Serri O, Li L, Mamputu JC, Beauchamp MC, Maingrette F, Renier G. The influences of hyperprolactinemia and obesity on cardiovascular risk markers: effects of cabergoline therapy. Clin Endocrinol. 2006;64:366–70.
Shelly S, Boaz M, Orbach H. Prolactin and autoimmunity. Autoimmun Rev. 2012;11:A465–70.
Shibli-Rahhal A, Schlechte J. The effects of hyperprolactinemia on bone and fat. Pituitary. 2009;12:96–104.
Sievertsen GD, Lim VS, Nakawatase C, Frohman LA. Metabolic clearance and secretion rates of human prolactin in normal subjects and in patients with chronic renal failure. J Clin Endocrinol Metab. 1980;50:846–52.
Skrabanek P, McDonald D, de Valera E, Lanigan O, Powell D. Plasma prolactin in amenorrhoea, infertility, and other disorders: a retrospective study of 608 patients. Ir J Med Sci. 1980;149:236–45.
Sluijmer AV, Lappöhn RE. Clinical history and outcome of 59 patients with idiopathic hyperprolactinemia. Fertil Steril. 1992;58:72–7.
Smith S. Neuroleptic-associated hyperprolactinemia. Can it be treated with bromocriptine? J Reprod Med. 1992;37:737–40.
Smith S. The impact of hyperprolactinaemia on sexual function in patients with psychosis. J Psychopharmacol. 2008;22:63–9.
Smith TP, Suliman AM, Fahie-Wilson MN, McKenna TJ. Gross variability in the detection of prolactin in sera containing big big prolactin (macroprolactin) by commercial immunoassays. J Clin Endocrinol Metab. 2002a;87:5410–5.
Smith S, Wheeler MJ, Murray R, O'Keane V. The effects of antipsychotic-induced hyperprolactinaemia on the hypothalamic-pituitary-gonadal axis. J Clin Psychopharmacol. 2002b;22:109–14.
Sobrinho LG. Familial prolactinoma. Clin Endocrinol. 1995;43:511.
Sobrinho LG. Emotional aspects of hyperprolactinemia. Psychother Psychosom. 1998;67:133–9.
Sonigo C, Bouilly J, Carré N, Tolle V, Caraty A, Tello J, Simony-Conesa FJ, Millar R, Young J, Binart N. Hyperprolactinemia-induced ovarian acyclicity is reversed by kisspeptin administration. J Clin Invest. 2012;122:3791–5.
Sorenson RL, Brelie TC, Hegie OD, Marshall S, Anaya P, Sheridam JD. Prolactin (in vitro) decreases the glucose stimulation threshold, enhances insulin secretion, and increases dye coupling among islet b cells. Endocrinology. 1987;121:1447–53.
Spitzer M, Sajjad R, Benjamin F. Pattern of development of hyperprolactinemia after initiation of haloperidol therapy. Obstet Gynecol. 1998;91:693–5.
St-Jean E, Blain F, Comtois R. High prolactin levels may be missed by immunoradiometric assay in patients with macroprolactinomas. Clin Endocrinol. 1996;44:305–9.
Svestka J, Synek O, Tomanová J, Rodáková I, Cejpková A. Differences in the effect of second-generation antipsychotics on prolactinaemia: six weeks open-label trial in female in-patients. Neuro Endocrinol Lett. 2007;28:881–8.
Szosland K, Stasiak M, Lewiński A. Hyperprolactinemia diagnostics-dilemmas over optimal selection of prolactinemia time points. Ann Agric Environ Med. 2015;22:332–7.
Takase M, Tsutsui K, Kawashima S. Effects of prolactin and bromocryptine on the regulation of testicular luteinizing hormone receptors in mice. J Exp Zool. 1990;256:200–9.
Takeyama M, Nagareda T, Takatsuka D, Namiki M, Koizumi K, Aono T, Matsumoto K. Stimulatory effect of prolactin on luteinizing hormone-induced testicular 5 alpha-reductase activity in hypophysectomized adult rats. Endocrinology. 1986;118:2268–75.

Tolis G, Hickey J, Guyda H. Effects of morphine on serum growth hormone, cortisol, prolactin and thyroid stimulating hormone in man. J Clin Endocrinol Metab. 1975;41:797–800.

Tollin SR. Use of the dopamine agonists bromocriptine and cabergoline in the management of risperidone-induced hyperprolactinemia in patients with psychotic disorders. J Endocrinol Investig. 2000;23:765–70.

Unnikrishnan AG, Rajaratnam S, Seshadri MS, Kanagasapabathy AS, Stephen DC. The "hook effect" on serum prolactin estimation in a patient with macroprolactinoma. Neurol India. 2001;49:78–80.

Vallette S, Serri K, Rivera J, Santagata P, Delorme S, Garfield N, Kahtani N, Beauregard H, Aris-Jilwan N, Houde G, Serri O. Long-term cabergoline therapy is not associated with valvular heart disease in patients with prolactinomas. Pituitary. 2009;12:153–7.

Vera-Lastra O, Jara LJ, Espinoza LR. Prolactin and autoimmunity. Autoimmun Rev. 2002;1:360–4.

Verhelst J, Abs R, Maiter D, van den Bruel A, Vandeweghe M, Velkeniers B, Mockel J, Lamberigts G, Petrossians P, Coremans P, Mahler C, Stevenaert A, Verlooy J, Raftopoulos C, Beckers A. Cabergoline in the treatment of hyperprolactinemia: a study in 455 patients. J Clin Endocrinol Metab. 1999;84:2518–22.

Volavka J, Czobor P, Cooper TB, Sheitman B, Lindenmayer JP, Citrome L, McEvoy JP, Lieberman JA. Prolactin levels in schizophrenia and schizoaffective disorder patients treated with clozapine, olanzapine, risperidone, or haloperidol. J Clin Psychiatry. 2004;65:57–61.

Vroonen L, Jaffrain-Rea ML, Petrossians P, Tamagno G, Chanson P, Vilar L, Borson-Chazot F, Naves LA, Brue T, Gatta B, Delemer B, Ciccarelli E, Beck-Peccoz P, Caron P, Daly AF, Beckers A. Prolactinomas resistant to standard doses of cabergoline: a multicenter study of 92 patients. Eur J Endocrinol. 2012;167:651–62.

Wakil A, Rigby AS, Clark AL, Kallvikbacka-Bennett A, Atkin SL. Low dose cabergoline for hyperprolactinaemia is not associated with clinically significant valvular heart disease. Eur J Endocrinol. 2008;159:R11–4.

Wallaschofski H, Donné M, Eigenthaler M, Hentschel B, Faber R, Stepan H, Koksch M, Lohmann T. PRL as a novel potent cofactor for platelet aggregation. J Clin Endocrinol Metab. 2001;86:5912–9.

Walsh JP, Pullan PT. Hyperprolactinaemia in males: a heterogeneous disorder. Aust NZ J Med. 1997;27:385–90.

Webster J, Piscitelli G, Polli A, Ferrari CI, Ismail I, Scanlon MF. A comparison of cabergoline and bromocriptine in the treatment of hyperprolactinemic amenorrhoea. Cabergoline Comparative Study Group. N Engl J Med. 1994;331:904–9.

Winters SJ, Troen P. Altered pulsatile secretion of luteinizing hormone in hypogonadal men with hyperprolactinaemia. Clin Endocrinol. 1984;21:257–63.

Wong A, Eloy JA, Couldwell WT, Liu J. Update on prolactinomas. Part 2: treatment and management strategies. J Clin Neurosci. 2015;22:1568–74.

Yavuz D, Deyneli O, Akpinar I, Yildiz E, Gözü H, Sezgin O, Haklar G, Akalin S. Endothelial function, insulin sensitivity and inflammatory markers in hyperprolactinemic pre-menopausal women. Eur J Endocrinol. 2003;149:187–93.

Yazigi RA, Quintero CH, Salameh WA. Prolactin disorders. Fertil Steril. 1997;67:215–25.

Yener S, Comlekci A, Arda N, Men S, Yesil S. Misdiagnosis due to the hook effect in prolactin assay. Med Princ Pract. 2008;17:429–31.

Yu-Lee LY. Prolactin modulation of immune and inflammatory responses. Recent Prog Horm Res. 2002;57:435–55.

Zanettini R, Antonini A, Gatto G, Gentile R, Tesei S, Pezzoli G. Valvular heart disease and the use of dopamine agonists for Parkinson's disease. N Engl J Med. 2007;356:39–46.

Zellweger R, Zhu XH, Wichmann MW, Ayala A, DeMaso CM, Chaudry IH. Prolactin administration following hemorrhagic shock improves macrophage cytokine release capacity and decreases mortality from subsequent sepsis. J Immunol. 1996;157:5748–54.

Zis AP, Haskett RF, Albala AA, Carroll BJ. Morphine inhibits cortisol and stimulates prolactin secretion in man. Psychoneuroendocrinology. 1984;9:423–7.

Neuroendocrinology of Energy Homeostasis

15

Valentina Lo Preiato, Valentina Vicennati, Silvia Garelli, and Uberto Pagotto

Contents

Abstract

Body weight regulation consists of a series of complex and occasionally redundant mechanisms principally involving food intake and energy expenditure. Many hormones, nutrients, and neurotransmitters are involved in signaling hunger and satiety. This multitude of signals is integrated by the hypothalamus, which in turn acts by changing the hormonal secretion pattern. In addition, the homeostatic system is strongly influenced by the brain circuits involved in pleasure, resulting in a food intake that is based on hedonistic choices, rather than just biological need. However, alterations in the mechanisms controlling the pleasure and gratification derived from food might also induce an increase in weight.

V. Lo Preiato · V. Vicennati · S. Garelli · U. Pagotto (✉)
Endocrinology Unit and Center for Applied Biomedical Research, Department of Medical and Surgical Sciences, University of Bologna – S. Orsola-Malpighi Hospital, Bologna, Italy
e-mail: uberto.pagotto@unibo.it

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_16

Keywords
Food intake · Hypothalamus · Arcuate nucleus

The Homeostatic Control of Food Intake

The brainstem and hypothalamus are the main brain areas involved in the homeostatic control of food intake. Signals from the gastrointestinal system and adipose tissue reaching these regions of the brain usually provide information on the nutritional tonic status (such as insulin and leptin), while other hormones secreted during meals are hypothesized as indicating that food is being consumed (such as cholecystokinin, glucagon-like peptide-1, and peptide YY). In addition, ghrelin, the gastric hormone stimulating hunger, acts on the hypothalamus and brainstem.

Brainstem

Within the brainstem, the dorsal vagal complex (DVC) plays a crucial role in the signal transmission from the vagus to the hypothalamus. Like the arcuate nucleus, the nucleus of the tractus solitarius (NTS) is located in an ideal position to integrate peripheral signals because in this area, the blood brain barrier (BBB) is permeable and incomplete. In fact, numerous peripheral hormones act on the NTS, such as ghrelin (Lawrence et al. 2002), cholecystokinin (Moriarty et al. 1997), leptin (Burdyga et al. 2002), and glucagon-like peptide-1 (Nakagawa et al. 2004). The importance of the brainstem in the energy balance is supported by numerous studies. Lesions of the area postrema (van der Kooy 1984) or vagotomy (Schwartz et al. 1978; Abbott et al. 2005a) cause a reduction in the peripheral hormone effects on food intake, and the sectioning of all vagal fibers leads to an increase in the food consumed and outage duration.

Hypothalamus

The hypothalamus is located in proximity of the third ventricle, at the base of the brain. It is divided into several nuclei involved in many important biological processes, including energy balance control. The hypothalamic nuclei receive numerous afferent signals through the vagus nerve and many gastrointestinal hormonal signals. These nuclei also have many neuronal interconnections. The hypothalamic nuclei involved in the homeostatic control of food include the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the ventromedial nucleus (VMN), the dorsomedial nucleus (DMN), and the lateral hypothalamic area (LHA).

Arcuate Nucleus

The ARC is located near the median eminence, where the BBB is incomplete and permeable. The ARC neurons are therefore widely exposed to hormonal and

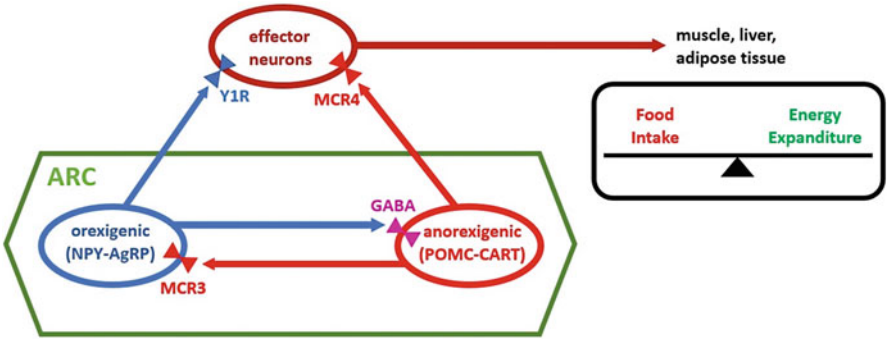


Fig. 1 Orexigenic and anorexigenic neurons of arcuate nucleus. *ARC* arcuate nucleus, *NPY* neuropeptide Y, *AgRP* agouti-related peptide, *POMC* proopiomelanocortin, *CART* cocaine- and amphetamine-regulated transcript, *Y1R* NPY receptor type 1, *MCR4* type 4 melanocortin receptor, *MCR3* type 3 melanocortin receptor, *GABA* gamma-aminobutyric acid.

metabolic nutrient signals due to their anatomical location. In the ARC, various populations of first-order neurons have been described (Fig. 1); neuropeptide Y (NPY) and agouti-related peptide (AgRP) that coexpress neurons stimulating food intake, and proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) that co-express neurons suppressing hunger (Rui 2013).

The orexigenic effect of AgRP/NPY neurons is explained through multiple mechanisms. In rats, the intracerebroventricular infusion of AgRP or NPY leads to an increase in food intake and delay of satiety (Stanley et al. 1985). AgRP released in the synaptic space acts on second-order neurons, and counteracts the anorexigenic effect of the α-melanocyte-stimulating hormone (α-MSH). In fact, AgRP is a potent and selective antagonist of type 3 (MC3R) and type 4 melanocortin receptor (MC4R) (Ollmann et al. 1997). In close analogy with AgRP, NPY also contributes to the orexigenic stimuli, acting on two (Y2 and Y5) out of the six specific receptors Y1–Y6 (Lin et al. 2005; Mashiko et al. 2009). AgRP/NPY neurons also release gamma-aminobutyric acid (GABA) and inhibit adjacent anorectic POMC neurons. The GABA-mediated AgRP/NPY action increases significantly in response to fasting, and stimulates food intake (Cowley et al. 2001).

AgRP/NPY neurons are stimulated by ghrelin, and inhibited by amylin, insulin, leptin, and serotonin (5-HT) (Harrold et al. 2012). They can also interact with the corticolimbic reward system, increasing the motivation to eat (Krashes et al. 2016).

POMC/CART neurons produce the large protein POMC, from whose cleavage many peptides are generated including α-MSH. This latter peptide binds to specific receptors (MC3R and MC4R) located on the second-order hypothalamic neurons, in particular at the level of PVN, resulting in a reduction in food intake and an increase in energy expenditure (Mountjoy 2015; Krashes et al. 2016). MC4R knock-out mice are characterized by hyperphagia and obesity (Huszar et al. 1997), and the stimulation of MC4R reduces food intake (Benoit et al. 2000). In humans, MC4R mutations cause severe hyperphagia and early-onset obesity (Krashes et al. 2016). In contrast,

MC3R null mice develop obesity even when food intake is normal, indicating its role in fat metabolism and energy expenditure (Chen et al. 2000). POMC neurons also project to the NTS, promoting satiety (Zheng et al. 2010) and to the VMN, where α-MSH stimulates the expression of anorexigenic brain-derived neurotrophic factor (BDNF) (Xu et al. 2003).

The POMC/CART neurons are connected with dopaminergic neurons in the nucleus accumbens (NAc), promoting the interaction between the homeostatic and hedonic systems to control food intake (Krashes et al. 2016). The release of α-MSH from POMC neurons is regulated by adiposity and nutrient signals. Leptin stimulates the expression of both POMC and CART (Kristensen et al. 1998), whereas glucose stimulates the α-MSH release (Parton et al. 2007). In addition, a subpopulation of POMC neurons (distinct from POMC neurons expressing leptin receptors) expresses the 5-hydroxy tryptamine subtype 2c receptor (5-HT2C), which binds the serotonin released from the raphe nuclei and increases α-MSH release (Heisler et al. 2002). In fact, some studies have shown that 5-HT2CR-deficient mice are hyperphagic and obese (Nonogaki et al. 1998).

POMC/CART neurons also produce CART, a peptide largely represented within the hypothalamus and NAc (Lau and Herzog 2014). The physiological role of CART in energy homeostasis is largely unknown, but may relate to a variety of mechanisms impacting on food intake. In rats, the blockade of CART action by the central infusion of anti-CART antibodies increases food intake (Lau and Herzog 2014), whereas CART injection in the NAc suppresses food intake (Yang and Shieh 2005). In addition, CART seems to exert an effect on the endocannabinoid and dopaminergic systems, suggesting a role in the hedonic regulatory circuit (Lage et al. 2015). Finally, CART appears to have a role in energy expenditure. In rats, CART injection into the ARC or PVN causes an upregulation of the uncoupling protein-1 transcription in brown adipose tissue, increasing energy dissipation (Wang et al. 2000; Kong et al. 2003).

Paraventricular Nucleus

PVN is an important integration center of hunger regulatory signals from ARC, NTS, and several other brain areas. It has long been known that PVN lesions cause hyperphagia and reduced energy expenditure (Shor-Posner et al. 1986). These observations indicate that the PVN exerts a catabolic effect through oxytocin, thyrotropin-releasing hormone (TRH), and corticotropin-releasing hormone (CRH) secretion (Rui 2013).

Oxytocin is produced in the PVN and also in the supraoptic nucleus. It is involved in many biological functions, such as stress regulation, analgesia, and hunger control (Onaka et al. 2012). Oxytocin increases the NTS sensitivity to satiety signals (Blevins et al. 2004) and reduces the AgRP neuronal action, causing hunger suppression. Indeed, in rodents, the central administration of oxytocin inhibits food intake (Onaka et al. 2012) and the block of oxytocin release causes hyperphagia (Zhang et al. 2011). Oxytocin levels are controlled by numerous mechanisms, including satiety signals arising from the gastrointestinal system resulting in an increase in oxytocin release (Onaka et al. 2012).

TRH induces an increase in the basal metabolic rate through the TSH-thyroid axis activation. TRH also stimulates GABAergic neurons, which in turn act on orexygenic LHA neurons, leading to hunger reduction (Zhang and van den Pol 2012).

Exactly how CRH neurons regulate hunger is still largely unknown. The CRH-ACTH-glucocorticoid system is involved in stress regulation. Generally, acute and/or intense stress leads to a reduction in hunger, while chronic stress tends to increase the intake of food in rodents, monkeys, and humans (Ulrich-Lai et al. 2007; Willner et al. 1996; Bartolomucci et al. 2009; Michopoulos et al. 2012; Rutters et al. 2009; Vicennati et al. 2011). Glucocorticoids also seem to be involved in food choices. Numerous studies in animals and humans have shown that highly palatable foods are favored in stressful times (George et al. 2010). Glucocorticoid effects are mediated by the activation of AgRP neurons and the inhibition of POMC neurons (Gyengesi et al. 2010). PVN also contains a distinct neuron population (parvocellular neurons) expressing MC3Rs and MC4Rs. These neurons project to the NTS glutaminergic neurons. Therefore, α-MSH alters vagal satiety inputs from the PVN neurons (Singru et al. 2012).

Dorsomedial Nucleus

The DMN contains abundant orexigenic NPY neurons projecting to the PVN and LHA. NPY administration into the DMN increases food intake (Bi et al. 2012), and NPY overexpression in this area leads to body weight gain in rats (Yang et al. 2009). In addition, when dietary intake is increased, an enhanced expression of NPY in the DMN occurs (Bi et al. 2003; Lee et al. 2013).

DMN perceives signals arising from the gastrointestinal system. In fact, DMN neurons express cholecystokinin 1 receptors, and the intracerebral injection of cholecystokinin decreases food intake (Bi et al. 2004).

Ventromedial Nucleus

The VMN receives neuronal projections from the ARC and, in turn, projects to the glutamatergic axons to the POMC neurons located in the ARC, to the LHA and to the brainstem. VMN neurons also produce the anorectic factor BDNF, which acts at the PVN level, increasing the production of CRH and MC4R expression (Toriya et al. 2010; Jeanneteau et al. 2012). VMN is a crucial area to maintain glucose homeostasis within the physiological ranges because VMN neurons act as sensors of glucose and leptin (Shimizu et al. 1987).

Lateral Hypothalamic Area

LHA neurons receive projections from NPY/AgRP and α-MSH neurons of the ARC. In addition, large populations of LHA neurons project to the mesolimbic area, connecting the homeostatic and hedonic systems of hunger regulation. LHA neurons mainly produce two neuropepetides; the melanin-concentrating hormone (MCH) and orexin (hypocretin).

MCH is an important endogenous orexigenic neurotransmitter; indeed, MCH overexpression is linked to obesity (Ludwig et al. 2001). Conversely, MCH or MCH-R-deficient transgenic mice are lean (Marsh et al. 2002).

