Contemporary Endocrinology
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Anthony C. Hackney
Naama W. Constantini *Editors*

Endocrinology of Physical Activity and Sport

Third Edition

Humana Press

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This book is dedicated to my mentor, the late Professor Atko Viru, and my family who have always provided unwavering support for me. My since thanks and loving gratitude to each of you.

– Anthony C. Hackney

I dedicate this book to my late parents, Prof. RJZ Werblowsky and his beloved wife, Aliza, who passed away since the last edition. I owe them both my family and career.

– Naama W. Constantini

While finalizing this edition, Dr. Barbara Drinkwater, the pioneer of female athlete sports medicine and science research, has passed away. She will always be remembered as the first woman president of the American College of Sports Medicine and a leader to so many of us, especially in the field of eating disorders, menstrual irregularities, and bone health. The chapters in this book dealing with female physiology and endocrinology are honorably dedicated to her.

– Naama W. Constantini and Anthony C. Hackney

Series Editor Foreword

With the twin epidemics of obesity and diabetes upon us, endocrinologists often counsel patients on the benefits of exercise. During our many years of training, however, relatively little time is spent learning about the relationship between exercise and the endocrine system. To address this deficit, the volume edited by Drs. Anthony C. Hackney and Naama W. Constantini and written by an illustrious international group of experts provides an immense wealth of information on the topic.

The relationship between exercise and the endocrine system is complicated and bidirectional: the effectiveness of exercise training in part depends on the state of the endocrine system, while all components of the endocrine system can be dramatically affected by exercise. The interactions between hormones and exercise extend well beyond the obvious (exercise and energy metabolism, exercise and diabetes) to include reproductive, adrenal and growth hormone axes, as well as bone metabolism, thyroid function, endogenous opiates, and circadian endocrine physiology, among others. Moreover, the relationship between the endocrine system and exercise evolves during the person's lifetime (from childhood to puberty to advanced age) and is affected by both type and intensity of exercise (e.g., acute vs. chronic, moderate exercise vs. Olympic athlete training, etc.).

Given these complexities, it is remarkable how clearly and completely the authors of this encyclopedic text have been able to cover their subject. The book is a true pleasure to read. It is exceptionally well referenced and, without a doubt, will become a valuable resource for anybody interested in human physiology. This most certainly includes endocrinologists, who, after becoming familiar with the content of this volume, will find themselves on a much firmer ground while advising their patients on the myriads of benefits, as well as some potential risks, of exercise.

New York, NY, USA — Leonid Poretsky, MD

Preface

The first edition of this book was entitled *Sports Endocrinology* edited by Michelle P. Warren and Naama W. Constantini and published in 2000. It was the first book with incursions into this complex and critically important topic area of exercise, sports, and hormones. It answered a recognized need and was well received by the scientific community. Twelve years later, the book took on a new expanded title, new editorship, and new authorship of chapter topics, and the second edition was published. It too was highly popular and a leading volume on the discipline. Now, after five additional years, a third edition has been developed with revised and updated content as well as new expanded materials. Nevertheless, over its evolution and multiple editions, the emphasis of the book has remained the same: to provide the reader with current, insightful discussion of the key elements of endocrinology as they relate to physical activity, exercise, and sports.

Endocrinology is a demanding scientific endeavor and when overlaid with the unique aspects of the physical stress of exercise and exercise training can become a daunting topic. The editors are profoundly grateful to the contributor's authors who have painstakingly and carefully crafted each of their discussions to aid the reader in overcoming what some might consider an insurmountable set of topics. The author's scholarship, devotion to the scientific method, and overall professionalism have allowed for a new edition that not only reflects the present state of knowledge on each of their topics but will undoubtedly serve as a stimulus for further advances in this highly dynamic, constantly evolving, and challenging subject. Our sincere thanks to each of them for their efforts. We hope the readers will enjoy this new edition and it spurs them to ask new research questions.

Chapel Hill, NC, USA — Anthony C. Hackney, PhD, DSc
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1 Methodological Considerations in Exercise Endocrinology

Anthony C. Hackney, Abbie E. Smith-Ryan, and Julius E. Fink

Introduction

Over the last several decades, an increasing number of exercise science investigations have incorporated measurements of endocrine function (e.g., hormones, cytokines) into their research designs and protocols [1, 2]. This approach has allowed for a heightened level of investigation into research which examines the physiological mechanisms associated with clinical and performance-related conditions found in individuals involved in exercise training.

Some exercise science investigations, however, have not always controlled certain critical factors (e.g., time of day for blood sampling, level of chronic training, etc.) that can influence many of the hormones associated with the human endocrine system. This lack of investigative control has often resulted in the resulting research findings to be inconsistent, contradictory, and sometimes extremely difficult to interpret. This insufficient control of biological experimental factors appears to be due in part to limited knowledge by exercise science researchers in the area of clinical endocrine methodology and techniques.

Experts suggest that the factors that influence hormonal measurements, and contribute to variance in experimental outcomes, can be categorized as consisting of two potential sources: factors affecting physiological variation (i.e., affiliated with the physiological function status of the subject) and factors affecting procedural-analytical variation (i.e., determined by the investigators conducting research) [1, 3]. Regardless of the source of variance, subject, or investigator derived, if it is not controlled or accounted for appropriately, the resulting hormonal measurements obtained can be compromised and thus call into question the scientific validity of a research study.

The focus of this chapter is to provide background information for exercise science researchers on those physiological-procedural-analytical factors that can potentially affect endocrine measurements. The intent is for this material to serve as an introductory "fundamental coverage" on this topic in hopes of improving the quality of research in exercise endocrinology.

The field of endocrinology uses numerous abbreviations for the many of the hormones that exist. To aid those researchers unfamiliar with

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Table 1.1 The following are abbreviations commonly used for various hormones seen in exercise science and sport medicine endocrinological research (see Ref. [4])

Name	Abbreviations
Adrenocorticotropic hormone	ACTH
Aldosterone	ALD
Antidiuretic hormone	ADH
Atrial natriuretic peptide	ANP
Arginine vasopressin	AVP
β-Endorphin	β-END
Catecholamines	Cats
Corticotropin-releasing hormone	CRH
Cortisol	CORT
Epinephrine	EPI
Estradiol-β-17	E_2
Follicle-stimulating hormone	FSH
Glucagon	GLU
Gonadotropin-releasing hormone	GnRH
Growth hormone	GH
Growth hormone-releasing hormone	GHRH
Insulin	IN
Insulin-like growth factor [1]	IGF_1
Leptin	LP
Luteinizing hormone	LH
Norepinephrine	NEPI
Parathyroid hormone	PTH
Progesterone	P
Prolactin	PRL
Reverse triiodothyronine	rT_3
Testosterone	TEST
Thyrotropin-releasing hormone	TRH
Thyroid-stimulating hormone	TSH
Thyroxine	T_4
Triiodothyronine	T_3

this lexicon, Table 1.1 lists those abbreviations for the most common hormones associated with the area of exercise science [4].

Physiological Factors

As mentioned, factors that can influence hormonal measurements can be categorized into two broad areas: "physiological" and "procedural-analytical." The physiological factors are those that are determined to be connected in some way to a biological function or status of the research subject (patient) at the time of the collection of the specimen (e.g., blood) to be analyzed. These are factors that can be viewed as pertaining to a variety of endogenous aspects of the subject/patient.

Sex/Gender

It appears that until the onset of puberty, there is little difference between males and females in their resting hormonal profile. Once puberty is reached though, there is increased androgenic steroid hormone production in the male, and the female starts the characteristic menstrual cycle pulsatile release of gonadotropin and sex steroid hormones [5–7]. Additionally, at puberty, resting leptin (an adipocyte cytokine; a low molecular weight protein that has endocrine-like actions on select physiological process such as the immune system [8]) levels tend to become increased in females, as compared to those in males [9]. In adulthood, the hormonal differences that begin to manifest at puberty tend to remain until females reach the postmenopausal period and males reach andropause [8, 9].

There are some sex-specific differences in the hormonal responses to exercise in males and females. These include an earlier and greater rise in testosterone and creatine kinase during exercise and up to 24 hours after exercise in males as compared to females [1, 10, 11] and a greater pre-exercise growth hormone response in females. Furthermore, the magnitude of the sex steroid hormonal response to exercise in females is influenced by the status and phase of their menstrual cycle [10, 12]. Interestingly, the menstrual cycle hormones can influence other hormones and their response to exercise (e.g., increased estradiol-β-17 → increases growth hormone levels) [10–13] (see later discussion concerning the menstrual cycle in this chapter, as well as Chap. 16 in this book). On the other hand, some hormones show little or no differences in response to exercise between the sexes (e.g., water balance hormones such as aldosterone and arginine vasopressin) [5, 10, 12].

Due to these potential differences in outcomes due to sex, the researcher should be cautious when using adult subject populations involving a mixture of males and females in their studies. To avoid confounding results, researchers need to be certain that the hormonal outcomes they are measuring are not influenced by sex/gender and stage of menstrual cycle, as will be discussed in subsequent sections of this chapter.

Age

If subjects are not matched for age and maturity level, whenever possible, variance in the outcomes can be potentially increased. For example, a prepubertal and postpubertal child (of the same gender) will not typically display the exact same hormonal exercise responses or relationships [14, 15]. This is illustrated by the well-documented increase in insulin resistance which is observed as an adolescent goes through puberty [16].

This concern should also be extended to the other end of the age spectrum. That is, a postmenopausal female or andropausal male could have drastically different hormonal responses when compared to a relative prepausal individual. For example, basal growth hormone and testosterone typically decrease with age, while cortisol and insulin resistance increase [17–19].

These types of age-related differences cannot only exist at rest, but also in response to exercise, and even after completing an exercise training program. As an illustration, a study by Brook et al. showed that the anabolic responses to resistance training are impaired within the elderly, possibly due to lower testosterone levels (young subjects = 367 ± 19; old subjects = 274 ± 19 ng•dl^{-1}), ribosomal biogenesis (RNA:DNA ratio and c-MYC induction; young = +4 ± 2-fold change; old = +1.9 ± 1-fold change), and/or translational efficiency S6K1 phosphorylation (young = +10 ± 4-fold change; old = +4 ± 2-fold change) (see Chap. 21 in this book concerning S6K1 [mTOR substrate S6 kinase 1]) [20]. For this reason, it is important to match subjects in research studies by chronological age and/or maturation level in order to increase the homogeneity of the responses and decrease interindividual variability, obviously, that is, unless the researcher is trying to study age-related changes among groups of individuals [3].

Ethnicity and Race

A variety of different humoral constituents are known to vary between people of different races and ethnic groups [1, 3]. For example, resting parathyroid hormone levels tend to be higher in Black compared to Caucasian individuals [21]. Caucasian females tend to have higher levels of estrogens than Asian females [1, 22]. Evidence also suggests that reproductive hormone levels during gestational periods may vary greatly across several races and ethnic groups (Caucasians, Blacks, Latinos, Asians, and Indians) [22–25]. Findings of greater resting insulin and degree of insulin resistance in certain Native American tribes (e.g., Pima Indians) have also been reported; however these differences may in fact be more related to obesity issues in these individuals [26]. Testosterone levels seem also to have differences among ethnicities, with Asian- and Indian-related ethnicities showing slightly lower levels as compared to other ethnicities [27].

Hormonal responses to exercise and exercise training related to race and ethnicity have not been well studied, and the limited available findings do not suggest drastically different response outcomes beyond basal differences. Further research is certainly necessary and warranted in this area [1, 25, 26].

Body Composition: Adiposity

The level of adiposity of the body can greatly influence the release of certain cytokines by adipose tissue [3, 8, 9]. These cytokines in turn can have autocrine-, paracrine-, and endocrine-like actions and influence aspects of metabolism, reproductive, and inflammatory function [2, 3, 8, 9]. For example, increase in adipose tissue raises the expression of aromatase, triggering higher conversion rates of testosterone to estradiol, triggering a negative feedback to the pituitary gonadotropin secretion, and ultimately resulting in lower testosterone levels [28]. Additionally, several of these cytokines have been directly linked to the promotion of increased hormonal levels (e.g., increased interleukin-6 → increased cortisol) [8]. This situation becomes compounded as adiposity reaches the level of obesity and subsequently affects many hormones to a far greater degree.

For example, insulin and leptin levels tend to be appreciably elevated at rest in many obese persons [29–33].

As levels of adiposity increase, the hormonal response to exercise and exercise training can change considerably from that of a normal-weight person. As an illustration, in obese persons, catecholamine and growth hormone response to exercise becomes blunted [33]. Cortisol responses to exercise seem to become elevated in some overweight-obese individuals, although isolated cases have shown cortisol responses have been shown to be blunted and reduced [32, 33]. Exercise training often allows a loss of body mass, in particular fat mass, which helps to normalize these hormones with levels observed in normal-weight people [33–37].

To ensure that varying levels of body composition of subjects will not confound hormonal outcomes, investigators need to match their subjects for adiposity as closely as possible and not just use body weight matching as criterion. Exactly how close of a match is needed is not known, but grouping normal-weight, overweight (body mass index (BMI) $\geq$ 25.0–< 30.0 kg/m^2), and obese (BMI $\geq$ 30.0 kg/m^2) individuals into the same subject group can most certainly complicate and add variance to some hormonal outcomes [1, 33].

Disease States

Several disease conditions such as HIV, testicular cancer, hormonal disorders (e.g., Cushing's syndrome, Graves' disease, etc.), infections, chronic liver or kidney diseases, type 2 diabetes, and obesity have been shown to affect hormones; e.g., lower sex hormone levels [38, 39]. Specifically, infection-type diseases may lead to testicular dysfunction, and metabolic conditions may lead to hypogonadism via increased adipose tissue and inflammation [28]. Indeed, in HIV-infected patients, lymphoma, or syphilis effects on the pituitary, can trigger or mimic apoplexy and meningeal or pituitary infection leading to fibrosis and ultimately dysfunction [40]. Increased adipose tissue often observed in obese and patients with type 2 diabetes leads to increased aromatization activity converting testosterone to estradiol, leading to a negative feedback to the pituitary gonadotropin secretion triggering hypogonadism [28]. Many individuals are unaware of these conditions when signing up for a study; therefore thorough medical screening is an important consideration.

Mental Health

Select mental health conditions and states are associated with high levels of anxiety and apprehension (e.g., posttraumatic stress disorder), which can lead to enhanced activity of the sympathetic nervous system and hypothalamic-pituitary-adrenal axis [41–43]. Subsequently, resting levels of circulating catecholamines, adrenocorticotropic hormone, β-endorphin, and cortisol can be elevated. In contrast, persons who are experiencing depression can have low arousal levels, and the abovementioned hormones could be suppressed. Moreover, depression is sometimes accompanied by low activity levels in the hypothalamic-pituitary-thyroid axis (i.e., low thyrotropin-releasing hormone, thyroid-stimulating hormone, thyroxine, and triiodothyronine) creating a euthyroid sick syndrome response [41–43]. These alterations in resting hormonal levels from such conditions can in turn result in altered hormonal responses to exercise and exercise training in individuals who have high levels of anxiety [44–46]. In some cases this can result in heightened responses (excessive) or diminished responses [44–46]. Evaluating the mental health status, via the completion of a screening questionnaire by a participant, can serve as an excellent tool to determine if a potential emotional or psychological problem exists which could confound hormonal measurements. A variety of such screening tools are available, and the reader is directed to several excellent references for overviews of this topic [47, 48]. Importantly, it is highly advisable that any such assessment be performed by a trained, qualified individual.

Menstrual Cycle

Menstrual status (eumenorrheic vs. oligomenorrheic vs. amenorrheic) and cycle phase (follicular, ovulation, luteal) in females can produce basal changes in key reproductive hormones such as estradiol-β-17, progesterone, luteinizing hormone (LH), testosterone, and follicle-stimulating hormone (FSH). These changes can be large and dramatic within select individuals. For example, the ovulatory and luteal phases result in increases in all of the aforementioned hormones above what is seen in the follicular phase (e.g., 2–10-fold greater in eumenorrheic female) [49]. These typical changes are depicted in Fig. 1.1. As noted earlier, select reproductive hormones (sex steroids) at rest can influence certain other nonreproductive hormones and nonreproductive physiological function such as estradiol-β-17 enhancing growth hormone release and thus subsequently increased lipid metabolism [11, 50, 51].

The menstrual status and cycle phase hormonal influences can carry over to have an impact on exercise and exercise training responses, too. Consequently, researchers may need to conduct exercise testing with females of similar menstrual status and/or in similar phases of their cycle. This precaution is also applicable to females who are using oral contraceptives, which can mimic some hormonal fluctuations similar to cycle phase changes [51, 52]. The precise impact of oral conceptive (OC) depends upon the composition of the OC used (mono-, bi-, or triphasic) and the dosage of the active estrogen and progestin agents in the pharmaceuticals.

It is also an important consideration when there is an absence of a menstrual cycle either due to amenorrhea (especially hypothalamic based) or due to pregnancy, this absence leads to changes in hormonal levels and exercise responses [1, 2, 24].

Circadian Rhythms

Over the course of a 24-h period, many hormonal levels will fluctuate and display circadian variations (see Chap. 20 of this book). In some cases these variances are due to pulse generator aspects, which is the spontaneous release of select hypothalamic hormonal releasing factors/hormones [53] within the endocrine regulatory axis. In other cases, variances are related to humoral stimuli, changes brought on by individual behavior or environmental factors, and these humoral stimuli influence hormonal release [54, 55]. Circadian hormones can display dramatic changes in levels due to their rhythm patterns, cortisol being a prime example. Morning cortisol levels are typically twice that of those found later

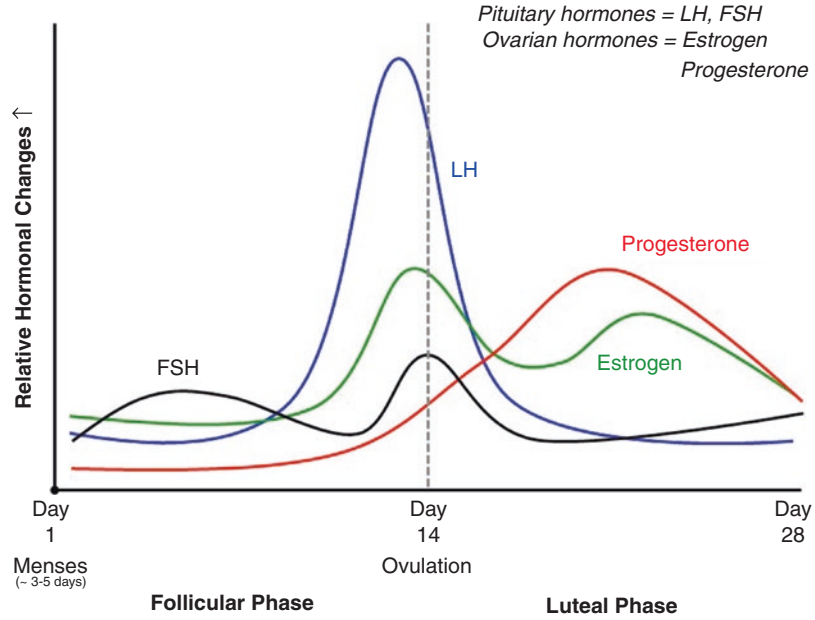

Fig. 1.1 Typical hormone changes (arbitrary scaling for concentration changes) associated with the menstrual cycle in eumenorrheic women

Table 1.2 Hormones that display discernable circadian patterns. The arrows indicate a relative direction for changes in concentration levels

Hormone	AM concentration	PM concentration	Remarks
ACTH	↑ Early AM	↓	Highest levels may be during sleep
Aldosterone	↓	↑	Highest levels may be during sleep
Cortisol	↑↑	↓↓	Influenced by food intake; highest levels may be during sleep
Growth hormone	↑ Early AM	↓	Only slight differences; highest levels may be during sleep
LH-FSH	↓↑	↓↑	Pulsatility or release and menstrual cycle phase override circadian pattern
Melatonin	↑ Early AM	↓	Highest levels may be during sleep
Parathyroid hormone	↓	↑	Highest levels may be during sleep
Prolactin	↑ Early AM ↓ Late AM	↑	Highest levels may be during sleep
Testosterone	↑	↓	Lessens with age

Arrows indicate an increase (↑) or decrease (↓) in hormone concentration

in the day [56–58]. Table 1.2 provides some reference on the circadian pattern seen in some key hormones.

These fluctuations and circadian variations need to be addressed when conducting exercise research. Studies demonstrate that the magnitude of exercise responses may not be similar at different times of the day, even if the exercise intensity and duration are held constant [1, 56]. Investigators should plan accordingly so as to more carefully control and replicate the time of day in which research evaluations are conducted and hormonal specimen collected [59, 60].

Total Versus Free Hormone Concentration

A number of hormones exist in the circulation as either in there free or total amounts forms, the latter being the sum of the free and the carrier-bound portion of the hormone. Steroid hormones are the principal example of this situation. Some investigators do not completely recognize this point and in conducting their research at times measure the wrong form of the hormone in question. A prime illustration of this is the hormone testosterone in which the free form is viewed as more biologically active.

That is, the major part of circulating testosterone binds to sex hormone-binding globulin (SHBG) and some to albumin, leaving only a small fraction of testosterone as free form. In order to be bioactive, testosterone has to be unbound, making only free and albumin-bound (weak bound easy to dissociate) available for tissue uptake. It has been debated for a long time which form of testosterone (total vs. free) is a better indicator for testosterone levels in subjects. A recent study demonstrated that even if total testosterone levels are normal, low free testosterone is associated with hypogonadal signs and symptoms. This suggests free testosterone might be a more accurate measure of androgen-related conditions as compared to total testosterone, although clinicians/researchers must make their own determination on which hormonal form they should be accessing [61].

Procedural-Analytical Factors

The second category of factors influencing hormonal measurements is made up of those factors that have procedural or analytical aspects to them. These factors are determined, selected, or in some way potentially controlled for by the investigators conducting or the participant involved with the research [1]. These factors can be viewed as exogenous relative to their influence.

Ambient Environment

When conducting research investigations, it is important to remember that excessive exposure to hot or cold ambient temperatures can stimulate the release of various hormones, e.g., those involved in water balance (aldosterone) or energy substrate mobilization (cortisol) [44, 62, 63]. Even elevated ambient relative humidity (water vapor) can induce this effect, primarily due to a compromised heat dissipation through reduced evaporative efficiency adding to the body core temperature [63]. These effects can be further augmented if hypoxemia is induced along with temperature extremes (e.g., mountain climbing), as can occur when moving to higher elevations and being exposed to greater degrees of hypoxia [64–66].

Many of the exercise and exercise training hormonal responses are tremendously impacted by environmental factors. In particular, catecholamines, growth hormone, aldosterone, arginine vasopressin, adrenocorticotropic hormone, and cortisol are all susceptible to changes in environmental conditions and show highly exacerbated responses in such varying conditions [1, 44, 62, 63].

To minimize these influences, it is critical to conduct exercise testing in controlled, standardized conditions such as in a laboratory. On the other hand, if conducting field research (where environmental standardization can be impossible), then it is important to measure/record environmental factors and convey them in any subsequent reporting of the data in the literature.

Nutrition

The nutritional status and practices of a research subject, including food composition, caloric intake, and timing of meals, can greatly impact the hormones associated with energy substrate mobilization and utilization (e.g., insulin, glucagon, epinephrine, growth hormone, insulin-like growth factor, cortisol) [1, 67, 68]. The exact nature of the effect (augmented or attenuation) depends on the interaction of the nutritional factors just mentioned and how severely the alterations are from the normal nutritional regimes of the individual [1, 33, 41].

The hormones noted above are critical during exercise to ensure that energy metabolism meets the demands of exercise. Thus, altered dietary practices and nutrition status of a subject can alter energy substrate (glycogen) storage and availability [68–70]. This in turn can cause the hormonal response to exercise to vary to some degree. For example, Galbo and associates demonstrated that the glucagon, epinephrine, growth hormone, and cortisol response to exercise were greater when a low-carbohydrate, high-fat diet is consumed (i.e., 4 days of consumption) compared to a normal mixed diet [67].

Normally in clinical settings, it is recommended that subjects be fasted prior to blood hormonal evaluations (e.g., 8 h). It is not always practical, however, for athletes to comply with such request due to their high demand for adequate caloric intake to maintain energy balance, anabolism, and muscle glycogen reserves. Therefore, a modified fasted approach may be necessary for this special population such as only a 4–6-h fast. Even with the constraints of working around an athlete's special needs, it is still advisable that exercise investigators try to control and standardize the dietary practices of their subjects as much as possible to mitigate the effects of differing diet between subjects, and within an individual subject's diet, if a repeated measures research design is being used [41, 67].

Nutrient Timing

The concept of nutrient timing is arguably one of the most important aspects to account for when designing a study and evaluating results [71]. The evaluation of timing food consumption has been shown to influence muscle morphology outcomes directly and indirectly by stimulating hormone secretion [72]. As coined by Dr. John Ivy, the nutrient timing system accounts for three phases: the energy phase, anabolic phase, and growth phase [71]. Additional consideration should be given to the pre-exercise phase, which can largely influence the endocrine response during and

post-exercise. Although most research protocols hold diet constant, considerations for what the subject consumes before and after, *ad libitum*, may have substantial influences on acute and chronic adaptations, in part due to the stimulation of hormones.

Pre-exercise Cortisol levels, which help to maintain the integrity of the immune system, are strongly influenced by glucose availability [73–75]. Additionally, acute carbohydrate intake can stimulate an increase in insulin and glucose levels, sparing muscle glycogen as well as reducing cortisol levels. Acute consumption of a glucose-electrolyte solution (GES) prior to exercise has been shown to significantly reduce cortisol levels, when compared to water. Allowing a subject to consume a carbohydrate drink before testing, independent of amount (e.g., 25 g vs. 200 g), may significantly maintain glucose and cortisol levels post-exercise, as well as stabilizing the neutrophil to lymphocyte ratio [75]. Pre-exercise vitamin consumption, or an antioxidant enhanced beverage, may protect against acute tissue damage augmenting exercise adaptations and when consumed chronically may maintain immune system markers [76].

Anabolic Phase (During Exercise) Carbohydrate supplementation during exercise has also been associated with a blunted cortisol, growth hormone, and cytokine response while also maintaining glucose levels and insulin stability [77, 78]. There is additional evidence demonstrating reduced T cell and NK cell levels with carbohydrate feeding during exercise [77]. Acute, uncontrolled feedings should be accounted for when establishing a study design as well as potential confounders when interpreting immune function results. Protein consumption during exercise blunts protein degradation and has a sparing effect on muscle glycogen [72].

Growth Phase (Post-exercise) Immediate post-workout fuel consumption has the potential to highly influence muscle machinery by utilizing the anabolic characteristics of insulin. Additionally, an increase in insulin post-exercise can enhance muscle glycogen resynthesis and is enhanced with a protein/carbohydrate combination [72, 79]. A carbohydrate-amino acid supplement influenced testosterone and cortisol levels 120 min after intake and exercise [80]. However, post-exercise nutrient consumption consumed later following exercise (i.e., 8–9 h) has demonstrated no hormonal influence [81]. Intake of carbohydrates + protein + vitamins post-exercise has been shown to reduce free radicals and maintain immune function. This may be a consideration for researchers evaluating exercise and immunology characteristics, as well as various aspect of overtraining.

Meal Frequency and Patterning

Meal frequency and overall caloric consumption may also influence metabolic-hormonal markers, such as C-reactive protein, fasting plasma glucose, insulin, as well as total cholesterol [82, 83]. In as much, investigators may consider questioning participants about food consumption patterns or utilize a food frequency questionnaire.

Eating Disorders

The eating disorder “anorexia nervosa” is a special concern relative to nutrition status due to its profound effect on the endocrine system [1, 52, 84]. Anorexics tend to have lower resting luteinizing hormone, follicle-stimulating hormone, and estradiol-β-17 levels [84]. Anorexia also affects the pituitary-thyroid-glandular axis. Specifically, the condition is associated with suppression of triiodothyronine, somewhat decreased thyroxine, elevation of reverse triiodothyronine, and, occasionally, decreases in thyroid-stimulating hormone [84]. Such a thyroidal state is referred to as the “euthyroid sick syndrome” and can accompany severe body weight loss [3, 52, 84]. There is also an effect on the adrenocortical axis, with higher levels of cortisol due to an increased liberation of corticotropin-releasing hormone [84]. Growth hormone is also increased, although insulin-like growth factor-1 levels (which facilitate the physiological actions of growth hormone) are suppressed in the anorexia condition [84]. Due to the psychological aspects of the anorexia

nervosa (see Refs. [85, 86]), this condition could, in the context of the organization of this chapter, be also discussed with mental health issues. Thus this factor could also be considered of a biological nature and consequently has powerful effects on a multitude of endocrine measurements.

Stress-Sleep

Emotional stress and/or sleep deprivation are each known to affect certain hormones within the endocrine system. For example, emotionally distraught individuals will typically have elevated basal catecholamine, growth hormone, cortisol, and prolactin levels [1, 87–89]. Those hormones with circadian patterns (see Circadian Rhythm section; e.g., luteinizing hormone, follicle-stimulating hormone, adrenocorticotropic hormone, cortisol) can be shifted in their characteristic pattern-rhythm by disruption of sleep cycles [43, 46, 87–91].

These types of factors (i.e., stress, sleep deprivation) can also influence the hormonal response to exercise and exercise training. Investigators must attempt to control these factors whenever possible. In fact, it is advisable to have a pre-exercise questionnaire completed by a subject to monitor and evaluate the level of these factors, and if a predetermined status is not obtained, then hormonal measures and exercise testing should be rescheduled.

As a footnote to this issue, many investigations in the exercise area use college students as research subjects. Such students can have high levels of emotional stress due to their education demands (e.g., examination periods, projects being due, oral reports). Care should be taken to not utilize student subjects when there are in high emotion stress periods as a multitude of hormones can potentially have very atypical values and responses [43].

Physical Activity

The proximity in time between exercise sessions can affect the hormonal profiles of individuals [92, 93]. If inadequate amounts of time have elapsed (lack of recovery), some hormonal responses at rest, or in the subsequent exercise testing, can be attenuated and others augmented. Furthermore, the magnitude of this effect can be influenced by the exertion required of the prior exercise (e.g., high-intensity intervals require longer recovery).

If possible, researchers may require a 24-h recovery prior to a subject reporting to the laboratory for testing. However, subjects who are athletes may find it difficult to reduce their training or miss a workout session for experimental purposes in research studies. A modified approach may be necessary, such as only a 12- or 8-h recovery period, because this could somewhat prevent stress and anxiety (which as noted can affect the endocrine system) in the athlete since they would be missing less training time [1, 93–95].

A powerful influence on resting and exercise hormonal response of a subject is the exercise training status—that is, trained vs. sedentary. The more "trained" a subject is, typically the greater the effect on the neuroendocrine system. Many hormones show attenuated resting and submaximal exercise responses in trained individuals, although some can actually be augmented (e.g., testosterone in resistance-trained individuals) in response to submaximal and maximal exercise [2, 96–100].

Besides acute effects of physical activity on hormonal levels, chronic endurance exercise such as distance running, cycling, race walking, and triathlon has been shown to put immense stress on the endocrine system, many times resulting in the suppression of some hormones [9]. This phenomenon may be related to the "overtraining syndrome" (see Chap. 27 of this book). The exact mechanism of hormonal reduction following chronic strenuous endurance exercise is not elucidated yet; however an impairment of the hypothalamic-pituitary regulatory axis due to energy decreases is postulated by some researchers [101].

An extensive dialogue on the influence of exercise training on hormonal profiles at rest and in response to exercise is beyond the scope of this

chapter, but the reader is directed to Refs. [2, 3] for more in-depth discussions.

Subject Posture-Position

There are changes in the plasma volume component of the blood as a subject changes position. Standing upright results in a reduction of plasma volume compared to a recumbent position [102]. These shifts in the plasma fluid are in response to gravitation effects as well as alterations in capillary filtration and osmotic pressures [102]. Large molecular size hormones, or ones bound to large weight carrier proteins, could be trapped in the vascular spaces; this means that a loss of plasma fluid would increase the concentration of these hormones (hemoconcentration). Conversely, a gain of plasma fluid would decrease the concentration of these hormones (hemodilution) [44, 103]. These adjustments in fluid volume to move in or out of the vascular space due to posture shifts typically require approximately 10–30 min [102, 103].

In exercise research situations where blood is drawn to assess hormones, it is recommended that the condition of specimen collection related to the subject's position be controlled and reported in publications. This type of information is most certainly necessary if a postural change is occurring for a 10-min or greater duration [58, 103].

Specimen Collection

Suitable precautions must be taken in the collection and storage of blood specimens to ensure they are viable for later hormonal analysis. In clinical and exercise-related blood work, venous blood is the specimen usually utilized. If the blood specimen is being obtained by venipuncture, it is important to not have the tourniquet on the subject's arm too long (~1 min or longer). Greater lengths of time can result in fluid movement from the vascular bed due to increased hydrostatic pressures [103]. Once collected, the blood sample should be centrifuged at ~ 4 °C in order to separate the plasma (collection tube contains anticoagulant) or allowed to clot (collection tube is sterile) then centrifuged for serum. If centrifugation cannot be done immediately, then the blood sample should be placed on ice, but it is more prudent to centrifuge without delay. Once separated, the plasma/serum should be aliquoted and stored at a temperature of −20 to −80 °C until later analysis. Care should be given to ensure certain plasma/serum is stored in airtight cryofreeze tubes (screw-cap type is recommended), which allow for a longer storage period. It is also advisable to split up specimens into several aliquots if multiple hormonal analyses are going to be conducted. Once a sample is thawed, it has a relatively short "shelf life" in a refrigerator, and repeated unthawing and refreezing cycles can degrade certain hormonal constituents and compromise the validity of the analysis [104–106]. Care should be taken to ensure that the assay procedures employed are specific for plasma or serum, as in some cases these cannot be used interchangeably in the assay (e.g., adrenocorticotropic hormone is measured in plasma). Furthermore, an examination of the research literature may be necessary to determine if one form of blood component is more popular or prevalently used in research.

In blood specimens, either plasma or serum is utilized for biochemical analysis, but some hormonal measures can also be made in urine and salivary samples. In general plasma and serum give very similar values for hormonal analytes, and seldom is one considered better than the other in blood analysis [105, 106]. Be aware, however, specific assay procedures do, in some situations, have a preferred blood fluid for analysis. Thus it is critical for the researcher to know what each hormonal assay requires as the analyte and then plan accordingly. This type of information is provided by the manufacturer of the analytical supplies-components used in the assay procedures.

With respect to urine and saliva, they are attractive as specimens to collect because of their noninvasive nature. They do, however, have cer-

tain drawbacks. Urine analysis tends to be limited primarily to steroid-based hormones, and there is usually a need to collect 24-h urine specimen. The collection of 24-h urine specimens can be a tedious and demanding process for the subject. Also, urine measurements may not always be reflective of "real-time" hormonal status either, as urine can sit in the bladder for hours before being voided. Saliva allows for easier sampling procedure and can reflect hormonal status in a more real-time fashion. However, saliva also primarily only allows for steroid hormonal assessments (i.e., constituents that can cross from the blood into the salivary gland) [107]. Furthermore, saliva is limited to free hormonal concentrations as the protein-bound constituents typically cannot pass through the salivary gland due to their large molecular size. Research does suggest that the blood and saliva levels of hormones can mirror each other in their relative changes, but not perfectly, as correlation coefficients of only 0.7–0.8 are typically found [1, 104, 107]. Researchers must determine if these limitations preclude the use of these biological fluids in their studies [104, 107–109].

Analytical Assays

A variety of biochemical analytical methods (i.e., "assays") exist for measuring hormones in biological specimens. Chromatographic, receptor, and immunological assays are all available. Perhaps the most prevalent contemporary technique in use is immunological assays, which have variations such as chemiluminescence immunoassay (CLIA), radioimmunoassays (RIA), enzyme immunoassays (EIA), enzyme-linked immunoassays (ELISA), and electrochemiluminescence immunoassays (ECLIA) [109–111]. Each of these techniques has its strengths and weaknesses, and the discussion of each is beyond the scope of this chapter, but the reader is directed to Refs. [112–114] for more background and explanation about this subject.

Researchers should always know the particular aspects of the hormonal assay techniques they plan to use in their studies. Specifically, it is important they be aware of the precision of the assay ("how accurate is it?"), sensitivity of the assay ("how small of a change can it detect?"), and the specificity of the assay ("how much cross-reactivity is there with similar looking chemical structures in the specimen?"). Ideally the researcher wants the most precise, highly sensitive, and specific assay they can obtain, but cost considerations can impact decision-making in these matters. It is advisable for the researcher to report precision, sensitivity, and cross-reactivity values in publications to allow readers to determine the quality of the analytical techniques and procedures of the assays that were used. Additionally, it is desirable to report in publications the coefficient of variation (CV) "within" an assay and "between" an assay for each respective hormone measured. This will allow the reader to determine how well the analytical technical procedures were carried out [114, 115]. One step to mitigate the potential between-assay CV is to collect and analyze your biological samples in batches of specimens and not as isolated specimens on a day-by-day basis. However, caution is necessary here as batches that are too large can influence your outcome by creating "end of run effects" within the assay. That is, running such a large number of samples in a single batch that the precision of the technician performing the assay may be compromised (i.e., procedural fatigue), or the kinetics of the specific assay may be influenced by the length of time it takes to pipette the various components in assay (i.e., in adding the chemical reagents to the first sample tubes vs. the last tubes; too much time has transpired, resulting in different lengths of time for chemical reactions to take place within the specimen tubes) [114, 115].

Data Transformations

Before conducting statistical analysis on hormonal data measured within the assays, it may be necessary to transform the data. Two of the most common endocrine transformations usually seen

in literature are (1) expressing the data as a percent change from some precondition (i.e., before exercise), basal value, and (2) conducting a logarithmic conversion of the data. The first is typically done to account for relative changes in hormonal concentrations when absolute magnitude of change may be misleading. For example, a cortisol change from 276 to 331 nmol/L is highly different from a 55–110 nmol/L (20% vs. 200%) even though the absolute magnitude is identical. A 200% increase in the hormonal concentration may have many more profound physiological effects than the smaller percentage. In the second form of transformation, logarithmic transformation is normally performed due to a large degree of variance in the subject data resulting in a nonnormal distribution. This can be due to sample size issues, variance with the analytical technique, or the physiological nature of the hormone being studied. Despite the transformation used, it is vital that the researcher report to the reader in the publication if and how the data were manipulated prior to conducting the statistical analysis (and what was the rationale for performing the transformation) [109, 116].

A third data transformation that is less frequently used is the area under-the-curve (AUC) procedure. This is carried out when there are serial specimen samples (repeated measures design) from a subject. These serial values are plotted, and then an integration of the area under the plotted responses curve is determined, thus collapsing numerous data values into one response and potentially eliminating some of the variability associated with having many hormonal measurements [117]. This approach is favored by some researchers; their rationale is the overall response of the hormone, and gland in question can be better quantified. Nonetheless, the procedure can be influenced by the number of serial samples collected to determine the response curve as well as the circadian rhythm of the hormonal release. The latter point results in the need for highly variable hormones (pulsatile) to be assessed using more frequent specimen sampling because misleading results can occur if the sampling is too infrequent [118].

Statistical Analysis

The statistical analytical procedures applied to any research study data are dictated by the design of that study. Most research in the exercise area tends to employee parametric analysis (e.g., *t*-test, one-way ANOVA, Pearson correlation). These analytical procedures work well with endocrine data, provided that the underlying assumptions for their use are not violated (see Ref. [119] for details). Furthermore, many North American journals prefer this form of analysis due to the robust nature of the techniques and the reduced likelihood of making a type I error (indicating findings are significant when they are in fact, not). Nevertheless, nonparametric analysis (e.g., Wilcoxon signed-rank test, Mann-Whitney *U* test, Friedman test) can be equally applicable for endocrine use when study designs are not excessively complex and sample sizes are relatively small [119]. It is important, however, to recognize the likelihood of increasing the occurrence of a type I error with small sample sizes. Regardless of whether parametric or nonparametric analyses are used, it is vital that the researcher report in a publication of their work what the specific statistical analysis being used is and what the rationale was for their usage [118–121].

Once assays are performed and statistical results are obtained, the researcher needs to try and understand their data in order to interpret the magnitude of treatment outcomes and physiological effects. In this interpretative process, many researchers focus intently only upon obtaining statistical significance, usually a probability level less than 0.05 ($p < 0.05$). Obtaining such significance is important; however, a key question that has to be addressed in the data is the issue of "statistical significance" vs. "practical (clinical) significance" for the hormonal findings. To address that question, the researcher must think about and take into account the smallest clinically important positive and negative response value levels of the effect being researched, that is, the smallest change value levels that matter. Studies can be statistically sig-

nificant yet largely insignificant clinically. It is important to note that large sample sizes can produce a statistically significant result even though there might be limited or no practical importance associated with the findings [122].

To this end, effect sizes (ES) are becoming an increasingly important index used to quantify the degree of practical significance of study results (see Ref. [123] for explanation to calculating the ES statistic). Once computed, the ES statistic can be a useful indicator of the practical-clinical importance of research results because it can be operationally defined; that is, it is possible to give the observed ES ratings such as "negligible-trivial," "moderate," or "important-very large" [124]. Such ratings allow the researcher to discern the form and quantity of significance they have obtained in their study findings. In addition, the ES statistic has two advantages over traditional statistical significance testing: (a) it is independent of the size of the sample, and (b) it is a scale-free index. Thus, ES can be uniformly interpreted in different studies regardless of the sample size and the original scales of the variables being examined [123, 124].

Summary and Conclusion

To conclude, over the last several decades, exercise researchers have steadily increased the number of studies conducted which have examined the hormones and the endocrine system. Unfortunately, not all investigators working in this area of research are entirely aware of the factors that must be accounted for, and controlled, in order to ensure that valid and accurate data are obtained. This chapter reviewed some of the key physiological and procedural-analytical factors that can confound endocrine data and add variance to hormonal findings and those steps to be taken to reduce the confounding factors. Implementation of these steps can greatly aid the researcher in the interpretation and understanding of their endocrine data and in turn make their research more scientifically sound.

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2 Endogenous Opiates and Exercise-Related Hypoalgesia

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Introduction (Endogenous Opiates)

Endogenous opiate-like substances were first discovered in the mid-1970s, when opioid receptors were identified and located within the brain and hypothalamus [135]. This led to the discovery that endogenous opioid-like molecules, enkephalins [69] and endorphins [9, 106], were produced within the CNS. Subsequently another class of opiate-like molecules known as dynorphins was identified within the body [14, 50]. The latest addition are nociception/orphanin FQ molecules which work on nociceptin opioid receptors (NOP) within the CNS and counteracts the analgesic effect of opiates. The endogenous opiates fall into four major classes of substances: *endorphins*, a peptide 31 amino acids in length; *enkephalins*, smaller peptide molecules that are five amino acids in length (denoted either as leu- or met-, based on the terminal carboxyl amino acid of the peptide); *dynorphins*, located in the posterior lobe of the pituitary gland [86, 107] and gastrointestinal tract [60] with a 13 amino acid length; and *nociception/orphanin FQ* molecules, a peptide of 17 amino acids, which binds to NOP receptors. Enkephalins were first noted in areas of the brain and parts of the endocrine system. The original studies noted that both endorphins and enkephalins were important regulators of pain [4, 106]. However, more recent studies have determined that enkephalins not only play an important role with pain regulation but affect cardiac function, cellular growth, immunity, ischemic tolerance, and certain behaviors. Various tissues (heart, smooth and skeletal muscle, kidney, and intestines) in animals and humans have recently been shown to have proenkephalin expression [26]. Recently, inflammatory cells were shown to produce and release these opiates, and endorphins seem to be involved not only in immune function [79, 81, 123], pain modulation [152], and the exercise pressor response [72, 125, 162] but also in metabolic control [71, 80, 110, 111, 171]. Therefore, numerous challenges remain to be clarified concerning the role of these endogenous opiates on these processes as they relate to exercise. This is especially true regarding the control of cellular functions not only under normal conditions but when acute and chronic exercise stress is imposed.

Beta-endorphins (βE) were first identified within specific brain regions and the hypothalamus and were found to bind to *mu*-opioid receptors

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(MOR). When MOR are activated there is a strong inhibition of acute pain [175]. βE within the circulation was first ascribed to βE release from the anterior pituitary gland after being activated by factors within the hypothalamus. These factors activate the anterior pituitary gland to synthesize the parent molecule pro-opiomelanocortin (PMOC) which can be cleaved into various active components, one of them being βE. POMC is also expressed in the arcuate nucleus as well as the nucleus of the solitary tract within the CNS. βE is therefore an important neurotransmitter within the brain and a neurohormone outside the CNS when released into the circulation, to act on *mu* receptors on target tissues throughout the body.

The molecule POMC, the precursor polypeptide for several factors that arise from the hypothalamus and the paraventricular nucleus (PVN) in the brain, can be stimulated by truncated active peptides. POMC has a section toward the C terminus known as β-lipotropin (1–89 amino acids) that is ultimately cleaved to β-lipotropin (1–56 amino acids) and βE (59–89 amino acids). Both βE and β-lipotropin molecules help to mobilize lipid molecules from adipose tissue. Originally the assays that were developed to measure these molecules did not effectively differentiate between β-lipotropin and βE, which were thus denoted as having both β-lipotropin/βE activities.

Neuroanatomical sites for opioid analgesia are present within the CNS and located on neurons within the dorsal root ganglia (DRG) originating from peripheral somatosensory DRG neurons that can transmit these activated processes through the spinal cord to the medulla [5]. It should be noted that opioid receptors are expressed on these somatosensory neurons passing through the DRG and have been reported to have the ability to inhibit or reduce pain perception [16]. Both *mu* and *delta* opioid receptors are located on DRG neurons, and when opioids are activated, a depressed neuropeptide release from these afferents to CNS neurons occurs. Recent research has suggested that myelinated mechanosensory neurons appear to regulate DRG hypersensitivity and chronic inflammatory pain [4]. Peripheral agonists that do not cross the blood brain barrier can produce analgesia through the DRG [164]. However, research which used knockout deletion of *mu* receptors and DRG nociceptors in the periphery but with intact CNS receptors reported these peripheral receptors were not obligatory for analgesia [23]. It was suggested that these receptors could be involved with adverse side effects related to tolerance and opioid-induced hyperalgesia (OIH) with chronic agonist treatments. However, brain regions can also contribute to both tolerance and OIH. These peripheral sensory processes appear to activate important aspects to initiate or modulate CNS pain circuits [82] and may be activated with exercise [21, 22, 91].

In addition, there is evidence that serotonin release which alters behavior is modified by activated opioid receptors to influence γ-aminobutyric acid (GABA) involvement [38]. Additionally, interactions have been suggested with MOR with serotonergic structures involved with both reuptake and release of serotonin [159]. Furthermore, numerous non-opioid analgesics may influence both acute and chronic pain stress [59] and some are related to cannabinoid action [10, 24, 53, 67, 161]. Therefore, caution should be taken when considering the interpretation of changes which only measure opioid-like agents without assessing alternative pain influencing agents. There are many circuits that can influence pain or its attenuation.

There is limited information related to exercise and brain βE modulation [66, 138, 149]. βE immunoactivity in cerebrospinal fluid (CSF) of spontaneously hypertensive rats was shown to be significantly higher (about twofold) in runners (5–6 weeks) than in controls [66]. This study also reported that CSF βE was elevated up to 48 hours after cessation of voluntary wheel running. It was suggested that this βE effect may be at least partially responsible for the beneficial effect of exercise on controlling blood pressure [66]. βE immunoactivity taken from CSF in dogs was shown to increase with low-intensity exercise but not with high-intensity exercise [138]. In contrast, circulating βE immunoactivity increased in these dogs at both intensities of

exercise [138]. This indicates that the βE level within the brain is not reflected by the amount of βE within the circulation. Rat brain receptor binding of [3H]diprenorphine, a βE analog, was not significantly elevated 1 hour following a swim but was increased in several brain regions (5 of 6) 2 hours after exercise [149]. It is unclear if this was related to changes in βE concentration or a change in receptor availability. Pain threshold increase that occurred with exercise was abolished when naloxone (a receptor antagonist for βE) was injected into brain ventricles after 5 weeks of exercise training [155]. This suggests that the opioids were involved in elevating pain threshold in response to exercise training in these rats. Clearly more work is needed in this area. Specific brain areas that might be involved with BE and pain regulation in response to different types of exercise still needs further investigation.

βE within the circulation has been implicated in a number of processes including immune function, pain modulation, and assisting in glucose and lipid homeostasis. The major function of these endogenous opiate-like molecules was first identified as modulators of pain and euphoria based on the receptors they activated. As a result of this, the phenomena known as "runner's high," "second wind," and "exercise dependency" were postulated to be related to this endogenous activity.

This chapter will summarize what is currently known about the stimulation of these endogenous opiates in response to exercise or physical activity, and how exercise may induce exercise-induced hypoalgesia (EIH). The influence of an acute bout of exercise on the βE response will be presented first as these studies were the impetus of the original research. The influence of training on βE will then be discussed. Then the influence of an acute bout of exercise on enkephalins will be presented followed by training influences on enkephalins. The physiological mechanisms responsible for activation and secretion of these substances will be briefly discussed when known and related to functional outcomes when possible. Finally the effects of EIH will then be presented.

Influence of Acute Exercise on β-Endorphin Levels

The initial studies that were conducted to examine the impact of exercise on endogenous βE levels utilized various modes of exercise. The original articles examined various activities such as running at various distances to determine if blood βE level was elevated [15, 21, 25, 170]. These studies noted elevated βE after the exercise activities which led to more controlled experiments utilizing incremental graded exercise tests in laboratories to ascertain the βE response [48, 61, 62, 120, 130, 139]. These studies suggested that blood βE can increase from 1.5- to 7-fold following these graded exercise tests. The large variation in the βE response was in part attributed to procedural methods for the exercise tests as well as methods to determine βE and possibly related to the subjects utilized.

Aerobic Exercise at Work Intensities Related to Percentage of VO_2 Max on β-Endorphin

Several studies determined whether there was an exercise intensity effect on blood βE level. McMurray et al. [118] was one of the first researchers to examine the βE response to a specific exercise intensity. Donevan and Andrew [28] noted that βE did not increase after 8 minutes of cycling at 25% and 50% maximal oxygen uptake (VO_2 max) but increased after 75% VO_2 max after similar duration. They also reported a greater increase in βE at 95% VO_2 max. Goldfarb et al., in that same year, examined the effects of cycling at several intensities of exercise (60%, 70%, and 80% VO_2 max) to determine if there was a critical exercise intensity needed to induce circulating βE [46]. βE concentration increased in the two higher exercise intensities but not at 60% VO_2 max. The time course of βE changes at these exercise intensities up to 30 minutes of exercise was examined with βE increases occurring earlier with the highest exercise intensity (by 5 minutes). Research comparing 60% VO_2 max and 80% VO_2 max as well as self-paced running

for 30 minutes noted only an increase after the 80% run [33]; however, they utilized βE/B-lipotropin immunoreactivity. A run at 60% VO_2 max for 60 minutes induced no change in βE [103]. Exercise at 80% VO_2 max for 30 minutes with or without naloxone increased βE with a greater augmented increase with naloxone [1]. These studies taken together suggested that circulating βE increases with an appropriate minimal exercise intensity (>60% VO_2 max), but this was not always the case. The time course information also suggested that higher intensities of exercise would result in βE increases more rapidly [33, 35, 49, 61]. Later it was reported that gender did not influence the βE response to either 60% or 80% VO_2 max [32, 61, 65, 140].

It was noted that menstrual cycle had minimal effects on the exercise βE response in women [45, 49]. However, other factors might have differed which could have contributed to the discrepancy in the literature such as nutritional status of the individuals, time of day, immune function, and training status. Farrell et al. noted that βE+/β-lipotropin levels in well-trained endurance athletes only increased at 92% VO_2 max whereas lower intensities did not elicit significant increases [33].

Instead of a critical intensity relative to one's maximal aerobic capacity, other studies related the increase in circulating βE to lactate threshold [148]. They plotted the change in lactate with increased work intensity and compared the βE response. Incremental increases in exercise intensity elevated circulating βE levels and showed a similar pattern of change as blood lactate. However, it should be noted that these similar changes are only for short-duration incremental exercise. For activities with longer duration, the βE increase does not coincide with lactate changes [46]. In addition, other factors such as diet, training status, and immune function can influence the βE response.

High-Intensity Bouts with an Anaerobic Component

Short bouts of highly intensive exercise (anaerobic exercise), consisting of various types of exercise from a few seconds up to several minutes duration, can induce an increase of βE. A few studies reported that βE concentration in the circulation can increase about 2–4-fold above resting with these high-intensity anaerobic exercise bouts [35, 120, 139, 148]. Schwarz et al. noted a significant increase in blood catecholamines that correlated with the maximal lactate concentrations in response to exercise. Stimulation of the HPA axis through sympathetic activation appears to be related to the release of βE into the circulation.

Investigations of resistance exercise as a stimulus to augment circulating βE concentration in humans includes a limited number of published studies. Equivocal results have been reported, and this may be related to differences in subjects, type of exercise intensity, workload volume, and time of measurement. Typically the resistance exercise was related to the person's 1-repetition maximum (1-RM), i.e., maximum weight that was lifted or pushed/pulled by a subject with maximal effort. Often the load is referenced as a percentage of the 1-RM. Circulating βE level increased in response to high total workloads [97]. These authors suggested that the total work, rest to work ratio, and total force needed most likely influenced the βE response. An increase in βE in 28 elite male weight lifters was demonstrated after a moderate- to high-intensity workload [98]. An increase in βE level also occurred after three sets of work at 85% 1-RM in females but was significantly elevated (3.7-fold) only when these women were in a negative energy balance [172]. An increased βE/β lipotropin level was reported in response to weight lifting in five males [31].

In contrast, Kraemer et al. conducted a study using low-volume resistance exercise as a stimulus and reported no change in βE levels [95]. Furthermore, blood βE level based on immunoreactivity decreased after exercise compared to at rest in ten male and ten female college-aged students who performed three sets of eight repetitions at 80% 1-RM on four exercises [136]. This same group had reported earlier that resistance-trained subjects (N = 6) showed no change in blood βE level compared to baseline after three sets of eight repetitions at 80% 1-RM [137]. Both resistance exercise and treadmill

exercise were reported to significantly increase circulating βE/B-lipotropin immunoreactivity [31]. Unfortunately the intensity and volume of exercise was not available. McGowan et al., however, noted a decrease in βE concentration after exercise at 80% 1-RM in 20 college-aged subjects (both genders) [117]. It appears that resistance exercise of sufficient intensity and volume (workload) can result in a transient βE increases within the circulation in both men and women, but this finding is sometimes equivocal.

Influence of Training on β-Endorphin Levels

The training status of the individual can influence the response to exercise for a number of reasons. One reason is related to the relative intensity of the exercise. Well-trained athletes can typically perform at a greater absolute workload and usually would exercise at a higher relative workload compared to an untrained individual. Therefore, when comparing the βE response one should compare the absolute workload and the relative intensity. In addition, other factors might influence the secretion of βE such as the diet or immune function which can be influenced by training. Typically one would expect a downregulation on the secretion of βE to a similar absolute workload. However, there could be an upregulation of the capacity of the hypothalamic–pituitary–adrenal (HPA) axis in trained individuals. Finally, the amount of free hormone and the number of binding receptors could be modulated to influence action on target tissues.

Influence of Endurance Training

Resting levels of βE in endurance-trained individuals were reported to be lower [66] with the vast amount of studies reporting no change [45, 61, 62, 68]. The studies that reported no changes were mostly cross-sectional studies. In contrast, the study that reported lower levels used an endurance training program and compared the βE level before and after the training program at rest [109]. In contrast, Heitkamp reported that women who trained three times per week for 30 minutes each time at their individualized lactate threshold did not have changes in their resting βE [61]. Harber and associates compared normal eumenorrheic sedentary to eumenorrheic-trained and amenorrheic-trained women and reported that βE varied considerably, but there was no menstrual cycle effect at rest on βE [56]. They also noted that resting βE levels were higher in the trained women compared to the sedentary women. Goldfarb et al. reported a trend for lower βE levels during the luteal phase of the menstrual cycle compared with the follicular phase, but this did not reach significance [49]. They also noted no significant difference in βE at rest between men and women. Therefore, there is currently no consensus in the literature as to the effect of endurance training on resting βE levels.

The βE response during exercise is in slightly better agreement when exercise intensities were controlled. One early study reported a higher βE concentration after 4 months of aerobic training six times per week [15]. They reported that the βE level was higher cycling at 85% max heart rate (HR) than before training. This occurred after 2 months of training with no further changes. It should be noted, however, that to elicit a similar 85% max HR, the subjects worked at a greater absolute workload.

Most of the other studies have reported no detectable differences in trained and untrained subjects regardless of whether it was a cross-sectional design [47] or longitudinal design [12, 32, 61, 62, 68]. Goldfarb et al. compared untrained ($N = 6$) and trained cyclists ($N = 6$) that cycled for 30 minutes at 60%, 70%, and 80% VO_2 max with subjects randomly assigned in a counterbalanced order [47]. There was no difference in the βE concentration for the trained and untrained at similar relative workloads despite higher absolute workloads for the trained group. Both untrained and trained groups responded with higher βE levels at both 70% and 80% workloads compared to rest and the 60% workload. Heitkamp et al. reported that after training the βE response was comparable but was obtained at higher absolute workload for the trained subjects [61]. They also reported that after training the

recovery βE was lower suggesting faster removal of βE. Howlett et al. also reported no difference in βE concentration after endurance training at maximal workloads but met-enkephalin concentration was reduced after 4 months of training [68]. Bullen et al. reported greater peak βE/β--lipotropin post-exercise after 8 weeks of cycling training in seven women [12]. Engfred reported similar βE increases after 5 weeks of cycling training at 70% VO_2 max cycling to exhaustion [32]. VO_2 max increased 12% following 5 weeks of training so a higher absolute workload after training was utilized. In conclusion, it appears that blood βE concentration in trained individuals will be similar to concentrations before training if the workload is at the same relative intensity of aerobic capacity. This would require a higher absolute workload for the trained individual.

Influence of Resistance Training on Circulating β-Endorphin

Unfortunately there are few studies that have examined the influence of resistance training on circulating βE. There are no published studies found which indicate that βE concentration would change at rest or at any specific workload or a percentage (%) of one's maximal capacity with resistance training. Fry and coworkers reported similar βE concentration after both 4 and 9 weeks of resistance training to baseline levels [40]. It is important to note that most of the resistance research typically utilized resistance-trained subjects. As noted above, higher total work volume with resistance exercise resulted in greater increases in circulating βE [97].

β-Endorphin and the Immune System

βE within the circulation has been implicated in a number of processes including modulation of immune function, pain modulation, blood pressure regulation, and assisting in glucose homeostasis. βE receptors have been identified in many locations within the body including nerves, adipose tissue, pancreas, and skeletal muscle. However, the exact role(s) βE may have on these tissues is still being elucidated.

The influence of βE on immune function has been investigated in vitro but has not been adequately investigated in vivo. βE in rats and humans was shown to stimulate T lymphocyte proliferation [63]. The data suggests that βE mode of action was not though a MOR. It was shown that synthetic βE could bind to non-opioid receptors on T lymphocytes, and this binding was not blocked by naloxone or met-enkephalin [126].

In vitro βE stimulated rat spleen lymphocytes in a dose-dependent manner by enhancing the proliferative response to several mitogens [44]. This binding was not blocked by naloxone. βE enhanced the proliferative response of splenocytes on T-cells from adult male F344 rats [165]. In addition, naloxone was not effective in blocking the βE effect. βE stimulated the proliferative effect on human T lymphocytes using the mitogen concanavalin A [131]. This βE-stimulated mitogen response demonstrated a bell-shaped curve indicating that too high a dose would actually inhibit the response. It was suggested that this response may change with time, dose, or mitogen used [121]. These authors also reported that the inhibition of the immune response to cortisol maybe partially reversed by βE. Therefore, the activation of βE may inhibit suppression of the immune response by acting on cortisol actions in vivo.