Orexin is present in two isoforms (A and B) that act in several brain areas, stimulating food intake (Parise et al. 2011). In fact, orexin receptors (OX_1R and OX_2R) are widely expressed in the brain and their activation results in an enhanced sense of hunger (Sakurai et al. 1998). In fact, LHA damage leads to weight loss and hypophagia. Orexin neurons are also involved in numerous biological functions, such as the control of blood pressure, body temperature, and sleep-wake cycles (Plazzi et al. 2011). In humans, hypocretin system impairment causes narcolepsy, a chronic sleep disorder characterized by cataplexy and excessive daytime sleepiness (Plazzi et al. 2011). In this disease, orexin signaling is weak, and obesity often occurs. Orexin neuronal activity is stimulated by the MCH neurons of the LHA (Tsujino and Sakurai 2009) and by low glucose blood concentrations (Burdakov et al. 2013).

Hedonic Control of Food Intake

The homeostatic control of hunger is heavily influenced by hedonic signals involving cognitive and emotional aspects. These neuronal mechanisms are complex and still largely unknown. The insula and the orbitofrontal cortex are the brain areas that play a major role in the emotional experience of pleasure (also known as "liking"). On the other hand, subcortical limbic structures such as the amygdala, ventrotegmental area (VTA), and NAc are involved in the motivational processes in relation to food ("wanting") (Nicola 2016). These dopaminergic neuronal circuits are excited by glutamate and inhibited by GABAergic fibers (Mameli-Engvall et al. 2006). Numerous studies support the idea that dopamine circuits are heavily involved in the control of food intake and in playing a major role in obesity development in humans and rodents (Volkow et al. 2011). To confirm this, the body mass index (BMI) has been shown to correlate inversely with the levels of dopamine D2 receptors (Haltia et al. 2007). In addition, the selective inactivation of tyrosine hydroxylase (causing dopamine reduction) induces fatal hypophagia in mice, and the central administration of dopamine abolishes this effect (Szczypka et al. 2001). In addition, some studies have shown a different activation of dopamine circuits following food intake between obese and lean subjects. One study using positron emission tomography (PET) suggested that the orbitofrontal activation is lower in obese individuals after meals (Del Parigi et al. 2002). One study using functional resonance magnetic imaging (fMRI) also shows that obese subjects had a greater activation of dopaminergic circuits in response to fat rich food and food smells (Bragulat et al. 2010).

The mesolimbic circuits are also modulated by peripheral hormones. In particular, leptin and insulin act on the dopaminergic neurons in the VTA to suppress feeding (Hommel et al. 2006). In contrast, ghrelin activates the corticolimbic reward system in humans and increases hedonic food-related cues (Malik et al. 2008).

The opioid and endocannabinoid systems are another important mechanism involved in hunger control. In the 1970s, much epidemiological evidence showed that exogenous cannabinoids (such as marijuana) influence many biological functions including food intake (Johansson et al. 1975). Later, the main endogenous

cannabinoids were found to be 2-arachidonylglycerol (2-AG) and anandamide (AEA), which are produced within the brain by the action of phospholipase D on N-arachidonoyl phosphatidylethanolamine (Devane et al. 1992). Exogenous and endogenous cannabinoids bind two specific receptors: cannabinoid receptor 1 (CB1), which is mainly expressed in the hypothalamus, cortex, and thalamus; and cannabinoid receptor 2 (CB2), which is expressed in immune cells. The effect of cannabinoids on food regulation is therefore mediated by CB1 receptors. In fact, the administration of endocannabinoids into the VMN of satiated rats causes hyperphagia (Jamshidi and Taylor 2001). In addition, anandamide injection increases NPY secretion (Gamber et al. 2005) and promotes "liking" reactions to sucrose (Mahler et al. 2007). In contrast, the selective CB1 antagonist, rimonabant, reduces body weight and food intake. It selectively inhibits the consumption of palatable food, alcohol and sweets (Cota et al. 2006; Arnone et al. 1997), thereby reducing NAc dopamine release (Melis et al. 2007).

Rimonabant has also been proposed as an anti-obesity drug in humans due to its positive results on body weight and the metabolic set. However, in 2008, rimonabant was withdrawn from the market due to the increased risk of depression and suicide detected in the obese population treated with this drug (Jones 2008). The endocannabinoid system is also affected by many signals involved in the homeostatic control of hunger (Wenger et al. 1997; Verty et al. 2009). Fasting causes increase in endocannabinoids in the NAc and the hypothalamus, and their levels decrease after refeeding (Kirkham et al. 2002). A similar increase has also been described under the influence of leptin (Di Marzo et al. 2001) and ghrelin (Kola et al. 2008).

Numerous evidences suggest that the endogenous opioid system is implicated in food-related sensory pleasure (Yeomans and Gray 2002). Palatable food consumption stimulates the release of beta-endorphins into the hypothalamus (Dum et al. 1983) and the administration of opioid antagonists reduces the highly palatable food intake (Cooper 1983). In humans, the administration of opioid antagonists, such as naloxone or naltrexone, causes a substantial reduction in the consumption of sweet foods in obese patients or subjects with binge-eating behavior (Drewnowski et al. 1992; Yeomans and Gray 1996). The impact of opioids on food intake is more significant in terms of feeding maintenance rather than the initiation of feeding itself (Rudski et al. 1994). This suggests that opioids act more on "liking" than "wanting" (Barbano and Cador 2007). The opioid mechanism of action is still partly unknown; however, it seems that POMC neurons are involved in the ARC, thereby producing an autoinhibitory circuit through the production of beta-endorphin, resulting from POMC cleavage (Greenway et al. 2009).

The Peripheral Hormones in the Control of Food Intake

The brain's regulation of food intake is influenced by many gut hormones. Some of these are secreted in relation to the food and have an episodic secretion pattern, while other hormones reflect the general nutritional status of the body (Halford and Blundell 2000).

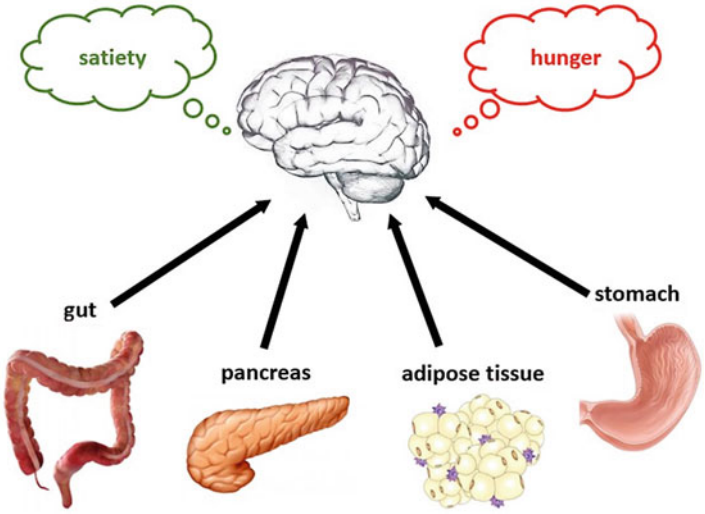


Fig. 2 Peripheral signals of food intake control

The smell and/or the appearance of the food lead to important organic changes in preparation of the food intake (McCrickerd and Forde 2016). Subsequently, the contact of the food with the oral mucosa is transmitted to the brainstem through the cranial nerves. Such perceptual signals (taste, smell) are modified by hormonal messages such as glucagon-like peptide-1 (GLP-1), insulin, and leptin (Soria-Gomez et al. 2014). In fact, GLP-1 stimulates the intake of sweet foods, reducing the intake of salty food (Martin et al. 2009). In addition, leptin reduces the perception of a sweet taste by acting on tongue cells (Shigemura et al. 2004), and modulates the food smell by changing the mucus production in olfactory cells (Badonnel et al. 2009). When food arrives in the stomach, gastric distension leads to the secretion of several hormones, which act in the brain by changing the sensation of hunger/satiety (Lean and Malkova 2016). In addition, these hormones act on the intestine with feedback mechanisms, resulting in delayed gastric emptying, and therefore increasing satiety signals (Fig. 2).

Gut Signals

GLP-1

GLP-1 is derived from the cleavage of its precursor pre-proglucagon, which is subjected to various tissue-specific cleavages. In the gut and the brain, pre-proglucagon is transformed into GLP-1, GLP-2, and oxyntomodulin (OXM) (Pocai 2012). In contrast, in the pancreas, pre-proglucagon undergoes the action of a different enzyme, thus stimulating the production of glucagon, while GLP-1 and GLP-2 are not separated, constituting a single inactive peptide (Cho et al. 2014).

GLP-1 has two biologically active forms: GLP-17-37 and GLP-17–36. Both show a short plasma half-life (1–2 min), in part due to the renal clearance, in part due to rapid degradation by dipeptidyl-peptidase IV (DPP-IV) (Cho et al. 2014).

In the intestinal cells, GLP-1 is co-secreted with peptide YY (PYY) in response to food ingestion, and particularly sugars and lipids. GLP-1 circulating levels are therefore low in fasting, whereas they increase in postprandial time and in anticipation of meals (Vahl et al. 2010).

GLP-1 acts through the activation of a G-protein-coupled receptor (GLP-1R), which is widely distributed in the gut, pancreas, ganglion nodosum, and in the brain, particularly in the hypothalamus, striatum, brainstem, substantia nigra, and amygdala (Holst 2007).

GLP-1, along with glucose-dependent insulinotropic polypeptide (GIP), is responsible for the "incretin effect," a mechanism through which insulin secretion is increased in response to oral but not to intravenous glucose administration (Campbell and Drucker 2013). In addition, GLP-1 inhibits glucagon secretion and reduces the gastric emptying rate, delaying the intestinal nutrient absorption (Deane et al. 2010; Campbell and Drucker 2013). This phenomenon is called the "ileal brake" which is a negative feedback mechanism that inhibits the motility of the upper gastrointestinal tract, when there are unabsorbed dietary components in the colon (Spreckley and Murphy 2015). The gastric emptying inhibition induced by GLP-1 explains the anorectic effect of this hormone, partly due to the activation of vagal afferents (Plamboeck et al. 2013), and also determined by the direct action of GLP-1 in the brain (De Silva et al. 2011; Sisley et al. 2014).

Finally, some studies using fMRI in obese and obese diabetic patients have shown that the intravenous infusion of GLP-1 agonists reduces the activation of brain regions involved in food reward mediation (van Bloemendaal et al. 2014). This thus supports the belief that GLP-1 has a role in the hedonistic control of food (Heppner and Perez-Tilve 2015).

GLP-2

GLP-2 is a 33 amino-acid peptide co-secreted with GLP-1 by L cells in response to a meal. Similar to GLP-1, it is rapidly degraded by DPP-IV and reduces gastric motility. In addition, GLP-2 reduces gastric acid secretion and induces crypt cell proliferation, improving the absorption of nutrients in the gut (Spreckley and Murphy 2015).

PYY

PYY is released from the gut L-cells in response to nutrients, especially protein-rich food (Batterham et al. 2006).

There are two forms of PYY: PPY1-36 and PYY3-36; the latter is the product of PYY1-36 degradation by DPP-IV.

PYY causes a decrease in gastric emptying, and consequently a reduction in food intake (Batterham et al. 2006). In addition, PYY appears to reduce acid secretion, and inhibits gallbladder contraction and exocrine pancreatic secretions (Suzuki et al. 2012). The anorectic effect of PYY appears to be in part mediated by the vagus

(Broberger et al. 1997; Abbott et al. 2005b), and also directly, through PYY action on the ARC. Finally, PYY also seems to act on the areas involved in the hedonistic control of food (Batterham et al. 2007).

OXM

OXM is produced by the intestinal L-cells and is rapidly degraded by DPP-IV. It is a dual agonist of GLP-1 and glucagon receptors, but with 10 to 100 times lower affinity than the native ligand. Therefore, as with GLP-1, OXM appears to decrease gastric acid secretion, delay gastric emptying and reduce food intake, causing weight loss in rodents (Dakin et al. 2001) and humans (Wynne et al. 2005). OXM also seems to increase energy expenditure, miming the glucagon effect on its receptor.

Cholecystokinin (CCK)

CCK is a 27 amino acid peptide secreted by the duodenum and jejunum in response to fat and protein. Its plasma levels increase within 15 min after a meal, and remain high for 3–5 h (Murphy and Bloom 2006).

CCK acts by binding to CCK-1 and CCK-2 receptors, otherwise distributed in peripheral and CNS (Moran et al. 1986).

CCK actions include an increase in pancreas enzymes, intestinal motility facilitation, gallbladder contraction stimulation, and a reduction in gastric acid secretion (Moran and Kinzig 2004). Numerous studies have also shown that the CCK infusion reduces the amount of food consumed by rodents, monkeys, and humans (Gibbs et al. 1973; Hirosue et al. 1993; Figlewicz et al. 1992; Kissileff et al. 1981). This anorectic effect seems to be mediated mainly, but not only, by CCK1 receptors on the vagus nerve (Beglinger et al. 2001). In fact, bilateral vagotomy reduces, but not suppresses, the anorectic action of CCK (Zhang and Ritter 2012). CCK-1 receptors are also found in the hypothalamus (Blevins et al. 2000), thus explaining the residual action of CCK after bilateral vagotomy.

Ghrelin

Ghrelin is an octanoylated peptide showing an orexigenic property (Tschöp et al. 2000). Indeed, the central or peripheral infusion of ghrelin stimulates food intake in rodents and humans (Wren et al. 2000; Wren et al. 2001). In addition, serum levels of ghrelin have been shown to decrease after meals, whereas they increase in response to fasting (Cummings et al. 2001).

Ghrelin is mainly secreted by the gastric oxyntic gland cells, but also by the intestine, pancreas, colon, and brain (Müller et al. 2015). Ghrelin is also expressed within the ARC and PVN, and thus may also act as a neurotransmitter (Cowley et al. 2003).

The action of ghrelin is mediated partly by the vagus (Page et al. 2007), and also directly on the central nervous system through the stimulation of NPY/AgRP neurons in the ARC (Wang et al. 2002). In addition, much recent evidence suggests that ghrelin also acts on the dopaminergic mesolimbic circuit, increasing the motivation for highly palatable food (Dickson et al. 2011). In fact, circulating ghrelin levels are negatively correlated with BMI (Lean and Malkova 2016), demonstrating

a strong correlation between obesity and alterations in the ghrelin secretory pattern. In addition, obese patients have shown lower ghrelin suppression after meals compared to lean subjects (English et al. 2002).

Adipocyte and Pancreas Signals

Unlike the gut hormones which provide information regarding the acute food intake, the pancreas and adipose tissue provide information on the chronic nutritional status. The circulating levels of leptin and insulin, therefore, show a positive correlation with the fat mass in the body (Belgardt and Brüning 2010).

Leptin

Leptin is produced by white adipose tissue as a signal that quantifies the body energy deposits. In fact, plasma leptin concentrations correlate with total body fat. Therefore, although obese subjects have high levels of leptin, these levels are not very efficient because of the leptin resistance, in particular in the brain (Caro et al. 1996). The mechanisms of this leptin resistance are not completely understood; however, several theories have been proposed. The leptin crosses the BBB through a saturable transport system; thus, leptin resistance may result from the saturation of these transporters (Wauman and Tavernier 2011). Moreover, the high levels of intrahypothalamic proinflammatory cytokines in obese subjects can affect the leptin transport, thus reducing its efficacy (de Git and Adan 2015).

Leptin actions are mediated by three receptors: the long form (OB-Rb), the secreted form (OB-Rc), and the short intracellular domain receptor (OB-Ra). The Ob-Rb receptor is highly expressed in the hypothalamus (Fei et al. 1997) and is heavily involved in appetite control. In fact, rodents and humans with mutations in the leptin gene or in this leptin receptor show severe early-onset obesity (Farooqi and O'Rahilly 2008).

Leptin induces an increase in POMC/CART and a decrease in NPY/AgRP neuronal activity leading to a reduced food intake and an increased energy expenditure (Schwartz et al. 2000; Sahu 2003). Leptin is also able to amplify the satiating effect of CCK (Peters et al. 2006). Finally, leptin influences eating behavior through the regulation of the mesolimbic and nigrostriatal dopamine pathways (Farooqi et al. 2007) and by activating the hippocampus, a brain area that controls learning and memory (Kanoski et al. 2011).

Insulin

Insulin is synthesized in pancreatic beta cells and secreted rapidly after a meal. It acts not only on the blood glycemic control, but also as an anorexigenic hormone in the brain. Insulin enters the central nervous system through a saturable receptor-mediated transporter (Baura et al. 1993). In the brain, insulin receptors are expressed in the hypothalamic nuclei such as PVN, ARC, and DMN, thus mediating the insulin anorectic effect (Corp et al. 1986). In addition, insulin is able to modify the mesolimbic centers involved in hedonic food control. Indeed, insulin directly

suppresses dopamine neurons in VTA, thus reducing the reinforcement systems of palatable foods (Labouebe et al. 2013).

The Regulation of Energy Expenditure

Body weight is maintained through a balance between food intake and energy expenditure.

Energy expenditure is mainly composed of three mechanisms: physical activity, basal metabolic rate, and adaptive thermogenesis. All these forms of energy dissipation are also controlled by the hypothalamus, thus the brain oversees the energy expenditure as well as the energy intake.

The hypothalamus controls basal metabolism mainly through the hypothalamic-pituitary-thyroid axis and the hypothalamic-pituitary-adrenal axis (Cerri and Morrison 2006). However, there are numerous other mechanisms that act on the energy expenditure and on the thermogenesis.

The brown adipocytes contain abundant mitochondria with the uncoupling protein-1 (UCP-1), a mitochondrial proton channel that uncouples respiration from the oxidative phosphorylation, thus producing heat. The activities of these mitochondria are modulated by sympathetic projections that act through beta adrenergic receptors. In fact, mice lacking all three types of beta adrenergic receptors show cold intolerance and obesity (Bachman et al. 2002). Sympathetic projections involved in the brown adipose tissue regulation are controlled by neurons located in the rostral raphe pallidus (rRPa) (Bamshad et al. 1999). The rRPa neurons receive both excitatory (glutamate) and inhibitory (GABA) signals from the DMN and the preoptic area, respectively (Cao and Morrison 2006). The rRPa also receives direct inputs from the NTS and from orexin neurons in the LHA (Kong et al. 2012; Tupone et al. 2011). In fact, during fetal life, the placenta production of orexin is indispensable for the correct neoformation of brown adipose tissue (Sellayah et al. 2011). In addition, the intracerebral injection of orexin or the stimulation of orexin-producing neurons activate the brown adipose tissue and an increase in energy expenditure (Tupone et al. 2011). PVN is also involved in the regulation of energy expenditure. It has been suggested that GABAergic PVN neurons do not directly innervate the rRPa, but they act on rRPa through inhibitory NTS neurons, which in turn inhibit rRPa neurons (Kong et al. 2012).

Another very important center in the regulation of energy expenditure is the ARC. In this brain area, there are also GABAergic neurons, which are distinct from AgRP and POMC neurons. GABAergic neurons are characterized by the expression of the rat insulin-2 promoter (Rip neurons). These neurons lead to an increase in energy expenditure, whereas the deletion of GABA signaling from Rip neurons in the ARC suppresses thermogenesis (Kong et al. 2012). AgRP/NPY neurons are also involved in thermogenesis and energy expenditure through the GABAergic inhibitory input (Cao et al. 2010). In fact, rats knockdown in the NPY of the DMN leads to a greater development of brown adipose tissue and increased expression of UCP-1. This is associated with cold tolerance and increased glucose metabolism and an improvement in insulin sensitivity

(Chao et al. 2011). POMC neurons also act on thermogenesis through the MC4R receptor. In mice, the loss of POMC neurons results in a reduction in energy expenditure (Claret et al. 2007).

Another thermogenesis regulation mechanism involves the endocannabinoid system. CB1 receptors lack mice showed decreased body weight, high β (3) adrenergic receptor, and UCP-1 mRNA levels in brown adipose tissue, without changes in the diet. These findings suggest that the CB1 receptor is a determinant key of energy expenditure at baseline (Cardinal et al. 2012).

In addition, the intracerebral administration of GLP-1 leads to an increase in UCP-1 expression in brown adipose tissue and a reduction in body weight, highlighting the involvement of GLP-1 in the control of energy expenditure (Lockie et al. 2012). Insulin and leptin also increase energy expenditure. Leptin acts on thermogenesis through multiple sites in both the hypothalamus and brainstem (Enriori et al. 2011; Rezai-Zadeh and Münzberg 2013).

In the last decade, the receptors through which leptin exerts its actions (LepRb) have been identified. These receptors have been detected in the preoptic area and dorsomedial hypothalamus, but not in the rRPa. LepRb-expressing DMN neurons were found to directly innervate rRPa neurons, stimulating thermogenesis (Zhang et al. 2011). An increase in thermogenesis also occurs by leptin-mediated enhancing GABAergic inputs onto the PVN neurons (Kong et al. 2012). Leptin stimulates TRH secretion both directly and indirectly via the melanocortin system (Guo et al. 2004).

Obesity

A chronic imbalance between energy intake and energy expenditure causes important changes in body weight. When the food intake exceeds daily calorie expenditure, obesity occurs. Obesity is one of the major health problems of the twenty-first century. It is associated with numerous comorbidities (heart disease, stroke, type 2 diabetes, sleep apnea syndrome, osteoarthritis, as well as bowel and other organ cancers), resulting in a significant increase in mortality (Flegal et al. 2005). Furthermore, all of these comorbidities therapies have a high cost for the health system.