The βE effect on to enhance natural killer (NK) cell function in vitro was reported to be a dose-dependent manner but was inhibited by naloxone [85]. This suggests that the mode of action on NK cells appears to be different than the enhancement of T lymphocyte function. The effect of βE concentration on NK cell activity (NKCA) and amount was examined after exercise [43]. Naltrexone treatment administered 60 minutes before a run at 65% VO_2 max which elevated blood βE levels at 90 and 120 minutes did not alter the exercise response in NKCA or amount. These authors suggested that βE may work independent of the MOR action to assist NKCA [79]. Chronic exercise (wheel running

for 5 weeks) in spontaneously hypertensive rats enhanced NKCA. The βE levels in CSF increased after the running and enhanced lymphoma cell clearance from the lungs. The delta-receptor antagonist naltrindole significantly but not completely inhibited the enhanced NKCA after 5 weeks of exercise. Neither α nor β receptor antagonists influenced the NKCA. These authors suggested that the endurance training mediated central receptor-mediated adaptations. However, if βE levels increased in the periphery via subcutaneous administration, this did not alter NKCA in vivo [79]. In contrast, NKCA after central injection of a delta opioid receptor agonist was depressed [3]. In addition, a single injection of a mu agonist into the intracerebral ventricle reduced NKCA activity. Furthermore, a single morphine injection into the periaqueductal area suppressed NKCA [173]. These findings suggest that central-mediated βE levels may act to modulate NKCA via both delta and mu receptors. Clearly more research with human models is needed, but this may be difficult as most of these actions appear to be centrally mediated.

Additional βE modes of action on the immune response include mononuclear cell chemotaxis [133, 166], immunoglobulin migration [146, 166], and lymphokine production [166]. Macrophages showed migration to βE levels injected into the cerebral ventricles in rats [166]. Human neutrophils demonstrated enhanced migration to β receptors when βE was infused, and this response was blocked by prior incubation with naloxone. Analogs of opioids appear to have different responses when injected into the cerebral ventricles [146]. Some may stimulate macrophages, and others may influence neutrophils. The chemotaxis response appears to be dose-dependent [133]. High doses of βE (10^{-3} M) inhibited the chemotaxis response whereas low concentrations stimulated upregulation of neutrophils. Since physiological βE concentration is below the high-dose level utilized even when elevated by exercise or other stressors, it is likely that βE at these low levels provides a stimulatory effect on this aspect of the immune system. It was also noted that endogenous opioids which may be elevated with exercise training induce a secondary antibody response in mice [83].

It was postulated that the opioid peptides such as βE and the enkephalins have a similar structural component to that of interleukin-2 [76]. Interleukin-2 and other interleukins are involved in the inflammatory response and are targets of βE levels and cortisol. It is highly likely that both βE levels and cortisol influence immune responses by interacting with interleukins [174]. The inhibitory response may act at a number of levels including the attenuation of production of both interleukin-1 and interleukin-6 in a dose-dependent manner.

It appears that βE may act on a number of immune factors both centrally and peripherally and may act through both opioid and non-opioid receptors. Additionally, βE action may work through direct inhibition of cortisol. Both βE and cortisol influence immune function with βE generally enhancing immune function and cortisol acting as an immunosuppressant. The interplay of βE and cortisol in regulating immune function in response to both acute and chronic exercise requires more research to clarify their contributions. Training adaptation effects also need further study. In addition, nutritional factors (i.e., carbohydrate level) have not been adequately examined in relation to both βE and cortisol influence on immune responses with exercise. It was reported that βE increased to a similar level after cycling to exhaustion (90% VO_2 max after cycling for 60 minutes at 65% VO_2 max) independent of a high or a low glycemic diet or placebo prior to the exercise [72]. More studies are needed to clarify the role of diet on the βE response to exercise.

Endogenous Opioids and Pain Perception

There are numerous citations that have implicated endogenous opioids and pain perception. A good number of these have suggested that endogenous opioids are involved in the processes of myocardial ischemia and or angina [74, 156]. It was reported that endorphins could modulate

adenosine-provoked angina pectoris-like pain in a dose-dependent manner in seven healthy subjects [156]. In contrast, met-enkephalin had no apparent effect on the pain. There may be a gender difference as angina pectoris pain induced by adenosine was attenuated by βE in males (both healthy and with coronary artery disease), but βE infusion did not modulate the pain nor did naloxone in females [144]. Increased plasma concentrations of βE were shown to alter peripheral pain threshold but did not alter angina threshold in patients with stable angina pectoris [74]. Therefore, peripheral pain may be influenced by βE, and the βE level may in part manifest some alteration in pain threshold. However, it is more likely that peripheral nerves which contain βE and/or immunocytes which release βE are involved with altering pain perception and reduction of damage [124].

Several studies have reported that exercise can modulate pain perception, and this has been attributed to endogenous opioids. Both acute and chronic exercise was reported to significantly enhance MOR expression in the hippocampal formation [27]. However, acute and chronic exercise had no significant effect on MOR expression in trained rats. Immunohistochemical techniques showed a higher number of MOR-positive cells after acute exercise compared to a control group. These authors noted that both acute and chronic exercise modulate MOR expression in the hippocampus region of rats. Higher pain thresholds for pain were reported in individuals who exercised for both finger and dental pulp stimulations [29]. Plasma βE levels increased after exercise to exhaustion as did cortisol and catecholamines, but pain threshold level changes did not correlate with plasma βE. Furthermore, naloxone failed to affect pain thresholds, despite the fact that with naloxone and exercise, βE levels increased to a greater extent. These authors suggested that the pain-related changes with exercise were not directly related to plasma βE. Janal et al. reported that after a 6.3 mile run at 85% VO_2 max, hypoanalgesic effects to thermal, ischemic, and cold-pressor pain occurred, together with elevated mood [73]. In this study, naloxone infusion partially inhibited some of the pain and mood effects with the exercise. This suggests that exercise can modulate pain, and it appears it is related to βE but may not be related to the plasma βE concentration.

Perception of pain in trained men (N = 17) after a run (12 minute for maximal distance) with either placebo or with naloxone was examined [134]. Post-exercise βE levels increased to a similar extent for both trials, but pain level was greater with the naloxone treatment. These authors concluded that the perception of pain associated with exhaustive exercise may be related to endogenous opiates, but this had no effect on performance. Low-intensity exercise reversed muscle pain in rats, and this was blocked by naloxone [6]. Microinjections of opiates into the periaqueductal gray matter in the brain of rats attenuated pain symptoms [152]. It was found that systemic and supraspinal opiates could suppress pain in rats [106]. These studies clearly suggest that pain can be altered by opiates and that exercise can modify pain; however, the alteration in pain does not appear to be related to circulating βE.

Neuropathy-induced mechanical hypersensitivity occurred in wild-type mice subjected to a chronic constriction injury of the sciatic nerve [102]. It was reported that T lymphocytes infiltrating the injury site (11% of total immune cells) released βE. Corticotropin-releasing factor (CRF) was applied at the injured nerve site and fully reversed the hypersensitivity. These authors suggested that the T lymphocytes which contain βE are crucial for not only immune function but also altered pain with peripheral nerves.

It is now clear that βE is found in parts of the immune system and can act both centrally and peripherally to help modulate pain. It is unclear how these different areas in the body respond to both acute and chronic exercise, but it appears that βE are involved. Part of the modulation of pain perception is clearly related to MOR within the brain, and more research is needed to understand the effects of both acute and chronic exercise on these receptors. In addition, circulating βE may increase, but this may not always be related to pain modification, and naloxone may not always block this effect. Therefore, the

peripheral-mediated βE effect on pain thresholds may not be related to the MOR in the periphery.

β-Endorphin and Glucoregulation

The opioid system has been implicated in the control of blood glucose concentration during rest [36, 142] and exercise [37, 71, 72]. βE and opiate receptors have been isolated from sites that are involved in glucoregulation [173]. Additionally, it has been reported that βE appears to play a role in metabolic regulation during exercise or muscle contraction [132, 137]. A bolus injection of βE followed by intravenous infusion of βE in rats raised βE levels 6–7-fold and resulted in higher plasma glucose levels at 60 and 90 minutes of exercise compared to saline infusion [37]. Lower insulin and higher glucagon levels were evident compared to saline infused rats at these times. Additionally βE exerts an effect on insulin and glucagon at rest [36, 132] in humans and animals. βE infusion without a bolus infusion of βE compared to saline infusion enhanced glucose homeostasis and exacerbated the glucagon rise in rats that were exercised [71]. This study reported that βE infusion independent of a βE bolus during exercise can attenuate blood glucose decline and increase glucagon levels in response to exercise. Additionally, βE infusion alone did not alter insulin, catecholamines, corticosterone, or FFA's response during exercise. It appears that βE infusion alone at a level to increase circulating βE at 2.5-fold greater than normal level does not inhibit insulin; however, if the βE level increased to greater than 2.5-fold (infusion and/or increase by exercise), inhibition of insulin occurred possibly related to help maintain blood glucose.

Influence of Acute Exercise on Enkephalins

There is some evidence that exercise can increase enkephalin concentration and or opioid receptor numbers in the brain [18, 27]. These alterations in the brain have been linked to changes in mood state [46], control of exercise blood pressure [7, 70, 75, 125], cardiac ischemia and angina [156], pain [154, 156], and immune function [13]. However, some of the actions of these opioid molecules may manifest themselves in other compartments such as vascular control. Research is unfolding regarding the actions of these enkephalins and enkephalin-like molecules. For example, proenkephalin peptide F which is primarily released from the adrenal gland and co-released with epinephrine has immune-modulating functions [13, 81, 160].

Met-enkephalin level was unchanged after a Nordic ski race determined in both highly trained (N = 11, 150 km/week with greater than 3 years of experience) or recreationally trained (N = 6, 20 km/week with no competitive experience) skiers [122]. The distance covered was 75.7 km, and subjects were allowed to have water and food ad libitum. Met-enkephalin plasma concentration was determined at rest prior to a graded treadmill exercise to exhaustion and after a run of 87.2 km (5 minutes post exercise). The basal level of enkephalin was 171.7 ± 7.16 fmol/mL and increased after the treadmill exercise to 265.8 ± 9.88 fmol/mL with a further increase after the run to 378.3 ± 15.16 fmol/mL. The authors suggested that the increase in met-enkephalin in plasma may be related to intensity and duration of exercise [153]. The same authors compared unfit (N = 24) and fit (N = 23) subjects exposed to a graded intensity treadmill run to exhaustion (4 minute stages of at least five stages). Plasma Met-enkephalin concentration was lower for the unfit compared to the fit (126.3 ± 5.3 fmol/mL vs. 156.7 ± 6.9 fmol/mL). Both groups demonstrated increased plasma met-enkephalin after the exercise with the fit group showing a greater response (unfit = 180.4 ± 5.3 fmol/mL vs. fit = 278 ± 6.58 fmol/mL) [154]. In contrast, Boone et al. reported that met-enkephalin was no different in trained and untrained subjects following 4 minutes of exercise at 70% VO_2 max and 2 minutes at 120% VO_2 max [8]. These authors noted that cryptic met-enkephalin (activated) was elevated similarly in both groups after the 70% VO_2 max and returned to baseline levels at the higher workload.

The response to exercise in met-enkephalin concentration in the plasma from trained and untrained subjects was reported to be similar [75]. Subjects rested for at least 15 minutes prior to collection of a resting blood sample and then performed a graded treadmill protocol to maximum, after which another blood sample was attained. There was no difference in the met-enkephalin concentration in plasma, red cells, cytoplasm, or ghosts when comparing pre- to post-exercise in both trained and untrained groups. However, the degradation rate was slower in the trained group compared to the untrained group independent of time (pre- and post-exercise). The authors suggested this may facilitate opioid responses and could provide tolerance for trained subjects.

One of the early investigations in this area examined leu-enkephalin activity in plasma both before and after a competitive run [34]. Blood samples were obtained from experience runners (9 males; 5 females) before and after a 10 mile road race (2–8 minutes). Resting leu-enkephalin was 22.2 ± 13.7 pmol/mL and increased ($p > 0.05$) to 26.1 ± 21.5 pmol/mL, a modest increase. The leu-enkephalin change was inconsistent and variable among the runners.

In conclusion, the influence of exercise on met-enkephalin is variable and appears to depend on assay method. There is inconsistency in the results, as some studies suggest enhanced levels and others no change. There is insufficient data to suggest that aerobic capacity or fitness level alters met-enkephalin level. Additionally, leu-enkephalin research suggests a modest increase in blood concentration with large individual variation responses with limited research.

There is limited information on exercise training programs with enkephalins. Chen et al. examined acute and chronic exercise training effects on leu-enkephalin in the caudate-putamen of rat brains and compared the levels to sedentary control rats [18]. The trained rats exercised for 5 weeks on a motorized treadmill with a progressive overload in time and speed and ran 5–7 days per week. Staining of leu-enkephalin was primarily in the PVN and the caudate-putamen region (CPR). Acute exercise increased staining in the CPR region and remained elevated in this region for up to 180 minutes post-exercise with a gradual decrease over time [18]. These results suggest that there is a central-mediated enkephalin response influencing the brain to the acute exercise in these brain regions. It also suggests that this response is transitory and reverts back to normal over time. Unfortunately, this study did not include a sedentary acute exercise group to determine whether the endurance training elicited different results than an acute exercise bout.

There is also limited information with regard to the influence of exercise on proenkephalin peptide F that is typically released from the adrenal medulla and co-released with epinephrine [108]. The influence of exercise intensity and training was examined in college-aged students [100]. The trained subjects were middle-distance runners ($N = 10$) and were compared to untrained individuals ($N = 10$). The subjects exercised on a cycle ergometer for 8 minutes stages that elicited 28%, 54%, and 84% VO_2 max and then exercised to VO_2 max. Peptide F levels at rest were twice as high in the trained group compared to untrained but were very low (<0.1 pmol/mL). Neither group demonstrated any change in peptide F at the lowest workload, but there was a significant increase at 54% workload in the trained group. Peptide F stayed at a fairly constant concentration at the higher work intensities (~0.4 pmol/mL). In contrast, the untrained group demonstrated an increase in peptide F at 100% VO_2 max that was similar to the level of the trained group. It is interesting to note that the epinephrine level for both groups showed a similar response. This suggests that peptide F level may be related to other factors than its release and epinephrine level.

The effect of fitness and intensity of exercise was examined in women to see if peptide F levels might be altered differently in women [160]. Women who were endurance trained (>3 times per week, 30–45 minutes/session) were compared to inactive women. They were tested on a cycle ergometer at 60% and 80% VO_2 max (15 minutes at each workload) during the early follicular phase of the menstrual cycle. Blood was collected at rest and 10 minutes into each intensity. Only the fit women demonstrated a sig-

nificant increase in peptide F at the 80% intensity workload. However, this increase was modest (0.046–0.056 pmol/mL). In contrast, untrained women showed a greater epinephrine level compared to the fit women. This again suggests dissociation in the amount of epinephrine and peptide F within the circulation.

The menstrual cycle effect on peptide F to maximal exercise was reported in eumenorrheic women ($N = 8$) [99]. There appeared to be a slight but insignificant (0.06) effect of menstrual cycle on plasma peptide F level at rest. In addition, there was no exercise main effect on plasma peptide F levels. These results suggest there may be fluctuations in peptide F levels over time as well as over the course of the menstrual cycle. This also suggests that the changes in the previous study with lower peptide F levels may be an anomaly. Clearly, more research studies with exercise on peptide F levels. Furthermore, many of the variables that might influence baseline peptide F levels should be considered.

Exercise-Induced Hypoalgesia

Physical activity is known to be critical for health, longevity, and high quality of life [129] and is an effective treatment as well as prevention of certain diseases [19, 77, 110, 145]. Chronic exercise has been shown to increase coordination and aerobic fitness, decrease risk of various cardiovascular diseases [112, 150], and enhance body image, self-efficacy, and emotional stability while alleviating depression and reducing anxiety and stress [54]. In addition, research has also indicated health benefits associated with acute exercise. Some forms of acute exercise influence pain perception by decreasing pain sensitivity in healthy individuals following a bout of exercise [88, 128]. This decrease in pain sensitivity, which is known as exercise-induced hypoalgesia (EIH), occurs during and after higher intensities and longer periods of aerobic exercise [66, 89]. Recent evidence suggests that signaling molecules carried in the circulation or through nerves are important for exercise-induced hypoalgesia. Jones et al. measured pressure pain thresholds in subjects 5 minutes after high-intensity cycling with one arm occluded and the other with normal blood flow [78]. The investigators reported that a reduced EIH effect occurred in the occluded arm. This analgesic phenomenon is of great interest as exercise regimens are becoming the focal point of most pain management programs [141]. A review of the literature on EIH reveals that healthy individuals will demonstrate hypoalgesia following most modalities of exercise including aerobic, isometric, and dynamic resistance exercise [2, 124]. However, there are differences in the degree to which each modality alters pain perception. According to the results from a meta-analysis, aerobic exercise produces EIH in response to both pressure and thermal pain stimuli and seemed to be the strongest when performed at moderate-to-high intensity [124]. Isometric exercise produced the largest effect size of the modalities, and this was consistent regardless of pain stimulus and exercise intensity. There is a paucity of findings [2, 39, 90] on dynamic resistance exercise, and the effect sizes were large when pain was assessed immediately after exercise. While these studies have provided great insight into the effects of dynamic resistance exercise on EIH, there are a few key aspects of these studies that should be addressed to elucidate the effects of dynamic resistance exercise on pain perception. The first important discrepancy in research methodology is the inconsistencies in time points in which pain was assessed after exercise. Koltyn and Arbogast [90] measured pain perception at 5 and 15 minutes post-exercise, whereas Focht and Koltyn [39] assessed pain at 1 and 15 minutes time points, and Baiamonte et al. [2] utilized all three time points (1, 5, and 15 minutes post exercise). In addition, the exercise protocol implemented in each study varied in terms of sets, repetitions, intensity, and duration. Baiamonte et al. utilized 9 lifts and participants were required to perform three sets of 12 repetitions at 60% 1-RM for 45 minutes with a 1:1 work to rest ratio, while the previous two studies consisted of only four movements of three sets of 10 repetitions at 75% 1-RM for 45 minutes where the work to rest ratio was unclear but appears to be longer rest [2]. The structure of the resistance

exercise protocol probably influences pain perception and the mechanisms responsible for EIH. In summary, all three modalities of exercise produced moderate-to-large effects in healthy individuals depending on the protocol. The EIH effects were transient with optimal resistance exercise dose along with mechanisms responsible for this phenomenon still unclear.

Aerobic exercise and resistance exercise are known to elicit EIH for a brief period [2, 124]. Since aerobic exercise [46, 47, 48] and resistance exercise [56] of high enough intensity [46, 95, 96] are known to enhance circulating endogenous opioids, it has been speculated that EIH is due to pain modulating substances such as beta-endorphin [88, 90, 118, 127]; however, this has not been fully supported. While the EIH findings have been consistent for these exercise modalities at higher intensities in healthy participants, the evidence supporting the effectiveness of exercise on chronic pain patients is limited. The EIH findings in healthy individuals are more consistent when compared to EIH in chronic pain populations, which produced variable outcomes with small-to-large effects in individuals with regional chronic pain conditions [124]. This variability could be explained by the exercise intensity, location of chronic pain condition in relation to experimental pain induction site, and severity of chronic pain condition. Interestingly, there was no evidence of EIH in patients with widespread chronic pain conditions and at times, exercise at moderate-to-high intensity exacerbated the pain. Moreover, it has been suggested that greater sensitivity to pain in response to pressure in muscle after static contractions in patients with fibromyalgia [92, 93, 129] suggests that patients with fibromyalgia possibly have dysfunction of endogenous analgesia during exercise compared with reduced pain sensitivity in healthy patients during exercise [94]. However, an acute exercise session by women with chronic neck pain was shown to reduce pain intensity and sensitivity which was associated with greater circulating beta-endorphin and cortisol concentrations [84].

Electroacupuncture has been used to treat chronic pain and studies have reported that electroacupuncture at 2 Hz will enhance release of beta-endorphin and encephalin [55]. There is evidence that patients with chronic low back pain have greater activity in pain-related areas of the brain, whereas there is reduced activity in analgesic regions of the brain [101]. Recent evidence for fibromyalgia patients suggests that after ten treatments of transcranial direct current stimulation, pain was reduced, mood was improved, and these changes were related to circulating concentrations of beta-endorphin [87].

Chronic pain disorders such as lower back pain [167] and fibromyalgia [11, 114] are often treated with exercise therapy [127]. Patients with low back pain have significant pain reduction following treatments of aquatic exercise [151], and unsupervised, low volume trunk exercises have also been shown to reduce pain in these individuals [57]; however, patients with long-term whiplash disorder do not show reductions in long-term pain after exercise treatments [52].

Research has indicated that stimulation of afferent A-delta and C fibers via muscle contractions during exercise will activate spinal and supraspinal inhibitory signaling to dampen pain perception [91, 158]. Both animal and human studies have verified this mechanism, but findings have been controversial. Most studies have utilized administration of opioid antagonists (naltrexone or naloxone) prior to exercise which should bind to the mu-opioid receptors and theoretically prevent or reduce EIH. In both human and animal studies, the results were mixed with attenuation of EIH with opioid antagonist prior to exercise in some studies and insensitivity to opioid antagonist in another [88]. Researchers have suggested that the equivocal findings are due in part to methodological differences which resulted in different exercise intensity, duration, and variations in opioid antagonist administration [91]. In fact, previous research has revealed that manipulation of the exercise protocol in animal research produce differences in EIH following opioid antagonist administration [22]. Therefore, animal research has indicated that there may be multiple mechanisms (both opioid and non-opioid systems) involved in EIH [67, 91]. Researchers have suggested involvement of the endocannabinoid system in EIH due to the

presence of CB1 receptors in pain processing areas [53, 64, 161] and evidence of increased endocannabinoid concentration after exercise [24, 41–43, 91]. A recent study by Crombie et al. [24] demonstrated an interaction between opioid system and endocannabinoid system. When participants were administered naltrexone, the endocannabinoid 2-arachidonoylglycerol (2-AG) increased significantly following exercise. Even more interesting, the endocannabinoid N-arachidonoylethanolamine (AEA) did not increase following administration of naltrexone and exercise. Therefore, increases in AEA typically observed after exercise were blocked by administration of an opioid antagonist, which suggests an interaction between the two systems. The recent work with MOR inhibition resulting in decreased voluntary wheel running in rats suggests this signaling in a dopamine-dependent manner supports complex regulation of pain at multiple levels within the brain [143]. This study noted that an overlap may at least partially explain why some individuals sense pleasure with exercise and others may not. Future research should focus on the complex interplay between the opioid and non-opioid systems on EIH rather than concentrating on each system independently. Investigation into the interaction between these two systems and probably other pathways should provide further evidence of the multiple mechanisms involved in EIH. This should provide insights into more appropriate treatments for prescribing dose and exercise intensity needed to take advantage of all the physical and mental benefits that exercise has to offer besides just EIH.

β-Endorphin and Pain in Clinical Populations

There are a number of studies that investigated the effects of exercise on βE in different clinical populations affected by pain. Circulating concentrations of several neuropeptides, steroid hormones and metabolites were assessed after exercise to determine if women with chronic neck/shoulder pain responded differently than healthy women [84]. The investigators used microdialysis to analyze substance P, βE, cortisol, glutamate, lactate, and pyruvate before and after an exercise training regimen. They also assessed pain intensity and pain threshold. Before the training regimen, women with neck/shoulder pain had higher circulating levels of glutamate and βE and lower cortisol concentration than healthy women. Following exercise training program, women with shoulder/neck pain had less circulating substance P (and possibly glutamate) and greater circulating concentrations on βE and cortisol as well as reduced pain intensity and higher pain pressure thresholds. The researchers suggested that exercise training could alter pain intensity and sensitivity as well as peripheral substances related to pain. This study provides more suggestive effects of opioid-mediated pain modification following exercise training.

In a study conducted on coronary artery bypass graft surgery patients, transcutaneous electrical nerve stimulation (TENS) or sham TENS was applied over the posterior cervical region (C7-T4) to access the stellate ganglion region, 5 days after surgery [20]. The treatment was conducted four times per day for 30 minutes per session. Patients who had TENS treatment reported less postoperative pain and had less opiate requirements with higher circulating βE. They also had greater limb blood flow during a sympathetic stimulation (cold pressor) procedure. Thus, TENS, which elicits muscle contractions, appears to increase circulating βE which could lead to pain reduction.

Advanced Techniques to Investigate β-Endorphin and Pain

Thermal heat pain challenges were employed before and after running and walking trials to determine the effects of exercise intensity on pain [155]. The pattern of pain-related activity in response to heat/pain treatment using fMRI analysis was compared. The medial and lateral pain systems and periaqueductal gray (PAG) were key areas of the descending antinociceptive pathway that were evaluated. Running reduced affective

pain ratings whereas walking did not. fMRI revealed that there was a reduction in pain activation in the PAG with decreases after running but pain activation was elevated after walking. For the pregenual anterior cingulate cortex and middle insular cortex there were similar trends of activation for running vs. walking. Importantly, the authors concluded that increased circulating βE levels that were noted with running, but not walking suggested involvement of the opioidergic system. Another study which utilized fMRI examined pain modulation in athletes both before and after running or walking [147]. They examined both PAG and pain ratings and noted enhanced antinociceptive mechanisms were attenuated by running (23 km, HR = 148) but not walking (10 km, HR = 84). Elevated plasma beta-endorphins were reported only after running. These results support previous studies that indicated sufficient exercise intensity and duration are needed to influence blood beta-endorphin levels. A recent fMRI study reported that resistance exercise training (twice per week for 15 weeks) in fibromyalgia patients did not significantly alter distraction-induced analgesia nor influence brain activity [115]. This group previously reported the influence of resistance exercise training in a larger cohort of subjects [105].

Role of Enkephalins

It is possible that enkephalin peptides play a role in altering pain sensation. Exercise- or ischemia-induced enkephalin release from selected tissues was examined in rat, mouse, pig, and human tissues [26]. Using real-time PCR, Western blot analysis, ELISA, and immunofluorescence microscopy, they reported extensive expression of preproenkephalin mRNA as well as enkephalin precursor protein proenkephalin. Isolated ex vivo tissue that were analyzed revealed that skeletal muscle, heart muscle, and intestinal tissue released enkephalins. The investigators concluded that non-neuronal tissues could aid in inducing local and systemic enkephalin effects.

From a physiological standpoint, most researchers fail to agree on the exact mechanism(s) responsible for pain reduction following bouts of exercise. The most commonly proposed mechanism includes activation of the endogenous opioid system, in particular the release of βE with the CNS [66] and from muscle contractions during intense exercise stimulating pain receptors in skeletal muscle which can stimulate the endogenous opioid system [163]. However, previous research on humans and animals has been unclear regarding the involvement of the endogenous opioid system in EIH after opioid antagonist administration [91]. In animal studies, the opioid antagonists attenuated the hypoalgesic effect of exercise whereas human studies have produced conflicting results [91]. Therefore, further investigation into the role of endorphins in EIH is warranted to address the inconsistent findings in the literature.

Recent Pain Models

In a recent review, it was determined that increased pain threshold following exercise was attributed to release of endogenous opioids [127]. More specifically, EIH was demonstrated in healthy participants due to activation of μ-opioid receptors both peripherally and centrally. However, evidence of EIH in individuals with chronic pain has been equivocal. Research has indicated that exercise of many modalities can decrease pain symptoms, resulting in improved daily function for individuals who suffer from chronic pain [17, 30, 51, 58, 113, 116, 147]. In contrast, exercise may not produce pain facilitation in certain chronic pain groups [127]. For example, patients with fibromyalgia, whiplash, and chronic fatigue all demonstrate pain sensitivity following exercise [52, 104, 119, 168, 169]. Nijs et al. suggested that these patients had "dysfunctional endogenous analgesia" in response to exercise resulting in abnormalities in the central pain modulation system, which includes βE [127]. While this topic has been extensively investigated over the last 20 years, research has mainly focused on the hypoalgesic effects of exercise following a single episode of exercise as a result of increases in endogenous opioids [89].

Current research should focus on the effects of repeated exercise on chronic pain and attempt to discover the mechanisms responsible. It has been hypothesized that regular aerobic exercise leads to sustained reversal of neuropathic pain by activating endogenous opioid-mediated pain modulatory systems [157]. Following nerve ligation, rats displayed thermal and mechanical sensitivities that were attenuated within 3 weeks of exercise training [155]. However, hyperalgesia returned 5 days after cessation of exercise. These authors provided evidence of βE and met-enkephalin involvement and injected naltrexone into the intracerebroventricular region which reversed EIH. Recent studies have indicated increased βE and met-enkephalin in the medulla and periaqueductal gray area, regions of the brain that are also involved in the descending pain pathway.

Conclusion

Exercise of sufficient intensity and duration can induce transient pain modification, but more evidence is needed to substantiate the role of agents that are involved in triggering the mechanisms of action in EIH. βE can bind to various opioid receptors within the CNS and can modify pain. However, it is unclear if these actions are solely dependent to induce the EIH and if these actions reside exclusively within the CNS or are also triggered by agents outside the CNS such as signals arising from exercising muscles to help alleviate pain. In addition, not all individuals may respond in a similar manner, thus the proposed mechanisms explaining why EIH may work in some and not others, needs further clarification.

Summary

In conclusion, exercise of sufficient intensity and duration may influence the endogenous opioids, but what is measured in the circulation does not necessarily reflect what occurs within the brain. Numerous factors such as sex, menstrual cycle, diet, plasma volume, carbohydrate level, and inflammation can influence the endogenous opioids. Furthermore, immune function and neural control can clearly alter endogenous opioid activity. Finally, a greater understanding of the influence of exercise on the endogenous opioid effects in the brain needs to be established.