The onset of obesity is determined by several factors, such as an increase in food intake, reduced physical activity, and the susceptibility determined by genetic factors. In the last few decades, there have been many changes in eating habits. There has been a particularly dramatic increase in the consumption of processed foods, which are high in fats and therefore rich in calories. In addition, palatable foods activate the reinforcement circuits causing overconsumption.

Altered Food Reward Processing

Overeating in obese subjects has some similarities with the compulsive and loss of control observed in drug-addicted people. A study using PET showed a reduction in striatal D2 receptors in obese patients which is similar to that presented by drug

addicts (Wang et al. 2004). In obese individuals, hyperactivity of the subcortical reward circuitry occurs (e.g., the striatum, hypothalamus, amygdala, hippocampus) in response to food signals. Furthermore, there is hypoactivity in the inhibitory cortical regions which are responsible for the voluntary control of the decision-making process (Holsen et al. 2012; Stice et al. 2008). These alterations cause hyperphagia and therefore weight gain.

Some obese subjects also show a higher activation of the amygdala, the anterior cingulate cortex, and orbitofrontal cortex in response to palatable foods (Gearhardt et al. 2012). These mechanisms are even more pronounced in people with binge eating disorder (BED), which is characterized by binge eating episodes, usually including high fat foods, without following purification. A study using PET shows that in binge eaters, the food stimuli significantly increase dopamine in the caudate and putamen, which does not happen in non-binge eaters. This dopamine increase is significantly correlated with the binge eating scores, but not with the BMI (Wang et al. 2011).

Another example of an important alteration in the feeding reinforcing mechanisms is the Prader Willi syndrome (PWS). This disorder is determined by the microdeletion or maternal uniparental disomy of chromosome 15, and is characterized by hypotonia at birth, mental retardation, and abnormalities of the hypothalamus which cause hypogonadotropic hypogonadism, short stature, temperature dysregulation, and obesity. In PWS, there are abnormal and obsessive behaviors, also toward foods. These patients are therefore characterized by violent hyperphagia (von Deneen et al. 2009). PWS subjects also have delayed meal termination and a shorter period of fullness after a meal than control groups (Holland et al. 1993).

A study using fMRI showed that PWS patients have a greater activity in the prefrontal cortex compared with lean controls when they are shown pictures of food. This type of response is in line with greater levels of food intake satisfaction, which involves a strong emotional food response which may be one of the causes of excessive hunger in PWS (Miller et al. 2007). The modifications in the prefrontal cortex are also supported by other studies, some of which have used PET instead of fMRI (Hinton et al. 2006).

As with PWS, individuals undergoing surgery involving the diencephalic and hypothalamic areas also show significant modifications of the controlling hunger circuits. In fact, patients operated for craniopharyngioma develop hyperphagia, which causes severe obesity (Page-Wilson et al. 2012). A recent study using fMRI shows that patients with craniopharyngioma have an altered perception of satiety. The activity of the NAc, insula, and medial orbitofrontal cortex was evaluated before and after a meal in four patients versus an age- and weight-matched control. The subjects with craniopharyngioma did not show the normal adaptive response, which consist in a reduction in activation of these specific brain areas by visual food cues after a meal (Roth et al. 2012).

Aberrant Energy Expenditure Mechanism

Energy expenditure (EE) consists of several components: resting energy expenditure (REE), activity energy expenditure (AEE), and diet-induced thermogenesis (DIT).

Some studies show that obese individuals have higher absolute EE and REE. However, when the body composition is taken into account (considering the lean mass percentage), these differences between obese and nonobese individuals disappear (Carneiro et al. 2016). This is not true for the AEE component; in fact, obese sedentary individuals show a reduction in AEE. In fact, physical exercise increases the transcription of muscle proteins, including irisin. This molecule promotes the transformation of the white adipose tissue into beige, increasing the transcription of UCP-1. Irisin shows increased levels in obese subjects, suggesting that weight gain and physical inactivity lead to irisin-resistance and therefore less effective AEE (Aydin 2014).

The alteration in energy expenditure in the development of obesity is particularly important in patients undergoing surgical procedures involving the hypothalamus area. Numerous studies have shown a reduction in the sympathetic system and hyperactivation of the vagal system after diencephalic area surgery (Holmer et al. 2010). These changes result in a reduction in REE, which favors the onset of severe obesity.

Modification of Adipose Tissue

Obesity leads to adipocyte hypertrophy. This modification of the adipocyte cells causes many metabolic changes, which result in insulin resistance (Klöting et al. 2010). In obese adipose tissue, the vascularization deficit occurs and causes hypoxia. In hypoxic conditions, the cells produce an up-regulation of the proinflammatory genes (Hosogai et al. 2007). In addition, hypoxia can determine fat necrosis which in turn favors the phlogistic state (Strissel et al. 2007). Moreover, hypertrophic adipocytes exhibit increased expression of proinflammatory cytokines, as tumor necrosis factor, monocyte chemoattractant protein-1, interleukin 6 and 8 (Jernås et al. 2006). This inflammation state causes phosphorylation of the insulin receptor, with the development of insulin resistance (Yuan et al. 2001).

Furthermore, inflamed adipose tissue increases the production of free fatty acids (FFAs) that accumulate specifically on the muscles and liver inducing lipotoxicity (Rutkowski et al. 2015). Some FFAs, such as palmitic and stearic acid, act directly on the innate immune cells, activating them and exacerbating inflammatory status (Shi et al. 2006).

Conclusion

Since the 1990s, there has been an increasing interest in the literature in understanding the mechanisms that regulate hunger and that are responsible for the development of obesity. Today, it is evident that the control of eating behavior involves complex mechanisms, not only including biological homeostasis, but also hedonistic mechanisms. The extensive use of highly palatable foods that trigger reinforcing mechanisms, overlooking the real biological needs, and the physical inactivity caused by lifestyle changes in the industrialized era, are mainly responsible for the

pandemic of obesity. Obesity and its severe comorbidities (cardiovascular disease, respiratory illness, musculoskeletal pathologies, and cancer) are the greatest causes of mortality and public health spending in the modern era. Continued research is therefore needed to further highlight the mechanisms involved in the regulation of hunger in order to help generate new targets to treat obesity.

References

Abbott CR, Monteiro M, Small CJ, et al. The inhibitory effects of peripheral administration of peptide YY 3-36 and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. Brain Res. 2005a;1044:127–31.

Abbott CR, Small CJ, Kennedy AR, et al. Blockade of the neuropeptide Y Y2 receptor with the specific antagonist BIIE0246 attenuates the effect of endogenous and exogenous peptide YY(3-36) on food intake. Brain Res. 2005b;1043:139–44.

Arnone M, Maruani J, Chaperon F, et al. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. Psychopharmacology. 1997;132(1):104–6.

Aydin S. Three new players in energy regulation: preptin, adropin and irisin. Peptides. 2014;56:94–110.

Bachman ES, Dhillon H, Zhang CY, et al. betaAR signaling required for diet-induced thermogenesis and obesity resistance. Science. 2002;297(5582):843–5.

Badonnel K, Durieux D, Monnerie R, et al. Leptin-sensitive OBP-expressing mucous cells in rat olfactory epithelium: a novel target for olfaction-nutrition crosstalk? Cell Tissue Res. 2009;338:53–66.

Bamshad M, Song CK, Bartness TJ. CNS origins of the sympathetic nervous system outflow to brown adipose tissue. Am J Phys. 1999;276(6 Pt 2):R1569–78.

Barbano MF, Cador M. Opioids for hedonic experience and dopamine to get ready for it. Psychopharmacology. 2007;191(3):497–506.

Bartolomucci A, Cabassi A, Govoni P, et al. Metabolic consequences and vulnerability to diet-induced obesity in male mice under chronic social stress. PLoS One. 2009;4:e4331.

Batterham RL, Heffron H, Kapoor S, et al. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metab. 2006;4:223–33.

Batterham RL, Ffytche DH, Rosenthal JM, et al. PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. Nature. 2007;450:106–9.

Baura GD, Foster DM, Porte D Jr, et al. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. J Clin Invest. 1993;92:1824–30.

Beglinger C, Degen L, Matzinger D, et al. Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger feelings in humans. Am J Physiol Regul Integr Comp Physiol. 2001;280:R1149–54.

Belgardt BF, Brüning JC. CNS leptin and insulin action in the control of energy homeostasis. Ann N Y Acad Sci. 2010;1212:97–113.

Benoit SC, Schwartz MW, Lachey JL, et al. A novel selective melanocortin-4 receptor agonist reduces food intake in rats and mice without producing aversive consequences. J Neurosci. 2000;20(9):3442–8.

Bi S, Robinson BM, Moran TH. Acute food deprivation and chronic food restriction differentially affect hypothalamic NPY mRNA expression. Am J Physiol Regul Integr Comp Physiol. 2003;285:R1030–6.

Bi S, Scott KA, Kopin AS, et al. Differential roles for cholecystokinin receptors in energy balance in rats and mice. Endocrinology. 2004;145:3873–80.

Bi S, Kim YJ, Zheng F. Dorsomedial hypothalamic NPY and energy balance control. Neuropeptides. 2012;46:309–14.
Blevins JE, Stanley BG, Reidelberger RD. Brain regions where cholecystokinin suppresses feeding in rats. Brain Res. 2000;860:1–10.
Blevins JE, Schwartz MW, Baskin DG. Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size. Am J Physiol Regul Integr Comp Physiol. 2004;287:R87–96.
Bragulat V, Dzemidzic M, Bruno C, et al. Food-related odor probes of brain reward circuits during hunger: a pilot fMRI study. Obesity (Silver Spring). 2010;18(8):1566–71.
Broberger C, Landry M, Wong H, et al. Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in proopiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. Neuroendocrinology. 1997;66:393–408.
Burdakov D, Karnani MM, Gonzalez A. Lateral hypothalamus as a sensor-regulator in respiratory and metabolic control. Physiol Behav. 2013;121:117–24.
Burdyga G, Spiller D, Morris R, et al. Expression of the leptin receptor in rat and human nodose ganglion neurons. Neuroscience. 2002;109:339–47.
Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab. 2013;17:819–37.
Cao WH, Morrison SF. Glutamate receptors in the raphe pallidus mediate brown adipose tissue thermogenesis evoked by activation of dorsomedial hypothalamic neurons. Neuropharmacology. 2006;51(3):426–37.
Cao WH, Madden CJ, Morrison SF. Inhibition of brown adipose tissue thermogenesis by neurons in the ventrolateral medulla and in the nucleus tractus solitarius. Am J Physiol Regul Integr Comp Physiol. 2010;299(1):R277–90.
Cardinal P, Bellocchio L, Clark S, et al. Hypothalamic CB1 cannabinoid receptors regulate energy balance in mice. Endocrinology. 2012;153(9):4136–43.
Carneiro IP, Elliott SA, Siervo M, et al. Is obesity associated with altered energy expenditure? Adv Nutr. 2016;7(3):476–87.
Caro JF, Kolaczynski JW, Nyce MR, et al. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. Lancet. 1996;348:159–61.
Cerri M, Morrison SF. Corticotropin releasing factor increases in brown adipose tissue thermogenesis and heart rate through dorsomedial hypothalamus and medullary raphe pallidus. Neuroscience. 2006;140(2):711–21.
Chao PT, Yang L, Aja S, et al. Knockdown of NPY expression in the dorsomedial hypothalamus promotes development of brown adipocytes and prevents diet-induced obesity. Cell Metab. 2011;13(5):573–83.
Chen AS, Marsh DJ, Trumbauer ME, et al. Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. Nat Genet. 2000;26:97–102.
Cho YM, Fujita Y, Kieffer TJ. Glucagon-like peptide-1: glucose homeostasis and beyond. Annu Rev Physiol. 2014;76:535–59.
Claret M, Smith MA, Batterham RL, et al. AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. J Clin Invest. 2007;117(8):2325–36.
Cooper SJ. Minireview. Benzodiazepine-opiate antagonist interactions in relation to feeding and drinking behavior. Life Sci. 1983;32(10):1043–51. Review
Corp ES, Woods SC, Porte D Jr, et al. Localization of 125I-insulin binding sites in the rat hypothalamus by quantitative autoradiography. Neurosci Lett. 1986;70(1):17–22.
Cota D, Tschoep MH, Horvath T, et al. Cannabinoids, opioids and eating behavior: the molecular face of hedonism? Brain Res Rev. 2006;51:85–107.
Cowley MA, Smart JL, Rubinstein M, et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature. 2001;411:480–4.
Cowley MA, Smith RG, Diano S, et al. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron. 2003;37:649–61.
Cummings DE, Purnell JQ, Frayo RS, et al. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes. 2001;50:1714–9.

Dakin CL, Gunn I, Small CJ, et al. Oxyntomodulin inhibits food intake in the rat. Endocrinology. 2001;142:4244–50.
de Git KC, Adan RA. Leptin resistance in diet-induced obesity: the role of hypothalamic inflammation. Obes Rev. 2015;16:207–24.
De Silva A, Salem V, Long CJ, et al. The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate brain activity in appetite centers in humans. Cell Metab. 2011;14:700–6.
Deane AM, Nguyen NQ, Stevens JE, et al. Endogenous glucagon-like peptide-1 slows gastric emptying in healthy subjects, attenuating postprandial glycemia. J Clin Endocrinol Metab. 2010;95:215–21.
Del Parigi A, Gautier JF, Chen K, et al. Neuroimaging and obesity: mapping the brain responses to hunger and satiation in humans using positron emission tomography. Ann N Y Acad Sci. 2002;967:389–97. Review
Devane WA, Hanus L, Breuer A, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992;258(5090):1946–9.
Di Marzo V, Goparaju SK, Wang L, et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature. 2001;410:822–5.
Dickson SL, Egecioglu E, Landgren S, et al. The role of the central ghrelin system in reward from food and chemical drugs. Mol Cell Endocrinol. 2011;340:80–7.
Drewnowski A, Krahn DD, Demitrack MA, et al. Taste responses and preferences for sweet high-fat foods: evidence for opioid involvement. Physiol Behav. 1992;51:371–9.
Dum J, Gramsch C, Herz A. Activation of hypothalamic beta-endorphin pools by reward induced by highly palatable food. Pharmacol Biochem Behav. 1983;18(3):443–7.
English PJ, Ghatei MA, Malik IA, et al. Food fails to suppress ghrelin levels in obese humans. J Clin Endocrinol Metab. 2002;87:2984.
Enriori PJ, Sinnayah P, Simonds SE, et al. Leptin action in the dorsomedial hypothalamus increases sympathetic tone to brown adipose tissue in spite of systemic leptin resistance. J Neurosci. 2011;31(34):12189–97.
Farooqi IS, O'Rahilly S. Mutations in ligands and receptors of the leptin-melanocortin pathway that lead to obesity. Nat Clin Pract Endocrinol Metab. 2008;4:569–77.
Farooqi IS, Bullmore E, Keogh J, et al. Leptin regulates striatal regions and human eating behavior. Science. 2007;317:1355.
Fei H, Okano HJ, Li C, et al. Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. Proc Natl Acad Sci USA. 1997;94:7001–5.
Figlewicz DP, Nadzan AM, Sipols AJ, et al. Intraventricular CCK-8 reduces single meal size in the baboon by interaction with type-A CCK receptors. Am J Phys. 1992;263:R863–7.
Flegal KM, Graubard BI, Williamson DF, et al. Excess deaths associated with underweight, overweight, and obesity. JAMA. 2005;293(15):1861–7.
Gamber KM, Macarthur H, Westfall TC. Cannabinoids augment the release of neuropeptide Y in the rat hypothalamus. Neuropharmacology. 2005;49(5):646–52.
Gearhardt AN, White MA, Masheb RM, et al. An examination of the food addiction construct in obese patients with binge eating disorder. Int J Eat Disord. 2012;45(5):657–63.
George S, Khan S, Briggs H, et al. CRH-stimulated cortisol release and food intake in healthy, non-obese adults. Psychoneuroendocrinology. 2010;35:607–12.
Gibbs J, Young RC, Smith GP. Cholecystokinin decreases food intake in rats. J Comp Physiol Psychol. 1973;84:488–95.
Greenway FL, Dunayevich E, Tollefson G, et al. Comparison of combined bupropion and naltrexone therapy for obesity with monotherapy and placebo. J Clin Endocrinol Metab. 2009;94(12):4898–906.
Guo F, Bakal K, Minokoshi Y, et al. Leptin signaling targets the thyrotropin-releasing hormone gene promoter in vivo. Endocrinology. 2004;145(5):2221–7. Epub 2004 Feb 5
Gyengesi E, Liu Z-W, D'Agostino G, et al. Corticosterone regulates synaptic input organization of POMC and NPY/AgRP neurons in adult mice. Endocrinology. 2010;151:5395–402.

Halford JC, Blundell JE. Separate systems for serotonin and leptin in appetite control. Ann Med. 2000;32:222–32.
Haltia LT, Rinne JO, Merisaari H, et al. Effects of intravenous glucose on dopaminergic function in the human brain in vivo. Synapse. 2007;61:748–56.
Harrold JA, Dovey TM, Blundell JE, et al. CNS regulation of appetite. Neuropharmacology. 2012;63:3–17.
Heisler LK, Cowley MA, Tecott LH, et al. Activation of central melanocortin pathways by fenfluramine. Science. 2002;297:609–11.
Heppner KM, Perez-Tilve D. GLP-1 based therapeutics: simultaneously combating T2DM and obesity. Front Neurosci. 2015;9:92.
Hinton EC, Holland AJ, Gellatly MS, et al. Neural representations of hunger and satiety in Prader-Willi syndrome. Int J Obes. 2006;30(2):313–21.
Hirosue Y, Inui A, Teranishi A, et al. Cholecystokinin octapeptide analogues suppress food intake via central CCK-A receptors in mice. Am J Phys. 1993;265:R481–6.
Holland AJ, Treasure J, Coskeran P, et al. Measurement of excessive appetite and metabolic changes in Prader-Willi syndrome. Int J Obes Relat Metab Disord. 1993;17(9):527–32.
Holmer H, Pozarek G, Wirfält E, et al. Reduced energy expenditure and impaired feeding-related signals but not high energy intake reinforces hypothalamic obesity in adults with childhood onset craniopharyngioma. J Clin Endocrinol Metab. 2010;95(12):5395–402.
Holsen LM, Lawson EA, Blum J, et al. Food motivation circuitry hypoactivation related to hedonic and nonhedonic aspects of hunger and satiety in women with active anorexia nervosa and weight-restored women with anorexia nervosa. J Psychiatry Neurosci. 2012;37(5):322–32.
Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev. 2007;87:1409–39.
Hommel JD, Trinko R, Sears RM, et al. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron. 2006;51:801–10.
Hosogai N, Fukuhara A, Oshima K, et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes. 2007;56(4):901–11.
Huszar D, Lynch CA, Fairchild-Huntress V, et al. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell. 1997;88:131–41.
Jamshidi N, Taylor DA. Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. Br J Pharmacol. 2001;134:1151–4.
Jeanneteau FD, Lambert WM, Ismaili N, et al. BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. Proc Natl Acad Sci USA. 2012;109:1305–10.
Jernås M, Palming J, Sjöholm K, et al. Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. FASEB J. 2006;20(9):1540–2. Epub 2006 Jun 5
Johansson JO, Jarbe TU, Henriksson BG. Acute and subchronic influences of tetrahydrocannabinols on water and food intake, body weight, and temperature in rats. TIT J Life Sci. 1975;5 (1–2):17–27.
Jones D. End of the line for cannabinoid receptor 1 as an anti-obesity target? Nat Rev Drug Discov. 2008;7(12):961–2.
Kanoski SE, Hayes MR, Greenwald HS, et al. Hippocampal leptin signaling reduces food intake and modulates food-related memory processing. Neuropsychopharmacology. 2011;36:1859–70.
Kirkham TC, Williams CM, Fezza F, et al. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. Br J Pharmacol. 2002;136:550–7.
Kissileff HR, Pi-Sunyer FX, Thornton J, et al. C-terminal octapeptide of cholecystokinin decreases food intake in man. Am J Clin Nutr. 1981;34:154–60.
Klöting N, Fasshauer M, Dietrich A, et al. Insulin-sensitive obesity. Am J Physiol Endocrinol Metab. 2010;299(3):E506–15.
Kola B, Farkas I, Christ-Crain M, et al. The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. PLoS One. 2008;3:e1797.

Kong WM, Stanley S, Gardiner J, et al. A role for arcuate cocaine and amphetamine regulated transcript in hyperphagia, thermogenesis, and cold adaptation. FASEB J. 2003;17:1688–90.
Kong D, Tong Q, Ye C, et al. GABAergic RIP-Cre neurons in the arcuate nucleus selectively regulate energy expenditure. Cell. 2012;151(3):645–57.
Krashes MJ, Lowell BB, Garfield AS. Melanocortin-4-receptor regulated energy homeostasis. Nat Neurosci. 2016;19:206–19.
Kristensen P, Judge ME, Thim L, et al. Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature. 1998;393:72–6.
Labouebe G, Liu S, Dias C, et al. Insulin induces long-term depression of ventral tegmental area dopamine neurons via endocannabinoids. Nat Neurosci. 2013;16:300–8.
Lage R, Parisi C, Seoane-Collazo P, et al. Lack of hypophagia in CB1 null mice is associated to decreased hypothalamic POMC and CART expression. Int J Neuropsychopharmacol. 2015;18 (9). pii: pyv011
Lau J, Herzog H. CART in the regulation of appetite and energy homeostasis. Front Neurosci. 2014;8:313. eCollection 2014
Lawrence CB, Snape AC, Baudoin FM, et al. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. Endocrinology. 2002;143:155–62.
Lean ME, Malkova D. Altered gut and adipose tissue hormones in overweight and obese individuals: cause or consequence? Int J Obes. 2016;40:622–32.
Lee SJ, Kirigiti M, Lindsley SR, et al. Efferent projections of neuropeptide Y-expressing neurons of the dorsomedial hypothalamus in chronic hyperphagic models. J Comp Neurol. 2013;521:1891–914.
Lin S, Boey D, Couzens M, et al. Compensatory changes in [125I]-PYY binding in Y receptor knockout mice suggest the potential existence of further Y receptor(s). Neuropeptides. 2005;39:21–8.
Lockie SH, Heppner KM, Chaudhary N, et al. Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. Diabetes. 2012;61 (11):2753–62.
Ludwig DS, Tritos NA, Mastaitis JW, et al. Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. J Clin Invest. 2001;107:379–86.
Mahler SV, Smith KS, Berridge KC. Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. Neuropsychopharmacology. 2007;32:2267–78.
Malik S, McGlone F, Bedrossian D, et al. Ghrelin modulates brain activity in areas that control appetitive behavior. Cell Metab. 2008;7:400–9.
Mameli-Engvall M, Evrard A, Pons S, et al. Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. Neuron. 2006;50(6):911–21.
Marsh DJ, Weingarth DT, Novi DE, et al. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. Proc Natl Acad Sci USA. 2002;99:3240–5.
Martin B, Dotson CD, Shin YK, et al. Modulation of taste sensitivity by GLP-1 signaling in taste buds. Ann N Y Acad Sci. 2009;1170:98–101.
Mashiko S, Moriya R, Ishihara A, et al. Synergistic interaction between neuropeptide Y1 and Y5 receptor pathways in regulation of energy homeostasis. Eur J Pharmacol. 2009;615:113–7.
McCrickerd K, Forde CG. Sensory influences on food intake control: moving beyond palatability. Obes Rev. 2016;17:18–29.
Melis T, Succu S, Sanna F, et al. The cannabinoid antagonist SR 141716A (Rimonabant) reduces the increase of extra-cellular dopamine release in the rat nucleus accumbens induced by a novel high palatable food. Neurosci Lett. 2007;419:231–5.
Michopoulos V, Toufexis D, Wilson ME. Social stress interacts with diet history to promote emotional feeding in females. Psychoneuroendocrinology. 2012;37:1479–90.

Miller JL, James GA, Goldstone AP, et al. Enhanced activation of reward mediating prefrontal regions in response to food stimuli in Prader-Willi syndrome. J Neurol Neurosurg Psychiatry. 2007;78(6):615–9. Epub 2006 Dec 8

Moran TH, Kinzig KP. Gastrointestinal satiety signals II. Cholecystokinin. Am J Physiol Gastrointest Liver Physiol. 2004;286:G183–8.

Moran TH, Robinson PH, Goldrich MS, et al. Two brain cholecystokinin receptors: implications for behavioral actions. Brain Res. 1986;362:175–9.

Moriarty P, Dimaline R, Thompson DG, et al. Characterization of cholecystokinin (A) and cholecystokinin (B) receptors expressed by vagal afferent neurons. Neuroscience. 1997;79:905–13.

Mountjoy KG. Pro-opiomelanocortin (POMC) neurones, POMC-derived peptides, melanocortin receptors and obesity: how understanding of this system has changed over the last decade. J Neuroendocrinol. 2015;27:406–18.

Müller TD, Nogueiras R, Andermann ML, et al. Ghrelin. Mol Metab. 2015;4:437–60.

Murphy KG, Bloom SR. Gut hormones and the regulation of energy homeostasis. Nature. 2006;444:854–9.

Nakagawa A, Satake H, Nakabayashi H, et al. Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. Auton Neurosci. 2004;110:36–43.

Nicola SM. Reassessing wanting and liking in the study of mesolimbic influence on food intake. Am J Physiol Regul Integr Comp Physiol. 2016;311:R811.

Nonogaki K, Strack AM, Dallman MF, et al. Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT2C receptor gene. Nat Med. 1998;4:1152–6.

Ollmann MM, Wilson BD, Yang YK, et al. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science. 1997;278:135–8.

Onaka T, Takayanagi Y, Yoshida M. Roles of oxytocin neurones in the control of stress, energy metabolism, and social behaviour. J Neuroendocrinol. 2012;24:587–98.

Page AJ, Slattery JA, Milte C, et al. Ghrelin selectively reduces mechanosensitivity of upper gastrointestinal vagal afferents. Am J Physiol Gastrointest Liver Physiol. 2007;292:G1376–84.

Page-Wilson G, Wardlaw SL, Khandji AG, et al. Hypothalamic obesity in patients with craniopharyngioma: treatment approaches and the emerging role of gastric bypass surgery. Pituitary. 2012;15(1):84–92.

Parise EM, Lilly N, Kay K, et al. Evidence for the role of hindbrain orexin-1 receptors in the control of meal size. Am J Physiol Regul Integr Comp Physiol. 2011;301:R1692–9.

Parton LE, Ye CP, Coppari R, et al. Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. Nature. 2007;449:228–32.

Peters JH, Simasko SM, Ritter RC. Modulation of vagal afferent excitation and reduction of food intake by leptin and cholecystokinin. Physiol Behav. 2006;89:477–85.

Plamboeck A, Veedfald S, Deacon CF, et al. The effect of exogenous GLP-1 on food intake is lost in male truncally vagotomized subjects with pyloroplasty. Am J Physiol Gastrointest Liver Physiol. 2013;304:G1117–27.

Plazzi G, Moghadam KK, Maggi LS, et al. Autonomic disturbances in narcolepsy. Sleep Med Rev. 2011;15:187–96.

Pocai A. Unraveling oxyntomodulin, GLP-1's enigmatic brother. J Endocrinol. 2012;215:335–46.

Praful S. Singru, Gábor Wittmann, Erzsébet Farkas, Györgyi Zséli, Csaba Fekete, Ronald M. Lechan. Refeeding-Activated Glutamatergic Neurons in the Hypothalamic Paraventricular Nucleus (PVN) Mediate Effects of Melanocortin Signaling in the Nucleus Tractus Solitarius (NTS). Endocrinology 2012;153(8):3804–3814

Rezai-Zadeh K, Münzberg H. Integration of sensory information via central thermoregulatory leptin targets. Physiol Behav. 2013;121:49–55.

Roth CL, Aylward E, Liang O, et al. Functional neuroimaging in craniopharyngioma: a useful tool to better understand hypothalamic obesity? Obes Facts. 2012;5(2):243–53.

Rudski JM, Billington CJ, Levine AS. Butorphanol increases food-reinforced operant responding in satiated rats. Pharmacol Biochem Behav. 1994;49(4):843–7.
Rui L. Brain regulation of energy balance and body weight. Rev Endocr Metab Disord. 2013;14:387–407.
Rutkowski JM, Stern JH, Scherer PE. The cell biology of fat expansion. J Cell Biol. 2015;208 (5):501–12.
Rutters F, Nieuwenhuizen AG, Lemmens SGT, et al. Acute stress-related changes in eating in the absence of hunger. Obesity (Silver Spring). 2009;17:72–7.
Sahu A. Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance. Front Neuroendocrinol. 2003;24:225–53.
Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell. 1998;92:573–85.
Schwartz TW, Holst JJ, Fahrenkrug J, et al. Vagal, cholinergic regulation of pancreatic polypeptide secretion. J Clin Invest. 1978;61:781–9.
Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature. 2000;404:661–71.
Sellayah D, Bharaj P, Sikder D. Orexin is required for brown adipose tissue development, differentiation, and function. Cell Metab. 2011;14(4):478–90.
Shi H, Kokoeva MV, Inouye K, et al. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest. 2006;116(11):3015–25.
Shigemura N, Ohta R, Kusakabe Y, et al. Leptin modulates behavioral responses to sweet substances by influencing peripheral tastestructures. Endocrinology. 2004;145:839–47.
Shimizu N, Oomura Y, Plata-Salamán CR, Morimoto M. Hyperphagia and obesity in rats with bilateral ibotenic acid-induced lesions of the ventromedial hypothalamic nucleus. Brain Res. 1987;416:153–6.
Shor-Posner G, Azar AP, Jhanwar-Uniyal M, et al. Destruction of noradrenergic innervation to the paraventricular nucleus: deficits in food intake, macronutrient selection, and compensatory eating after food deprivation. Pharmacol Biochem Behav. 1986;25:381–92.
Sisley S, Gutierrez-Aguilar R, Scott M, et al. Neuronal GLP-1R mediates liraglutide's anorectic but not glucose-lowering effect. J Clin Invest. 2014;124:2456–63.
Soria-Gomez E, Bellocchio L, Marsicano G. New insights on food intake control by olfactory processes: the emerging role of the endocannabinoid system. Mol Cell Endocrinol. 2014;397:59–66.
Spreckley E, Murphy KG. The L-cell in nutritional sensing and the regulation of appetite. Front Nutr. 2015;2:23.
Stanley BG, Chin AS, Leibowitz SF. Feeding and drinking elicited by central injection of neuropeptide Y: evidence for a hypothalamic site(s) of action. Brain Res Bull. 1985;14:521–4.
Stice E, Spoor S, Bohon C, Small DM. Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. Science. 2008;322:449–52.
Strissel KJ, Stancheva Z, Miyoshi H, et al. Adipocyte death, adipose tissue remodeling, and obesity complications. Diabetes. 2007;56(12):2910–8.
Suzuki K, Jayasena CN, Bloom SR. Obesity and appetite control. Exp Diabetes Res. 2012;2012:824305.
Szczypka MS, Kwok K, Brot MD, et al. Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice. Neuron. 2001;30:819–28.
Toriya M, Maekawa F, Maejima Y, et al. Long-term infusion of brain-derived neurotrophic factor reduces food intake and body weight via a corticotrophin releasing hormone pathway in the paraventricular nucleus of the hypothalamus. J Neuroendocrinol. 2010;22:987–95.
Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature. 2000;407:908–13.
Tsujino N, Sakurai T. Orexin/hypocretin: a neuropeptide at the interface of sleep, energy homeostasis, and reward system. Pharmacol Rev. 2009;61:162–76.

Tupone D, Madden CJ, Cano G, Morrison SF. An orexinergic projection from perifornical hypothalamus to raphe pallidus increases rat brown adipose tissue thermogenesis. J Neurosci. 2011;31(44):15944–55.
Ulrich-Lai YM, Ostrander MM, Thomas IM, et al. Daily limited access to sweetened drink attenuates hypothalamic-pituitary-adrenocortical axis stress responses. Endocrinology. 2007;148:1823–34.
Vahl TP, Drazen DL, Seeley RJ, et al. Meal-anticipatory glucagon-like peptide-1 secretion in rats. Endocrinology. 2010;151:569–75.
van Bloemendaal L, IJzerman RG, Ten Kulve JS, et al. GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. Diabetes. 2014;63:4186–96.
van der Kooy D. Area postrema: site where cholecystokinin acts to decrease food intake. Brain Res. 1984;295:345–7.
Verty AN, Boon WM, Mallet PE, et al. Involvement of hypothalamic peptides in the anorectic action of the CB receptor antagonist rimonabant (SR 141716). Eur J Neurosci. 2009;29:2207–16.
Vicennati V, Pasqui F, Cavazza C, et al. Cortisol, energy intake, and food frequency in overweight/obese women. Nutrition. 2011;27:677–80.
Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. Trends Cogn Sci. 2011;15:37–46.
von Deneen KM, Gold MS, Liu Y. Food addiction and cues in prader-willi syndrome. J Addict Med. 2009;3(1):19–25.
Wang CF, Billington CJ, Levine AS, Kotz CM. Effect of CART in the hypothalamic paraventricular nucleus on feeding and uncoupling protein gene expression. Neuroreport. 2000;11:3251–5.
Wang L, Saint-Pierre DH, Tach Y. Peripheral ghrelin selectively increases Fos expression in neuropeptide Y—synthesizing neurons in mouse hypothalamic arcuate nucleus. Neurosci Lett. 2002;325:47–51.
Wang GJ, Volkow ND, Telang F, et al. Exposure to appetitive food stimuli markedly activates the human brain. NeuroImage. 2004;21(4):1790–7.
Wang GJ, Geliebter A, Volkow ND, et al. Enhanced striatal dopamine release during food stimulation in binge eating disorder. Obesity (Silver Spring). 2011;19(8):1601–8.
Wauman J, Tavernier J. Leptin receptor signaling: pathways to leptin resistance. Front Biosci. 2011;16:2771–93.
Wenger T, Jamali K, Juaneda C, et al. Arachidonyl ethanolamide (anandamide) activates the parvocellular part of hypothalamic paraventricular nucleus. Biochem Biophys Res Commun. 1997;237:724–8.
Willner P, Moreau JL, Nielsen CK, et al. Decreased hedonic responsiveness following chronic mild stress is not secondary to loss of body weight. Physiol Behav. 1996;60:129–34.
Wren AM, Small CJ, Abbott CR, et al. Ghrelin causes hyperphagia and obesity in rats. Diabetes. 2000;50:2540–7.
Wren AM, Seal LJ, Cohen MA, et al. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab. 2001;86:5992.
Wynne K, Park AJ, Small CJ, et al. Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. Diabetes. 2005;54:2390–5.
Xu B, Goulding EH, Zang K, et al. Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci. 2003;6:736–42.
Yang SC, Shieh KR. Differential effects of melanin concentrating hormone on the central dopaminergic neurons induced by the cocaine- and amphetamine-regulated transcript peptide. J Neurochem. 2005;92:637–46.
Yang L, Scott KA, Hyun J, et al. Role of dorsomedial hypothalamic neuropeptide Y in modulating food intake and energy balance. J Neurosci. 2009;29:179–90.
Yeomans MR, Gray RW. Selective effects of naltrexone on food pleasantness and intake. Physiol Behav. 1996;60:439–46.

Yeomans MR, Gray RW. Opioid peptides and the control of human ingestive behaviour. Neurosci Biobehav Rev. 2002;26(6):713–28.

Yuan M, Konstantopoulos N, Lee J, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science. 2001;293(5535):1673–7.

Zhang J, Ritter RC. Circulating GLP-1 and CCK-8 reduce food intake by capsaicin-insensitive, nonvagal mechanisms. Am J Physiol Regul Integr Comp Physiol. 2012;302:R264–73.

Zhang X, van den Pol AN. Thyrotropin-releasing hormone (TRH) inhibits melanin-concentrating hormone neurons: implications for TRH-mediated anorexic and arousal actions. J Neurosci. 2012;32(9):3032–43.

Zhang G, Bai H, Zhang H, et al. Neuropeptide exocytosis involving synaptotagmin-4 and oxytocin in hypothalamic programming of body weight and energy balance. Neuron. 2011;69:523–35.

Zheng H, Patterson LM, Rhodes CJ, et al. A potential role for hypothalamo-medullary POMC projections in leptin-induced suppression of food intake. Am J Physiol Regul Integr Comp Physiol. 2010;298:R720–8.

Neuroendocrine Control of Carbohydrate Metabolism

16

Sebastian J. Neggers and Aart J. van der Lely

Contents

Abstract

The neuroendocrine control of metabolism is of paramount importance for life. The control over appetite and satiety are very important for the brain to sustain homeostasis of the body, its weight, and the metabolic processes within. In the past, not so much attention was payed towards separate control systems in the brain that selectively control uptake and metabolism of the main constituents of food, namely, proteins, carbohydrates, and fat.

This chapter tries to address the complex systems that control metabolism of especially carbohydrates, including the effects of carbohydrate intake on the brain.

S. J. Neggers · A. J. van der Lely (✉)
Department of Internal Medicine, Erasmus University MC, Rotterdam, The Netherlands
e-mail: a.vanderlelij@erasmusmc.nl

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_17

Keywords
Carbohydrate · Appetite · Addiction · Hedonic system · Neuroendocrine

Introduction

In the Western world, consumption of soft drinks has increased the last three decades and is partly responsible for the epidemic-like increase in obesity. Soft drinks, originally sweetened by sucrose, are now sweetened by other caloric sweeteners, such as fructose. In a study by Lindqvist et al., they investigated the short-term effect of sucrose, glucose, or fructose solutions on food intake and body weight in rats, and on peripheral and central appetite signals (Lindqvist et al. 2008). All rats offered the sugar solutions increased their total caloric intake. The increased caloric intake occurred even though the rats offered either of the sugar solutions consumed less chow. Because of the increased caloric intake, the sugar-drinking rats had elevated serum levels of free fatty acids, triglycerides, and cholesterol (Lindqvist et al. 2008).

Stanhope and coworkers also addressed these effects of high sugar intake (Stanhope et al. 2008). High-fructose corn syrup (HFCS) has replaced sucrose as the predominant sweetener in beverages. They compared the metabolic/endocrine effects of HFCS with sucrose and, in a subset of subjects, with pure fructose and glucose by studying 34 men and women who consumed three isocaloric meals with either sucrose- or HFCS-sweetened beverages (Stanhope et al. 2008). Eight of the male subjects were also studied when fructose- or glucose-sweetened beverages were consumed. Unexpectedly, postprandial triglycerides (TG) profiles after HFCS or sucrose were not intermediate but comparably high as after pure fructose (Stanhope et al. 2008). Apparently, short-term consumption of sucrose and HFCS results in postprandial TG responses comparable to those induced by fructose (Stanhope et al. 2008).

Nonalcoholic fatty liver disease (NAFLD) is the most frequent liver disease worldwide and is commonly associated with the metabolic syndrome. Secular trends in the prevalence of these diseases may be associated with the increased fructose consumption observed in the Western diet. NAFLD is characterized by two steps of liver injury: intrahepatic lipid accumulation (hepatic steatosis) and inflammatory progression to nonalcoholic steatohepatitis (NASH) (the "two-hit" theory) (Lim et al. 2010). Diet is an important contributor to the pathogenesis of NAFLD. In a recent review by de Wit et al., they focused on recent publications reporting on the effect of macro- and micronutrients on development and progression of NAFLD (de Wit et al. 2012). In general, saturated fat and fructose seem to stimulate hepatic lipid accumulation and progression into NASH, whereas unsaturated fat, choline, antioxidants, and high-protein diets rich in isoflavones seem to have a more preventive effect. In two recent and very informative reviews, the link between simple sugar intake and fatty liver disease has been further addressed. In the first "hit," hepatic metabolism of fructose promotes de novo lipogenesis and intrahepatic lipid, inhibition of mitochondrial beta-oxidation of long-chain fatty acids, triglyceride formation and steatosis, hepatic and skeletal muscle insulin resistance, and hyperglycemia (Lim et al. 2010). In the second "hit," owing to the molecular instability of its

five-membered furanose ring, fructose promotes protein fructosylation and formation of reactive oxygen species (ROS), which require quenching by hepatic antioxidants (Lim et al. 2010). Many patients with NASH also have micronutrient deficiencies and do not have enough antioxidant capacity to prevent synthesis of ROS, resulting in necroinflammation (Lim et al. 2010). Lim et al. postulated that excessive dietary fructose consumption may underlie the development of NAFLD and the metabolic syndrome (Lim et al. 2010). Furthermore, they also stated that NAFLD and alcoholic fatty liver disease share the same pathogenesis (Lim et al. 2010).

Yki-Jarvinen also concluded in her review that cross-sectional increased intake of fructose and simple sugars characterizes patients with NAFLD compared with weight-matched controls (Yki-Jarvinen 2010). Increased fructose intake is also associated with hepatic insulin resistance and fibrosis severity in non-alcoholic steatosis hepatis (NASH) (Tappy et al. 1986). Intake of saturated fat may also be increased in NAFLD (Yki-Jarvinen 2010). Dietary intervention studies have shown that liver volume and fat content changes significantly within a few days in response to caloric restriction or excess despite no or small changes in body weight (Tappy et al. 1986). Therefore, maintenance of normal body weight and avoidance of intake of excess lipogenic simple sugars would seem beneficial for prevention of NAFLD and its metabolic consequences (Tappy et al. 1986).

How Objective Are the Studies that Report on the Link Between Carbohydrate Intake and Diabetes and Obesity?

The outcomes of recent regulatory initiatives, tax measures, and federal nutritional guidance designed to curb consumption of sugar-sweetened beverages (SSBs) have hinged on whether these beverages are a proven cause of obesity and diabetes (Schillinger et al. 2016). The SSB industry has opposed such initiatives, claiming that causation is scientifically controversial. Schillinger et al. comprehensively surveyed the literature to determine whether experimental studies that found no association between SSBs and obesity- and diabetes-related outcomes (negative studies) are more likely than positive studies to have received financial support from this industry (Schillinger et al. 2016). They searched PubMed from January 2001 to July 2016 for English-language experimental studies on the effects of SSB consumption on obesity- and diabetes-related outcomes, augmented by hand-searching recent reviews (Schillinger et al. 2016). They classified articles as having positive or negative associations versus no associations. They also identified whether articles were independently funded or were funded by, or had authors with financial conflicts with, the SSB industry (Schillinger et al. 2016). They identified 60 studies (28 trials and 32 systematic reviews/meta-analyses of trials) that examined the effects of SSB consumption on obesity- and diabetes-related outcomes. Twenty-six articles (8 trials and 18 systematic reviews/meta-analyses) described no associations, and 34 articles (20 trials and 14 systematic reviews/meta-analyses) described positive associations (Schillinger et al. 2016). Studies funded by the SSB industry were significantly more likely to find no associations than independently funded ones; 26 of 26 negative

studies (100%) had funding ties to this industry, whereas only 1 of 34 positive studies (2.9%) had such ties (Schillinger et al. 2016). Apparently, experimental studies that have financial conflicts with the SSB industry are much more likely than independently funded ones to find no relationship between SSB consumption and metabolic outcomes (Schillinger et al. 2016). The SSB industry seems to be manipulating contemporary scientific processes to create controversy and advance their business interests at the expense of the public's health (Schillinger et al. 2016).