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3 The Effect of Exercise on the Hypothalamic-Pituitary-Adrenal Axis

David H. St-Pierre and Denis Richard

The HPA Axis

Introduction

Over the last decades, important discoveries have allowed exercise science to bloom as a research field. Practical applications in kinesiology influence a wide range of populations including individuals with diverse degrees of disabilities to high-performance athletes. Important advances include the optimization of training techniques, biomechanics, motor skills, periodization, and injury prevention. Sports psychology is another emerging discipline recognized to have a profound impact on active individuals in terms of adherence and compliance to a training program as well as physical improvements and raw performance. As for injury prevention, it is now generally accepted that physical activity must be performed in an equilibrated way in order to maximize the desire to pursue while reducing the risks of nonadherence, of non-compliance, and of developing psychological disorders. Since its discovery, the hypothalamic-pituitary-adrenal (HPA) axis was shown to play a major role in the control of anxiogenic and depressive behaviors. A growing evidence indicate that exercise exerts acute and chronic effects on the HPA axis. However, the mechanisms through which it influences the HPA axis, and vice versa, remain to be clarified. To add to the complexity, a wide range of HPA axis responses are reported in different populations. These are generally proposed to depend on the type of physical activity, the intensity, and the volume at which it is achieved. Hence, overtraining and the dynamic progression of performance could also influence the relationship between exercise and the HPA axis. The present chapter will review the current state of knowledge to clarify how exercise influences the HPA axis.

Defining the HPA Axis

The HPA axis consists of three structurally independent components including the hypothalamus, the anterior pituitary, and the adrenal cortex (see Fig. 3.1). These structures are intimately interacting through the release of neuroendocrine messengers. In the medial parvocellular and the magnocellular parts of the paraventricular nucleus of the hypothalamus (PVH), corticotropin-releasing factor [CRF, a 41-amino acid (aa) peptide] and arginine vasopressin (AVP, expressed in approximately half of the CRF neurons) are synthesized [1]. CRF neurons project

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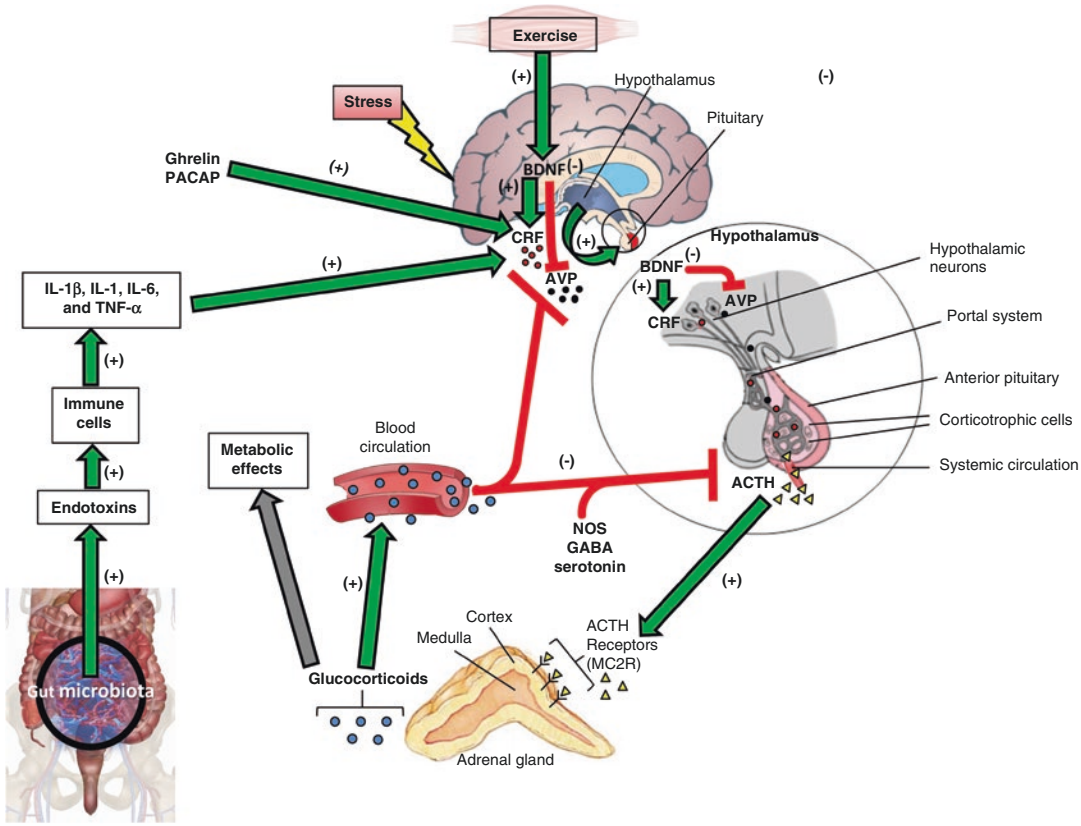

Fig. 3.1 The three structurally independent components of the hypothalamic-pituitary-adrenal (HPA) which include the hypothalamus, the anterior pituitary, and the adrenal cortex

to the exterior layer of the median eminence and release CRF into the portal circulation until they subsequently reach corticotroph cells from the anterior pituitary to stimulate the secretion of adrenocorticotropic hormone (ACTH). In turn, ACTH is released and transported via the general circulation to activate the adrenal secretion of glucocorticoids. Importantly, it is known that glucocorticoids negatively control pituitary corticotrophs and PVH CRF neurons through direct or hippocampus-mediated feedback inhibition mechanisms [2, 3].

In mammals, the CRF system is not limited to PVH CRF neurons. The system also comprises two CRF receptor types (CRF-R1 and CRF-R2) [4], a CRF-binding protein [5] and endogenous CRF receptor ligands, that include mammalian peptides CRF [6], urocortin (UCN) [7], UCN II [8, 9], and UCN III [9, 10]. In the brain, the broad distribution of CRFergic cells, UCNergic neurons, and CRF receptors is compatible with the main functions attributed to the CRF system [11]. Central administration of CRF evokes autonomic responses [2, 3], general arousal [12], as well as anxiety-like behaviors [3, 13]. Furthermore, central CRF injections also activate the sympathetic while inhibiting the parasympathetic branches of the autonomic nervous system by stimulating cardiorespiratory functions [14] and reducing the activity of the digestive system [15]. Because of their selectivity for CRF-R2, UCN II and UCN III [10] (also referred to stresscopin in humans) have been described as "stress-coping" peptides capable of exerting anxiolytic effects [9].

AVP is a 9-amino acid (aa) peptide with a disulfide bridge that is mainly secreted from the magnocellular cells of the supraoptic nucleus and the PVH and transported to the circulation to

exert its effects on kidneys and blood vessels [16, 17]. In addition, AVP's expression is also reported in the parvocellular neurons of the bed nucleus of the stria terminalis, the medial amygdala, the suprachiasmatic nucleus, and the PVH [18–20]. Three major types of AVP receptors are known: AVPR1a, AVPR1b, and AVPR2 [21, 22]. The activation of AVPR1b in the anterior pituitary stimulates the release of ACTH [23], while AVPR1a and AVPR2 are mainly expressed in the kidneys and blood vessels [24].

ACTH is a 39 aa peptide derived from the proteolytic cleavage of the proopiomelanocortin (POMC) gene [25–27]. The expression of ACTH is modulated positively by CRF and AVP, naloxone, interleukins (IL) IL-1 and IL-6, as well as leukemia inhibitory factor (LIF), but negatively by glucocorticoids [28–32]. However, other factors such as pituitary adenylate cyclase-activating peptide (PACAP), catecholamines, ghrelin, nitric oxide synthase (NOS), dihydroxyphenylalanine (DOPA), serotonin, and γ-aminobutyric acid (GABA) are also suspected to influence ACTH secretion through still ill-defined mechanisms [33–35]. ACTH is released in a pulsatile manner and has been shown to be regulated through a calcium-dependent mechanism [36]. It is subsequently transported in the circulation to activate the melanocortin type 2 receptor (MC2R) from the adrenal glands [37, 38] and, ultimately, stimulate species-specific glucocorticoid (either cortisol in human, nonhuman primates, pigs, and dogs or corticosterone in laboratory rodents such as rats and mice) synthesis and secretion [39]. In a matter of seconds to minutes, the release of glucocorticoids from adrenal glands will activate glucocorticoid receptors (GR), stimulate annexin 1 (ANXA1) production, and, consequently, block CRF-induced ACTH secretion [40, 41]. It is however suggested that the level of complexity of the direct and indirect mechanisms through which glucocorticoids exert their repressive effects on the HPA is much higher than what was anticipated during the 1980s [1].

Other mediators of the HPA axis were identified over the last decades. For instance, the gut microbiota is now proposed to influence anxiogenic and depressive behaviors via its effects on the HPA axis. Germ-free (absence of gut microbiota) chronically restrained mice display anti-anxiety behaviors but increased CRF, ACTH, cortisol, and aldosterone levels in hypothalamic tissues compared to specific pathogen-free microbiota mice [42–45]. Although the microbial mechanisms influencing these effects remain ill-defined, it is proposed to regulate glucocorticoid receptor sensitivity (Fkbp5), steroidogenesis (MC2R, StaR, Cyp11a1), and catecholamine synthesis (TH, PNMT) [46]. Hence, colon expression of 11-β hydroxysteroid dehydrogenase 1 (11HSD-1), CRF, urocortin II and its receptor, and CRFR2 as well as cytokines TNFα, INFγ, IL-4, IL-5, IL-6, IL-10, IL-13, and IL-17 is also reported to be modulated by the microbiota.

As recently evidenced, there is an intimate link between the regulation of the HPA axis and inflammatory cytokines [47]. For instance, interleukin 1β (IL-1β) is reported to influence the release of CRF in the hypothalamus, ACTH in the pituitary, and glucocorticoids in the adrenal cortex [48–53]. It was also reviewed that IL-6 and TNF-α promote the activation of the HPA axis [54]. Some of these effects are mediated through the activation of cyclooxygenase enzymes (prostaglandins) as well as by brain nitric oxide, noradrenaline, and serotonin production [55]. Interestingly, the translocation of endotoxins (derived from Gram-negative microbial components such as lipopolysaccharides/LPS and others) was previously shown to activate the HPA axis through the release of IL-1, IL-6, and TNF-α [56]. This reinforces the existence of an intimate relation between the gut (and the microbiota) and the brain for the regulation of the HPA axis.

Brain-derived neurotrophic factor (BDNF) is another factor with an influence on the HPA axis. For example, a single bout of exercise was shown to stimulate hippocampal BDNF expression in mice [57]. In humans, carriers of the Val66Met BDNF allele (prevalence of up to 50% and 32% in Asians and Caucasians, respectively [58]) were shown to display increased HPA axis activity through a higher cortisol response to stress [59, 60]. Expression of BDNF is co-localized with CRF and AVP in the PVH and the lateral

ventricle [61]. Hence, BDNF administrations increased the expression of CRF while exerting the opposite effect on AVP in the parvocellular and magnocellular PVH portions. Hence this treatment was likely to promote CRF secretion since its levels were decreased, while those of AVP were higher in the hypothalamus. This hypothesis is supported by the fact that the administration of BDNF also upregulated ACTH and corticosterone plasma concentrations.

The HPA Axis and Exercise

Endurance Training

The effect of endurance training on the activation of the HPA axis has been investigated extensively in animal and human models. In pigs submitted to a high-fat diet, a 200% increase in free fatty acid (FFA) levels is related to a 40% decrease in ACTH concentrations in response to stress [62]. In the same study, pigs submitted to an endurance training program displayed a 60% increase in ACTH following a stress challenge; this effect was associated with a 56% decrease in FFA without other changes in body composition and insulin sensitivity. In another study, rats confined to a cage that allowed voluntary wheel running, corticosterone responses to various stimulatory challenges of the HPA axis were shown to be significantly higher than in untrained animals [63, 64]. Interestingly, this enhanced adrenal sensitivity to ACTH was completely restored to normal following 5–8 weeks of exercise training. In an ovine model, ACTH levels were found to rise in response to exercise, even though the animals had been previously submitted to a CRF infusion [65]. The latter suggests that ACTH release could be stimulated by other factor than CRF, and the authors suggested AVP as a plausible candidate. Endurance training upregulated mRNA expression of BDNF and its receptor TrkB in the hippocampus, midbrain, and striatum while increasing BDNF levels in the hippocampus and striatum in rats [66]. On the other hand, sprint interval training was more effective to enhance BDNF brain content than intensive endurance training in rats [67]. These increased BDNF levels in the brain were also shown to be associated with reduced anxiety- and depression-like behaviors in tested animals.

In human studies, the activation of the HPA axis in response to physical activity has been abundantly reported. For instance, individuals submitted to chronic endurance training displayed higher hair cortisol [68]. In endurance-trained men, after a day without physical exercise, ACTH and cortisol concentrations were similar to those of untrained controls [69]. For most of these athletes, dexamethasone (a synthetic agonist of the glucocorticoid receptor) was not found to influence the activity of the HPA axis; however, in contrast to untrained subjects, a subsequent administration of CRF was shown to increase cortisol levels. On the other hand, obese adolescents submitted to a chronic physical activity program displayed a marginal decrease in glucocorticoid sensitivity and increased levels of glucocorticoid receptor-α (GR-α) expression in blood mononuclear cells [70]. In young men who were previously undergoing a strength training program, cortisol responses were significantly increased when submitted to higher frequencies of endurance training [71]. Twenty weeks of endurance training were also shown to decrease basal cortisol levels [72]. Hence, the magnitude of the reduction in cortisol levels was significantly associated with increases in local skeletal muscle endurance. As observed in animals, endurance training also significantly upregulated basal BDNF circulating levels in healthy sedentary or physically active males, and the authors suggest that this effect could promote brain health in these populations [73, 74].

The influence of an acute bout of endurance exercise on HPA axis activity has also been investigated in a multitude of studies. In response to a walk on a treadmill until exhaustion at 40 °C, circulating levels of cortisol were higher in trained than in untrained individuals, while those of ACTH were not different [75]. Interestingly, in response to the same challenge in trained and untrained individuals, ACTH, norepinephrine, and dehydroepiandrosterone-sulfate (DHEA-S) levels were significantly increased, while those

of growth hormones (GHs), aldosterone and epinephrine, were initially elevated but reached a maximal value (plateau) at 38.5 °C. In athletes submitted to a strenuous exercise, CRF and cortisol responses to HPA activation were not blunted by physiological endogenous hypercortisolism, and this suggests that pituitary sensitivity is decreased in response to the feedback inhibition induced by cortisol [76]. As noted, acute physical activity has been reported to influence HPA axis activity; however, the relevance of considering other physiological conditions should not be neglected. In fasting subjects submitted to physical exhaustion, ACTH and cortisol levels significantly increased in hypoglycemic conditions, but this effect was abolished when pretest glycemic levels were maintained [77]. This also suggests the relevance of further examining the HPA axis activation under hypoglycemia.

While the abovementioned information indicates that HPA axis activity is modulated by chronic and acute training, it is also important to evaluate the effect of a recuperation phase. In runners, it has been observed that cortisol and ACTH levels are significantly lower 2 days following a marathon, while whole body 11β-HSD-1 and ghrelin levels are upregulated [78]. Also, the suppression of cortisol in response to a dexamethasone challenge is strongly increased after 6 weeks of reduced training.

Resistance Training

Although the effect of endurance training on the activation of the HPA axis is abundantly described, fewer studies have evaluated the effect of resistance training. Resistance training can be defined as any exercise program using one or multiple training strategies (own body mass, free weights, or diverse exercising machines), to enhance health, fitness, and performance [79]. In healthy untrained men submitted to acute resistance training, cortisol concentrations were not modulated [80]. However, in the same subjects, catecholamines, lactate, TNF-α, IL-2, and epidermal growth factor (EGF) levels increased, while monocyte chemotactic protein-1 (MCP-1) concentrations decreased. Furthermore, a positive correlation was observed between the concentrations of cortisol and TNF-α. Interestingly, the type and the intensity at which resistance training is performed are suggested to influence the HPA axis. In competitive athletes performing in muscular power disciplines (alpine ski, bodybuilding, and volleyball), an isokinetic exercise induced higher acute increases in ACTH, cortisol, and lactate than in endurance athletes (marathon, triathlon, cross-country skiing, and rowing) [81]. However, this effect was not observed during the recovery period. The type of training is reported to influence the activation of the HPA axis; however the effects of the intensity and volume of resistance training needed to be clarified. Interestingly, significantly lower cortisol levels were measured after a single bout of high-intensity resistance training (HIT) then after performing a traditional 3-set protocol in male college students [82].

Age, gender, circadian rhythm, and body composition are other factors that are often reported to influence hormonal secretions (see Copeland, Chap. 23 in this book). Studies were conducted to clarify the effects of age, gender, circadian rhythm, and body composition on the activation of the HPA axis. Young and middle-aged men were submitted to an 8-week resistance training program which was shown to decrease both basal cortisol and ACTH levels [83]. However, age did not have a significant influence on the results. In contrast, 9 weeks of combined endurance and resistance training was shown to increase cortisol levels by 23% in young sedentary women, but this effect was not observed in their male counterparts [84]. This suggested that women undergoing physical training are more sensitive to the activation of the HPA axis than males. To determine the role of the circadian rhythm on the activation of the HPA axis, trained subjects were instructed to perform the same resistance training session at three time periods over different days. Cortisol levels were higher in the morning but decreased 3 min and up to 48 h after performing their bout of exercise [85]. This indicated the importance of considering the time at which blood samples are collected before, during, and

after undergoing a session of resistance training. Contrastingly, after submitting untrained young males to 11 weeks of resistance training, the time of the day at which exercises were performed did not influence the levels of hormones of the HPA axis [86]. However, the same authors reported that postexercise cortisol levels were lower than basal concentrations. To determine the effects of body composition on the activation of the HPA axis, normal weight and obese individuals were submitted to resistance training. Cortisol levels were significantly different between normal weight and obese individuals [87]. This suggested that body composition may also modulate the HPA axis.

Different types of resistance training promote skeletal muscle hypertrophy or strength. Untrained young male and female adults were recruited to clarify the different effects of the two types of resistance training on the HPA axis. While performing the experimental protocol, significantly higher BDNF levels were measured during the exercise designed to promote hypertrophy than the one intended to increase strength [88]. In trained men, BDNF levels increased similarly in response to the different intensity and volume levels of resistance training [89]. In older adults submitted to various loads of resistance training, BDNF levels increased in male participants, while no effects could be detected in female individuals [90]. These data support the hypothesis that the HPA axis activation might be influenced by the type of training, the intensity, the post-training period, and body mass but not by age or the time of the day at which it is performed. These latter issues are in need of further investigations to clarify aspects of the contradictory results.

Intensity of Physical Activity and HPA Axis Activation

It is profusely reported that the HPA axis is activated in response to physical activity, and different levels of exercise intensity were also shown to have an important impact. In mice submitted to acute psychological stress, high-intensity physical activity increased cortisol, IL-1β, IL-2, and IL-6 while decreasing ACTH-positive cells in the pituitary [91]. Although collected in animals, these results indicated the relevance of considering the intensity of physical activity, and this was also investigated in human models. For instance, it was initially proposed that cortisol levels are increased by 60 min of running on a treadmill at a threshold intensity of 60% of the VO_{2max} [92]. Moderately trained men also displayed a significant increase in cortisol after performing 30 min of exercise at 60% and 80% of their VO_{2max}, while ACTH levels were only elevated at the highest intensity [93]. In endurance-trained males, 30 min of exercise on a cycle ergometer, significant increases in cortisol were only observed at 80% of the VO_{2max} both in saliva serum [94]. Interestingly, the same authors observed that peak cortisol levels were only monitored 30 min after the cessation of the physical activity. When compared to low-intensity, high-intensity cycling caused similar increases in BDNF and cortisol levels in both participants with or without depression [95]. In trained athletes submitted to a prolonged high-intensity exercise, increased plasma concentrations of cortisol, ACTH, CRF, and AVP were observed [96]. It was also reported that the rise in osmolality observed during exercise correlates with increases in plasma AVP. Furthermore, for a given type of physical activity, high-intensity and prolonged duration respectively increased AVP and CRF levels. In healthy participants administered with dexamethasone (4 mg), performing physical activity at the highest intensity (90% vs. 100% maximal aerobic capacity) caused a significant raise in ACTH, cortisol, and AVP circulating levels [97]. Interestingly, this response was shown to be amplified in women with regard to the one observed in men. Interestingly, high-intensity interval training was shown to increase BDNF levels to a higher magnitude than continuous moderate-intensity exercises in obese individuals [98]. This suggests that short and intense bouts of exercise could exert beneficial effects to individuals intending to design and/or perform physical activity programs.

While the effect of exercise intensity was evaluated in response to distinct physical activities,

another group compared occupational differences between workers performing high-intensity duties (slaughterhouse workers) and others achieving low-intensity tasks (office workers) [99]. Slaughterhouse workers displayed higher levels of ACTH, total peroxides, antioxidant capacity, oxidative stress index, and c-reactive protein (CRP), while their levels of endogenous peroxidase activity, polyphenols, and BDNF were reduced. These results were even affected by the duration of the work shifts in slaughterhouse workers since higher CRP and lower BDNF levels were measured after completing 12 h vs. 8 h shifts.

Results presented in this section clearly indicate that the intensity and the volume of a physical activity, the fitness level, and the type of exercise performed by an individual have a direct impact on the activation of the HPA axis. In turn, this should be taken into consideration when elaborating training programs.

Highly Trained and Elite Athletes

Overall, the increased activity of the HPA axis in highly trained athletes could have important implications on their somatic and mental health. During a progressive stress test until exhaustion on a treadmill, cortisol levels were higher from baseline to the initiation of recuperation in professional athletes than in controls [100]. Interestingly, hormonal levels were regularized over the recuperation period. In ultramarathon runners, cortisol levels were at their highest at the completion of a 622 km race, and levels were only normalized after 6 days of recovery [101]. In highly trained athletes, the morning surge in ACTH and cortisol was observed earlier, and ACTH levels were significantly higher than in normal individuals [102]. In addition, the stimulation of CRF and ACTH release was more pronounced in highly trained athletes than in untrained individuals following the administration of the nonselective opioid receptor antagonist naloxone [32]. Altered HPA axis functions were also observed in elite athletes. For instance, artistic gymnasts competing at the European Championships displayed higher salivary cortisol concentrations and more important levels of psychological stress than controls [103]. In addition, higher psychological stress and saliva cortisol levels were also observed in female vs. male athletes. In elite junior soccer players, nonfunctional overreaching performances were associated with higher scores of depression and angriness, whereas resting GH and ACTH concentrations after maximal effort were diminished [104]. These observations could be associated with the decreased expression of GR-α mRNA in highly trained individuals and with lower increases in atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels in response to exercise [105–110]. These elements suggest the influence of the HPA axis on stress and emotional status. Ultimately these factors could also have a major incidence on sportive performances in elite athletes.

Overtraining

The available information regarding altered HPA axis functions in athletes suggests the relevance of considering potentially for pathological conditions such as overtraining. In rats submitted to daily swimming bouts of 45 min 5 days per week for 2, 4, or 6 weeks, corticosterone gradually increased. In parallel both basal ACTH and corticosterone plasma levels increased until they reached a plateau after 6 weeks of swimming [111]. Hence, in the PVN and the pituitary of the same animals, mRNA expression of the glucocorticoid receptor decreased, while the one of CRF transiently increased. While these results are interesting, it is difficult to determine whether the important volume of exercise to which rats were submitted can be considered as overtraining. These results raise important questions since cortisol levels were significantly below normal in overtrained Standardbred racehorses [112, 113]. Interestingly, these discrepancies may be species-specific or be related to the duration overtraining in the animals. In other words, rats submitted to swimming may still have the capacity to produce corticosterone, while Standardbred

racehorses may have been submitted to a chronic overactivation of the HPA axis which led to impairments in their capacities to secrete cortisol before being diagnosed. In different populations of human athletes, several alternative methods such as a CRF stimulation test (evaluation of basal ACTH concentrations and GH pulsatility), free testosterone over cortisol concentrations, low basal cortisol levels, as well as HPA responses to two standardized exercise tests were proposed for the diagnostic of overtraining [114–116]. For instance, in response to two acute bouts of exercise, increased prolactin (PRL) levels and decreased ACTH concentrations are reported in overtrained athletes [117–119]. These effects could be mediated by the repetitive occurrence of muscle and skeletal trauma resulting in local inflammation and, consequently, in a systemic inflammatory responses which, in turn, could yield to impairments of athletic performances [115].

Postexercise Recuperation

Depending on the type of physical activity and its intensity and volume, it is critical to allow the body to recuperate, replenish its energy reserves, and resynthesize injured tissues in order to improve athletic performance. Recuperation is well-characterized in nutrition and physiology; however, it is another factor to take into consideration when considering the effects of exercise on the HPA axis. For instance, it was shown that the carbohydrate/electrolyte consumption right after performing a bout of high-intensity physical activity significantly reduced blood cortisol levels in male athletes [120]. However, the hydration status, per se, was not associated with an alteration of circulating cortisol concentrations [121]. In rugby players submitted to a magnesium supplementation, significantly higher ACTH but decreased cortisol levels were observed compared to the same type of participants given a placebo [122]. In addition, magnesium supplementation abolished the post-game increase in IL-6 while reducing the increase in neutrophil/lymphocyte ratio.

Memory, Defeat, Fear, and Cognitive Functions

During a physical activity, the capacity to remember how to optimally perform an exercise as well as the bad feelings and the fear of defeat or mishaps occurring during the event may have profound effects on an individual's performance. Because of obvious ethical reasons, this is difficult to investigate in humans. However, rodent models were used to investigate the effect of the HPA axis on memory, defeat, and fear. In rats administered with metyrapone (a corticosterone synthesis disruptor), impaired traces of fear conditioning have been observed [123]. A number of studies also evaluated the effects of CRF on defeat conditioning as well as on memory. The central administration of anti-sauvagine-30 (a CRF-R2 receptor antagonist) reduced submissive and defensive behaviors induced by territorial aggression conditioning in Syrian hamsters [124]. However this effect was not observed in response to neither metyrapone nor CP-154,526 (a CRF-R1 antagonist) administrations in the lateral ventricle of rats increased spatial memory through a β-adrenergic-dependent mechanism [125]. Also, central administrations of NBI30775 (CRF-R1 antagonist) prevented stress-induced hippocampal dendritic spine loss while restoring stress-impaired cognitive functions [126]. This suggests that stress-induced central effects are mediated through the activation of CRF-R1. These discoveries are important since they could allow the implementation of targeted interventions or pharmacological treatments to reduce the fear of defeat or the occurring of an injury in individuals or athletes who previously encountered such negative experiences. In turn, this would allow preventing the adverse outcomes on their athletic performances.

Conclusion

The last paragraphs have underlined the importance of the HPA axis on the regulation of moods and behaviors in animals and humans undergoing physical activity. Depending on the population of

interest and the objectives to be reached, it is critical to adapt training programs to maximize their benefits while minimizing the risk of developing anxiety and depression. This has to be applied to athletes as well as other populations with different levels of fitness and/or degrees of disabilities. For instance, professional or Olympic athletes are particularly at risk of overtraining, and their moods, cognitive functions, and confidence levels have an important impact on their performances. Physical activity is also an important element of a healthy lifestyle to prevent and/or counteract the dreadful effects of obesity and ensuing metabolic dysfunctions that have reached epidemic levels in North American populations. In obese individuals, adherence and compliance to training programs remain major obstacles. For athletes or obese individuals, a better understanding on how exercise modulates the HPA axis will provide essential tools to develop novel training approaches. As one consequence of this, it will be essential to exhaustively characterize individuals undergoing an exercise program in order to determine their levels of fitness; the type, intensity, and volume of physical activity required; as well as the window of time over which objectives need to be achieved. It is also important to constantly monitor exercising individuals since adaptation to the training planification will be required as soon as anxiogenic or depressive behaviors will be present. Hence, distinct factors of the HPA axis may be used as sensitive biomarkers to detect disorders before clinical symptoms can be detected. Globally, this indicates the relevance of including parameters of the HPA axis as modulators of anxiety and depressive behaviors in exercising individuals. In sum, while it remains a precarious equilibrium, it suggests that elements of the HPA axis must be taken into consideration along with the assessment of an individual's physical capacities when designing a training program. Consequently, while the planification and periodization must be optimized, it is important to adapt the program accordingly in function of early signs of anxiogenic or depressive behaviors. Ultimately, this will be more effective at yielding improvements in athletic performance and health benefits than the simple addition of strenuous exercises that could provoke the premature interruption or to slow down physical training program for various clinical reasons.

Our current knowledge of the relationship between the HPA axis and physical exercise reviewed in the above paragraphs clearly highlights the importance of adequate preparation for exercise. Also, a number of data indicate that complex molecular and cellular mechanisms intervene in highly trained athletes that do not occur in normal individuals. As a whole, the above information suggests that the HPA axis importantly influences stress-induced functions and that the intensity of HPA axis activation is intimately related to the type of training, the intensity, and the volume at which it is performed. This strongly suggests the relevance of considering the impact of the HPA axis when elaborating training programs in different types of individuals.

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4 Impact of Chronic Training on Pituitary Hormone Secretion in Humans

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Introduction

The impact of chronic training on pituitary function is best understood by a basic appraisal of the neuroendocrine physiology of any given individual axis and the more complex interactive pathophysiology among axes [1–12]. Interaxes interactions have received relatively little attention. Even evaluating a single neuroendocrine axis in its dynamic state is a complicated challenge, given combined feedforward and feedback activities among the key control loci within any given axis [13, 14]. For example, in the case of the growth hormone (GH) and insulin-like growth factor 1 (IGF-1) axis, hypothalamic GH-releasing hormone (GHRH) secreted by arcuate nuclei stimulates pituitary GH secretion acutely, whereas the somatostatinergic system originating in the paraventricular nuclei opposes GHRH action [15]. These two neuronal inputs are reciprocally interconnected by intrahypothalamic synapses and common impinging neuromodulator pathways [14]. In addition, secreted GH feeds back on brain GH receptors, stimulating somatostatin secretion and possibly inhibiting GHRH release. Available GH secreted into the bloodstream triggers IGF-1 production in various target tissues, and circulating IGF-1 is capable of inhibiting pituitary GH secretion indirectly and directly (*see* Fig. 4.1). Such feedforward (GHRHs driving GH secretion) and feedback (GHs inhibiting its own secretion, IGF-1 s inhibiting GH secretion, and so forth) dynamic control mechanisms in principle can be modified by the effects of exercise at one or more levels within the axis. Moreover, multiple determinants modulate neuroendocrine responses to training, such as the body composition of the individual, concurrent stress and/or weight loss, gender, diet and energy balance, concomitant drug or hormone use, age, puberty, pregnancy, and/or lactational status [16–18].

Here, we will examine the neuroendocrine determinants of pituitary responses to exercise training, explore some of the confounding issues (e.g., species differences, varying modes of neurohormone secretion, within- and between-axis regulation, and so on), and explore the overall notion of neuroendocrine axes as feedback and feedforward control systems capable of within-axis as well as between-axes interactions. Finally, metabolic mechanisms, although likely multifactorial, will be examined briefly, and their clinical implications underscored.

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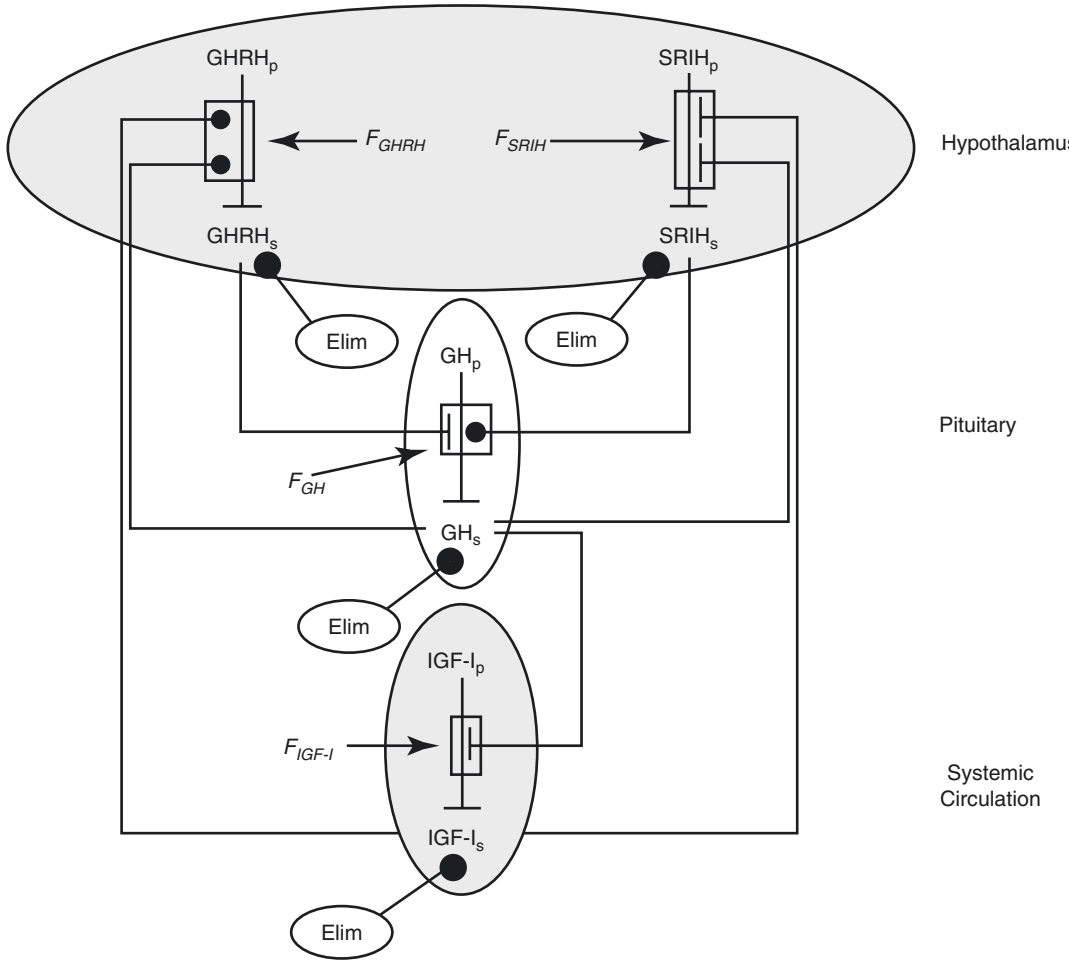

Fig. 4.1 EU eumenorrheic athletes; AM amenorrheic athletes; ANX anorexics. Amenorrheic athletes have endocrine profiles (i.e., decreased thyroid hormones) similar to anorexics with chronic energy deficiency. (Data taken from Refs. [43, 47]). ∗Eumenorrheic means are significantly different from amenorrheic and anorexic means ($p < 0.05$). Panel A = total T3 (triiodothyronine) and panel B = total T4 (thyroxine)

Multiple Determinants of Pituitary Responses to Exercise Training

Among other determinants of neuroendocrine responses to exercise training is the acuteness vs. chronicity of the training or exercise stimulus [2, 5, 11, 12, 19–22]. In particular, numerous studies demonstrate that acute exercise induces a variety of short-term changes in multiple hypothalamo–pituitary axes, including the nearly immediate secretion of GH and adrenocorticotrophic hormone (ACTH), β-endorphin, and cortisol, whereas the results of chronic training are not necessarily identical [20, 21, 23–30]. Moreover, stress or acute exercise imposed in an untrained individual will elicit endocrine responses potentially distinct from those observed in a highly physically trained subject [3, 8, 9, 11, 31–40]. Thus, many studies are confounded in part by the nature of the prior or concomitant training regimen, its duration, and its intensity. Finally, extreme physical exertion, "overreaching," often evokes neuroendocrine disturbances that are not typical of either short-term submaximal exertion or chronic training [5, 9, 41–43].

Neuroendocrine axes are exquisitely sensitive to nutrient intake, body composition, and total (and percentage) body fat [44–51]. Recent studies of the GH axis document unequivocally that percentage body fat, and in particular visceral (intra-abdominal) fat accumulation [52], negatively influences pulsatile GH secretion by suppressing the mass of GH secreted per burst and shortening the half-life of GH in the circulation [44, 45, 53–56]. The reciprocal relationship between visceral fat mass and GH secretion is illustrated in Fig. 4.2. Impaired GH secretion and more rapid GH removal jointly serve to reduce 24-h pulsatile serum GH concentrations in otherwise healthy but relatively more (viscerally) obese individuals. In contrast, acute weight loss or nutrient deprivation potently stimulates GH secretion in the human (while suppressing it in the rat) by 3–10-fold, with augmentation in both men and women of GH secretory pulse amplitude and mass and, to a lesser degree, burst frequency [47, 57, 58]. Consequently, nutrition, body weight, and body composition are prime determinants of pituitary (GH) secretory activity, which likely condition responses to exercise [59]. In addition, in men, as well as more recently recognized in women, body mass index (relative obesity) is a negative correlate of LH pulse amplitude [49, 60] and of the serum testosterone concentration in middle-aged men [49].

Gender distinctions also strongly influence the secretory output of several neuroendocrine axes. Foremost, the gonadotropin-releasing hormone–luteinizing hormone (GnRH–LH) follicle-stimulating hormone (FSH)–sex steroid axes in men and women exhibit clarion differences, particularly at the level of the so-called positive feedback, which is mechanistically required to achieve a preovulatory LH surge in women [61]. The GH–IGF-1 axis is also strongly sexually dimorphic in the human (as well as in the rat, as reviewed earlier [15]). For example, in healthy premenopausal men and women, GH secretion differs quantitatively by way of a nearly twofold greater mean (24-h) serum GH concentration, higher plasma IGF-1 level, greater mass of GH

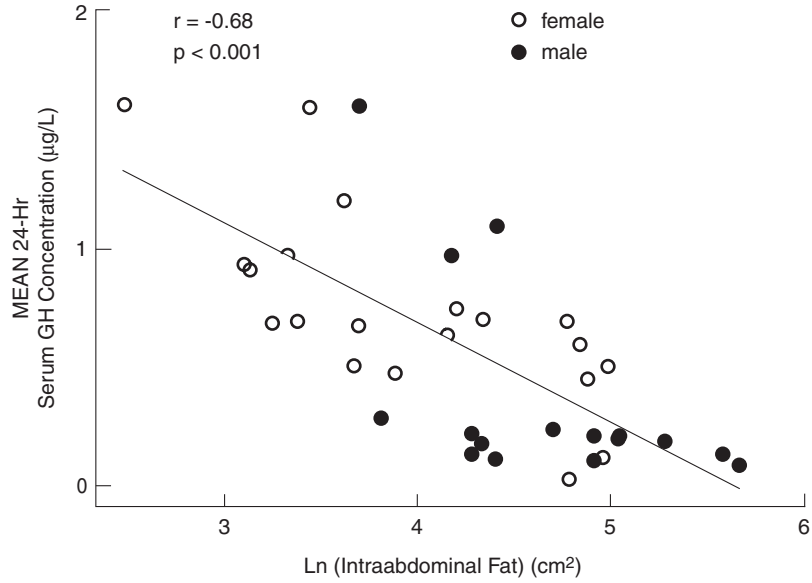

Fig. 4.2 Negative relationship between 24-h mean serum GH concentration and intra-abdominal (visceral) fat mass, as determined by computerized axial tomographic scanning of the abdomen, in a cohort of healthy middle-aged men and women. GH concentrations were determined by 20-min blood sampling for 24 h and subsequent assay by immunofluorometry. The *solid circles* denote male subjects, and the *open circles* females. The *regression line* shows a strongly negative relationship between the natural logarithm of intra-abdominal adiposity and daily GH secretory activity in both men and women. In multiple linear regression analyses, intra-abdominal fat mass accounted for the majority of the variability in integrated serum GH concentrations, exceeding that owing to age and gender in this population. (Redrawn with permission from Vahl et al. [56])

secreted per burst, and a more disorderly pattern of GH release in women compared to men [62]. In addition, the individual negative impact of age, body mass index, or percentage body fat on GH secretion is 1.5–2-fold more evident in men than women [48]; the positive effect of physical conditioning (increased VO_2max) on GH release is also more prominent in the male [48] (Fig. 4.3).

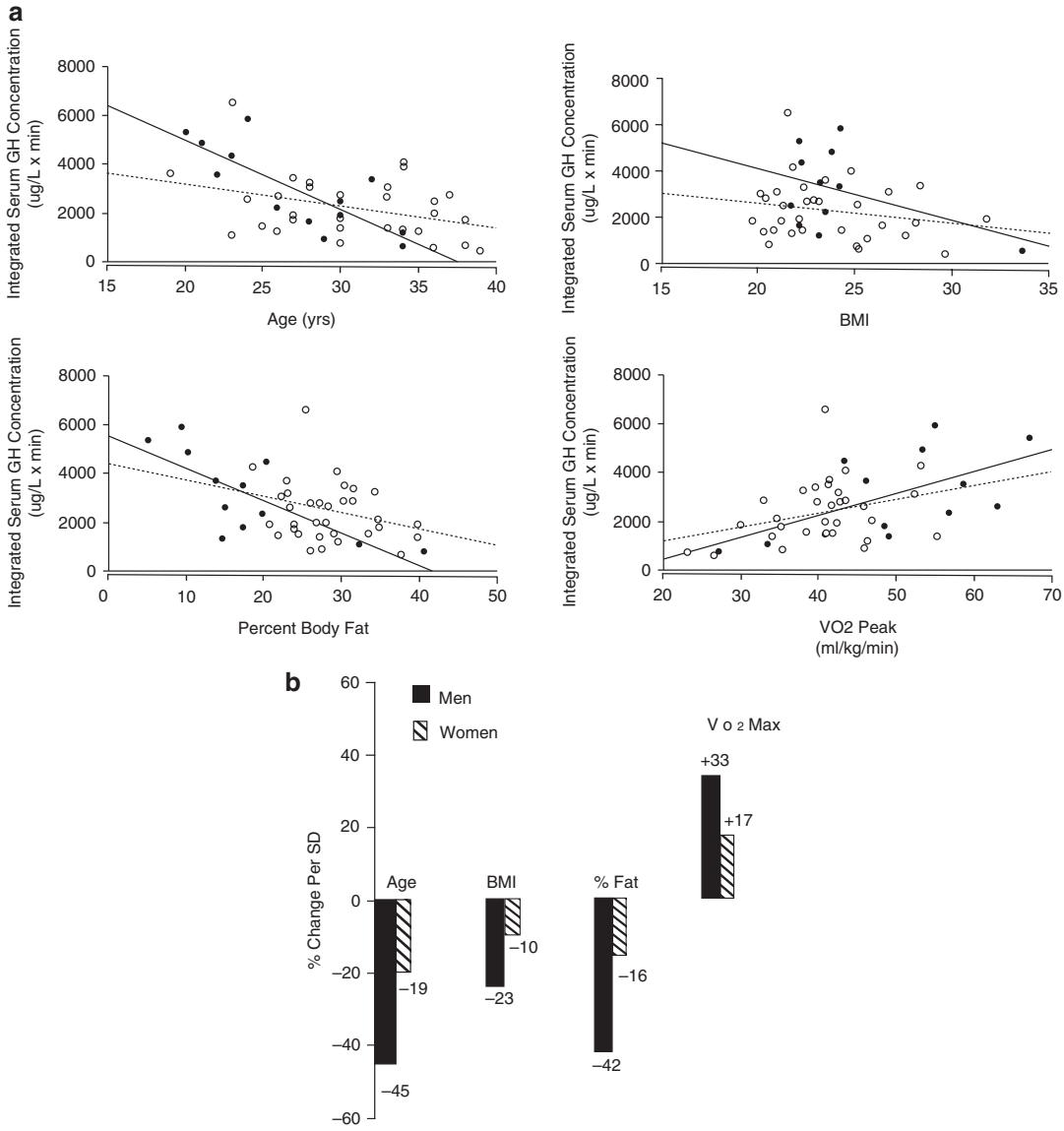

Fig. 4.3 (**a**) Impact of gender on the effects of age, adiposity as measured by body mass index (BMI) or percentage body fat, and physical fitness as quantitated by maximal oxygen consumption (VO_2max peak or max) on integrated (24-h) serum GH concentrations in normal men (*filled circles*, $N = 12$) and women (*open circles*, $N = 32$). Linear regression plots are given for each sex. The *solid lines* denote regression in men, and the *interrupted lines* depict women's data. (**b**) Approximately twofold greater impact of age, BMI, percentage body fat, and VO_2max on 240-h mean serum GH concentrations in men than women. Data are means ± SEM expressed as standardized regression coefficients for the regression lines in (**a**). The gender-specific standardized regression coefficient is the slope of the linear relationship (given as a percentage) adjusted per unit standard deviation (SD) of the male or female group as pertinent. (Redrawn with permission from Weltman et al. [48])

The tissue responses to GH also may be sex-specific in part, since estrogen can antagonize GH-driven IGF-1 production by the liver [15]. Consequently, gender must be identified as a major determinant of neuroendocrine responses in the GH–IGF-1 axis. Exercise-stimulated GH secretion may be less gender-dependent [63].

A lesser gender difference is observed for the corticotropin-releasing hormone (CRH)–arginine vasopressin (AVP)/ACTH–cortisol axis, where in the female, relatively increased expression of the CRH gene and increased adrenal responsiveness to ACTH are proposed [64]. However, the orderliness of individual 24-h ACTH and cortisol release (approximate entropy) or their relative synchrony (crossentropy) in men and women is similar [65].

Another significant confounding influence on neuroendocrine axes is age. For example, in the case of the LH–testosterone axis in men, there is progressive deterioration of LH or testosterone's individual orderliness of release over 24 h and of LH–testosterone coupling or synchrony, when assessed by either cross-correlation analysis (indicating diminished feedforward control) [66] or cross-approximate entropy (indicating decreased pattern synchrony within the reproductive axis' feedback system) [67]. The regularity of GH or ACTH/cortisol release also deteriorates with age in men and women [65, 68]. In addition, in both men and women, there are marked quantitative decreases in overall GH axis secretory activity, with a progressive fall in plasma IGF-1 and daily GH secretion rates with aging, especially in men compared to women of premenopausal age [44, 45, 48, 54].

Concurrent drug and/or hormone use can also markedly modify several pituitary-target tissue axes. For example, prescribed or self-use of anabolic steroids will profoundly suppress LH and FSH release and reduce levels of endogenous sex steroids while potentially stimulating the GH–IGF-1 axis (if aromatizable androgens are employed) [13, 63, 69–71]. Likewise, the use of birth control pills in young women stimulates GH secretion significantly and may produce some alterations in body composition [72]. At puberty, when sex steroid hormone secretion changes more dramatically [73, 74], the individual's GH–IGF-1 and/or GnRH–LH axis may be uniquely susceptible to the impact of exercise training (at least prior to pubertal onset), resulting in a significant delay in sexual maturation and adolescence and possibly reduced predicted adult height [75] (*see Chap.* 17; First edition).

We infer that an array of important factors, such as exercise intensity and duration, its acuteness vs. chronicity, associated weight loss and/or stress (discussed further below), diet and energy balance, body composition, gender, age, and maturational status (e.g., prepubertal vs. pubertal), may all codetermine the neuroendocrine and pituitary responses to a stress perturbation, such as exercise.

Other Confounding Issues

One confounding issue experimentally in evaluating the impact of acute or chronic physical training on pituitary function is species differences. For example, in the rat, physical exertion reduces GH secretion [15], whereas in the human acute and chronic exercise, both increase GH secretion significantly, the former within 15–30 min and the latter following sustained exercise at an intensity above the individual lactate threshold [15, 20, 21, 24, 76, 77]. Indeed, chronic physical training in women results in a doubling of the 24-h mean serum GH level even on days when exercise is not undertaken [21] (*see* Fig. 4.4 [20]). Consequently, many experiments carried out in the rodent do not find applicability, especially for the GH–IGF-1 axis, to human studies. Moreover, the gender differences in the GH axis in the rat and human are readily distinguishable mechanistically in the two species, with a greater mean amplitude (and mass) of GH secretory bursts in women than men (but the converse occurs in the rat) [62]. A similarity in the two species is a more disorderly pattern of GH release in the female [78].

Further complicating interpretation and analysis of pituitary secretion are the multifold temporal modes of physiological pituitary hormone release:

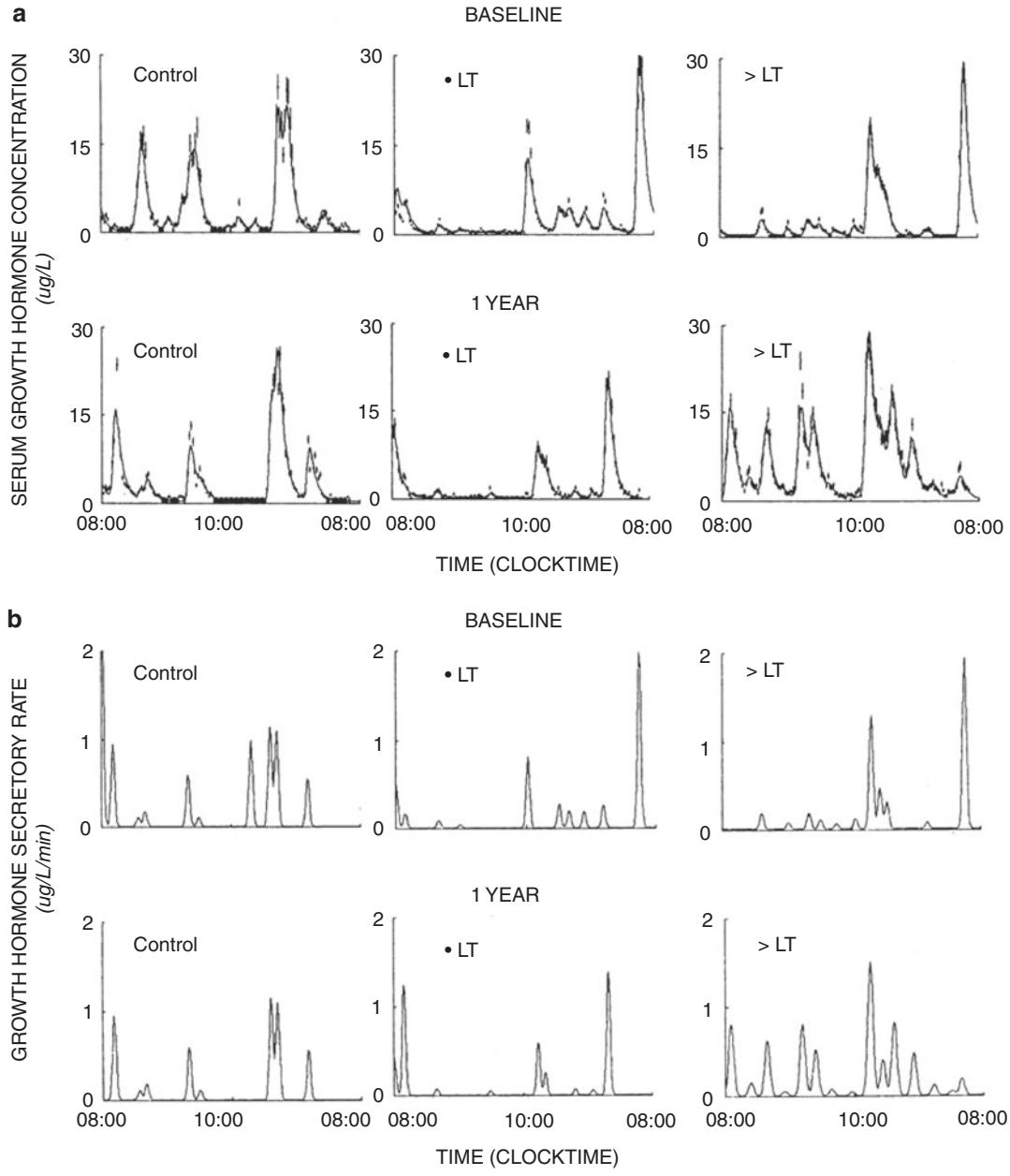

Fig. 4.4 The 24-hour serum GH concentration (**a**) or secretion rate (**b**) profiles in three different premenopausal women each studied twice: control (left; no exercise training, sedentary volunteer); before (baseline) and after 1 year of exercise training below or at the individually determined lactate threshold (LT) (middle panel; exercise volunteer #1), and exercise training above the LT (right panel; exercise volunteer #2). (Adapted with permission from Ref. [20])

1. Pulsatile.
2. Nyctohemeral or circadian.
3. Entropic, or moment-to-moment variations in the orderliness of secretion [67, 79–81].

Pulsatile hormone secretion typically mirrors episodic neural input that acts via intermittent secretagog delivery to a responsive pituitary cell population in the absence of significant inhibitory input concurrently. Indeed, a pulse of pituitary hormone secretion can be viewed as a collection of secretory rates, centered about some moment in time. This concept is illustrated in Fig. 4.5.

In contrast to the foregoing episodic (pulsatile) secretory mode are less rapid, 24-h variations in serum hormone concentrations, which are well established for ACTH, LH, GH, thyroid-stimulating hormone (TSH), prolactin, cortisol, and so forth [82]. These nyctohemeral (night–day) variations constitute only a small part of the total variation in daily neurohormone release. True circadian rhythms are so-called free-running with a periodicity of 24 h, temperature-compensated, and susceptible to zeitgebers or specific phase-entraining cues [83]. Not all human 24-h neuroendocrine rhythms conform to this definition, which would denote true

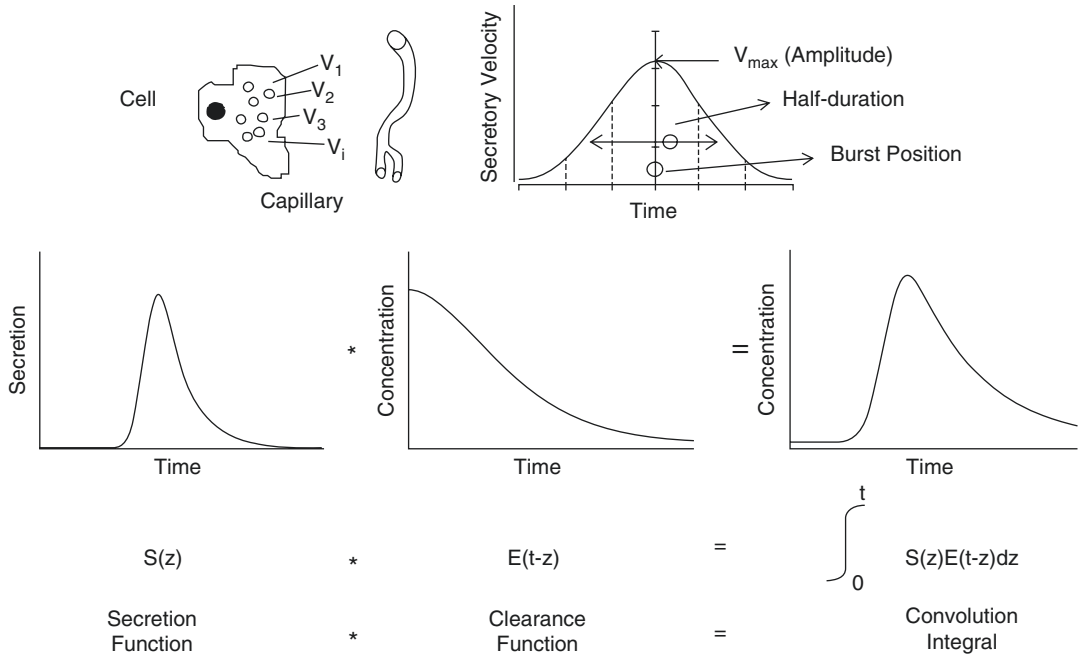

Fig. 4.5 Schematized illustration of a model-specific deconvolution concept implemented to quantitate (GH) secretion. The *upper* landscape depicts an intuitive formulation of a hormone secretory burst, as arising from (multi-)cellular discharge of individual hormone molecules more or less in concert temporally, each at its own particular secretory rate (velocity). A secretory burst (or pulse) is visualized as an array of such molecular secretory velocities centered about some moment in time and dispersed around this center with a finite standard duration (SD) or half-width. The burst event may or may not be symmetric over time. The *lower* landscape with the algebraic subheads shows the mathematical notion, whereby a plasma hormone concentration peak (*far right*) is viewed as developing from a burst-like secretory process (*far left*) and a finite hormone-specific removal rate (half-life of elimination). The so-called convolution (intertwining or interaction) of the simultaneous secretory and elimination functions creates a resultant (skewed) plasma concentration pulse. Deconvolution analysis consists of mathematically estimating the constituent underlying secretory features (and/or associated half-life), given (a series of) blood hormone concentration peaks as the starting point. A variety of model-independent (waveform-invariant) deconvolution strategies can also be applied, if a priori knowledge of the pertinent (biexponential) hormone elimination rate process is available. (Adapted with permission from Ref. [125])

(suprachiasmatic nucleus-driven) circadian activity. Based on sleep-reversal studies, and so forth, circadian rhythmicity clearly does exist for ACTH/cortisol release in the human and GH secretion (approx 50% of the 24-h GH rhythm is sleep- and activity-entrained, and 50% is circadian) [15, 84].

Neurohormone release also exhibits features of minute-to-minute patterning, serial orderliness, or relative regularity, which can be quantified by an approximate entropy statistic [67, 78]. Higher values of approximate entropy denote greater disorderliness of hormone release and are a feature of female GH secretory patterns (compared to male), healthy aging of the human insulin, GH, LH, and ACTH/cortisol axes [54, 65, 67, 78, 85, 86], as well as aldosteronomas [87], tumoral pituitary hormone secretion (acromegaly, Cushing's disease, and prolactinomas [65, 88]), and insulin release in type II diabetes mellitus [89, 90]. Thus, entropy measures can identify secretory disturbances complementary to pulsatile or circadian variations.

The complex mode of pituitary hormone secretion imposes the need for appropriately rigorous sampling intensity and duration to capture the pulsatile, circadian, and entropic features, followed by application of relevant analytical tools appropriately validated under those conditions of study. Such technical issues have been reviewed recently [80, 91–93].

Further confounding in the literature arises because biochemically measurable endocrine changes do not always imply definite biological or clinical sequelae. For example, studies of the thyrotropin-releasing factor (TRH)–TSH–thyroidal axis have revealed numerous biochemically measurable changes during acute or chronic exercise, but their clinical sequelae are not known [94]. Similarly, in relation to the male reproductive axis, a variety of pituitary–gonadal changes are well established in response to chronic exercise, such as diminished LH pulse frequency at least in a subset of men, and relatively decreased spermatogenesis (e.g., a 30–50% decline in sperm number). However, clinical signs and symptoms of androgen deficiency rarely, if ever, occur, and male infertility is not known to be associated with chronic physical training [5, 32–35, 43, 95–100]. Finally, multiple hormones are produced by the anterior pituitary gland, and, as discussed further below, the corresponding individual axes may evince significant interactions.

Neuroendocrine Axes as Feedback and Feedforward Control Systems

As intimated in the Introduction, neuroendocrine axes should be viewed as dynamic feedforward and feedback control systems. The term feedforward defines the ability of a secreted agonist to act on a remote or proximal tissue and evoke a typically sigmoidal (e.g., log-logistic) dose–response curve, e.g., as anticipated for GHRHs acting on somatotrope cells in the anterior pituitary gland, GnRHs acting on gonadotrope cells, and so forth [15, 101]. Conversely, feedback denotes the ability of a secreted product from a target tissue to inhibit the production of the agonistic signal, e.g., testosterone feeds back on hypothalamic GnRH secretion in the male, IGF-1 feeds back on pituitary somatotrope secretion of GH, l-thyroxine feeds back on TSH secretion at the pituitary and hypothalamic levels, and so forth. As highlighted in Figs. 4.1 and 4.6, both the GHRH–somatostatin/GH–IGF-1 axis [14] and the GnRH–LH/FSH/sex steroid [101] axes should be viewed as complex feedback and feedforward control systems [13, 14, 79, 101–103]. This concept is physiologically critical, since most pathophysiological stimuli impinge on several points within the feedback control system, thus impacting on the overall dynamics. Such system-level responses cannot be observed readily when separated components are studied individually. Similarly, the stress-responsive ACTH–adrenal axis comprises CRH–AVP/ACTH–cortisol, with corresponding feedforward and interactive feedback mechanisms inherent [3, 40, 104].