Food Addiction

"Food addiction" has become a focus of interest for researchers attempting to explain certain processes and/or behaviors that may contribute to the development of obesity (Hebebrand et al. 2014). Although the scientific discussion on "food addiction" is in its nascent stage, it has potentially important implications for treatment and prevention strategies (Hebebrand et al. 2014). As such, it is important to critically reflect on the appropriateness of the term "food addiction," which combines the concepts of "substance-based" and behavioral addiction. The currently available evidence for a substance-based food addiction is poor, partly because systematic clinical and translational studies are still at an early stage (Hebebrand et al. 2014). Hebebrand et al. do, however, view both animal and existing human data as consistent with the existence of addictive eating behavior (Hebebrand et al. 2014). Accordingly, they stress that like other behaviors, eating can become an addiction in thus predisposed individuals under specific environmental circumstances (Hebebrand et al. 2014). In a review, they introduced diagnostic and neurobiological concepts of substance-related and non-substance-related addictive disorders and highlight the similarities and dissimilarities between addiction and overeating (Hebebrand et al. 2014). Via that review process, they concluded that "food addiction" is a misnomer because of the ambiguous connotation of a substance-related phenomenon (Hebebrand et al. 2014). They instead proposed the term "eating addiction" to underscore the behavioral addiction to eating (Hebebrand et al. 2014).

The Hedonic Rewards System

The learning function is mediated by neuronal reward prediction error signals which implement basic constructs of reinforcement learning theory (Schultz 2015). These signals are found in dopamine neurons, which emit a global reward signal to striatum and frontal cortex, and in specific neurons in striatum, amygdala, and frontal cortex projecting to select neuronal populations. The approach and choice functions involve subjective value, which is objectively assessed by behavioral choices eliciting internal, subjective reward preferences (Schultz 2015). Although all reward, reinforcement, and decision variables are theoretical constructs, their neuronal signals constitute measurable physical implementations and as such confirm the validity of these concepts. The neuronal reward signals provide guidance for

behavior while constraining the free will to act (Schultz 2015). The brain responds to macronutrients via intricate mechanisms. Tulloch et al. reviewed how the brain's neural systems implicated in homeostatic control of feeding and hedonic responses are influenced by the ingestion of specific types of food (Tulloch et al. 2015).

Obesity and substance abuse during adolescence have reached epidemic proportions, and both are among the leading major public health problems in the United States. There is a significant amount of weight and BMI gain in adolescent ex-addicts during supervised and confirmed abstinence from drugs and alcohol (Hodgkins et al. 2007).

Xue et al. focused on recent findings elucidating nutrient-related epigenetic changes linked to obesity (Xue and Ideraabdullah 2016). They highlighted studies demonstrating that obesity is a complex disease linked to disruption of epigenetically regulated metabolic pathways in the brain, adipose tissue, and liver. According to Xue, these pathways regulate (1) homeostatic and hedonic eating behaviors, (2) adipocyte differentiation and fat accumulation, and (3) energy expenditure (Xue and Ideraabdullah 2016). By compiling these data, they illustrated that obesity-related phenotypes are repeatedly linked to disruption of critical epigenetic mechanisms that regulate key metabolic genes.

The sensory experience of eating is an important determinant of food intake control, often attributed to the positive hedonic response associated with certain sensory cues (McCrickerd and Forde 2016). However, palatability is just one aspect of the sensory experience. Sensory cues based on a food's sight, smell, taste, and texture are operational before, during, and after an eating event (McCrickerd and Forde 2016). McCrickerd and coworkers considered the role of visual and odor cues in identifying food in the near environment, guiding food choice and memory for eating, and highlight the ways in which tastes and textures influence meal size and the development of satiety after consumption (McCrickerd and Forde 2016).

Carbohydrate intake is regulated by metabolic, neuronal, and hedonic factors, and gene polymorphisms are involved in determining sugar preference (Leturque et al. 2012). Genetic diseases linked to mutations in the disaccharidase genes (sucrase-isomaltase, lactase) and in sugar transporter genes (sodium/glucose cotransporter 1, glucose transporters 1 and 2) severely impact carbohydrate intake (Leturque et al. 2012). These diseases are revealed upon exposure to food containing the offending sugar, and withdrawal of this sugar from the diet prevents disease symptoms, failure to thrive, and premature death (Leturque et al. 2012).

Ventura et al. conducted a review of the neurobiologic basis for carbohydrate craving (Ventura et al. 2014). They reported that research on the basis of carbohydrate craving is varied but may be grouped into five main areas: the serotonergic system, palatability and hedonic response, the motivational system, stress response systems, and gene-environment interaction (Ventura et al. 2014).

A primary behavioral pathology in addiction is the overpowering motivational strength and decreased ability to control the desire to obtain, e.g., carbohydrates (Kalivas and Volkow 2005). While dopamine is critical for acute reward and initiation of addiction, end-stage addiction results primarily from cellular adaptations in anterior cingulate and orbitofrontal glutamatergic projections to the nucleus

accumbens (Kalivas and Volkow 2005). Mainly cellular adaptations in prefrontal glutamatergic innervation of the accumbens promote the compulsive character of seeking in addicts by decreasing the value of natural rewards, diminishing cognitive control (choice), and enhancing glutamatergic drive in response to drug-associated stimuli (Kalivas and Volkow 2005).

There is increasing evidence that the pathological overeating underlying some forms of obesity is compulsive in nature and therefore contains elements of an addictive disorder (Brown et al. 2015). Brown et al. sought to establish whether the propensity to diet-induced obesity (DIO) is associated with addictive-like behavior, as well as synaptic impairments in the nucleus accumbens core considered hallmarks of addiction in rats (Brown et al. 2015). They found that propensity to develop DIO is linked to deficits in the ability to induce long-term depression in the nucleus accumbens, as well as increased potentiation at these synapses as measured by AMPA/*N*-methyl-D-aspartate currents (Brown et al. 2015). Their results show overlap between the propensity for DIO and the synaptic changes associated with facets of addictive behavior, supporting partial coincident neurological underpinnings for compulsive overeating and drug addiction (Brown et al. 2015).

High-Carbohydrate Diets and the Hedonic System

In Fig. 1, the three most important regulators of food intake are depicted, the homeostatic system with its main physiological messengers, the psychological factor, and hedonic factors that all together determine what somebody eats, or wants to eat. High-carbohydrate meal or a glucose injection increases NPY mRNA in the ARC of rats, as

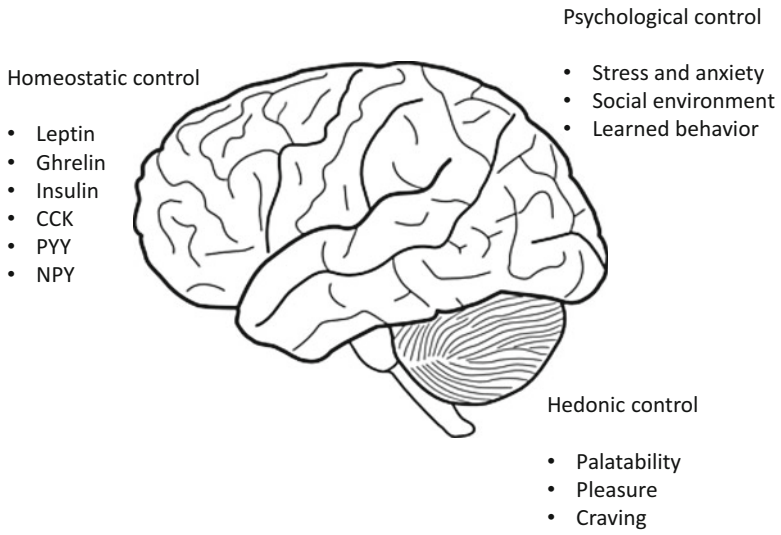


Fig. 1 The multiple factors that regulate food intake

well as levels of NPY protein in the ARC and PVN, compared with either a diet high in fat or a moderate-carbohydrate diet (Tulloch et al. 2015; Wang et al. 1999). Prior studies have demonstrated that chronic consumption over several weeks of a high-carbohydrate (65%) diet, compared to a moderate-carbohydrate (45%) or low-carbohydrate (15%) diet, potentiates the expression, synthesis, and release of hypothalamic NPY. This effect occurs specifically in neurons of the arcuate nucleus (ARC) which project to the paraventricular nucleus (PVN). After a high-carbohydrate meal compared to a moderate-carbohydrate or high-fat meal, NPY gene expression examined via in situ hybridization is found to be significantly enhanced in the ARC (Wang et al. 1999). After a high-carbohydrate meal, levels of glucose, together with corticosterone and insulin, are significantly elevated, while nonesterified fatty acids are reduced (Wang et al. 1999). A possible effect of circulating glucose on hypothalamic NPY is further suggested by the finding that the consumption or a single injection of a glucose solution at the onset of the feeding cycle similarly elevates NPY mRNA and peptide immunoreactivity in the ARC and PVN (Wang et al. 1999). The results of Wang et al. demonstrate that hypothalamic NPY can change rapidly in response to dietary carbohydrate (Wang et al. 1999).

Hajnal showed that the effects of sucrose on brain reward circuitry indicate that sweet taste has a role in hedonic-based eating. Sucrose licking increases release of dopamine in the nucleus accumbens (Hajnal and Norgren 2001).

Tulloch et al. in their review mentioned several studies that investigated the effects of carbohydrate intake on brain responses in humans (Tulloch et al. 2015). Carbohydrate intake appears to decrease the activation and cerebral blood flow of hypothalamic regions in healthy adults, while increasing the activation of regions associated with reward and motivation (Stice et al. 2013). Fat caused greater activation of the caudate and oral somatosensory regions than did sugar, while sugar caused greater activation in the putamen and gustatory regions than did fat (Stice et al. 2013). Increasing sugar intake caused greater activity in gustatory regions, while increasing fat did not affect the activation (Stice et al. 2013). The results of Stice et al. imply that sugar more effectively recruits reward and gustatory regions, suggesting that policy, prevention, and treatment interventions should prioritize reductions in sugar intake (Stice et al. 2013).

So carbohydrates appear to excite reward-associated regions and can have an acute inhibitory effect in hypothalamic regions associated with appetite. However, studies did produce mixed findings, perhaps due to differences in procedural and outcomes measures (Tulloch et al. 2015).

Differences Between Fructose and Glucose Metabolism

In a recent study by Lustig et al., they observed that isocaloric fructose restriction improved surrogate metabolic parameters in children with obesity and metabolic syndrome irrespective of weight change (Lustig et al. 2016). Changes in dietary composition associated with the Western diet are responsible for biochemical alterations known collectively as metabolic syndrome (Lin et al. 2016; Tappy and Le 2015).

Others could not confirm this relation, however (Angelopoulos et al. 2016; Rippe and Angelopoulos 2015).

Fructose has attracted particular attention, due to several unique metabolic and neuroendocrine properties (Lustig et al. 2016): it is metabolized almost exclusively in the liver and it serves as a substrate for de novo lipogenesis and drives hepatic triglyceride synthesis and accumulation (Lim et al. 2010). Fructose also engages in non-enzymatic fructation and ROS formation which causes cellular dysfunction (Schalkwijk et al. 2004). On top of that, it does not suppress ghrelin, resulting in excessive consumption (Van Name et al. 2015).

Basaranoglu et al. investigated whether increased consumption of fructose is linked to the increased prevalence of fatty liver (Basaranoglu et al. 2013). As high-fat diet alone produces obesity, insulin resistance, and some degree of fatty liver with minimal inflammation and no fibrosis, the fast food diet which includes fructose and fats produces a gene expression signature of increased hepatic fibrosis, inflammation, endoplasmic reticulum stress, and lipoapoptosis (Basaranoglu et al. 2013). Several other reviews addressed this relationship (Bantle 2009; Elliott et al. 2002; Kelishadi et al. 2014; Segal et al. 2007). Elliott et al. explored whether fructose consumption might be a contributing factor to the development of obesity and the accompanying metabolic abnormalities observed in the insulin resistance syndrome (Elliott et al. 2002). The per capita disappearance data for fructose from the combined consumption of sucrose and high-fructose corn syrup have increased by 26%, from 64 g/d in 1970 to 81 g/d in 1997 (Elliott et al. 2002). Both plasma insulin and leptin act in the central nervous system in the long-term regulation of energy homeostasis. Because fructose does not stimulate insulin secretion from pancreatic beta cells, the consumption of foods and beverages containing fructose produces smaller postprandial insulin excursions than does consumption of glucose-containing carbohydrate (Elliott et al. 2002). The combined effects of lowered circulating leptin and insulin increase the likelihood of weight gain and its associated metabolic sequelae (Elliott et al. 2002). In addition, fructose, compared with glucose, is preferentially metabolized to lipid in the liver. Fructose consumption induces insulin resistance, impaired glucose tolerance, hyperinsulinemia, hypertriacylglycerolemia, and hypertension in animal models (Rebollo et al. 2012). The data in humans are less clear, however (Elliott et al. 2002). Although there are existing data on the metabolic and endocrine effects of dietary fructose that suggest that increased consumption of fructose may be detrimental in terms of body weight and adiposity and the metabolic indexes associated with the insulin resistance syndrome, much more research is needed to fully understand the metabolic effect of dietary fructose in humans (Elliott et al. 2002).

The Role of Uric Acid

Fructose is distinct from other sugars in its ability to cause intracellular ATP depletion, nucleotide turnover, and the generation of uric acid (Johnson et al. 2013). Fructose is metabolized primarily in the liver. When it is taken up by the liver, ATP decreases rapidly as the phosphate is transferred to fructose in a form that

makes it easy to convert to lipid precursors. Fructose intake enhances lipogenesis and the production of uric acid. By worsening blood lipids, contributing to obesity, diabetes, fatty liver, and gout, fructose in the amounts currently consumed is hazardous to the health of some people (Bray 2013).

Several reviews address the potential role of uric acid in the metabolic syndrome and NAFLD (Johnson 2015; Kanbay et al. 2016; Lima et al. 2015; Lombardi et al. 2016; Sun et al. 2016). Petrie et al. reports on the cellular mechanisms by which uric acid interferes with hepatocyte function (Petrie et al. 2013). Plasma levels of uric acid, the final product of purine degradation in humans, are elevated in metabolic syndrome and are strongly associated with insulin resistance and nonalcoholic fatty liver disease (NAFLD) (Petrie et al. 2013). Hepatic and blood levels of purine metabolites (inosine, hypoxanthine, and xanthine) are also altered in pathophysiological states. Petrie and coworkers optimized a rat hepatocyte model to test the hypothesis that the production of uric acid by hepatocytes is a potential marker of compromised homeostasis of hepatocellular inorganic phosphate (Pi) and/or ATP (Petrie et al. 2013). The basal rate of uric acid production from endogenous substrates in rat hepatocytes was comparable to that in human liver and was $<10\%$ of the maximum rate with saturating concentrations of purine substrates (Petrie et al. 2013). It was marginally (~20%) decreased by insulin and increased by glucagon but was stimulated more than twofold by substrates (fructose and glycerol) that lower both cell ATP and Pi, and by inhibitors of mitochondrial respiration (complexes I, III, and V) that lower ATP but raise cell Pi. Clearance of inosine and its degradation to uric acid were also inhibited by cell Pi depletion. Apparently, uric acid production by hepatocytes is a very sensitive index of ATP depletion irrespective of whether cell Pi is lowered or raised. This suggests that raised plasma uric acid may be a marker of compromised hepatic ATP homeostasis (Petrie et al. 2013).

The discovery that fructose-mediated generation of uric acid may have a causal role in diabetes and obesity provides new insights into pathogenesis and therapies for this important disease (Johnson et al. 2013).

Aspects of Human Evolution on Obesity and Sugar Intake

Uricase is an enzyme involved in purine catabolism and is found in all three domains of life (Kratzer et al. 2014). Curiously, uricase is not functional in some organisms despite its role in converting highly insoluble uric acid into 5-hydroxyisourate (Kratzer et al. 2014). Of interest is the observation that apes, including humans, cannot oxidize uric acid, and it appears that multiple, independent evolutionary events led to the silencing or pseudogenization of the uricase gene in ancestral apes (Kratzer et al. 2014).

Uric acid is the highly insoluble end-product of purine metabolism in humans. Serum levels exceeding the solubility threshold can trigger formation of urate crystals resulting in gouty arthritis (Tan et al. 2016). Uric acid is primarily excreted through the kidneys with 90% reabsorbed back into the bloodstream through the uric

acid transporter URAT1 (Tan et al. 2016). This reabsorption process is essential for the high serum uric acid levels found in humans. Tan et al. discovered that URAT1 proteins from humans and baboons have higher affinity for uric acid compared with transporters from rats and mice (Tan et al. 2016). This difference in transport kinetics of URAT1 orthologs, along with inability of modern apes to oxidize uric acid due to loss of the uricase enzyme, raised the question whether these events occurred concomitantly during primate evolution (Tan et al. 2016). Ancestral URAT1 sequences were computationally inferred and ancient transporters were resurrected and assayed, revealing that affinity for uric acid was increased during the evolution of primates. This molecular fine-tuning occurred between the origins of simians and their diversification into New- and Old-World monkey and ape lineages. Remarkably, it was driven in large-part by only a few amino acid replacements within the transporter (Tan et al. 2016). This alteration in primate URAT1 coincided with changes in uricase that greatly diminished the enzymatic activity (Tan et al. 2016). These results suggest that the modifications to URAT1 transporters were potentially adaptive and that maintaining more constant, high levels of serum uric acid may have provided an advantage to our primate ancestors.

Kratzer et al. applied evolutionary models to understand the history of primate uricases by resurrecting ancestral mammalian intermediates before the pseudogenization events of this gene family (Kratzer et al. 2014). Resurrected proteins reveal that ancestral uricases have steadily decreased in activity since the last common ancestor of mammals gave rise to descendent primate lineages. They were also able to determine the 3D distribution of amino acid replacements as they accumulated during evolutionary history by crystallizing a mammalian uricase protein (Kratzer et al. 2014).

Various arguments have been made to suggest why natural selection would allow the accumulation of uric acid despite the physiological consequences of crystallized monosodium urate acutely causing liver/kidney damage or chronically causing gout. In fact, all humans are double knockouts. Humans lack the ability to synthesize vitamin C due to a mutation in L-gulono-lactone oxidase that occurred during the late Eocene, and humans have higher serum uric acid levels due to a mutation in uricase that occurred in the mid Miocene (Johnson et al. 2010). In the review by Johnson et al. they investigated the hypothesis that these mutations have in common the induction of oxidative stress that may have had prosurvival effects to enhance the effects of fructose to increase fat stores (Johnson et al. 2010). Fructose was the primary nutrient in fruit which was the main staple of early primates, but this food likely became less available during the global cooling that occurred at the time of these mutations (Johnson et al. 2010). However, today the intake of fructose, primarily in the form of added sugars, has skyrocketed, while the intake of natural fruits high in vitamin C has fallen (Johnson et al. 2010). They suggest that it is the interaction of these genetic changes with diet that is responsible for the obesity epidemic today (Johnson et al. 2010). Hence, Johnson also proposes that Neel's thrifty gene hypothesis is supported by these new insights into the mechanisms regulating fructose metabolism (Johnson et al. 2010).

Is There Specific Role for Ghrelin?

It is now more than 17 years since ghrelin was identified as the ligand of the growth hormone secretagogue receptor type 1a (GHSR-1a). The story of unacylayed ghrelin (UAG) also began in 1999, when Kojima and co-workers described this peptide in the same report that introduced ghrelin to the scientific world (Kojima et al. 1999). The preproghrelin gene-derived peptides include acyl ghrelin (AG), UAG, and obestatin. AG is produced mainly by the stomach and exerts its central and peripheral effects through the GHSR-1a (Kojima et al. 1999).

Ghrelin is a 28-amino-acid peptide that was identified in 1999 as the ligand of the growth hormone secretagogue receptor (GHSR) (Kojima et al. 1999). Produced mainly by the stomach, AG is acylated by the enzyme ghrelin O-acyl transferase (GOAT) (Gutierrez et al. 2008; Yang et al. 2008). UAG does not bind with high-affinity to the GHSR (Kojima et al. 1999) and was initially considered to be a degradation product of AG without intrinsic biological activities (Kojima et al. 1999). However, UAG overexpression increases circulating UAG and reduces epididymal and perirenal fat in transgenic animals with improved glucose tolerance attributable to increased insulin sensitivity (Zhang et al. 2008). In animal models of diabetes and obesity, administration of UAG improves glucose and lipid metabolism (Delhanty et al. 2013). In healthy volunteers, UAG administration improves glucose metabolism and inhibits lipolysis (Benso et al. 2012). In overweight patients with type 2 diabetes (T2D), continuous overnight infusion of UAG decreases postprandial blood glucose following a standard breakfast meal while insulin sensitivity is increased (Ozcan et al. 2014).

The most striking example of neuroendocrine control of appetite is Prader-Willi syndrome (PWS). PWS is a rare genetic neurodevelopmental disorder arising from the lack of expression of paternally imprinted genes in the 15q1–q12 chromosomal region (Kalsner and Chamberlain 2015). This syndrome is characterized by various nutritional phases, from suckling deficit with failure to thrive in infancy to early onset of obesity with hyperphagia (Miller et al. 2011). The mechanisms driven those different phases are not yet unraveled. In addition to enhance growth hormone secretion, ghrelin stimulates appetite and increase adiposity (Tschop et al. 2000). PWS patients have very high AG with normal UAG levels, resulting in an elevated AG/UAG ratio, suggesting an intrinsic defect in the ghrelin regulation (Kuppens et al. 2015). Compared to adiposity-matched control subjects, hyperphagia in PWS is not related to a lower postprandial GLP-1 or PYY response. Elevated ghrelin levels in PWS are consistent with increased hunger and are unrelated to insulin levels (Bizzarri et al. 2010; Haqq et al. 2008; Purtell et al. 2011).

Recently, Beauloye and coworkers demonstrated normal circulating AG and increased UAG levels in PWS infants compared to age-matched controls thus driving a low AG/UAG ratio, independently from their body mass index. This finding supports the concept of an UAG dependent anorexia in the early phases of the disease and may drive the switch from failure to thrive to obesity.

Parker et al. examined in humans the relative contributions of small intestinal and gastric nutrient exposure to postprandial suppression of ghrelin. They observed that

although the primary source of ghrelin is the gastric mucosa, that small intestinal nutrient exposure is sufficient for food-induced plasma ghrelin suppression in humans, and that gastric nutrient exposure is not necessary for suppression (Parker et al. 2005).

Maffeis et al. explored the changes in ghrelin levels induced by a mixed meal and their relationship with postprandial substrate oxidation rates in overweight and obese children with different levels of insulin sensitivity (Maffeis et al. 2006). The test meal induced a rapid decrease in ghrelin levels (Maffeis et al. 2006). Apparently, a relevant association between postprandial insulin-mediated glucose metabolism and ghrelin secretion in children with different levels of overweight exists (Maffeis et al. 2006).

Ghrelin, through the GHS-R1a, exerts a variety of metabolic functions including stimulation of appetite and weight gain and suppression of insulin secretion. Esler et al. examined the effects of novel small-molecule GHS-R1a antagonists on insulin secretion, glucose tolerance, and weight loss. They demonstrate that GHS-R1a antagonists have the potential to improve the diabetic condition by promoting glucose-dependent insulin secretion and promoting weight loss (Esler et al. 2007).

Ozcan et al. studied the effects of continuous overnight infusion of UAG on ghrelin levels and glucose and insulin responses to a standard breakfast meal (SBM) in 8 overweight patients with type 2 diabetes (Ozcan et al. 2013). Further, in the same patients plus two additional subjects, the effects of UAG infusion on AG concentrations and insulin sensitivity during a hyperinsulinemic-euglycemic clamp (HEC) were assessed (Ozcan et al. 2013). They reported that UAG administration improves glycemic control in obese subjects with type 2 diabetes (Ozcan et al. 2013). UAG might therefore be a good candidate for the development of compounds in the treatment of metabolic disorders or other conditions with a disturbed AG/DAG ratio, such as type 2 diabetes mellitus or Prader-Willi syndrome.

Summary

So, fructose-containing sugars are a focus of attention as a public health target for their putative role in obesity and cardiometabolic disease including diabetes. However, the fructose moiety is singled out to be the primary driver for the harms of sugars due to its unique endocrine signal and pathophysiological role. The point is that this is only supported by ecological studies, animal models of overfeeding and select human intervention studies with supraphysiological doses or lack of control for energy. Fructose-containing sugars can only lead to weight gain and other unintended harms on cardiometabolic risk factors insofar as the excess calories they provide. Prospective cohort studies, which provide the strongest observational evidence, have shown an association between fructose-containing sugars and cardiometabolic risk including weight gain, cardiovascular disease outcomes, and diabetes only when restricted to sugar-sweetened beverages and not for sugars from other sources, e.g., fruits. So, sugar content should not be the sole determinant of a healthy diet. There are many other factors in the diet – some providing excess

calories while others provide beneficial nutrients. Rather than just focusing on one energy source, we should consider the whole diet for health benefits.

What remains interesting is the specific metabolism of humans regarding their inability to synthesize vitamin-C and their lack of clearing uric acid. As today the intake of fructose, primarily in the form of added sugars, has skyrocketed, while the intake of natural fruits high in vitamin C has fallen, it is the interaction of these genetic changes with diet that might also be responsible for the obesity epidemic today. Finally, food addiction might be a misnomer because of the ambiguous connotation of a substance-related phenomenon. The recently proposed term eating addiction probably better underscores the behavioral addiction to eating.

References

Angelopoulos TJ, Lowndes J, Sinnett S, Rippe JM. Fructose containing sugars at normal levels of consumption do not effect adversely components of the metabolic syndrome and risk factors for cardiovascular disease. Nutrients. 2016;8(4):179. https://doi.org/10.3390/nu8040179.

Bantle JP. Dietary fructose and metabolic syndrome and diabetes. J Nutr. 2009;139(6):1263s–8s. https://doi.org/10.3945/jn.108.098020.

Basaranoglu M, Basaranoglu G, Sabuncu T, Senturk H. Fructose as a key player in the development of fatty liver disease. World J Gastroenterol. 2013;19(8):1166–72. https://doi.org/10.3748/wjg.v19.i8.1166.

Benso A, St-Pierre DH, Prodam F, Gramaglia E, Granata R, van der Lely AJ, … Broglio F. Metabolic effects of overnight continuous infusion of unacylated ghrelin in humans. Eur J Endocrinol. 2012;166(5):911–6. https://doi.org/10.1530/eje-11-0982.

Bizzarri C, Rigamonti AE, Luce A, Cappa M, Cella SG, Berini J, … Salvatoni A. Children with Prader-Willi syndrome exhibit more evident meal-induced responses in plasma ghrelin and peptide YY levels than obese and lean children. Eur J Endocrinol. 2010;162(3):499–505. https://doi.org/10.1530/EJE-09-1033. EJE-09-1033 [pii].

Bray GA. Energy and fructose from beverages sweetened with sugar or high-fructose corn syrup pose a health risk for some people. Adv Nutr. 2013;4(2):220–5. https://doi.org/10.3945/an.112.002816.

Brown RM, Kupchik YM, Spencer S, Garcia-Keller C, Spanswick DC, Lawrence AJ, … Kalivas PW. Addiction-like synaptic impairments in diet-induced obesity. Biol Psychiatry. 2015. https://doi.org/10.1016/j.biopsych.2015.11.019.

de Wit NJ, Afman LA, Mensink M, Muller M. Phenotyping the effect of diet on non-alcoholic fatty liver disease. J Hepatol. 2012. https://doi.org/10.1016/j.jhep.2012.07.003.

Delhanty PJ, Huisman M, Baldeon-Rojas LY, van den Berge I, Grefhorst A, Abribat T, … van der Lely AJ. Des-acyl ghrelin analogs prevent high-fat-diet-induced dysregulation of glucose homeostasis. FASEB J. 2013;27(4):1690–700. https://doi.org/10.1096/fj.12-221143.

Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. Am J Clin Nutr. 2002;76(5):911–22.

Esler WP, Rudolph J, Claus TH, Tang W, Barucci N, Brown SE, … Sweet LJ. Small-molecule ghrelin receptor antagonists improve glucose tolerance, suppress appetite, and promote weight loss. Endocrinology. 2007;148(11):5175–85. https://doi.org/10.1210/en.2007-0239.

Gutierrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, … Hale JE. Ghrelin octanoylation mediated by an orphan lipid transferase. Proc Natl Acad Sci U S A. 2008;105(17):6320–5. https://doi.org/10.1073/pnas.0800708105.

Hajnal A, Norgren R. Accumbens dopamine mechanisms in sucrose intake. Brain Res. 2001;904(1):76–84.

Haqq AM, Grambow SC, Muehlbauer M, Newgard CB, Svetkey LP, Carrel AL, ... Freemark M. Ghrelin concentrations in Prader-Willi syndrome (PWS) infants and children: changes during development. Clin Endocrinol (Oxf). 2008;69(6):911–20. https://doi.org/10.1111/j.1365-2265.2008.03385.x. CEN3385 [pii].

Hodgkins C, Frost-Pineda K, Gold MS. Weight gain during substance abuse treatment: the dual problem of addiction and overeating in an adolescent population. J Addict Dis. 2007;26 (Suppl 1):41–50. https://doi.org/10.1300/J069v26S01_05.

Hebebrand J, Albayrak O, Adan R, et al. Eating addiction, rather than food addiction, better captures addictive-like eating behavior. Neurosci Biobehav Rev 2014;47:295–306.

Johnson RJ. Why focus on uric acid? Curr Med Res Opin. 2015;31(Suppl 2):3–7. https://doi.org/10.1185/03007995.2015.1087979.

Johnson RJ, Andrews P, Benner SA, Oliver W. Theodore E. Woodward award. The evolution of obesity: insights from the mid-Miocene. Trans Am Clin Climatol Assoc. 2010;121:295–305; discussion 305–8.

Johnson RJ, Nakagawa T, Sanchez-Lozada LG, Shafiu M, Sundaram S, Le M, ... Lanaspa MA. Sugar, uric acid, and the etiology of diabetes and obesity. Diabetes. 2013;62(10):3307–15. https://doi.org/10.2337/db12-1814.

Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry. 2005;162(8):1403–13. https://doi.org/10.1176/appi.ajp.162.8.1403.

Kalsner L, Chamberlain SJ. Prader-Willi, Angelman, and 15q11-q13 duplication syndromes. Pediatr Clin N Am. 2015;62(3):587–606. https://doi.org/10.1016/j.pcl.2015.03.004.

Kanbay M, Jensen T, Solak Y, Le M, Roncal-Jimenez C, Rivard C, ... Johnson RJ. Uric acid in metabolic syndrome: from an innocent bystander to a central player. Eur J Intern Med. 2016;29:3–8. https://doi.org/10.1016/j.ejim.2015.11.026.

Kelishadi R, Mansourian M, Heidari-Beni M. Association of fructose consumption and components of metabolic syndrome in human studies: a systematic review and meta-analysis. Nutrition. 2014;30(5):503–10. https://doi.org/10.1016/j.nut.2013.08.014.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999;402(6762):656–60.

Kratzer JT, Lanaspa MA, Murphy MN, Cicerchi C, Graves CL, Tipton PA, ... Gaucher EA. Evolutionary history and metabolic insights of ancient mammalian uricases. Proc Natl Acad Sci U S A. 2014;111(10):3763–8. https://doi.org/10.1073/pnas.1320393111.

Kuppens RJ, Diene G, Bakker NE, Molinas C, Faye S, Nicolino M, ... Hokken-Koelega AC. Elevated ratio of acylated to unacylated ghrelin in children and young adults with Prader-Willi syndrome. Endocrine. 2015;50(3):633–42. https://doi.org/10.1007/s12020-015-0614-x.

Leturque A, Brot-Laroche E, Le Gall M. Carbohydrate intake. Prog Mol Biol Transl Sci. 2012;108:113–27. https://doi.org/10.1016/b978-0-12-398397-8.00005-8.

Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. Nat Rev Gastroenterol Hepatol. 2010;7(5):251–64. https://doi.org/10.1038/nrgastro.2010.41.

Lima WG, Martins-Santos ME, Chaves VE. Uric acid as a modulator of glucose and lipid metabolism. Biochimie. 2015;116:17–23. https://doi.org/10.1016/j.biochi.2015.06.025.

Lin WT, Chan TF, Huang HL, Lee CY, Tsai S, Wu PW, ... Lee CH. Fructose-rich beverage intake and central adiposity, uric acid, and pediatric insulin resistance. J Pediatr. 2016;171:90–6.e91. https://doi.org/10.1016/j.jpeds.2015.12.061.

Lindqvist A, Baelemans A, Erlanson-Albertsson C. Effects of sucrose, glucose and fructose on peripheral and central appetite signals. Regul Pept. 2008;150:26.

Lombardi R, Pisano G, Fargion S. Role of serum uric acid and ferritin in the development and progression of NAFLD. Int J Mol Sci. 2016;17(4):548. https://doi.org/10.3390/ijms17040548.

Lustig RH, Mulligan K, Noworolski SM, Tai VW, Wen MJ, Erkin-Cakmak A, ... Schwarz JM. Isocaloric fructose restriction and metabolic improvement in children with obesity and metabolic syndrome. Obesity (Silver Spring). 2016;24(2):453–60. https://doi.org/10.1002/oby.21371.

Maffeis C, Bonadonna RC, Consolaro A, Vettor R, Banzato C, Silvagni D, ... Tato L. Ghrelin, insulin sensitivity and postprandial glucose disposal in overweight and obese children. Eur J Endocrinol. 2006;154(1):61–8. https://doi.org/10.1530/eje.1.02055. 154/1/61 [pii].

McCrickerd K, Forde CG. Sensory influences on food intake control: moving beyond palatability. Obes Rev. 2016;17(1):18–29. https://doi.org/10.1111/obr.12340.

Miller JL, Lynn CH, Driscoll DC, Goldstone AP, Gold JA, Kimonis V, ... Driscoll DJ. Nutritional phases in Prader-Willi syndrome. Am J Med Genet A. 2011;155a(5):1040–9. https://doi.org/10.1002/ajmg.a.33951.

Ozcan B, Neggers SJ, Miller AR, Yang HC, Lucaites V, Abribat T, ... Van der Lely AJ. Does des-acyl ghrelin improve glycemic control in obese diabetic subjects by decreasing acylated ghrelin levels? Eur J Endocrinol. 2013. https://doi.org/10.1530/EJE-13-0347.

Ozcan B, Neggers SJ, Miller AR, Yang HC, Lucaites V, Abribat T, ... van der Lely AJ. Does des-acyl ghrelin improve glycemic control in obese diabetic subjects by decreasing acylated ghrelin levels? Eur J Endocrinol. 2014;170(6):799–807. https://doi.org/10.1530/EJE-13-0347.

Parker BA, Doran S, Wishart J, Horowitz M, Chapman IM. Effects of small intestinal and gastric glucose administration on the suppression of plasma ghrelin concentrations in healthy older men and women. Clin Endocrinol (Oxf). 2005;62(5):539–46. https://doi.org/10.1111/j.1365-2265.2005.02254.x. CEN2254 [pii]

Petrie JL, Patman GL, Sinha I, Alexander TD, Reeves HL, Agius L. The rate of production of uric acid by hepatocytes is a sensitive index of compromised cell ATP homeostasis. Am J Physiol Endocrinol Metab. 2013;305(10):E1255–65. https://doi.org/10.1152/ajpendo.00214.2013.

Purtell L, Sze L, Loughnan G, Smith E, Herzog H, Sainsbury A, ... Viardot A. In adults with Prader-Willi syndrome, elevated ghrelin levels are more consistent with hyperphagia than high PYY and GLP-1 levels. Neuropeptides. 2011;45(4):301–7. https://doi.org/10.1016/j.npep.2011.06.001.

Rebollo A, Roglans N, Alegret M, Laguna JC. Way back for fructose and liver metabolism: bench side to molecular insights. World J Gastroenterol. 2012;18(45):6552–9. https://doi.org/10.3748/wjg.v18.i45.6552.

Rippe JM, Angelopoulos TJ. Fructose-containing sugars and cardiovascular disease. Adv Nutr. 2015;6(4):430–9. https://doi.org/10.3945/an.114.008177.

Schalkwijk CG, Stehouwer CD, van Hinsbergh VW. Fructose-mediated non-enzymatic glycation: sweet coupling or bad modification. Diabetes Metab Res Rev. 2004;20(5):369–82. https://doi.org/10.1002/dmrr.488.

Schillinger D, Tran J, Mangurian C, Kearns C. Do sugar-sweetened beverages cause obesity and diabetes? Industry and the manufacture of scientific controversy. Ann Intern Med. 2016;165(12):895–7. https://doi.org/10.7326/l16-0534.

Schultz W. Neuronal reward and decision signals: from theories to data. Physiol Rev. 2015;95(3):853–951. https://doi.org/10.1152/physrev.00023.2014.

Segal MS, Gollub E, Johnson RJ. Is the fructose index more relevant with regards to cardiovascular disease than the glycemic index? Eur J Nutr. 2007;46(7):406–17. https://doi.org/10.1007/s00394-007-0680-9.

Stanhope KL, Griffen SC, Bair BR, Swarbrick MM, Keim NL, Havel PJ. Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup-, sucrose-, fructose-, and glucose-sweetened beverages with meals. Am J Clin Nutr. 2008;87(5):1194–203.

Stice E, Burger KS, Yokum S. Relative ability of fat and sugar tastes to activate reward, gustatory, and somatosensory regions. Am J Clin Nutr. 2013;98(6):1377–84. https://doi.org/10.3945/ajcn.113.069443.

Sun DQ, Wu SJ, Liu WY, Lu QD, Zhu GQ, Shi KQ, ... Zheng MH. Serum uric acid: a new therapeutic target for nonalcoholic fatty liver disease. Expert Opin Ther Targets. 2016;20(3):375–87. https://doi.org/10.1517/14728222.2016.1096930.

Schillinger D, Tran J, Mangurian C, Kearns C. Do Sugar-Sweetened Beverages Cause Obesity and Diabetes? Industry and the Manufacture of Scientific Controversy. Ann Intern Med 2016; 165(12):895–7.

Tan PK, Farrar JE, Gaucher EA, Miner JN. Coevolution of URAT1 and uricase during primate evolution: implications for serum urate homeostasis and gout. Mol Biol Evol. 2016;33(9):2193–200. https://doi.org/10.1093/molbev/msw116.

Tappy L, Randin JP, Felber JP, et al. Comparison of thermogenic effect of fructose and glucose in normal humans. Am J Physiol 1986;250(6 Pt 1):E718–E24.

Tappy L, Le KA. Health effects of fructose and fructose-containing caloric sweeteners: where do we stand 10 years after the initial whistle blowings? Curr Diab Rep. 2015;15(8):54. https://doi.org/10.1007/s11892-015-0627-0.

Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature. 2000;407(6806):908–13. https://doi.org/10.1038/35038090.

Tulloch AJ, Murray S, Vaicekonyte R, Avena NM. Neural responses to macronutrients: hedonic and homeostatic mechanisms. Gastroenterology. 2015;148(6):1205–18. https://doi.org/10.1053/j.gastro.2014.12.058.

Van Name M, Giannini C, Santoro N, Jastreboff AM, Kubat J, Li F, ... Caprio S. Blunted suppression of acyl-ghrelin in response to fructose ingestion in obese adolescents: the role of insulin resistance. Obesity (Silver Spring). 2015;23(3):653–61. https://doi.org/10.1002/oby.21019.

Ventura T, Santander J, Torres R, Contreras AM. Neurobiologic basis of craving for carbohydrates. Nutrition. 2014;30(3):252–6. https://doi.org/10.1016/j.nut.2013.06.010.

Wang J, Dourmashkin JT, Yun R, Leibowitz SF. Rapid changes in hypothalamic neuropeptide Y produced by carbohydrate-rich meals that enhance corticosterone and glucose levels. Brain Res. 1999;848(1–2):124–36.

Xue J, Ideraabdullah FY. An assessment of molecular pathways of obesity susceptible to nutrient, toxicant and genetically induced epigenetic perturbation. J Nutr Biochem. 2016;30:1–13. https://doi.org/10.1016/j.jnutbio.2015.09.002.

Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. Cell. 2008;132(3):387–96.

Yki-Jarvinen H. Nutritional modulation of nonalcoholic fatty liver disease and insulin resistance: human data. Curr Opin Clin Nutr Metab Care. 2010;13(6):709–14. https://doi.org/10.1097/MCO.0b013e32833f4b34.

Zhang W, Chai B, Li JY, Wang H, Mulholland MW. Effect of des-acyl ghrelin on adiposity and glucose metabolism. Endocrinology. 2008;149(9):4710–6.

Neuroendocrinology of Bone Metabolism

17

Gherardo Mazziotti, Mauro Doga, Annamaria Formenti, Stefano Frara, Filippo Maffezzoni, and Andrea Giustina

Contents

Abstract

Neuroendocrinology of bone is a new area of research based on the evidence that pituitary hormones may directly modulate bone remodeling and metabolism. As a matter of fact, skeletal fragility associated with high risk of fractures is a common complication of pituitary diseases characterized by either hypo- or hyperfunction of the pituitary gland. This chapter deals with physiological, pathophysiological, clinical, and therapeutic aspects concerning the effects of pituitary hormones on skeletal health.