An important notion in future studies of chronic exercise effects on the pituitary will be to limit isolation of individual components of the axis and rather study the overall axis dynamics. Technology, such as approximate entropy

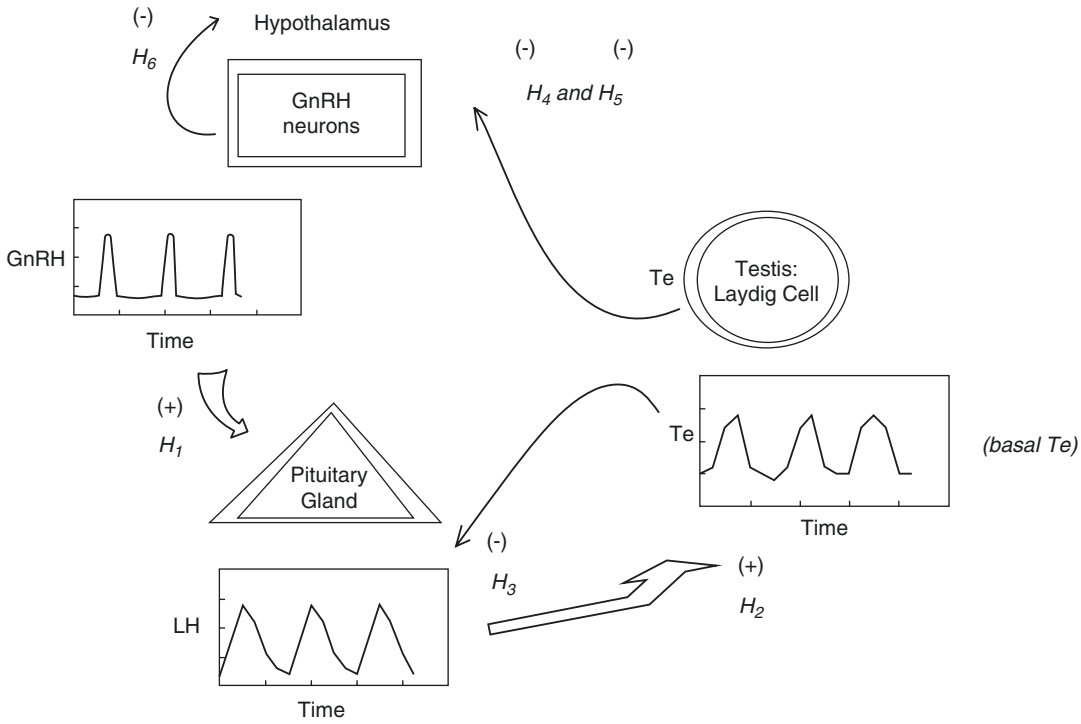

Fig. 4.6 Schematic illustration of the time-delayed negative feedback (−) and positive feedforward (+) within the human male GnRH–LH–testosterone (Te) axis. The *broad arrows* indicate feedforward (+) stimulus-secretion linkages, and the *narrow arrows* denote feedback (−) inhibition. The "H" functions are developed further in Ref. [101] and serve to define the dose–response relationships at each feedback interface within the axis. (Adapted with permission from Ref. [101])

[67, 105] and network analysis [14, 101], for accomplishing the latter is just beginning to emerge. To date, the vast majority of published literature (as discussed throughout this volume) has enunciated changes at individual control points, which unfortunately subdivides the feedback system artificially and limits insights into its interactive properties, which function from minute to minute and day to day.

Introductions Among Neuroendocrine Axes

Foremost among the challenges to be addressed in investigative and clinical neuroendocrine pathophysiology are the nature and mechanisms of interaction between two, or among three or more, neuroendocrine axes. For example, in relation to chronic exercise or other stressors in experimental animals, alterations occur not only in hypothalamic GHRH and somatostatin gene expression but also in the GnRH neuronal ensemble and neuropeptide Y (NPY)- and CRH-secreting neurons [104, 106]. In conjunction with concurrent changes in dietary intake, activity of TRH neurons in the hypothalamus may also be suppressed (reviewed in Ref. [107]). Relevantly, these multiple neuronal pathways are directed by corresponding families of neurotransmitters (e.g., norepinephrine, serotonin, acetylcholine, and so forth), as well as various potent neuromodulators (e.g., NPY, galanin, and so on). Thus, a major focus in understanding the whole-body neuroendocrine responses of an intact organism to chronic exercise training must eventually include the articulation of not only individual neuronal pathway changes but also their collective and interconnected alterations owing to common neuromodulatory

inputs. For example, infusion of leptin, the product of the *ob* gene in adipocytes, is capable of rescuing suppressed hypothalamic TRH secretion in fasting; relieving inhibited GnRH gene expression in certain stress models; and stimulating GH secretion in the fasted male rat (presumptively by reducing hypothalamic somatostatin gene expression). Thereby, leptin may integrate a complex response pattern via concerted hypothalamic actions that supervise diverse pituitary hormone secretory activities [107–109]. However, in the human, leptin levels correlate inversely (rather than directly, as in the rat) with GH axis secretory activity, as illustrated in Fig. 4.7 [55].

Metabolic Mechanisms

The exact metabolic mechanisms that subserve hypothalamo–pituitary responses to exercise training are not known. Among those extensively considered are free fatty acids, which clearly can inhibit GH secretion [15]. On the other hand, any direct role of free fatty acids in modifying the GnRH–LH–gonadal axis is not evident. Similarly, both insulin and free IGF-1 can inhibit GH secretion directly at the anterior pituitary level and indirectly via hypothalamic effects under several conditions in certain species [15].

Moreover, prolonged nutrient and/or glucose deprivation can arrest puberty in the immature

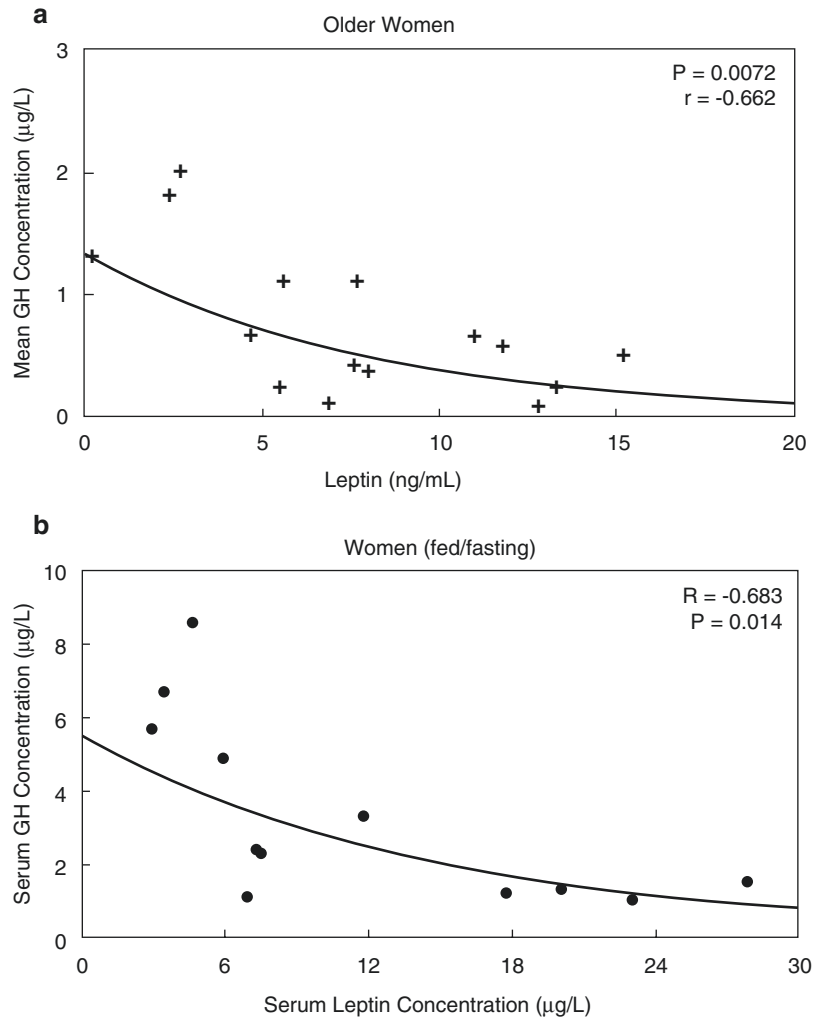

Fig. 4.7 (**a**) Inverse log-linear relationship between fasting serum leptin concentrations and integrated 24-h serum GH concentrations in 15 healthy postmenopausal women [55]. (**b**) Similar inverse (exponential) regression between serum leptin and GH output in young women fed or fasted [58]. *P* and *r* values for the linear regressions are shown. Adapted with permission

sheep and modify hypothalamic peptide secretion (e.g., stimulate CRH and/or AVP, while inhibiting GnRH, secretion) [104]. In contrast, carbohydrate ingestion during exercise in one study in the human seemed to increase cortisol and decrease gonadotropin release [110], whereas maintenance of euglycemia in another study abolished exercise-induced ACTH–cortisol release in nearly exhaustively exercised volunteers [111]. Finally, as intimated above, the peptide leptin can modify somatostatin, GnRH, TRH, and NPY gene expression, among other hypothalamic responses to the stress of fasting [55, 58]. Overall, we postulate that such multifactorial metabolic cues and the sex steroid milieu significantly codetermine neuroendocrine responses to exercise training [112–114]. In addition, under the most severe exercise stimulus, overall "final-common-pathway" stressor responses may prevail, such as secretion of reproductively inhibitory CRH and endogenous opioids, with consequent suppression of GnRH–LH secretion and conversely (in a species-specific manner) stress-driven alterations in the GH–IGF-1 axis [10, 15, 38, 115–123].

Implications

Among other implications of chronic training are favorable nonendocrine adaptations of hemodynamic and cardiovascular function. These changes are likely to be important in long-term health risk. Moreover, body compositional changes, motivated in part by the above neuroendocrine alterations, would be predicted to have a propitious impact on population-wide morbidity and mortality [12, 117]. In contrast, alterations in bone density accompanying chronic exercise have bipotential implications, e.g., with putatively increased fracture risk owing to sex steroid deprivation (amenorrhea) and possibly reduced total (height) growth potential [75] and, conversely, variably decreased fracture risk owing to increased bone density associated with the stress–strain mechanism of enhanced bone apposition accompanying sustained physical training [22, 124–126]. However, other confounding factors, such as concurrent estrogen status, activity of the GH–IGF-1 axis, ethnicity, and gender, can also modify bone density and fracture risk. For example, we recently observed that black men and women show increased bone mass over their Caucasian counterparts but that only in men is the higher bone density in blacks associated with correspondingly increased GH secretion [127]. The mechanisms underlying such ethnic differences are also not yet understood, nor are possible ethnic differences in endocrine responsiveness to exercise stress well investigated.

Summary

The impact of chronic exercise training on the neuroendocrine control of the anterior pituitary gland, and its feedback and feedforward inputs, is complex. Multiple determinants influence adaptive hypothalamo–pituitary secretory responses to physical stress, namely, training intensity and duration, including overreaching exercise, concurrent weight loss, diet and energy balance, other associated stressors (both psychological and physical), body composition, gender, age, the sex steroid milieu, and developmental/maturational status. Confounding variables include interspecies differences, the complexity of neurohormone secretion (pulsatile, circadian, and entropic rhythms), the difficulty in interpreting earlier cross-sectional studies (with possible ascertainment bias) compared to longitudinal data, and the distinction between biochemical changes in and clinically significant sequelae of neurohormonal alterations with exercise. We emphasize that measurable pituitary responses to exercise should be viewed as part of a feedforward and feedback control system, as exemplified for the GH–IGF-1, GnRH–LH, CRH–AVP–ACTH, and other axes, with yet additional between-axes interactions. Although the final metabolic mechanisms that direct neuroendocrine changes in chronic training are not known definitively (e.g., free fatty acids, insulin, IGF-1, glucose, sex hormones, leptin, and/or others), their nature is likely multifactorial. In response to extremely strenuous exercise, stress-like neuro-

endocrine reactivity may predominate, whereas with appropriately modulated exercise intensity and volume, favorable clinical benefits, such as augmented GH secretion, cardiovascular conditioning, improved sense of well-being, and preserved reproductive function and bone density, likely ensue.

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Exercise and the GH-IGF-I Axis

5

Alon Eliakim and Dan Nemet

Introduction

Physical activity and exercise play an important role in tissue anabolism, growth, and development, but the mechanisms that link patterns of physical activity with tissue anabolism are not completely understood. The anabolic effects of exercise are not limited to participants in competitive sports since substantial anabolic stimulus arises even from relatively modest physical activities [1].

The exercise-associated anabolic effects are age and maturity dependent. Naturally occurring levels of physical activity are significantly higher during childhood, and during adolescence there is a simultaneous substantial increase in muscle mass and strength. Thus, the combination of rapid growth, high levels of physical activity, and spontaneous puberty-related increases in anabolic hormones (growth hormone [GH], insulin-like growth factor-I [IGF-I], and sex steroids) suggests the possibility of integrated mechanisms relating exercise with anabolic responses. In contrast, participation of young athletes in intense competitive training, especially if associated with inadequate caloric intake, may be associated with health hazards and may reduce growth potential [2].

Training efficiency depends on the exercise intensity, volume, duration, and frequency and on the athlete's ability to tolerate it. An imbalance between the training load and the individual's tolerance may result in under- or overtraining. Therefore, efforts are made to develop objective methods to quantify the fine balance between training load and the athlete's tolerance. The endocrine system, by modulation of anabolic and catabolic processes, seems to play an important role in the physiological adaptation to exercise training [3]. In recent years changes in circulating components of the GH-IGF-I axis, a system of growth mediators that control somatic and tissue growth [4], have been used to quantify the effects of training [5]. Interestingly, exercise is also associated with remarkable changes in catabolic hormones and inflammatory cytokines [i.e., interleukin-6 (IL-6)], and the exercise-related response of these markers can be also used to gauge exercise load [6, 7]. Anabolic response dominance will eventually lead to increased muscle mass and improved fitness, while prolonged dominance of the catabolic response, particularly if combined with inadequate nutrition, may

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ultimately lead to overtraining. Therefore, the evaluation of changes in these antagonistic circulating mediators may assist in quantifying the effects of different types of single and prolonged exercise training and recovery modalities.

This chapter demonstrates the effects of exercise on the GH-IGF-I axis, with an emphasis on the unique relationships between the exercise-related anabolic response and exercise-associated changes in inflammatory mediators. An important goal of this chapter is to show how exercise-induced changes in the GH-IGF-I-inflammatory axis can be used by elite athletes and their accompanying staff to evaluate training load throughout the competitive season and in the preparation for competition in "a real-life" setting. Finally, the chapter demonstrates new data on the possible use GH-IGF-I genetics in sports selection and prediction of excellence.

The GH-IGF-I Axis

The GH-IGF-I axis is composed of hormones, growth factors, binding proteins (BP), and receptors that regulate essential life processes. The axis starts at the central nervous system where several neurotransmitters (e.g., catecholamines, serotonin, cholinergic agents, etc.) stimulate the hypothalamus to synthesize growth hormone-releasing hormone (GHRH) and somatostatin (SMS). GHRH stimulates the anterior pituitary to secrete GH, while SMS inhibits GH secretion.

GH is the major product of the axis. One of GH most important functions is the stimulation of hepatic IGF-I synthesis. However, some GH effects on metabolism, body composition, and tissue differentiation are IGF-I independent. Tissue GH bioactivity results from interaction between GH and its receptor. The GH receptor is composed of intra- and extracellular transmembrane domains. The extracellular domain is identical in structure to GH-binding protein (GHBP) [8]; thus, measuring circulating GHBP levels reflects GH receptor number and activity.

IGF-I is one of the insulin-related peptides. Some of IGF-I effects are GH dependent, but the majority of its actions occur due to autocrine or paracrine secretion and regulation, which are only partially GH dependent. IGF-I is responsible for most, but not all, anabolic and growth-related effects of GH. IGF-I stimulates SMS secretion and inhibits GH by a negative feedback mechanism [9].

The bulk of circulating IGF-I is bound to IGFBPs. The most important circulating BP is IGFBP-3, which accounts for 80% of all IGF binding. Some IGFBPs are GH dependent (e.g., IGFBP-3), while others (IGFBP-1 and IGFBP-2) are insulin dependent (being high when insulin level is low). The interaction between IGF-I and its BPs is even more complicated since some BPs stimulate (e.g., IGFBP-5), while others inhibit (e.g., IGFBP-4) IGF-I anabolic effects [10].

Some hormones in the GH-IGF-I axis (i.e., GHRH and GH) have a pulsatile secretion pattern, and it has been shown that GH pulsatility is important for growth rate acceleration [11]. In contrast, IGF-I and IGFBPs level are relatively stable throughout the day.

Furthermore, several components of the axis are age and maturity dependent. GH, GHBP, IGF-I, and IGFBP-3 reach their peak levels during puberty [12] and decrease with aging [13]. These changes are partially sex hormone mediated. Nutritional status influences the GH-IGF-I axis as well. Prolonged fasting and malnutrition increase GH secretion, yet despite elevated GH, IGF-I levels remain low due to reduced levels of GH receptors [14]. All these factors must be taken into account when studying the effect of exercise on the GH-IGF-I axis.

Optimizing Training Modalities

Aerobic Training

The majority of the current knowledge regarding the importance of the GH response to exercise is based on studies examining the effect of aerobic-type exercise in individualized sports [15, 16]. To this end, when exercise is performed at the same absolute intensity, the GH response is greater in *less fit* subjects [17]. Yet, when subjects perform exercise at the same *absolute*, rather than *relative*

intensity, some individuals exercise below, while others exercise above, their lactic/anaerobic threshold (LAT). This is important since circulating GH levels increase only in response to aerobic exercise intensity above the LAT and because exercise loads of 75–90% of the maximal aerobic power yielded greater GH increase than milder loads. Therefore, results of studies in which the GH response to exercise was tested at an *absolute* work rate demonstrate simply that as individuals become fitter, the stress associated with exercise at an absolute work rate is reduced. The obvious implication for athletes is that as they become more physically fit, a more intense exercise should be performed to stimulate GH secretion. This is consistent with the common coaching modality of training cycles with workloads of increased intensity throughout the training season.

The duration of aerobic exercise for the stimulation of GH secretion should be at least 10 min [18]. The exercise-induced GH peak occurs 25–30 min after the start of exercise (slightly earlier in females compared to males), irrespective to its duration [19, 20]. Thus, when the exercise task is brief (e.g., 10 min), GH peak is reached after the cessation of exercise, while, when exercise is long (e.g., 60 min), GH peak is reached while the individual is still exercising. The important possible implication for athletes is that brief training sessions can be enough to stimulate the GH-IGF-I axis and to achieve a "training effect" (i.e., relative to this hormone and its response).

Pituitary refractoriness, a time in which the normal pituitary gland will not respond sufficiently to any stimulus for GH release, could also influence the GH response to exercise. For example, the GH response to exercise was inhibited if a spontaneous, early morning, GH pulse had occurred within 1 hour prior to the exercise test [20]. A refractory period of at least 1 hour was also shown following exercise-induced GH secretion (i.e., the subsequent GH response to exercise was attenuated) [21]. GH auto-inhibition, exercise-induced elevation in free fatty acids, or alterations in parasympathetic-sympathetic tone can explain the development of pituitary refractoriness. A recovery from pituitary refractoriness to GH secretion was seen if a second bout of high-intensity endurance exercise was performed 3 hours after the first session [22]. Consistent with this report, integrated 1.5 hours GH concentrations were significantly greater if differences between the exercise bouts (30 min, 70% VO_2 max [maximal oxygen uptake]) were 3.5 hours and not when 1 hour apart [23]. The practical application for athletes should therefore be that in order to achieve optimal GH secretion, the rest interval between multiple daily training sessions should be long enough (probably more than 3 hours) to allow pituitary recovery.

Anaerobic Exercise

A major progress was achieved in recent years in the understanding of the effects of anaerobic exercise on the GH-IGF-I axis. Stokes et al. [24] studied the effect of a single supramaximal 30 sec sprint on a cycle ergometer against different levels of resistance workloads. They found that the increase in GH levels was significantly greater when resistance was 7% (faster cycling) and not 9% (slower cycling) of body mass. Consistent with that, it was shown that when heavier loads were lifted, more total work was performed, and higher IGF-I levels were found using faster compared to slower tempo resistance training [25]. The possible implication for athletes is that lower levels of resistance and/or faster anaerobic efforts may better stimulate the GH-IGF-I axis and thus preferred by coaches and athletes.

Interval training is currently one of the most frequent training methods used in anaerobic and aerobic-type sports [26]. The intensity of such training depends on the running distance (sprint versus long distance), running speed (percent of maximal speed), the number of repetitions, and the length of the rest interval between the runs. In addition, coaches and athletes often change the style of the interval training and use constant running distances (e.g., 6 × 200m), increasing distance interval session (e.g., 100 m–200 m–300 m–400 m), decreasing distance interval session (e.g., 400 m–300 m–200 m–100 m), or a combination of increasing-decreasing distance interval session

(e.g., 100 m–200 m–300 m–200 m–100 m). While these style differences may seem negligible, they may involve different physiological demands, since in the increasing distance protocol, metabolic demands (e.g., lactate levels) increase gradually and are highest toward the end of the session, while in the decreasing distance protocol, the metabolic demands are higher from the beginning of the session [27], if the intensity of the intervals is appropriate and able to be maintained by the athlete.

A significant increase in GH and IL-6 levels was demonstrated following a typical constant distance (4 × 250m) interval training [28]. Consistent with previous findings in aerobic exercise, changes in the GH-IGF-I axis following the brief sprint interval exercise suggested exercise-related anabolic adaptations. The increase in IL-6 probably indicates its important role in muscle tissue repair following anaerobic exercise [29]. It was suggested that changes in the anabolic/catabolic/inflammatory balance can be used as an objective tool to gauge the training intensity of different types of anaerobic exercises and training periods as well.

More recently, we evaluated the effect of increasing (100–200–300–400 m) and decreasing distance (400–300–200–100 m) sprint interval training protocols, two other common types of sprint interval training, on the balance between anabolic, catabolic, and inflammatory mediators [27]. Both types of sprint interval trainings led to a significant increase in lactate and the anabolic factors GH and IGF-I. Both types of sprint interval sessions led to a significant increase in the circulating inflammatory mediators (IL-6). Interestingly, the lactate and GH area under the curve was significantly greater in the decreasing distance session. In contrast, rate of perceived exertion (RPE) was higher in the increasing distance session. Thus, despite similar running distance, running speed, and total resting period in the two interval training sessions, the decreasing distance interval was associated with a greater metabolic (lactate) and anabolic (GH) response (see Fig. 5.1). Interestingly, these greater metabolic and anabolic responses were not accompanied by an increase in RPE suggesting that physiological and psychological responses to interval training do not necessarily correlate. When the athletes were asked to explain why the increasing distance protocol was perceived as more intense, they replied that the fact that the longest and hardest run (400 m) was only at the end of the session was very difficult to tolerate. Coaches and athletes should be aware of these differences and, as a consequence, of the need for

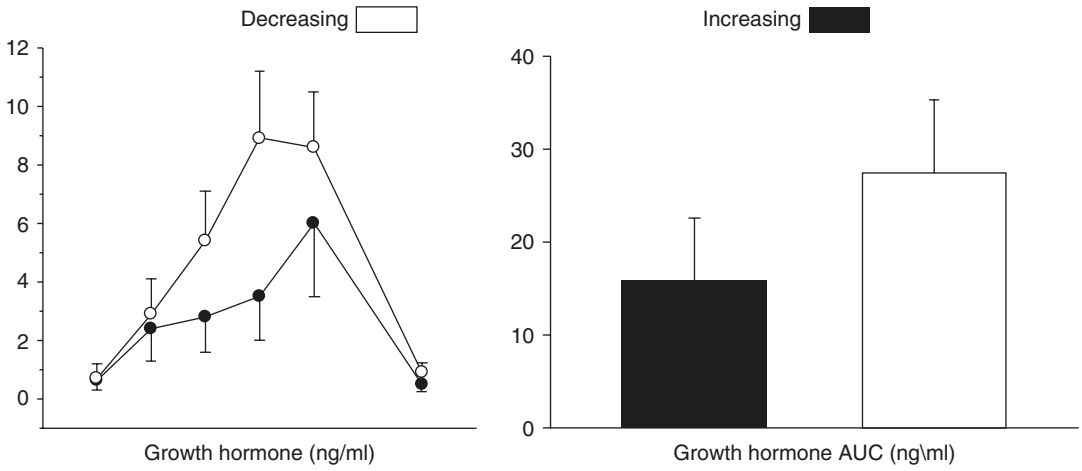

Fig. 5.1 The effect of decreasing and increasing distance of sprint interval exercise on GH and GH area under the curve responses. The decreasing distance interval was associated with a greater anabolic [GH (left) and GH AUC (right)] response

specific recovery adaptations after different types of interval training sessions. Differences in physiological and psychological responses to competitive sport training, and their influence on the training course and recovery process, should also be better addressed in future research work.

Finally, in contrast to the observation that both aerobic and anaerobic exercise require a high metabolic demand in order to stimulate GH secretion, we previously demonstrated a small but significant GH response to an exercise input that was perceived as difficult by the participants (i.e., 10 min of unilateral wrist flexion, a small and relatively unused muscle group) but which had no effect on heart rate or circulating lactate levels [30, 31]. This suggests that factors like the individual's perceived exertion and associated psychological stress play an important role in the activation of the hypothalamic-pituitary axis and to GH release even in exercise protocols involving small muscle groups.

Resistance Exercise

Previous studies have demonstrated increases in GH following a session of resistance exercise in adolescent and prepubertal boys. Children and adolescents demonstrate a lower GH response to resistance exercise compared with adults, presumably due to higher baseline GH levels [32]. As mentioned before, growth hormone is secreted in a pulsatile manner, with highest secretion during deep sleep, especially in children. Interestingly, Nindl et al. [33] reported that in men, an afternoon resistance training session affected the GH secretion pattern during resting states. Specifically, while mean GH secretion did not change, a lower rate of secretion in the first half of sleep and a higher rate of secretion in the second half of sleep were detected. This could be a direct effect of the resistance exercise on GH secretion, or an indirect effect on sleep quality. Considering the importance of nighttime GH secretion for linear growth, resistance training in adolescent athletes during different times of day (e.g., morning vs afternoon) may have different effects on sleep quality and/or directly on GH secretion pattern. Likewise, effects on the pulsatile secretion pattern may potentially even have effects on their linear growth. There is certainly a need for further research in this area.

Team Sports

Very few studies examined the effect of exercise on the GH-IGF-I axis in team sports. We previously demonstrated an increase in GH, testosterone, and IL-6 levels following a typical volleyball practice in adolescent national team level male and female players [34]. Interestingly, one of our most important findings was the effect of training on the endocrine response to a single practice. The hormonal response to a typical 60 min volleyball practice was assessed before and after 7 weeks of training during the initial phase of the season in elite national team level male and female players. In male players [35], training resulted in significantly greater GH increase along with significantly reduced IL-6 response to the same relative intensity volleyball practice. In female players [36], training resulted in significantly lower cortisol and IL-6 increase to the same relative intensity volleyball practice. The results suggest that along with the training-associated improvement of power, anaerobic and aerobic characteristics, part of the adaptation to training is that a single practice becomes more anabolic and less catabolic/inflammatory as training progresses during the initial phases of the training season (Fig. 5.2). Hormonal measurements therefore may assist athletes and their coaching staff in assessing the training program adaptation throughout different stages of the competitive season.

Finally, higher social position was associated with higher levels of IGF-I in both men and women, independent of wide range of known confounders such as age, ethnicity, body weight, and nutrition [37]. Along this line, Bogin et al. [38] studied high-level male and female competitive athletes from different university team sports (men, lacrosse, handball, rugby, and volleyball;

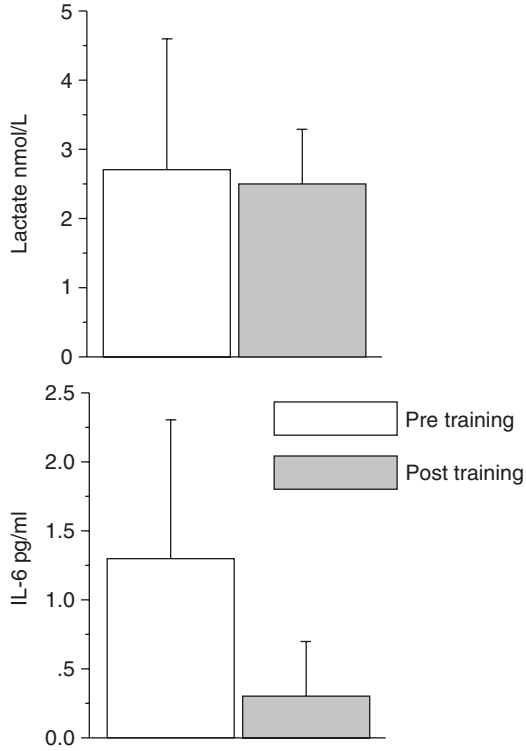

Fig. 5.2 The effect of training on the hormonal response to a single volleyball practice in male adolescent players. Same level of training (i.e., lactate response) leads to a reduced inflammatory response (IL-6)

women, football, rugby, netball, and volleyball) and assumed that what determines the social position in this social network is the level of success in sports (and not the economic status). Therefore the athletes were divided into winners and losers. The main finding of the study was that both pre- and post-competition IGF-I levels were about 11% greater among winners. There was no difference in the competition-related changes in IGF-I levels between the groups, suggesting that it is the baseline levels of IGF-I and not the change in IGF-I levels during the competition that may contribute to winning. This is the first study that related IGF-I levels with winning. It seems that IGF-I levels integrate the multiple genetic, nutritional, social, and emotional influences to a coherent signal that regulates growth and possibly athletic performance. This suggests a novel cycle: both single practice and prolonged training increase IGF-I levels, which in turn increase the chances of an athlete to win (see proposed model in Fig. 5.3). However, future larger studies that analyze other types of team sports and individual sports and that provide better control for nutritional, training, and doping status are needed to confirm this very interesting finding.

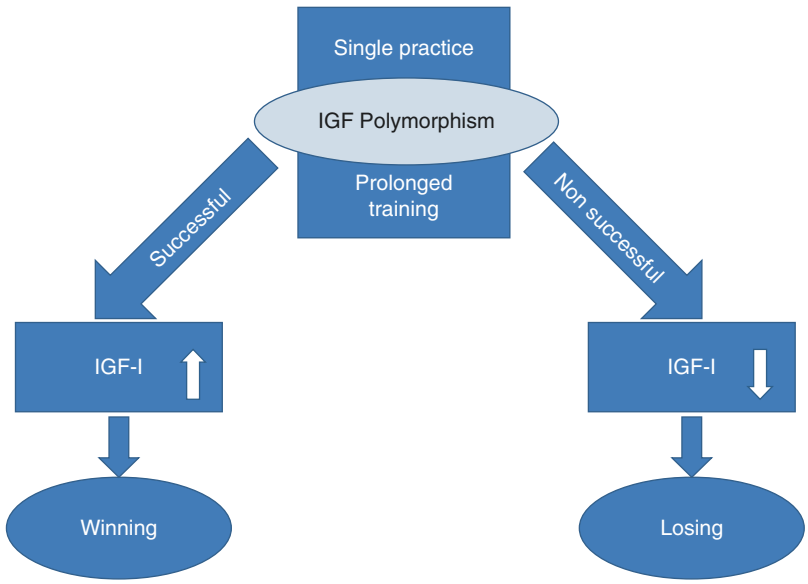

Fig. 5.3 A proposed exercise-training-IGF-I cycle. With proper training, both single practice and prolonged training increase IGF-I levels, which in turn may increase the chances of an athlete to win

"Real-Life" Exercise Studies

The majority of studies on the effect of exercise on the GH-IGF-I axis are laboratory-based. There is no doubt that laboratory-based science is important for understanding the exercise-related endocrine response. However, the translation of this knowledge to everyday use of competitive athletes is complicated, and there is a severe lack of "real-life" setting studies on the endocrine effect of exercise training. One of the main obstacles of executing "real-life" training studies is exercise standardization. We recently compared previous reports on the effect of "real-life" typical field individual (i.e., cross-country running and wrestling – representing combat versus non-combat sports) and team sports practice (i.e., volleyball and water polo – representing water and land team sports) on GH levels [39]. In this study, we were unable to control for the participants' fitness level or for each practice's intensity. In order to achieve some standardization, however, participants did not train during the day before the study, the duration of each practice was limited to 60–90 min, and the practice was performed during the initial phases of the training season when athletes are in relatively lower fitness level. All practice sessions were performed in the morning hours, and each typical practice included warm-up, main training segment, and cooldown. Blood samples were collected immediately before and at the end of practice, and the effect of the typical practice on hormonal and cytokine levels was expressed as percent change. Despite some limitations within the study, several important observations and conclusions could be drawn about the "real-life" training-related GH response from this unique comparison. These include the following: (1) cross-country running practice and volleyball practice in both males and females were associated with significant increases of GH, (2) the magnitude (percent change) of the GH response to the different practices was determined mainly by what were the pre-exercise GH levels, (3) there was no difference in the GH response between individual and team sports practices, and (4) interestingly, the GH response to the typical practices was not influenced by the practice-associated lactate change.

Cryotherapy, Recovery, and the GH-IGF-I Axis

The development of methods to enhance the recovery of elite athletes from intense training and/or competition has been a major target of athletes and their accompanying staff for many years. Cryotherapy is widely used to treat sports-associated traumatic injuries and as a recovery modality following training and competition that may cause some level of traumatic muscle injury [40, 41]. However, evidence regarding the effectiveness and appropriate guidelines for the use of cryotherapy are limited. To this end, Nemet et al. studied the effect of cold ice pack application following a brief sprint interval training on the balance between anabolic, catabolic, and circulating pro- and anti-inflammatory cytokines evaluated in 12 male, elite junior handball players [42]. The interval practice (4 × 250m) was associated with a significant increase in GH and IL-6 levels. Local cold-pack application was associated with significant decreases in the anabolic factors IGF-I and IGF-binding protein-3 during the recovery from exercise, supporting some clinical evidence of possible negative effects of cryotherapy on hormonal responses. These results, along with no clear detected effect on muscle damage or delayed onset muscle soreness (DOMS), may suggest that the use of cold packs should probably be reserved for traumatic injuries or used in combination with active recovery and not with complete rest. However, the findings of this study illustrate how exercise-induced changes in the GH-IGF-I axis and other catabolic and inflammatory markers may be used as an aid in competitive training. Further studies are needed to explore the beneficial use of anabolic, catabolic, and inflammatory markers measurement in the "monitoring" of recovery from exercise.

Nutrition, Performance, and the GH-IGF-I Axis

Nutritional factors may intervene with the GH response to exercise. For example, intravenous administration of the amino acid arginine is a strong stimulator of GH release and therefore used, for example, as one of the more common provocation tests for GH secretion in the diagnosis of some clinical states (e.g., short stature). Recently, it was demonstrated that oral arginine stimulates GH secretion as well [43]. Therefore, it is possible that the ingestion of arginine prior to exercise may attenuate the exercise-related GH response, most probably due to induction of a refractory period [44]. Along these lines, ingestion of a lipid-rich meal 45–60 min prior to an intermittent 30 min cycle ergometer exercise resulted in a significant more than 40% reduction in the exercise-induced GH elevation in healthy children [45]. The effect of prior high-fat meal ingestion appeared to be GH selective, as other counter-regulatory hormone responses to exercise, such as glucagon, cortisol, and epinephrine, were not affected. Similarly, administration of high-fat meal attenuated the magnitude of GH response to exercise also in adults, and this inhibition was correlated with circulating levels of SMS [46]. Interestingly, a high carbohydrate meal with a similar caloric content was also associated with a small decrease in GH response to exercise; however, this decrease was not statistically significant. These studies indicate that food consumption prior to exercise or sporting practice should be carefully selected, since a consumption of high-fat meal may affect the hormonal response to a training session.

Very few studies have examined the effects of nutrition on longer periods of training (i.e., weeks or months) primarily due to logistical issues. The timing of nutritional supplementation may also affect the training-associated response of the GH-IGF-I axis. For example, the combination of post-exercise essential amino acids and carbohydrate supplementation was accompanied by significant increases in free IGF-I during 3 weeks of high-intensity interval training [47] (compared to carbohydrate only or placebo). Protein supplementation 1 hour before and after practice during 10 weeks of resistance training (four times/week) were more effective than carbohydrate placebo in increasing muscle mass and muscle strength and were associated with greater increases in IGF-I and IGF-I mRNA in untrained males [48]. Consistent with these findings, twice daily protein compared to carbohydrate supplementation during 6 months of strength and conditioning training (five times/week) was associated with greater increase in IGF-I levels in untrained late pubertal and young adult males and females [49]. These results as well as others suggest a beneficial effect for protein supplementation during prolonged period of resistance training, but more research is needed on this topic to provide specific amount and type of protein supplementation.

Amenorrhea, Performance, and the GH-IGF-I Axis

The inhibitory effect of exercise training, in particular, when associated with nutritional deprivation, on the pulsatile release of hypothalamic GnRH and pituitary LH and FSH secretion is well established and will be discussed in more detail in other chapters of this book (see Chaps. 4, 7, and 8). This inhibition results in increased risk of athletic amenorrhea and hypoestrogenism [50]. To this end, it was shown that the exercise-associated GH release is attenuated in amenorrheic athletes. The mechanism for the attenuated amenorrhea-associated exercise-induced GH response is not completely understood. However, it was found recently [51] that low estrogen leads to decreased post-exercise type 1 deiodinase (an enzyme that converts T4 to the more active thyroid hormone T3), reduced T3 levels, and in turn a blunted GH response. This is particularly relevant to the adolescent female athlete, since the prevalence of amenorrhea among these athletes is 4–20 times higher than the general population [52]. “Athletic amenorrhea” appears mainly in younger athletes and is associated with sports activities where leanness provides a competitive

advantage (e.g., aesthetic-type sports, long-distance running, etc.) and, in particular, where intense training is accompanied by inadequate nutrition [50].The reduced exercise-induced GH response in these athletes should be considered critically important since it indicates probably reduced training effectiveness and performance. Consistent with that Vanheest et al. [53] showed that reduced energy intake and availability that was associated with ovarian suppression was also accompanied by lower T3 and IGF-I levels and by a 9.8% decline in 400 m swim velocity compared to 8.2% improvement among female swimmers without ovarian suppression at the end of 12 weeks of training (N.B., in total, 18% difference!). This occurred despite similar training protocols and while the ovarian suppressed swimmers were still menstruating (although less regularly). This is important because some coaches and young athletes promote energy-restrictive practices with the belief that it improves competitive performance [54]. The results of this study emphasize that athletes can maintain chronic energy deficit for varied periods with continued success in sport; however, prolonged negative energy balance results in training maladaptation and reduced performance. This may be particularly relevant for athletes during adolescence, a time with greater energy needs for growth and maturation.

Preparation for Competition

Measurements of hormones and in particular IGF-I levels can also assist athletes and coaches in the training preparation for selected competitions. For example, in one study, the effect of 4 weeks of training on fitness, self-assessment physical conditioning scores, and circulating IGF-I were determined in elite professional handball players [55] during their preparation for the junior world championships. Training consisted of 2 weeks of intense training followed by 2 weeks of relative tapering. Circulating IGF-I and physical conditioning scores decreased initially and returned to baseline levels at the end of tapering. There was a significant positive correlation between the changes in circulating IGF-I and self-assessed physical conditioning scores suggesting that the player's self-assessment may be a somewhat reliable tool when laboratory assessment is unavailable (see Fig. 5.4). Consistent with these findings, a follow-up of IGF-I levels during a training season in elite adolescent wrestlers showed an initial decrease in IGF-I level during periods of heavy training and return to baseline during tapering down and prior to the competition season [56]. Interestingly, changes in the pro-inflammatory mediators IL-6 correlated negatively with changes in IGF-I, being high when IGF-I level was low, and normalized

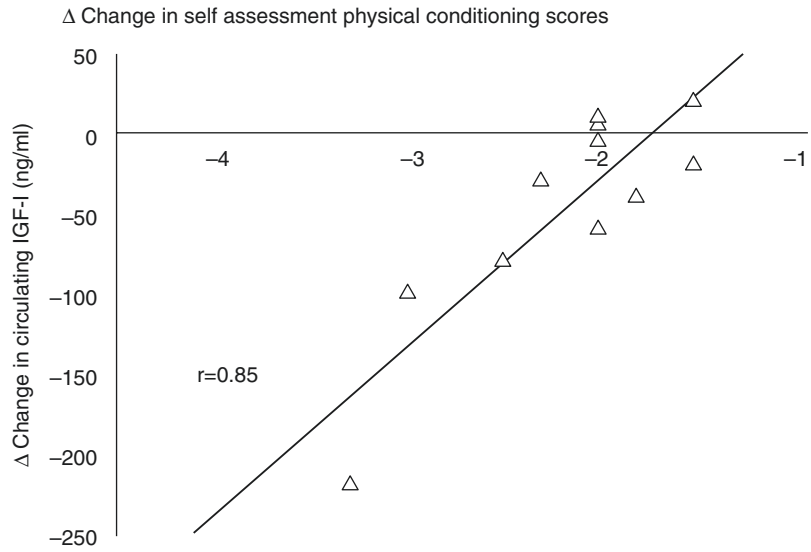

Fig. 5.4 Relationship between changes in self-assessment physical conditioning scores and change in circulating IGF-I during 2 weeks of intense training in handball players. There was a significant correlation between self-assessment scores and change in circulating IGF-I

when IGF-I levels normalized, emphasizing their potential contributing role for the training-associated change in IGF-I.

Tapering training intensity prior to the competition is a well-known training methodology used to help the athlete to achieve their best performance (i.e., increased rest leading to a psychophysiological restoration) [57]. Interestingly, this strategy is indeed associated with a parallel increase in circulating IGF-I levels. Therefore, measurements of IGF-I may assist coaches and athletes in their training preparations and provide a clue whether the tapering is being effective. Interestingly, in sports that do not plan their training for a specific targeted date of peak performance, like many of the team sports that train in the same relative intensity throughout a regular season (e.g., handball, soccer, etc.), changes in IGF-I level and its major binding protein IGFBP-3 are not typically found [58].

In optimal conditions, during the tapering of training intensity, IGF-I level will increase above baseline levels and will be associated with improved performance; however, this does not occur always. Since IGF-I can be reduced by nutritional imbalance and weight loss, it is possible that a deliberate decrease in body weight in athletes who participate in weight category sports (e.g., judo, wrestling), or even in team sport players prior to major tournaments, may prevent further increase in this anabolic hormone and will be associated "only" with a significant return to baseline values [56, 59]. This emphasizes the importance of proper nutritional counseling all throughout the training season. Previous studies demonstrated in athletes a training-associated negative correlation between circulating IGF-I and ghrelin, a hormone that is secreted by the stomach and pancreas and known to stimulate hunger [60]. Moreover, decreases of ghrelin and leptin, both known to mediate energy balance, were found following a 3-month preseason preparatory training in young female handball and basketball players [61]. All together this suggests that hormonal relationships, as one would expect, play a mediating role in training-induced associated energy balance, appetite, body composition, and muscle performance changes.

Interestingly, despite decreases in circulating IGF-I during period of intense training, physical fitness may still improve, as muscle mass does [62–65]. This suggests that while changes in circulating IGF-I are good markers of the general condition and energy balance of the athlete, they are not necessarily good indicators of the athlete's performance level. Probably, it is the local muscle levels of these hormones, and their autocrine or paracrine secretion, that are or could be more indicative of skeletal muscle performance [66, 67]. Nonetheless, tapering of the training intensity, was found to be associated with both increased IGF-I level and with further improvement of exercise performance of the athletes [57, 68].

It is still unknown what should be the below baseline permitted decrease of IGF-I levels during periods of heavy training, or what should be the optimal increase of this substance during periods of tapering and reduced training intensity (i.e., what magnitude of change is detrimental versus beneficial). However, the inability to increase circulating IGF-I levels before the target competition may suggest inappropriate recovery and suggest to the athlete and his/her coach that the athlete's general condition is not optimal. Collection of baseline and training-related hormonal changes, with a comparison to the hormonal response in previous seasons, and the knowledge and experience of the past success may prove to be of a very significant relevance as well.

IGF-I Genetics, Sports Selection, and Sports Excellence

The potential use of genetic single nucleotide polymorphisms (SNPs) of hormone genes, as a tool to assist in predicting future athletic performance, is currently an extremely challenging topic, mainly because each possible gene makes only a small contribution to the overall heritability. The majority of previous reports of hormonal gene polymorphism and athletic performance in professional athletes studied variations in the IGF-I polymorphism. The polymorphism of IGF-I promoter frequency

was significantly greater in athletes (9.2%) compared to controls (2.4%) and in particular among strength (11%) compared to athletes participating in team sports (7.8%) [69]. Our research group previously demonstrated [70] a higher frequency of the IGF-IC1245T T/T IGF-I promoter polymorphism among Israeli athletes (4.8%), compared to controls (nonexistence). Interestingly, while T/T polymorphism carriers were both endurance and power athletes, endurance athletes were of a national level, but the power athletes were top-level international and Olympic athletes. This suggests that the IGF1 T/T polymorphism may be more beneficial for power sports performance at the elite level. Along these lines, we also recently assessed [71] the frequency of another polymorphism of the IGF-I gene (i.e., IGF-Irs7136446) and demonstrated that the frequency of carrying the GG genotype was significantly greater among sprinters compared to weight lifters (see findings depicted in Fig. 5.5). Taken all together, this may suggest that among certain power sports activities, the IGF-I polymorphism is more important for speed rather than strength. In addition, we showed that the IGF2 (rs680) GG genotype frequency was significantly greater among sprinters compared to weight lifters [72], suggesting that carrying this IGF2 polymorphism may also be beneficial mainly for speed-related and not for strength sports. Circulating IGF2 levels were lower among individuals homozygous for the G allele [73], and higher levels of plasma IGF-I were found in individuals carrying the IGF2 GG genotype [74]. It is possible that the beneficial effect of the IGF2 rs680 polymorphism on speed performance is not necessarily mediated through its influence on circulating IGF2, but via its effect on IGF-I levels. This point, however, needs much further investigation.

Interestingly, it has been previously demonstrated that in contrast to elite track and field athletes, single nucleotide polymorphisms of IGF1, IGF1 receptor, and IGF2 were not frequent among swimmers [72, 75, 76]. These results possibly suggest that the insulin-like growth factor system is less significant for elite swimming than

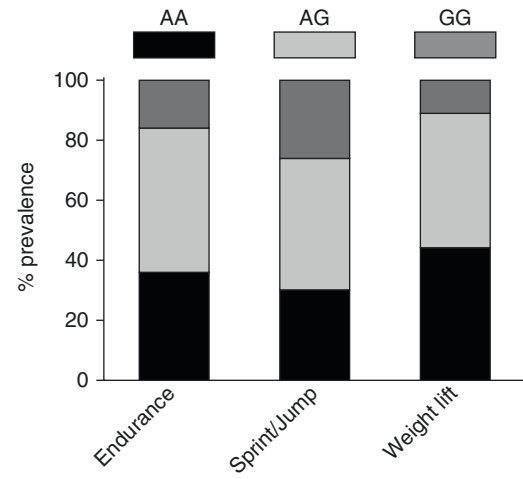

Fig. 5.5 The prevalence of the A/G IGF-I rs7136446 polymorphism among national and top-level Israeli endurance athletes, short-distance runners/jumpers and among weight lifters (p = 0.036, for GG genotype frequency, sprinters vs weight lifters)

for running performance. The mechanism for this discrepancy is currently unknown and in need of study. A possible explanation is that swimming excellence is mainly affected by the swimmer's physical attributes (particularly limb length) and swimming technique [77], possibly masking physiologic, metabolic, and muscle mass differences and enabling tall and technically skilled swimmers to excel in the majority of swimming distances.

These results indicate that extreme caution should be done before pooling different types of sport in genetic research because despite seemingly similar metabolic characteristics, athletes from different sport disciplines carry different genetic polymorphisms. Whether a multipotent athlete who wants to develop a competitive career and carries a beneficial IGF system polymorphism should prefer track and field over swimming is currently speculative and hence must be interpreted with caution. Moreover, one should always keep in mind that while a favorable genetic predisposition is essential, psychological features and environmental aspects, including training equipment and facilities, nutrition, familial support, and motivational issues, are also critically essential for top-level sports performance success.

Summary

In recent years there has been a significant research progress in the field of exercise endocrinology. It is now clear that monitoring changes in the balance of anabolic (GH→IGF-I system) and catabolic hormones and related inflammatory mediators following different types of exercise training and during different stages of the training season may help elite athletes and their coaching staff in developing a more "optimal" training program and in the preparation for competition. In addition, the use of hormonal genetic polymorphisms may serve as an additional assisting tool for talent identification and sports selection and perhaps also for building effective (i.e., more precise) training programs for athletes. Further research is needed, however, to better clarify the complex relationship of training, hormonal responses, nutrition, genetics, and optimal athletic performance in competitive sports.

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Exercise and Thyroid Function

6

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Introduction

Thyroid hormone receptors are present in virtually every tissue in the body, thereby permitting an important physiologic role for the two thyroid hormones, thyroxine (T4) and triiodothyronine (T3). Skeletal and cardiac muscle function, pulmonary performance, metabolism, and the neurophysiologic axis are only a few of the important areas affected by thyroid hormone level [1]. Any abnormality in thyroid function causing either an excess or deficiency in circulating thyroid hormone levels can lead to changes in body function at rest and during exercise. The presence of thyroid disease can have a major impact on exercise tolerance resulting in reduced performance of strenuous activities. On the other hand, exercise itself may have direct or indirect effects on thyroid function, either secondary to acute alterations in the integrity of the pituitary thyroid axis or to more long-lasting changes. In well-trained athletes, alterations in thyroid function can be viewed as an adaptive mechanism associated with enhanced performance possibly serving to provide a better balance between energy consumption and expenditure. Underlying energy balance does appear to play an important role in the effects that exercise may have on the hypothalamus–pituitary–thyroid axis. Reports in the literature indicate that athletes with excessive weight loss may exhibit a "low T3 syndrome" accompanied by amenorrhea (in women) as well as other alterations in pituitary function [2]. Fortunately, thyroid diseases usually can be treated effectively, and most individuals with thyroid disorders should expect to obtain resolution of their thyroid-related symptoms, including those associated with a negative impact on their exercise tolerance. The track athlete, Gail Devers, who has been very public about her experience with Graves' disease, is a well-known sprinter who went on to win Olympic fame following treatment for her Graves' disease and may act as a case in point.

After a brief overview of normal thyroid physiology, this chapter will provide a survey of the literature describing effects of abnormal thyroid hormone levels on exercise tolerance, with a special focus on alterations in cardiac, muscle, and respiratory function. The chapter will conclude with a review of existing data on the response of the pituitary–thyroid axis to varying levels and types of exercise.

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Thyroid Physiology

All steps in thyroid hormone (TH) biosynthesis are driven by thyrotropin (TSH) and are intimately linked to iodine metabolism. Dietary iodine is reduced to iodide, is absorbed by the small intestine, and then enters the circulation. Iodide "trapped" by the thyroid gland subsequently undergoes oxidation by thyroid peroxidase (TPO), iodinating tyrosyl residues in the storage protein, thyroglobulin, to form the iodothyronines, monoiodotyrosine (MIT), and diiodotyrosine (DIT). MIT and DIT molecules can then couple to form either tetraiodothyronine (T4) or triiodothyronine (T3), which are the two major thyroid hormones. T4 and T3 are bound within thyroglobulin and stored in thyroid follicles. Under control of TSH, thyroglobulin undergoes endocytosis and proteolytic digestion, releasing T4 and T3 into the circulation. The feedback loop is completed at the hypothalamic level where declining levels of circulating T4 or T3 will prompt secretion of thyrotropin-releasing hormone (TRH), which stimulates synthesis and secretion of TSH. After binding to its specific receptor on the thyroid cell membrane, TSH leads to stimulation of T4 and T3 production. Only 20% of circulating T3 is derived from thyroid secretion, whereas 80% is derived from the monodeiodination of T4 by 5′-deiodinase (type I and type II) in the periphery (see Fig. 6.1) [3]. Since T3 is some 10–15 times more biologically potent than T4, this latter conversion has been termed the "activating" pathway of thyroid hormone metabolism. Alternatively, in certain physiologic and pathologic states, the deiodination of T4 proceeds via a 5-deiodinase (type I and type III), which leads instead to reverse T3 (rT3). Since rT3 is a biologically inactive compound [3], this route of metabolism has been termed the "inactivating" pathway. A precise metabolic role for rT3 has not been described, but diversion of T4 metabolism from the activating to the inactivating pathway serves a nitrogen-sparing and protective effect for the body during times of stress and has been viewed as homeostatic. After binding to a cellular receptor, the thyroid hormones have both genomic and nongenomic effects, the former leading to modulation in expression of nuclear actions, whereas the latter appears to involve plasma membrane/mitochondrial responses [4] (Table 6.1).

Thyroid Hormone Effects

Hyper- and hypothyroidism, associated with either excess or deficiency of TH, respectively, may have a negative impact on exercise performance. Although TH has pervasive effects on virtually

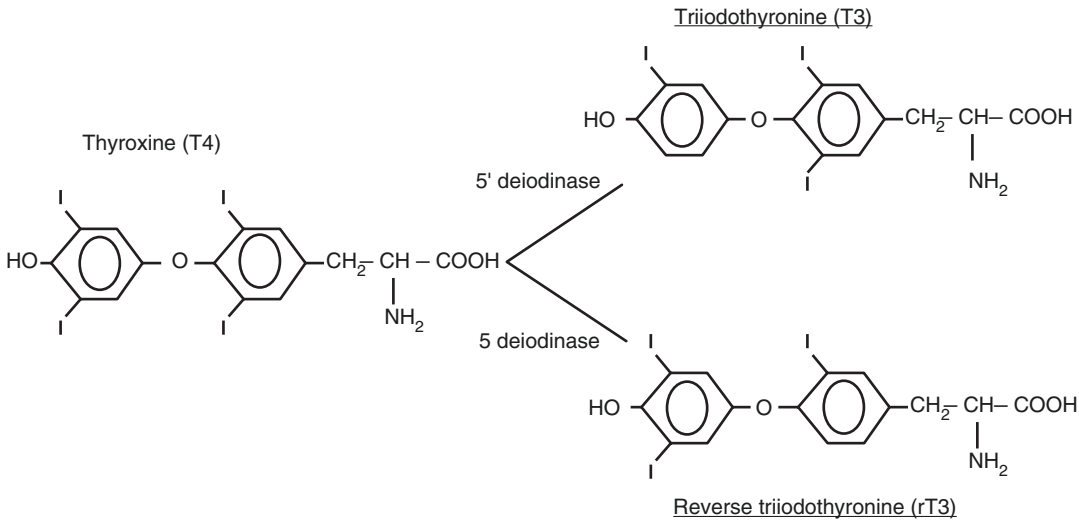

Fig. 6.1 Thyroxine, triiodothyronine, and reverse triiodothyronine

Table 6.1 Genomic and nongenomic actions of thyroid hormones

Genomic actions of thyroid hormones
-Positive regulation
Sarcoplasmic reticulum calcium adenosine triphosphatase
Myosin heavy chain α
β1-adrenergic receptors
Sodium/potassium adenosine triphosphatase
Voltage-gated potassium channels (Kv1.5, Kv4.2, Kv4.3)
Adenine nucleotide translocator 1
-Negative regulation
T3 nuclear receptor α1
Myosin heavy chain β
Phospholamban
Sodium/calcium exchanger
Adenylyl cyclase types V,VI
Nongenomic actions of thyroid hormones
Conductivity of sodium, potassium, and calcium channels
Actin polymerization status
Activation of PI3K/Akt/mTOR signaling pathway
Deiodination and decarboxylation of T4 resulting in thyronamine synthesis

all functions of the body, the following discussion emphasizes thyroid-related influences on exercise tolerance as mediated via involvement with cellular metabolism and the function of skeletal muscle and the cardiac, vascular, and pulmonary systems.

Cardiovascular Effects of Thyroid Hormones

Cardiac performance is dependent on the contractility of the heart as well as systemic vascular resistance. Resting tachycardia is very common in hyperthyroidism, and many patients complain of having a "racing" or "pounding" heart. The heart, being itself a muscle, is affected by thyroid hormone levels as is skeletal muscle. The heart relies mainly on serum T3 because there is no significant myocyte intracellular deiodinase activity [5].

TH can affect cardiac action via direct genomic and nongenomic effects on cardiac myocytes and hemodynamic alterations in the periphery that result in increased cardiac filling and modification of cardiac contraction [6]. TH mediates the expression of both structural and regulatory genes in the cardiac myocyte [5]. Thyroid hormone-responsive cardiac genes include sarcoplasmic reticulum calcium/adenosinetriphosphatase ($[Ca^{2+}]$/ATPase) and its inhibitor phospholamban, which are involved in regulation of calcium uptake by the sarcoplasmic reticulum during diastole [7], α- and β-myosin heavy chains, the ion channels coordinating the electrochemical responses of the myocardium: sodium/potassium ATPase (Na^+/K^+-ATPase), voltage-gated potassium channels (Kv1.5, Kv4.2, Kv4.3), and sodium/calcium exchanger [6]. TH increases the expression of β1-adrenergic receptors and downregulates TRα1 receptors [8, 9].

In summary, the genomic action of TH on the heart involves genes which are largely responsible for enhanced contractile function and diastolic relaxation. Thus, T3 markedly shortens diastolic relaxation, i.e., the hyperthyroid heart relaxes with a higher speed (lusitropic activity), whereas diastole is prolonged in hypothyroid states.

The nongenomic effects of TH on the cardiac myocyte and on the systemic vasculature tend to occur rapidly. Schmidt et al. documented that T3-enhanced myocardial contractility and reduced systemic vascular resistance occur within 3 min [10]. These rapid T3-mediated effects include changes in membrane ion channels for sodium, potassium, and calcium; effects on actin polymerization; adenine nucleotide translocator 1 in the mitochondrial membrane; and a variety of intracellular signaling pathways in the heart and vascular smooth muscle cells [11, 12]. The actions on channels may determine set points of myocardial excitability and duration of the action potential and contribute to development of tachyarrhythmias [13].

Additional mechanism of T3 actions observed in vitro includes rapid activation of phosphoinositide 3-kinases (PI3K) leading to protein kinase B (Akt) phosphorylation that in turn translocates to the nucleus and promotes mammalian target of rapamycin (mTOR) phosphorylation [14]. As mTOR is important to regulate ribosomal biogenesis and protein translation, the signaling pathway described in these studies may underlie at least one of the nongenomic mechanisms by which T3 regulates cardiac growth and hypertrophy.

Moreover, it has been discovered that deiodination and decarboxylation of T4 could generate a biologically active metabolite, thyronamine, which is characterized by actions opposite to those of TH [15, 16]. It has been demonstrated that thyronamine reduces cardiac output, heart rate, systolic pressure, and coronary flow in isolated heart within minutes [16]. Conceivably, a balance between T3 and thyronamine might be responsible for maintaining cardiac homeostasis. Changes in this equilibrium might contribute to the cardiovascular alterations that occur in patients with thyroid disease [17].

In Vivo Animal Studies on the Role of Abnormal Thyroid Function in the Regulation of Cardiac Response to Exercise

It has been believed that one of the main mechanisms of increased cardiac work during hyperthyroidism was the sensitization to catecholamines. However, Hoit et al. in a study on thyrotoxic baboons refuted a role of βl- or β2-adrenergic receptors in any cardiac response to hyperthyroidism [18]. Interestingly, abnormal cardiac response to exercise has been described as being due to an inefficient use of chemical energy stored in adenosine triphosphate (ATP). In hyperthyroid hearts, a larger fraction of energy goes to heat production, whereas in euthyroid animals more is spent for useful contractile energy. Finally, TH modifies the secretory activity of the heart—i.e., T3 has been found to increase mRNA and protein levels of atrial natriuretic factor [19].