G. Mazziotti
Endocrine Unit, Department of Medicine, A.S.S.T. Carlo Poma, Mantova, Italy

M. Doga · S. Frara · A. Giustina (✉)
San Raffaele Vita-Salute University, Milan, Italy
e-mail: a.giustina@libero.it

A. Formenti · F. Maffezzoni
Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4_18

Keywords
Pituitary diseases · Osteoporosis · Bone mineral density · Fractures · Growth hormone · Prolactin · Cortisol · Sex steroids

Introduction

Pituitary hormones are involved in the regulation of skeletal physiology, and bone loss commonly occurs in pituitary disorders (Mazziotti et al. 2015a). The traditional paradigm is that pituitary-derived hormones exert their biological effects on bone by peripheral mediators produced by target glands under their stimulation. However, over the recent years, there has been a growing evidence to suggest that pituitary hormones may bypass traditional endocrine organs to exert remarkable direct effects on the skeleton. This chapter will deal with physiological, pathophysiological, clinical, and therapeutic aspects of direct and indirect effects of pituitary hormones on skeletal health.

Pituitary Hormones and Bone: Physiological and Pathophysiological Aspects

The skeleton is an extremely dynamic tissue with a continuous remodeling process guided by bone-forming osteoblasts and bone-resorbing osteoclasts (Canalis et al. 2007a). The balance between bone resorption and bone formation is crucial to guarantee skeletal homeostasis, whereas osteoporosis develops when bone resorption exceeds bone formation (Mazziotti et al. 2012). Pituitary diseases may affect bone remodeling either by increasing bone resorption or inducing impairment in bone formation (Mazziotti et al. 2015a).

Growth hormone (GH)-insulin-like growth factor-1 (IGF-I) axis has an important role in the regulation of bone growth and bone metabolism during lifespan (Giustina et al. 2008). GH stimulates the proliferation of cells of the osteoblastic lineage and affects the fate of mesenchymal precursors favoring osteoblastogenesis and chondrogenesis and opposing adipogenesis (Giustina et al. 2008). Specifically, GH downregulates the expression of fetal antigen-1, which is the soluble form of delta-like 1 or Pref-1, and as a consequence may regulate adipogenesis (Abdallah et al. 2007). GH also stimulates the expression of bone morphogenetic proteins, which are important for the differentiation of osteoblasts and for bone formation (Kassem et al. 1993). In addition to its effects on the differentiation of osteoblasts, GH stimulates, either directly or indirectly through IGF-1, the differentiated function of mature osteoblast (Giustina et al. 2008). GH also stimulates the carboxylation of osteocalcin, which is a marker of osteoblastic function (Hubina et al. 2004). Most of the effects of GH on mature osteoblasts are mediated by systemic IGF-I (Digirolamo et al. 2007). Interestingly, when synthesized by peripheral tissues, IGF-I expression is controlled by diverse hormones (prevalently GH) and by other growth factors (Giustina and Veldhuis 1998). In osteoblasts, synthesis of IGF-I is induced by

parathyroid hormone (PTH), thyroid hormones, and estrogens, whereas the effects of GH seem to be modest (Giustina et al. 2008). In addition to the effects on osteoblastogenesis and bone formation, GH and IGF-I modulate bone resorption by regulating synthesis of osteoprotegerin and receptor activator of nuclear factor-B ligand (RANKL) by osteoblasts (Giustina et al. 2008). In fact, GH was shown to stimulate production of osteoprotegerin and its accumulation in the bone matrix, whereas IGF-I induces RANKL synthesis and, as a consequence, osteoclastogenesis (Rubin et al. 2002; Ueland et al. 2002; Mrak et al. 2007). Besides the direct effects on bone remodeling, GH and IGF-I regulate calcium and phosphate metabolism (Kamenický et al. 2014). Specifically, GH and IGF-I were shown to stimulate the conversion of 25-hydroxy vitamin D to 1,25-dihydroxy vitamin D by the calcium-dependent activation of renal 1-alpha-hydroxylase, increasing the resorption of calcium and phosphate in the intestine and kidney (Kamenický et al. 2014).

Gonadotropin-gonadal sex steroids axis plays a key role in maintaining bone health throughout life, and secondary hypogonadism is an important factor involved in the pathogenesis of skeletal fragility of patients with pituitary diseases (Riggs et al. 2002). Over the recent years, there has been evidence that follicle-stimulating hormone (FSH) may have direct pro-resorptive effects on mature osteoclasts and low FSH values were suggested to attenuate the negative effects of hypogonadism on the skeleton in experimental models of osteoporosis (Sun et al. 2006; Iqbal et al. 2012). In humans, the possible involvement of FSH in the pathogenesis of osteoporosis was suggested by the observation that amenorrheic women with higher FSH levels had greater bone loss than those with lower values in face of near-equal estrogen levels (Devleta et al. 2004). Moreover, in perimenopausal women increases in bone resorption markers and decrease in bone mineral density (BMD) were better correlated with serum FSH than estrogen levels (Zaidi 2007). However, clinical models of gonadotropin-releasing hormone agonist therapy and in vitro fertilization procedure failed to demonstrate a clinically significant effect of FSH on bone remodeling (Drake et al. 2010; Omodei et al. 2013). Therefore, possible consequences of low FSH values on the skeleton in patients with pituitary diseases have to be considered currently only as a working hypothesis.

Hypogonadism is traditionally considered the main mechanism causing skeletal fragility in patients with prolactinomas (Klibanski et al. 1988). However, there is also evidence that prolactin (PRL) may have sex hormone-independent effects on bone remodeling. In fact, PRL receptor was demonstrated in osteoblasts, and PRL was shown to decrease in vitro osteoblast proliferation with a secondary impairment of bone formation and mineralization (Seriwatanachai et al. 2009; Coss et al. 2000). Moreover, PRL induced increase in RANKL/osteoprotegerin expression in osteoblasts leading to an increase in bone resorption (Seriwatanachai et al. 2008a, b). The direct effects of PRL on skeletal remodeling may play a role in determining bone loss in postmenopausal women and eugonadal males with hyperprolactinemia (Mazziotti et al. 2011a, b).

Thyrotropin (TSH)-thyroid axis is important for the control of longitudinal growth, since thyroid hormones have physiological stimulatory effects on bone remodeling and bone mineralization (Gogakos et al. 2010). However, when thyroid hormones increase, bone remodeling is excessively stimulated with consequent bone

loss and decrease in skeletal strength (Vestergaard and Mosekilde 2003). The effects of thyroid hormones on bone may be modulated at different levels by TSH and GH (Mazziotti et al. 2015a). The peripheral deiodination and activation of thyroxine is stimulated by GH (Martins et al. 2007), and this effect may explain why hypopituitary patients treated for GHD are predis posed to the negative effects of thyroid hormone overtreatment (Mazziotti et al. 2014). Over the recent years, there has been convincing evidence that TSH may have direct inhibitory effect on bone resorption. In animal models, the lack of TSH signal was shown to increase bone resorption leading to osteoporosis regardless of the effects of thyroid hormones (Abe et al. 2003). Also in humans, TSH was clearly shown to exert direct effects on bone remodeling with inhibition of bone resorption (Mazziotti et al. 2005), and TSH levels in the low-normal range were found to be associated with high risk of vertebral fractures in postmenopausal women with osteopenia or osteoporosis (Mazziotti et al. 2010a). Based on this assumption, one could argue that low TSH values may favor skeletal fragility in patients with hypopituitarism, although the evidence to support such a hypothesis is still lacking.

Skeletal fragility is a frequent and well-known complication of glucocorticoid excess, as it occurs in patients with corticotropin (ACTH)-secreting adenomas (i.e., Cushing disease) (Mazziotti et al. 2016a) as well as in those treated with exogenous corticosteroids (Mazziotti et al. 2010b). ACTH has been shown to stimulate bone formation (Isales et al. 2010), but the potential skeletal effects of relatively high ACTH values in patients with Cushing disease are still unknown. The central pathophysiological mechanism of bone loss during long-term use of glucocorticoids is reduced bone formation, due to actions that affects osteoblast differentiation and function (Mazziotti et al. 2006a). However, during the first phase, a significant increase in bone resorption (ultimately leading to the observed early increase of risk of fractures) may occur (Mazziotti et al. 2006a). Besides the direct effects on bone cells, glucocorticoids may also have indirect effects mediated by derangements in neuroendocrine signals in the pituitary gland. Glucocorticoids modulate GH by various and competing effects on the hypothalamus and pituitary gland, with final effects depending on hormone concentrations and time of exposure (Mazziotti and Giustina 2013a). Exposure to chronic glucocorticoid excess, even if mild as in "subclinical hypercortisolism," during treatment with inhaled corticosteroids and overtreatment of hypoadrenalism, causes the increase in hypothalamic somatostatin tone with consequent impairment of GH secretion which may play a pathophysiological role in glucocorticoid-induced osteoporosis contributing to the development of a more severe impairment of bone quality with increased risk of fractures (Mazziotti et al. 2016b). As a matter of fact, both exposure to glucocorticoid excess and GHD are associated with a "low-turnover osteoporosis." Interestingly, there is a cross talking between glucocorticoids and GH-IGF-I axis, since the latter may modulate the activation of corticosteroids at peripheral tissues (Giavoli et al. 2004). In fact, GH stimulates the peripheral inactivation of cortisol in cortisone; this effect explains why patients with untreated GHD are particularly predisposed to negative effects of glucocorticoid excess on bone (Mazziotti et al. 2010c). Glucocorticoids may have also neuroendocrine effects on sex hormone production. Specifically,

glucocorticoids inhibit the release of gonadotropins with consequent secondary hypogonadism which may contribute to increased risk of fractures by impairment of skeletal remodeling and muscle function (Canalis et al. 2007b).

Oxytocin and vasopressin were shown to exert direct effects on bone via specific receptors on osteoblasts and osteoclasts (Tamma et al. 2009, 2013). Both osteoblasts and osteoclasts express oxytocin receptors, whose stimulation enhances bone mass. Consistent with this, mice deficient in oxytocin or its receptor display profoundly impaired bone formation with consequent low-bone-turnover osteoporosis (Tamma et al. 2009). In contrast, bone resorption remains unaffected in oxytocin deficiency because, even while oxytocin stimulates the genesis of osteoclasts, it inhibits their resorptive function (Tamma et al. 2009). Vasopressin was instead shown to have a double effect on bone remodeling, with stimulating effects on bone resorption and inhibitory effects on bone formation (Tamma et al. 2013). However, the role of oxytocin and vasopressin in skeletal fragility of patients with pituitary diseases is still unknown.

Skeletal Fragility in Pituitary Diseases: Clinical and Therapeutic Aspects

Measurement of biochemical markers of bone turnover may be useful in the clinical management of skeletal fragility in patients with pituitary diseases. Markers of bone formation are direct or indirect products of active osteoblasts expressed during various phases of their development and reflect different aspects of osteoblast function. Type I collagen is an important component of bone matrix, and osteoblasts secrete its precursor procollagen molecule during bone formation, whereas degradation products of type 1 collagen are released during bone resorption. As a matter of fact, serum procollagen type I N propeptide (PINP) and carboxy-terminal cross-linking telopeptide of type I collagen (sCTX) are recommended as reference markers of bone formation and resorption, respectively, to be used in the clinical practice (Vasikaran et al. 2011). In the clinical practice, PINP and CTX are generally used for monitoring treatment of osteoporosis with bone-active drugs (Vasikaran et al. 2011). In pituitary diseases, such as in other forms of secondary osteoporosis, measurement of biochemical markers of bone turnover may also provide information on the type of skeletal disorder (i.e., increase or decrease in bone turnover) caused by pituitary hormone excess or defect.

In clinical practice, measurement of BMD at the lumbar spine, total hip, and femoral neck by dual-energy X-ray absorptiometry (DXA) is the mainstay for diagnosis of osteoporosis and prediction of fracture risk (Schousboe et al. 2013). Skeletal demineralization is graded according to the World Health Organization criteria based on comparisons of patient's BMD with the average for young adults, after adjusting for race and gender. A T-score less than or equal to −2.5 SD at the hip or spine is defined as osteoporosis, whereas osteopenia is defined as a T-score between −1 and −2.5 SD (Schousboe et al. 2013). These densitometric definitions are applicable only for postmenopausal women and men aged 50 and older, whereas for younger subjects the Z-score (i.e., the number of standard deviations from

age-matched controls) of -2.0 or lower is used to define a BMD "below the expected range for age" (Schousboe et al. 2013). Since pituitary diseases often occur in men under 50 and premenopausal women, diagnosis of osteoporosis cannot be easily performed on the basis of BMD alone in this clinical setting. Moreover, patients with pituitary diseases were shown to fracture even in the presence of normal BMD, consistently with the concept that bone quality more than bone quantity is affected by pituitary hormone excess and defect (Mazziotti et al. 2015a). This is a clinically relevant finding that prompted to the search for alternative diagnostic tools better reflecting quality of bone in these patients, such as quantitative ultrasonometry, high-resolution peripheral quantitative computed tomography, or measurement of trabecular bone score by DXA (Griffith and Genant 2012; Ulivieri et al. 2014). However, the feasibility and reliability of these methods in the clinical setting of pituitary diseases are still uncertain.

Vertebral fractures are the hallmark of osteoporosis, being the most common fragility fractures (Cooper et al. 1993; Wasnich 1996). In more than 50% of the cases, spine fractures occur without specific clinical symptoms, and the radiological and morphometric approach has emerged as the method of choice for evaluating the true prevalence and incidence of these fractures in the clinical practice (Griffith and Genant 2012). Vertebral fractures are identified marking the vertebral body with six points to describe the vertebral shape and heights. According to the quantitative morphometric approach, vertebral fractures are defined mild, moderate, and severe based on a height ratio decrease of 20–25%, 25–40%, and more than 40%, respectively (Genant et al. 1996). Quantitative morphometry is usually performed on spinal X-ray images, although quantitative approach may also be applied to images of the spine acquired by DXA (Clark et al. 2014).

Hypopituitarism

Adult patients with hypopituitarism have generally marked reduction in bone turnover as predominant effect of GH deficiency (GHD) on bone remodeling regardless of coexistent other pituitary hormone deficiencies (Kaufman et al. 1992). However, about half of hypopituitary patients with GHD have normal vertebral BMD (Giustina et al. 2008). In childhood-onset GHD, vertebral BMD is reduced with T-scores often between −1 and −2; about one third of the patients have T-scores of −2.5 or less (Molitch et al. 2006). In contrast, patients with adult-onset GHD have variable BMD values in relationship with age (e.g., lower BMD in younger patients) and duration and the severity of the disease (e.g., lower BMD in longer lasting and more severe GHD) (Murray et al. 2006). The reasons for the different degrees of bone loss may be that childhood-onset GHD occurs before the achievement of peak bone mass and due to its longer disease duration. However, some authors also argued that lower BMD in childhood-onset disease may be due to an underestimation of BMD related to low size and volume of bones in patients with GHD and short stature (Högler and Shaw 2010).

The rate of non-vertebral fractures is increased about threefold in untreated GHD patients (Rosen et al. 1997; Wuster et al. 2001). Using a radiological and

morphometric approach, prevalent vertebral fractures were found in more than one half of adult patients with GHD, and in about one third of them, fractures were moderate-severe causing back pain and functional impairment (Mazziotti et al. 2006b). The prevalence of vertebral fractures was related to the duration of GHD and did not seem to be significantly affected by the presence of other pituitary hormone deficiencies (Mazziotti et al. 2006b, 2008a). Vertebral fractures were shown to occur even in hypopituitary patients with normal BMD (Mazziotti et al. 2006b).

The effects of recombinant human GH (rhGH) replacement therapy on bone have been widely studied over the last 20 years. Replacement therapy with rhGH led to a dose-dependent increase in bone turnover markers (Abrahamsen et al. 2002). Indeed, the effects of rhGH on bone remodeling are biphasic, since after an initial (6–12 months) predominance of bone resorption, stimulation of formation became predominant when treatment was continued for longer period of time (Ohlsson et al. 1998). Therefore, the positive effects of rhGH on bone were shown to be evident after 12 months of treatment (Barake et al. 2014), whereas shorter-term trials revealed decrease or no change in BMD (Davidson et al. 2004). The beneficial effects of rhGH on BMD have been reported to persist after withdrawal of GH (Biller et al. 2000). A few studies reported densitometric outcomes when rhGH was given for a period equal or longer than 10 years (Arwert et al. 2005; Elbornsson et al. 2012).

Consistently with former observation that fracture rate was lower in treated GHD as compared to untreated patients (Mazziotti et al. 2006b), recent prospective studies reported a significant decrease in incident vertebral (Mazziotti et al. 2016c) and non-vertebral (Mo et al. 2015) fractures in adult GHD patients undergoing treatment with rhGH, suggesting that skeletal integrity could be an emerging critical end point in the decision-making process to initiate GH replacement in hypopituitary patients with GHD (Giustina and Mazziotti 2015).

Replacement therapies of central hypoadrenalism and hypothyroidism were shown to influence the fracture rate in patients with hypopituitarism (Mazziotti et al. 2010c, 2014). As a matter of fact, an overtreatment with these hormones may be frequent in patients with hypopituitarism since replacement therapies do not completely mirror the endogenous hormonal production and their monitoring is also made difficult by the lack of good biomarkers of their action. Higher prevalence of vertebral fractures was demonstrated in hypopituitary patients treated with hydrocortisone doses higher than 28 mg per day (Mazziotti et al. 2010c) and thyroxine doses higher than 1.35 μg/Kg per day (Mazziotti et al. 2014). The negative effects of glucocorticoid overtreatment were shown to be more evident in patients with untreated GHD, whereas the negative skeletal effects of thyroxine overtreatment were more evident in patients with replaced GHD (Mazziotti et al. 2015a).

GH-Secreting Adenomas

Consistently with the concept that GH excess stimulates bone remodeling, markers of bone formation and resorption are increased in patients with active acromegaly,

whereas data on BMD are rather variable in relation to the skeletal site, activity of disease, and gonadal status (Mazziotti et al. 2015b). As a matter of fact, low BMD is a relatively uncommon clinical finding in patients with acromegaly (Kayath and Vieira 1997), whereas several studies reported either increased (Kotzmann et al. 1993; Kaji et al. 2001) or similar (Longobardi et al. 1998) bone mass in acromegaly patients as compared to control subjects. The discrepancy resulted to be much more evident at the lumbar spine as compared to the femoral neck (Mazziotti et al. 2015b). Different factors may be involved in determining this variability. Firstly, patients with acromegaly are frequently affected by osteoarthritis with structural modifications of the spine consisting in osteophyte formation and facet-joint hypertrophy which may lead to an overestimation of BMD measured at lumbar spine (Claessen et al. 2016). Moreover, there is evidence that GH and IGF-I excess may exert deleterious effect on trabecular microarchitecture, whereas cortical bone density tends to be increased as effect of GH on periosteal ossification (Ueland et al. 2006). DXA does not distinguish between cortical and trabecular bone, and densitometric results are greatly influenced by the variable distribution of these two compartments in the different skeletal sites (Diamond et al. 1989).

Although BMD is not generally decreased, recent studies have demonstrated that GH excess may cause abnormalities in bone microstructure (Madeira et al. 2013; Maffezzoni et al. 2016), predisposing patients with acromegaly to develop a specific bone metabolic disease, i.e., "acromegalic osteopathy," characterized by high bone turnover, deterioration of bone microarchitecture, and high risk of vertebral fractures (Mazziotti et al. 2017). Using a radiological and morphometric approach, increased prevalence of vertebral fractures was demonstrated in postmenopausal women (Bonadonna et al. 2005) and males (Mazziotti et al. 2008b) with acromegaly. This finding was confirmed by other cross-sectional studies (Mazziotti et al. 2017), and more recently two independent prospective studies provided evidence for an increased risk of vertebral fractures in male and female patients with acromegaly (Mazziotti et al. 2013a; Claessen et al. 2013). The occurrence of vertebral fractures in acromegaly correlated with the duration of active disease and serum IGF-I levels, but not with BMD, since they were found to develop even in patients with normal or minimally decreased BMD (Mazziotti et al. 2017). Biochemical control of acromegaly improves skeletal health (Mazziotti et al. 2015b), although the risk of vertebral fractures may persist high in some patients with well-controlled or cured acromegaly in relationship with preexistent vertebral fractures and untreated hypogonadism (Mazziotti et al. 2013a; Claessen et al. 2013). Therefore, guidelines for the diagnosis and follow-up of acromegaly complications now include not only DXA (Giustina et al. 2003) but also morphometric spine X-ray evaluation (Melmed et al. 2013).

PRL-Secreting Adenomas

Patients with prolactinomas have high-bone-turnover osteoporosis (Mazziotti et al. 2015a). Bone loss occurs predominantly at the lumbar spine (Naliato et al. 2008) in close relationship with the duration of disease, serum values of PRL, and

bone turnover markers (Mazziotti et al. 2015a). Patients with prolactinomas may develop vertebral and non-vertebral fractures (Mazziotti et al. 2011a, b; Vestergaard et al. 2002a). High prevalence of vertebral fractures was reported even in postmenopausal women with prolactinomas (Mazziotti et al. 2011a) and in men with normal testosterone values (Mazziotti et al. 2011b), supporting the hypothesis that PRL excess per se may contribute to skeletal fragility regardless of gonadal status. The frequency of vertebral fractures was significantly associated with duration of disease independently of the effects of hypopituitarism, age of patients, and serum PRL levels (Mazziotti et al. 2015a). Patients with fractures were shown to have lower BMD as compared to those without fractures, but only a minority of patients had either osteoporosis or BMD below the expected range for age (Mazziotti et al. 2011a, b).

Improvement of BMD was reported during medical treatment of prolactinomas with dopaminergic drugs (Klibanski and Greenspan 1986), although a partial recovery of osteopenia and osteoporosis was observed in some patients with prolactinomas (Di Somma et al. 1998). Few data from cross-sectional studies suggest that correction of hyperprolactinemia may lead to a significant decrease of fracture risk in women with prolactinomas (D'Sylva et al. 2015), although there is also evidence that fracture risk may remain high in some patients, especially if males and/or with long-standing hyperprolactinemia, independently of medical treatment (Mazziotti et al. 2011a, b).

ACTH-Secreting Adenomas

Skeletal fragility is a frequent complication of Cushing disease (Mazziotti et al. 2016a). At the diagnosis of Cushing disease, the skeletal phenotype is usually characterized by low-bone turnover and normal or low-normal BMD (Mazziotti et al. 2016a). However, fracture risk increases rapidly after few months of exposure to endogenous hypercortisolism, and fragility fractures may be the first clinical manifestation of Cushing disease (Abdel-Kader et al. 2012). Fractures involve more frequently the vertebrae, and they may occur in 30–50% of patients with Cushing disease in close relationship with the severity of hypercortisolism (Vestergaard et al. 2002b; Trementino et al. 2014). Moreover, vertebral fractures were shown to occur more frequently in males as compared to females (Valassi et al. 2011).

Bone health does not always completely recover after correction of endogenous hypercortisolism (Scillitani et al. 2014). In fact, some patients may experience an increase in bone formation soon after resolution of glucocorticoid excess with secondary improvement of BMD and decrease in fracture risk (Mancini et al. 2010; Szappanos et al. 2010; Randazzo et al. 2012), whereas in other patients the risk of fracture may persist elevated long-term after the cure of disease (Faggiano et al. 2001). Therefore, a single-case evaluation is often needed for the therapeutic management of osteoporosis induced by endogenous hypercortisolism also because specific guidelines are not available and data of the literature do not allow an evidence-based approach (Mazziotti et al. 2016b).

Summary

Pituitary hormones may negatively impact bone health. Pathophysiological and clinical relevance of these actions is well depicted by the often severe skeletal damage which is observed in pituitary diseases characterized by either hypo- or hyperfunction of the gland. Based on these findings, a novel area of research and of clinical activity has developed over the last years which has been defined "neuroendocrinology of bone." Contribution of neuroendocrine axes to pathophysiology of bone loss outside the classic field to pituitary diseases is still unknown but currently under active investigation.

References

Abdallah BM, Ding M, Jensen CH, et al. Dlk1/FA1 is a novel endocrine regulator of bone and fat mass and its serum level is modulated by growth hormone. Endocrinology. 2007;148:3111–21.

Abdel-Kader N, Cardiel MH, Navarro Compan V, et al. Cushing's disease as a cause of severe osteoporosis: a clinical challenge. Reumatol Clin. 2012;8:278–9.

Abe E, Marians RC, Yu W, et al. TSH is a negative regulator of skeletal remodeling. Cell. 2003;115:151–62.

Abrahamsen B, Hangaard J, Horn HC, et al. Evaluation of the optimum dose of growth hormone (GH) for restoring bone mass in adult-onset GH deficiency: results from two 12-month randomized studies. Clin Endocrinol. 2002;57:273–81.

Arwert LI, Roos JC, Lips P, et al. Effects of 10 years of growth hormone (GH) replacement therapy in adult GH-deficient men. Clin Endocrinol. 2005;63:310–6.

Barake M, Klibanski A, Tritos NA. Effects of recombinant human growth hormone therapy on bone mineral density in adults with growth hormone deficiency: a meta-analysis. J Clin Endocrinol Metab. 2014;99:852–60.

Biller BMK, Sesmilo G, Baum HBA, Hayden D, Schoenfeld D, Klibanski A. Withdrawal of long-term physiological growth hormone (GH) administration: differential effects on bone density and body composition in men with adult-onset GH deficiency. J Clin Endocrinol Metab. 2000;85:970–6.

Bonadonna S, Mazziotti G, Nuzzo M, et al. Increased prevalence of radiological spinal deformities in active acromegaly: a cross-sectional study in postmenopausal women. J Bone Miner Res. 2005;20:1837–44.

Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. N Engl J Med. 2007a;35:905–16.

Canalis E, Mazziotti G, Giustina A, Bilezikian JP. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporos Int. 2007b;18:1319–28.

Claessen KM, Kroon HM, Pereira AM, et al. Progression of vertebral fractures despite long-term biochemical control of acromegaly: a prospective follow-up study. J Clin Endocrinol Metab. 2013;98:4808–15.

Claessen KM, Mazziotti G, Biermasz NR, Giustina A. Bone and joint disorders in acromegaly. Neuroendocrinology. 2016;103:86–95.

Clark EM, Carter L, Gould VC, et al. Vertebral fracture assessment (VFA) by lateral DXA scanning may be cost-effective when used as part of fracture liaison services or primary care screening. Osteoporos Int. 2014;25:953–64.

Cooper C, Atkinson EJ, O'Fallon WM, Melton LJ III. Incidence of clinically diagnosed vertebral fractures: a population-based study in Rochester, Minnesota, 1985–1989. J Bone Miner Res. 1993;7:221–7.

Coss D, Yang L, Kuo CB, et al. Effects of prolactin on osteoblast alkaline phosphatase and bone formation in the developing rat. Am J Physiol Endocrinol Metab. 2000;279: E1216–25.
D'Sylva C, Khan T, Van Uum S, Fraser LA. Osteoporotic fractures in patients with untreated hyperprolactinemia vs. those taking dopamine agonists: a systematic review and meta-analysis. Neuro Endocrinol Lett. 2015;36:745–9.
Davidson P, Milne R, Chase D, et al. Growth hormone replacement in adults and bone mineral density: a systematic review and meta-analysis. Clin Endocrinol. 2004;60:92–8.
Devleta B, Adem B, Senada S. Hypergonadotropic amenorrhea and bone density: new approach to an old problem. J Bone Miner Metab. 2004;22:360–4.
Di Somma C, Colao A, Di Sarno A, et al. Bone marker and bone density responses to dopamine agonist therapy in hyperprolactinemic males. J Clin Endocrinol Metab. 1998;83:807–13.
Diamond T, Nery L, Posen S. Spinal and peripheral bone mineral densities in acromegaly: the effects of excess growth hormone and hypogonadism. Ann Intern Med. 1989;111:567–73.
Digirolamo DJ, Mukherjee A, Fulzele K, et al. Mode of growth hormone action in osteoblasts. J Biol Chem. 2007;282:31666–74.
Drake MT, McCready LK, Hoey KA, Atkinson EJ, Khosla S. Effects of suppression of follicle-stimulating hormone (FSH) secretion on bone resorption markers in postmenopausal women. J Clin Endocrinol Metab. 2010;95:5063–8.
Elbornsson M, Gotherstrom G, Bosaeus I, et al. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. Eur J Endocrinol. 2012;166:787–95.
Faggiano A, Pivonello R, Filippella M, et al. Spine abnormalities and damage in patients cured from Cushing's disease. Pituitary. 2001;4:153–61.
Genant HK, Jergas M, Palermo L, et al. Comparison of semiquantitative visual and quantitative morphometric assessment of prevalent and incident vertebral fractures in osteoporosis. The Study of Osteoporotic Fractures Research Group. J Bone Miner Res. 1996;11:984–96.
Giavoli C, Libé R, Corbetta S, et al. Effect of recombinant human growth hormone (GH) replacement on the hypothalamic-pituitary-adrenal axis in adult GH-deficient patients. J Clin Endocrinol Metab. 2004;89:5397–401.
Giustina A, Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev. 1998;19:717–97.
Giustina A, Casanueva FF, Cavagnini F, et al. Diagnosis and treatment of acromegaly complications. J Endocrinol Invest. 2003;26:1242–7.
Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev. 2008;29:535–59.
Giustina A, Mazziotti G. Growth hormone replacement therapy and fracture risk. Lancet Diabetes Endocrinol. 2015;3:307–8.
Gogakos AI, Duncan Bassett JH, Williams GR. Thyroid and bone. Arch Biochem Biophys. 2010;503:129–36.
Griffith JF, Genant HK. New advances in imaging osteoporosis and its complications. Endocrine. 2012;42:39–51.
Högler W, Shaw N. Childhood growth hormone deficiency, bone density, structures and fractures: scrutinizing the evidence. Clin Endocrinol. 2010;72:281–9.
Hubina E, Lakatos P, Kovacs L, et al. Effects of 24 months of growth hormone (GH) treatment on serum carboxylated and undercarboxylated osteocalcin levels in GH-deficient adults. Calcif Tissue Int. 2004;74:55–9.
Iqbal J, Blair HC, Zallone A, et al. Further evidence that FSH causes bone loss independently of low estrogen. Endocrine. 2012;412:171–5.
Isales CM, Zaidi M, Blair HC. ACTH is a novel regulator of bone mass. Ann N Y Acad Sci. 2010;1192:110–6.
Kaji H, Sugimoto T, Nakaoka D, et al. Bone metabolism and body composition in Japanese patients with active acromegaly. Clin Endocrinol. 2001;55:175–81.

Kamenický P, Mazziotti G, Lombès M, Giustina A, Chanson P. Growth hormone, insulin-like growth factor-1, and the kidney: pathophysiological and clinical implications. Endocr Rev. 2014;35:234–81.
Kassem M, Blum W, Ristelli J, et al. Growth hormone stimulates proliferation and differentiation of normal human osteoblast-like cells in vitro. Calcif Tissue Int. 1993;52:222–6.
Kaufman JM, Taelman P, Vermeulen A, Vandeweghe M. Bone mineral status in growth hormone-deficient males with isolated and multiple pituitary deficiencies of childhood onset. J Clin Endocrinol Metab. 1992;74:118–23.
Kayath MJ, Vieira JG. Osteopenia occurs in a minority of patients with acromegaly and is predominant in the spine. Osteoporos Int. 1997;7:226–30.
Klibanski A, Greenspan SL. Increase in bone mass after treatment of hyperprolactinemic amenorrhea. N Engl J Med. 1986;315:542–6.
Klibanski A, Biller BM, Rosenthal DI, et al. Effects of prolactin and estrogen deficiency in amenorrheic bone loss. J Clin Endocrinol Metab. 1988;67:124–30.
Kotzmann H, Bernecker P, Hubsch P, et al. Bone mineral density and parameters of bone metabolism in patients with acromegaly. J Bone Miner Res. 1993;8:459–65.
Longobardi S, Di Somma C, Di Rella F, et al. Bone mineral density and circulating cytokines in patients with acromegaly. J Endocrinol Invest. 1998;21:688–93.
Madeira M, Neto LV, de Paula Paranhos Neto F, et al. Acromegaly has a negative influence on trabecular bone, but not on cortical bone, as assessed by high-resolution peripheral quantitative computed tomography. J Clin Endocrinol Metab. 2013;98:1734–41.
Maffezzoni F, Maddalo M, Frara S, et al. Cone beam tomography analysis of bone microarchitecture in patients with acromegaly and vertebral fractures. Endocrine. 2016;54:532–42.
Mancini T, Porcelli T, Giustina A. Treatment of Cushing disease: overview and recent findings. Ther Clin Risk Manag. 2010;6:505–16.
Martins MR, Doin FC, Komatsu WR, et al. Growth hormone replacement improves thyroxine biological effects: implications for management of central hypothyroidism. J Clin Endocrinol Metab. 2007;92:4144–53.
Mazziotti G, Sorvillo F, Piscopo M, et al. Recombinant human TSH modulates in vivo C-telopeptides of type-1 collagen and bone alkaline phosphatase, but not osteoprotegerin production in postmenopausal women monitored for differentiated thyroid carcinoma. J Bone Miner Res. 2005;20:480–6.
Mazziotti G, Angeli A, Bilezikian JP, et al. Glucocorticoid-induced osteoporosis: an update. Trends Endocrinol Metab. 2006a;17:144–9.
Mazziotti G, Bianchi A, Bonadonna S, et al. Increased prevalence of radiological spinal deformities in adult patients with GH deficiency: influence of GH replacement therapy. J Bone Miner Res. 2006b;21:520–8.
Mazziotti G, Bianchi A, Cimino V, et al. Effect of gonadal status on bone mineral density and radiological spinal deformities in adult patients with growth hormone deficiency. Pituitary. 2008a;11:55–61.
Mazziotti G, Bianchi A, Bonadonna S, et al. Prevalence of vertebral fractures in men with acromegaly. J Clin Endocrinol Metab. 2008b;93:4649–55.
Mazziotti G, Porcelli T, Patelli I, et al. Serum TSH values and risk of vertebral fractures in euthyroid post-menopausal women with low bone mineral density. Bone. 2010a;46:747–51.
Mazziotti G, Canalis E, Giustina A. Drug-induced osteoporosis: mechanisms and clinical implications. Am J Med. 2010b;123:877–84.
Mazziotti G, Porcelli T, Bianchi A, et al. Glucocorticoid replacement therapy and vertebral fractures in hypopituitary adult males with GH deficiency. Eur J Endocrinol. 2010c;163:15–20.
Mazziotti G, Mancini T, Mormando M, et al. High prevalence of radiological vertebral fractures in women with prolactin-secreting pituitary adenomas. Pituitary. 2011a;14:299–306.
Mazziotti G, Porcelli T, Mormando M, et al. Vertebral fractures in males with prolactinoma. Endocrine. 2011b;39:288–93.
Mazziotti G, Bilezikian J, Canalis E, et al. New understanding and treatments for osteoporosis. Endocrine. 2012;41:58–69.

Mazziotti G, Giustina A. Glucocorticoids and the regulation of growth hormone secretion. Nat Rev Endocrinol. 2013a;95:265–76.
Mazziotti G, Bianchi A, Porcelli T, et al. Vertebral fractures in patients with acromegaly: a 3-year prospective study. J Clin Endocrinol Metab. 2013b;98:3402–10.
Mazziotti G, Mormando M, Cristiano A, et al. Association between l-thyroxine treatment, GH deficiency, and radiological vertebral fractures in patients with adult-onset hypopituitarism. Eur J Endocrinol. 2014;170:893–9.
Mazziotti G, Chiavistelli S, Giustina A. Pituitary diseases and bone. Endocrinol Metab Clin North Am. 2015a;44:171–80.
Mazziotti G, Biagioli E, Maffezzoni F, et al. Bone turnover, bone mineral density, and fracture risk in acromegaly: a meta-analysis. J Clin Endocrinol Metab. 2015b;100:384–94.
Mazziotti G, Delgado A, Maffezzoni F, Formenti AM, Giustina A. Skeletal fragility in endogenous hypercortisolism. Front Horm Res. 2016a;46:66–73.
Mazziotti G, Formenti AM, Adler RA, et al. Glucocorticoid-induced osteoporosis: pathophysiological role of GH/IGF-I and PTH/vitamin D axes, treatment options and guidelines. Endocrine. 2016b;54:603–11.
Mazziotti G, Doga M, Frara S, et al. Incidence of morphometric vertebral fractures in adult patients with growth hormone deficiency. Endocrine. 2016c;52:103–10.
Mazziotti G, Maffezzoni F, Frara S, Giustina A. Acromegalic osteopathy. Pituitary. 2017;20:63–9.
Melmed S, Casanueva FF, Klibanski A, et al. A consensus on the diagnosis and treatment of acromegaly complications. Pituitary. 2013;16:294–302.
Mo D, Fleseriu M, Qi R, et al. Fracture risk in adult patients treated with growth hormone replacement therapy for growth homone deficiency: a prospective cohort study. Lancet Diabetes Endocrinol. 2015;3:331–8.
Molitch ME, Clemmons DR, Malozowski S, et al. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2006;91:1621–34.
Mrak E, Villa I, Lanzi R, Losa M, Guidobono F, Rubinacci A. Growth hormone stimulates osteoprotegerin expression and secretion in human osteoblast-like cells. J Endocrinol. 2007;192:639–45.
Murray RD, Adams JE, Shalet SM. A densitometric and morphometric analysis of the skeleton in adults with varying degrees of growth hormone deficiency. J Clin Endocrinol Metab. 2006;91:432–8.
Naliato EC, Violante AH, Caldas D, et al. Bone density in women with prolactinoma treated with dopamine agonists. Pituitary. 2008;11:21–8.
Ohlsson C, Bengtsson BA, Isaksson OG, et al. Growth hormone and bone. Endocr Rev. 1998;19:55–79.
Omodei U, Mazziotti G, Donarini G, et al. Effects of recombinant follicle-stimulating hormone on bone turnover markers in infertile women undergoing in vitro fertilization procedure. J Clin Endocrinol Metab. 2013;981:330–6.
Randazzo ME, Grossrubatscher E, Dalino Ciaramella P, et al. Spontaneous recovery of bone mass after cure of endogenous hypercortisolism. Pituitary. 2012;15:193–201.
Riggs BL, Khosla S, Melton LJ 3rd. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev. 2002;23:279–302.
Rosen T, Wilhelmsen L, Landin-Wilhelmsen K, et al. Increased fracture frequency in adult patients with hypopituitarism and GH deficiency. Eur J Endocrinol. 1997;137:240–5.
Rubin J, Ackert-Bicknell CL, Zhu L, et al. IGF-I regulates osteoprotegerin (OPG) and receptor activator of nuclear factor-kappaB ligand in vitro and OPG in vivo. J Clin Endocrinol Metab. 2002;87(9):4273.
Schousboe JT, Shepherd JA, Bilezikian JP, et al. Executive summary of the 2013 International Society for Clinical Densitometry Position Development Conference on bone densitometry. J Clin Densitom. 2013;16:455–66.
Scillitani A, Mazziotti G, Di Somma C, et al. Treatment of skeletal impairment in patients with endogenous hypercortisolism: when and how? Osteoporos Int. 2014;25:441–6.

Seriwatanachai D, Charoenphandhu N, Suthiphongchai T, et al. Prolactin decreases the expression ratio of receptor activator of nuclear factor kappaB ligand/osteoprotegerin in human fetal osteoblast cells. Cell Biol Int. 2008a;32:1126–35.

Seriwatanachai D, Thongchote K, Charoenphandhu N, et al. Prolactin directly enhances bone turnover by raising osteoblast-expressed receptor activator of nuclear factor kappaB ligand/osteoprotegerin ratio. Bone. 2008b;42:535–46.

Seriwatanachai D, Krishnamra N, van Leeuwen JP. Evidence for direct effects of prolactin on human osteoblasts: inhibition of cell growth and mineralization. J Cell Biochem. 2009;107:677–85.

Sun L, Peng Y, Sharrow AC, et al. FSH directly regulates bone mass. Cell. 2006;125:247–60.

Szappanos A, Toke J, Lippai D, et al. Bone turnover in patients with endogenous Cushing's syndrome before and after successful treatment. Osteoporos Int. 2010;21:637–45.

Tamma R, Colaianni G, Zhu LL, et al. Oxytocin is an anabolic bone hormone. Proc Natl Acad Sci U S A. 2009;106:7149–54.

Tamma R, Sun L, Cuscito C, et al. Regulation of bone remodeling by vasopressin explains the bone loss in hyponatremia. Proc Natl Acad Sci U S A. 2013;110:18644–9.

Trementino L, Appolloni G, Ceccoli L, et al. Bone complications in patients with Cushing's syndrome: looking for clinical, biochemical, and genetic determinants. Osteoporos Int. 2014;25:913–21.

Ueland T, Bollerslev J, Flyvbjerg A, et al. Effects of 12 months of growth hormone (GH) treatment on cortical and trabecular bone content of insulin like growth factors (IGF) and osteoprotegerin in adults with acquired GH deficiency: a double-blind, randomized, placebo-controlled study. J Clin Endocrinol Metab. 2002;87:2760–3.

Ueland T, Fougner SL, Godang K, Schreiner T, Bollerslev J. Serum GH and IGF-I are significant determinants of bone turnover but not bone mineral density in active acromegaly: a prospective study of more than 70 consecutive patients. Eur J Endocrinol. 2006;155:709–15.

Ulivieri FM, Silva BC, Sardanelli F, et al. Utility of the trabecular bone score (TBS) in secondary osteoporosis. Endocrine. 2014;47:435–48.

Valassi E, Santos A, Yaneva M, et al. The European Registry on Cushing's syndrome: 2-year experience. Baseline demographic and clinical characteristics. Eur J Endocrinol. 2011;165:383–92.

Vasikaran S, Eastell R, Bruyère O, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int. 2011;22:391–420.

Vestergaard P, Mosekilde L. Hyperthyroidism, bone mineral, and fracture risk – a meta-analysis. Thyroid. 2003;13:585–93.

Vestergaard P, Jørgensen JO, Hagen C, et al. Fracture risk is increased in patients with GH deficiency or untreated prolactinomas – a case-control study. Clin Endocrinol. 2002a;56:159–67.

Vestergaard P, Lindholm J, Jørgensen JO, et al. Increased risk of osteoporotic fractures in patients with Cushing's syndrome. Eur J Endocrinol. 2002b;146:51–6.

Wasnich RD. Vertebral fracture epidemiology. Bone. 1996;18:179S–83S.

Wuster C, Abs R, Bengtsson BA, et al. The influence of growth hormone deficiency, growth hormone replacement therapy, and other aspects of hypopituitarism on fracture rate and bone mineral density. J Bone Miner Res. 2001;16:398–405.

Zaidi M. Skeletal remodeling in health and disease. Nat Med. 2007;13:791–801.

Index

F. F. Casanueva, E. Ghigo (eds.), *Hypothalamic-Pituitary Diseases*, Endocrinology,
https://doi.org/10.1007/978-3-319-44444-4