Several studies have indicated overactivation of the renin–angiotensin–aldosterone (RAA) system in hyperthyroid animals, documenting increased plasma renin [20, 21] and upregulated synthesis and secretion of angiotensinogen [22] in hyperthyroid rats. In contrast, the plasma renin activity is reduced in experimental hypothyroidism [20]. There is also an evidence of tissue-specific regulation of RAA. TH activates some components of cardiac RAA, and hyperthyroidism can promote an increase in cardiac levels of renin, stimulate Ang II generation [23], and raise levels of AT1 and AT2 receptors [20]. In the heart, Ang II exhibits growth-promoting effects by inducing hypertrophy and fibrosis, mediated by the AT1 receptor [24]. Although most of the effects of Ang II related to cardiac remodeling have been attributed to the AT1 receptor, the AT2 receptor is also involved in the development of some cardiac hypertrophy models [25]. There are several literature reports showing that AT1 receptor blockade and ACE inhibition attenuate or prevent the development of cardiac hypertrophy induced by TH in vivo [21, 26, 27]. Some authors suggest that the mechanism of action of these compounds is associated with the alterations in calcium handling [28], while others suggest that these drugs may inhibit AT1 receptor-induced activation of PI3K/Akt/mTOR pathway [29, 30].

In hyperthyroidism structural remodeling such as hypertrophy, left ventricular fibrosis, myocyte lengthening, chamber dilatation, and decreased relative wall thickness have been observed and have been considered as likely to contribute to global left ventricular functional impairment [31].

Clinical Findings

In thyroid disease, cardiac structures and function may remain normal at rest; however, impaired left ventricular (LV) function and cardiovascular adaptation to effort become unmasked during exercise [32].

Hypothyroidism

Hypothyroidism has been associated with a decrease in intravascular volume, stroke volume, and cardiac index and an increase in systemic vascular resistance, resulting in diastolic hypertension (Table 6.2) [33]. In patients with transient hypothyroidism owing to thyroidectomy, radionuclide ventriculography and right heart catheterization revealed lower cardiac output, stroke volume, and end-diastolic volume at rest, but increased systemic peripheral resistance [34]. In the same individuals, during exercise, heart rate, cardiac output, end diastolic volume, and stroke volume were higher when the patients were euthyroid than when they were hypothyroid.

Table 6.2 Cardiovascular changes observed in hyper- and hypothyroidism

	Hyperthyroidism	Hypothyroidism
Heart rate	↑ NC	↓NC
Vascular volume	↑	↓
Stroke volume	↑	↓
Cardiac output	↑	↓
SVR	↓	↑NC
LVEF		
Rest	↑↓NC	↓NC
Exercise	↓	↓
Diastolic blood pressure	↓	↑NC
Systolic blood pressure	↑NC	↓NC
LV pre-ejection period	↓	↑
LV ejection time	↓	↑

↑ increased, ↓ decreased, *NC* no change, *SVR* systemic vascular resistance, *LVEF* left ventricular ejection fraction, *LV* left ventricular

The baseline LV ejection fraction (LVEF) and peak LVEF were shown to be lower in hypothyroid subjects compared with their euthyroid state, although with exercise, the rise of LVEF in the two states was similar [35]. As assessed by radionuclide-gated pool ventriculography in a younger group (average age 24 years), there was no noticeable change in LVEF with hypothyroidism, although exercise tolerance did improve after levothyroxine (LT4) replacement [36]. Even hypothyroidism of brief duration of only 10 days was associated with an impaired LVEF response to exercise; LVEF response returned to normal with restoration of the euthyroid state [37]. Of interest, the patients still achieved the same workload in either state.

Interesting observations have been found in patients with subclinical hypothyroidism (Sc-HypoT) defined as mild elevations of TSH with normal levels of T4, fT4, T3, and fT3. It has been a matter of investigative interest whether the mild hypofunction associated with subclinical hypothyroidism affected any measureable cardiac parameters.

An accurate assessment of left ventricular function performed by Doppler echocardiography in patients with stable Sc-HypoT showed no changes in left ventricle morphology. However, the prolonged isovolumic relaxation time and a reduced early-to-late ratio of the transmitral peak flow velocities are suggestive of impaired diastolic function in the sense of slowed relaxation [38].

In the same study, ten randomly selected patients were re-evaluated after achieving euthyroidism by means of 6 months of LT4 administration. The treatment caused no change in the parameters of left ventricle morphology, whereas it normalized systolic and diastolic function. Interestingly, although systemic vascular resistance was comparable in untreated patients and control subjects, it was significantly decreased after LT4 therapy. Similar findings have been documented by Kahaly et al. [39], who assessed cardiac function on effort and physical exercise capacity showing no abnormalities in various cardiac parameters at rest, either before or after LT4 treatment. However, stroke volume, cardiac index, and peak aortic flow velocity were significantly lower, and the pre-ejection period was significantly prolonged during exercise in the untreated patients versus controls. Other authors confirmed early myocardial dysfunction unveiling a difference in longitudinal systolic and diastolic function reserve indexes during exercise in Sc-HypoT patients compared to controls [40]. However, in a large-scale study, structural changes were not observed when comparing patients with normal TSH with patients with TSH > 5 mIU/L [41]. Tadik et al. performing 3-dimensional echocardiography in 94 subjects observed significantly reduced LV cardiac output and ejection fraction in patients with Sc-HypoT compared to both controls and the same patients 1 year after treatment [42]. Furthermore, when women with Sc-HypoT perform physical activity, a slower HR kinetics (intended as time to reach 63% of the HR at steady state) has been observed in the transition from rest to exercise compared with euthyroid women [43].

Evidence supporting reversible left ventricle diastolic dysfunction in patients with subclinical hypothyroidism was documented employing radionuclide ventriculography [44]. The authors found that the time to peak-filling rate was prolonged in ten patients with Sc-HypoT compared

to ten normal control subjects. This accurate index of diastolic function normalized after achieving euthyroidism with LT4 therapy.

Abnormal diastolic function may impair coronary flow reserve. Hypothyroid individuals may have a form of reversible coronary dysfunction as found in a study of six patients undergoing stress testing before and after LT4 replacement therapy. Prior to replacement therapy, SPECT scanning revealed notable regional perfusion defects in four of six patients, which resolved within 8 weeks of LT4 therapy [45]. Similarly, Oflaz et al. [46] found that coronary flow reserve was lower in patients with Sc-HypoT than in euthyroid subjects. On the contrary, Owen et al. [47] using stress echocardiography with i.v. dobutamine found no differences in resting global, regional left ventricular function or regional myocardial velocities during maximal dobutamine stress between patients and controls or in patients treated with replacement therapy compared with baseline values.

To summarize, the vast majority of clinical studies show impaired LV systolic and diastolic function during exercise in patients with both overt and subclinical hypothyroidism.

Hyperthyroidism

The effects of hyperthyroidism on cardiac function both during rest and exercise are numerous (see Table 6.2) [33]. In thyrotoxicosis, the extent of the various cardiac responses to excess TH is somewhat dependent on the duration and severity of the disorder. Resting tachycardia, a slow decline in postexercise heart rate (HR), atrial fibrillation, decreased exercise tolerance, and, rarely, congestive heart failure (CHF) are seen in thyrotoxic patients. Cardiac complications from hyperthyroidism tend to occur in patients with a history of prior cardiac disease. Atrial fibrillation, atrial enlargement, and CHF are more common in patients over 60 years old with toxic multinodular goiter. Instead, cardiac valve involvement, pulmonary arterial hypertension, and specific cardiomyopathy are more common in Graves' disease [48]. Augmented blood volume and blood flow to the skin, muscles, and kidneys are seen and may be owing to vasodilators released secondary to increased cellular respiration [49]. A rise in cellular oxygen consumption leads to a higher demand for oxygen and the need to get fuel to the peripheral tissues [49]. An increase in the velocity of cardiac muscle contraction is present, as well as a rise in myosin ATPase activity [50]. Evaluation of systolic time intervals in thyrotoxic subjects reveals a shortening of the LV pre-ejection period along with quicker LV ejection time and isovolumetric contraction [33, 51].

Kahaly et al. analyzed alterations of cardiovascular function and work capacity using stress echocardiography as well as spiroergometry in subjects with untreated thyroid dysfunction, then again after restoration of euthyroidism. At rest, LVEF, stroke volume, and cardiac indices were significantly increased in hyperthyroidism, but exhibited a blunted response to exercise, which normalized after restoration of euthyroidism. During exercise, negative correlations were found between free T3 (fT3) and diastolic blood pressure, maximal workload, HR, and LVEF. This impaired cardiac response to exercise was specifically apparent in older subjects [52–54].

Of note, combined oral LT4/LT3 overdosage has been reported to cause ST wave depressions with treadmill stress testing that resolve with the euthyroid state [55]. In general, diagnostic treadmill testing is best delayed until patients are euthyroid.

"Subclinical" hyperthyroidism (Sc-HyperT) is a term that has been applied to patients with undetectable levels of serum TSH, but with normal levels of T4, fT4, T3, and fT3. In one study, there was no difference in LVEF at rest and exercise between Sc-HyperT and controls, whereas overt hyperthyroid subjects had a reduction in LVEF with exercise, increased HR, and cardiac output at both rest and exercise [56]. Supporting evidence was provided by a study performed in 1112 subjects with a 5-year follow-up in which left ventricular mass divided by height did not differ between subjects with and without Sc-HyperT [57].

However, studies by Kaminski et al. indicated worse physical capacity in subjects with Sc-HyperT and the possibility of improvement after therapy. Compared with results after treat-

ment, the end-diastolic and end-systolic volume indexes, stroke volume index, and cardiac index were significantly larger in patients with Sc-HyperT. Stroke volume index was negatively correlated with TSH and positively with fT4 and fT3 values, and cardiac index was positively correlated with fT4 and fT3 levels in Sc-HyperT [58].

Analysis of the Framingham Heart Study revealed that TSH was related to left ventricular contractility in women with TSH < 0.5 mU/L TSH [41]. Furthermore, thicker left ventricular posterior wall, higher HR, and a lower achieved maximum workload have been reported in women with nontoxic multinodular goiter treated with mildly suppressive levothyroxine therapy compared to women not under treatment [59].

To summarize, LVEF, stroke volume and cardiac index, may be greater at rest in hyperthyroidism, but the lack of an increase in LVEF with exercise seems to be a reproducible finding.

Effects on Systemic Vascular Resistance (SVR)

TH causes decreased resistance in peripheral arterioles through a direct effect on vascular smooth muscle and decreased mean arterial pressure, which, when sensed in the kidneys, activates the RAA system and increases renal sodium absorption. T3 also increases erythropoietin synthesis, which leads to an increase in red cell mass. The combination of both leads to an increased blood volume and preload. In hyperthyroidism, these effects increase cardiac output by 50–300%, while a 30–50% reduction is seen in hypothyroidism [5].

In the vascular smooth muscle cell, TH-mediated effects are the result of both genomic and nongenomic actions. Nongenomic actions target membrane ion channels and endothelial nitric oxide (NO) synthase, which serves to decrease SVR [60, 61]. Indeed, it was recently reported that the PI3K/Akt signaling pathway plays a role in T3-induced NO production by vascular smooth muscle cells and by endothelial cells [11, 62].

Furthermore, T3 has been shown to inhibit vascular remodeling via the inhibition of the cAMP response element binding protein, a nuclear transcription factor involved in the remodeling process [63]. It seems also that voluntary exercise training can improve long-lasting endothelial dysfunction resulting from transient thyroid hormone deficiency in early life [64].

Clinical Findings

Hypothyroidism

Vascular control mechanisms may be abnormal in hypothyroidism with blunted vasodilatation secondary to reduced endothelium-dependent vasodilatation [65, 66]. In overt hypothyroidism, arterial compliance is reduced, which leads to increased arterial stiffness with higher central augmentation pressure and lower pulse wave velocities. These abnormalities were reversible with adequate LT4 treatment [67, 68]. However, in subclinical hypothyroidism, the study results have been equivocal. Several studies have not found any association between Sc-HypoT and blood pressure at rest [69–71]. In one cross-sectional study [69], Sc-HypoT was not associated with increased resting blood pressure. Similar results were observed in the cross-sectional Busselton thyroid study [70] that included 105 subjects with Sc-HypoT and 1859 euthyroid controls from Western Australia. On the other hand, two large population-based studies with 5872 [72] and 30,728 [73] subjects reported a modest association between high-normal serum TSH levels and resting blood pressure. This observation has been confirmed in other studies, suggesting that mild thyroid hormone deficiency also may affect vascular tone [74–77]. Several studies documented an improvement of SVR after LT4 replacement [38, 78]. Endothelial dysfunction in patients with hypothyroidism, borderline hypothyroidism, and those with high-normal TSH values using flow-mediated arterial dilation (FMD) has been demonstrated with TSH levels correlating inversely to endothelium-dependent dilatation [77]. Impaired endothelium-dependent

vasodilatation as a result of a reduction in nitric oxide availability has been demonstrated in Sc-HypoT by Taddei et al. [79].

Studies have also shown that FMD is associated with plasma osteoprotegerin levels in hypothyroid patients [80]. Osteoprotegerin is a member of the tumor necrosis factor (TNF) receptor family involved in vasculature regulation and related with increased cardiovascular mortality. In vitro studies suggest that TH and TSH are involved in regulation of osteoprotegerin expression [81].

Hyperthyroidism

Endothelium-dependent arterial dilatation is increased in hyperthyroid patients and is reversible after subtotal thyroidectomy [82]. Ojamaa et al. [83] demonstrated vascular relaxation due to the action of excess TH on the vascular smooth muscle cells. Conceivably, an inability to lower SVR during exercise in the hyperthyroid state might lead to impaired exercise tolerance [84]. In this regard, phenylephrine administration was associated with an increase in SVR and a decrease in cardiac output not seen in euthyroid subjects [85]. On the contrary, a case-control study of 42 patients with untreated overt hyperthyroidism documented similar systolic and diastolic blood pressures during maximal exercise as in 22 healthy controls. Moreover, no changes in systolic and diastolic blood pressure responses to exercise were observed in these patients after restoration of euthyroidism during 6-month follow-up [52]. Similar findings hold true for the patients with Sc-HyperT. In a recent population-based prospective cohort study, Völzke et al. [86] found that Sc-HyperT is not associated with changes in blood pressure, pulse pressure, or incident hypertension. Some smaller studies have reported similar results [52, 87].

Effects in Muscles

TH plays a critical role in maintaining homeostasis and influencing the rate of metabolism and energy expenditure. Skeletal muscles contribute to about 20–30% of resting metabolic rate [88]. TH control the expression of myocyte-specific genes coding for myosin isoforms [32], the Na^+–K^+ ATPase pumps, and the Ca–ATPase canals of the sarcoplasmic reticulum. This explains the increase of contractility and relaxation of skeletal muscles observed in hyperthyroidism, as opposed to hypothyroidism. In both cases muscle performance is reduced, with accumulation of lactic acid at exercise. This is because of defective pyruvate oxidation and proton expulsion in hypothyroidism and of acceleration of glycolysis in hyperthyroidism. Muscle glycolysis exceeds mitochondrial oxidation enhancing the shunting of pyruvate to lactate, thus leading to an increased lactic acid concentrations resulting in intracellular acidosis. Furthermore, TH increases fast myosin and fast-twitch fibers in skeletal muscle, which are less economic in oxygen utilization during contraction than slow-twitch muscle fibers explaining impaired exercise tolerance.

In Vivo Animal Studies on the Role of Abnormal Thyroid Function in the Regulation of Muscle Response to Exercise

Animal studies of hypothyroidism reveal that glycogen levels in muscle appear to be normal to increased at rest, whereas during exercise, muscle utilization of glycogen rises as may lactate production [89, 90].

In hypothyroidism, studies reveal a reduction in flow to the fast-twitch type II fibers of high-oxidative type muscles [91] compromising exercise capacity via reduced oxygen delivery and endurance through decreased delivery of blood-borne substrates [92, 93]. Additionally, decreased mobilization of free fatty acids (FFA) from adipose tissue leads to reduced lipid delivery to skeletal muscle [94]. After exercise the rate of glycogenolysis exceeded those in controls, showing diminished oxidative capacity resulting in lowering the ATP content. Thus, inadequate fuel utilization may be considered as a factor limiting ability for heavy exercise in hypothyroidism [89] probably triggering compensatory mechanisms in gene expression resulting in a slower striated

muscle phenotype [95, 96]. Moreover, in distinction to hypothyroid individuals, muscle blood flow is enhanced in hyperthyroid subjects including fast-twitch sections of muscle [94].

In induced hyperthyroidism, compared to euthyroid control rats, the energy cellular potential was increased during exercise, and it remained higher after the recovery period [97] testifying for an impaired cellular energy. THs also promote expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1a), which mediates mitochondrial biogenesis and oxidative capacity in skeletal muscle. Acute exercise increases deiodinase-2 expression in skeletal muscle accelerating conversion of T4 to T3 which induces PGC-1a and its downstream effect on mitochondria [98].

Whether physical activity can be recommended in hyperthyroidism is questionable. The effect of T3-induced thyrotoxicosis on exercise tolerance has been studied, with increases noted in resting oxygen uptake and increased lactic acid levels, protein breakdown, and loss of lean body mass [99]. However, Venditti et al. demonstrated in vivo that moderate training attenuated T3-induced increases in hydrogen peroxide (H_2O_2) production and, therefore, oxidative damage increasing antioxidant protection and decreasing the reactive oxygen species (ROS) flow from the mitochondria to the cytoplasmic compartment [100]. Another study of leucine supplementation in hyperthyroid rats demonstrated a positive effect in physical performance compared to the non-treated group [101].

Clinical Findings

Hypothyroidism

Hypothyroidism is characterized by a decrease in Ca^{2+} uptake and ATP hydrolysis by sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA; see Table 6.3) [102]. At least mild elevations in creatine kinase levels are seen in about 90% of hypothyroid patients [103]. In hypothyroid subjects, the alterations in lipid, protein, and carbohydrate metabolism in muscle may have pronounced effects on muscle function.

Table 6.3 Muscle changes observed in hyper- and hypothyroidism

	Hyperthyroidism	Hypothyroidism
Muscle strength	↓	↓
Type II fibers	↑	↓
Lactate: exercise response	↑	↑
Sarcoplasmic reticulum Ca^{2+} uptake	↑	↓
PCr/ Pi ratio—exercise	↑	↓
PCr recovery rate	↑	↓

↑ increased, ↓ decreased, *PCr/Pi* phosphocreatine/inorganic phosphate, *PCr* phosphocreatine

Exercise may exacerbate this situation and be associated with rhabdomyolysis [104]. Several cases of rhabdomyolysis have been reported [105, 106], and a relation to a reversible defect in muscle glycogenolysis has been suggested [107].

In Hoffmann's syndrome, another muscle disorder related to hypothyroidism, abnormalities include increased muscle mass, muscle stiffness and weakness, creatine kinase of as much as >10 times normal levels, and repetitive positive waves on electromyography (EMG) [108]. Resolution of symptoms is expected with thyroid hormone replacement. EMG patterns that can be seen with hypothyroidism include fibrillations, increased polyphasic waves, unusual high-frequency discharges, and reduced motor unit recruitment [108].

An abnormal increase in lactate during exercise but not at rest has been described in subclinical hypothyroidism [109]. It was hypothesized that mitochondrial oxidative dysfunction was present and that this dysfunction worsens with length of disease; glycolysis may exceed pyruvate oxidation explaining the lactate buildup.

Phosphorous nuclear magnetic resonance spectroscopy (MRS) has been extensively used to investigate noninvasively the energy metabolism of human muscle. It allows tracking of real-time changes in the relative concentrations of the metabolites that are involved in high-energy phosphate metabolism [110]. A study by Kaminsky et al. performing MRS in hypothyroid women subdivided into either moderate hypothyroidism, subacute thyroid deficiency, or severe/

chronic hypothyroidism demonstrated dysfunction of muscle bioenergetics with even mild TH deficiencies [111]. Khushu et al. documented similar abnormalities in the bioenergetic profile in 32 hypothyroid patients [110]. Similarly, Bose et al. showed shifting of equilibrium of ATP breakdown to ADP and inorganic phosphate (Pi) after exercises confirming impaired oxidative phosphorylation in mitochondria [112].

Haluzik et al. compared metabolic changes in 12 hypothyroid women with those in 6 hyperthyroid and 12 euthyroid women. Compared to healthy subjects, hypothyroidism was associated with significantly decreased noradrenaline and glycerol concentrations, whereas the opposite is applied to hyperthyroid patients. These findings suggest altered adrenergic and lipolytic activities in thyroid disorders [113].

Whether the changes occurring in hypothyroidism are observed in subclinical hypothyroidism has been investigated. Changes in phosphometabolites (increased phosphodiester levels and Pi concentration) were similar in patients with overt hypothyroidism compared to Sc-HypoT. However, impaired muscle oxidative metabolism was not observed in Sc-HypoT patients [114].

Sc-HypoT in 3799 otherwise healthy subjects was associated with a lower resting HR and a significantly lower recovery HR [115]. While Reuters et al. observed no changes in muscle functional capacity in Sc-HypoT, symptoms of cramps, weakness, and myalgia were more frequent compared to controls [116], and a lower HR after exercise was observed [115]. Furthermore, Tanriverdi et al. observed Sc-HypoT subjects to have a higher arterial stiffness and lower physical activity duration with a significant difference in neuromuscular symptoms, muscle strength, and functional exercise capacity assessed by a 6-min walk test [117].

Hyperthyroidism

Hyperthyroid subjects also have impairment in cellular respiration and reduced exercise endurance [109]. Excess heat generation from the elevated metabolic activity associated with thyrotoxicosis and secondary hyperthermia may adversely impact heat dissipation during exercise and exercise tolerance. However, despite a baseline temperature increase of 0.5 °C in thyrotoxic subjects, exercise-induced temperature rise has been observed not to differ from that in controls [118]. Reduced duration of action potentials and increased polyphasic potentials can be seen with thyrotoxicosis [119]. Muscle weakness is a common complaint in patients with TH excess, and a variety of investigations have addressed muscle changes secondary to hyperthyroidism. Hyperthyroidism is associated with an increase in fast and a decrease in slow-twitch muscle fibers. Thyrotoxicosis appears to induce an oxidative muscular injury secondary to an increase in mitochondrial metabolism and a decrease in glutathione peroxidase, which may be protective against such injury [120]. Glycogen is lower at baseline in thyrotoxicosis and is utilized at a faster rate with an associated increase of serum lactate [121]. According to studies of Ribeiro et al., glycogen storage in hyperthyroidism can be differently distributed in tissues with lower levels in the heart, liver, and soleus and higher levels in mixed fiber type of gastrocnemius during regular swimming [122].

Thyrotoxic periodic paralysis (TPP) is an unusual complication of hyperthyroidism more typically seen in thyrotoxic Asian subjects, although not exclusively so. Patients with TPP suffer from attacks of para- or quadriplegia incited by exercise, high-carbohydrate meals, or high-salt intake [123].

The muscular function of these patients may appear grossly normal before and between episodes, although some patients have a prodrome of muscle stiffness and aching. The pathophysiology revolves around an imbalance in the Na^+/K^+ pump. EMG studies reveal that the muscle has reduced excitability during TPP episodes, and low-amplitude muscle action potentials are seen following a paralytic episode [124]. Decreased compound motor action potential amplitudes are found postexercise in TPP [125] and improve following treatment [126]. Of note, muscle fiber conduction velocity measured in two patients with TPP was within normal limits during paralysis episodes, although muscle strength

was reduced by 40% during an attack [127]. A comparison of the electrophysiologic response to prolonged exercise between thyrotoxic patients with and without TPP demonstrated a preexisting latent abnormal excitability of the muscle membrane in TPP [128]. TH regulates muscle membrane excitability by increasing Na^+/K^+ pump-dependent potassium influx [129]. Adding to our insight into the pathophysiology of TPP is the recent discovery of KCNJ18 gene mutations in a third of TPP patients which alter the function of an inwardly rectifying potassium channel named Kir2.6 [130].

There are also a few case reports documenting rhabdomyolysis as a complication of hyperthyroidism [131–133].

Some authors describe significant metabolic changes in exercising muscle exposed to excess TH. Reduced metabolic efficiency of skeletal muscle energetic with decreased phosphocreatine (PCr) in hyperthyroid patients has been documented by MRS [134]. Under thyrotoxic conditions, ATP is promptly depleted, and myopathy easily develops, as the intramuscular glycogen content decreases due to the suppression of glycogenesis and glycogenolysis. During vigorous exercise, glycogen is rapidly consumed, and ATP consumption by the skeletal muscles increases more than the ATP supply. At that time, the compensatory mechanisms include involvement of purine catabolism as a source of energy [135, 136]. Fukui et al. compared the levels of glycolytic metabolites (lactate and pyruvate) as well as purine metabolites (ammonia and hypoxanthine) in treated and untreated Graves' disease patients vs. normal controls [137]. The study revealed that glycolysis and purine catabolism were remarkably accelerated in hyperthyroidism and thyrotoxic myopathy could be closely related to the acceleration of purine catabolism, which can be normalized only after long-lasting euthyroidism. Moreover, such acceleration of the purine nucleotide cycle is thought to be in part a protective mechanism against a rapid collapse of the ATP energy balance in exercising muscles of patients with hyperthyroidism [137, 138].

Another important question facing clinicians is the effect of treatment with suppressive doses of LT4 necessary in some patients with differentiated thyroid cancer. Vigario et al. [139] addressed this question and documented that muscle mass was lower in the patients on suppressive LT4 treatment than in euthyroid control subjects, but aerobic training, twice a week, during 3 months partially reversed this deteriorating effect of excess TH on muscle mass. Greater attention should be paid to elderly men with subclinical hyperthyroidism who may have accelerated poor physical performance. Also in euthyroid man, higher FT4 was predictive of a lower Short Physical Performance Battery score at the 3-year follow-up [140].

Effects on Pulmonary Function

Performance of any strenuous activity especially of endurance training requires the ability of the respiratory system to augment oxygen utilization. Exercise capacity, the maximal capacity for oxygen consumption (VO_2 max), and endurance, the ability to perform prolonged exercise at 75% VO_2 max, are the two main components of exercise tolerance [141].

Clinical Findings

Large goiters, especially firm, nodular substernal goiters, can cause an extrathoracic tracheal obstruction, which can limit air flow to the lungs [142].

Hypothyroidism

Altered TH levels can lead to impairment in optimal pulmonary function. Myxedema or profound hypothyroidism is associated with alveolar hypoventilation related to a reversible reduction in hypoxic ventilatory drive [143]. Reductions in lung volumes are seen and include vital capacity, total lung capacity, functional residual capacity, and expiratory reserve volume, as well as a decrease in diffusing capacity for carbon monoxide (DLCO) [144]. LT4 replacement therapy is associated with resolution of the aforementioned changes, but a concomitant reduction in

patient weight may also be an important factor in pulmonary function improvement [145]. Frank respiratory failure is unusual. During exercise, hypothyroid subjects were characterized by reduced forced vital capacity and tidal volume at the anaerobic threshold [146]. Also, the increment of minute ventilation and oxygen uptake was significantly lower.

A study in women with subclinical hypothyroidism demonstrated a slower VO2 kinetics (defined as the time needed to reach 63% of change in VO_2) in both the onset and recovery of exercise and a higher oxygen deficit compared to euthyroid subjects [147]. Conceivably therefore, it seems that levothyroxine treatment of mild or subclinical hypothyroidism can decrease oxygen uptake, improve minute ventilation and cardiopulmonary exercise performance, and improve the ability in these patients to carry out activities of daily life [148].

Hyperthyroidism

Thyrotoxicosis has been implicated as a primary cause of decreased cardiorespiratory exercise tolerance [52, 149, 150]. In hyperthyroidism, already at rest, cardiorespiratory capacity is maximally increased, leading to a limited functional reserve, which may explain the inadequate response of ventilation [53]. Dyspnea on exertion is a common complaint although the causes of this symptom remain unclear and may vary from one patient to another [151]. In hyperthyroidism, the respiratory systems adjust to the increased oxygen demand by increasing respiratory rate and minute ventilation [149]. Alveolar ventilation remains normal, but a rise in dead space ventilation is seen, and also the amount of oxygen diffusion from alveoli to the blood may be reduced during periods of strenuous exercise in thyrotoxicosis [152].

Pulmonary function is dependent on not just intrinsic lung function but also the accessory muscles for respiration. Pulmonary compliance and airway resistance tend to remain unchanged, whereas vital capacity and expiratory reserve volume are reduced, implicating respiratory muscle weakness [153]. Other supporting evidence for respiratory muscle dysfunction in thyrotoxic patients is the reduction of maximal inspiratory and expiratory efforts, which are seen to resolve on restoration of euthyroidism [154]. It appears that ventilation increase beyond the oxygen uptake is related to the dead space ventilation [155]. These changes also appear to resolve with appropriate therapeutic intervention [155]. Furthermore, changes in TH levels modify diaphragmatic function as well as muscle fiber type. Goswami et al. documented a more marked functional weakness of the diaphragm in Graves' disease during maximal respiratory maneuvers, indicating a diminished diaphragmatic reserve that could cause dyspnea on exertion. These changes were reversible after achieving euthyroidism [156].

With cardiac and muscular function being adversely affected by excess TH, one would postulate that work capacity must be reduced in hyperthyroid individuals. A study of maximum power output in hyperthyroid individuals with measurements of work capacity both while thyrotoxic and then euthyroid revealed a 19% increase from a low maximum power output during the thyrotoxic phase compared to the euthyroid state 3 months later. A subset of patients were retested 12 months later, and maximum power output in comparison to controls was in the low normal range and represented a +13% rise from the 3-month test [157]. Oxygen uptake at maximal effort was low during thyrotoxicosis and did not increase at 3 and 12 months. Net mechanical efficiency was also low at baseline and returned back up to normal only by 12 months. Kahaly et al. showed reduced forced vital capacity, 1-second capacity, and increased respiratory resting oxygen uptake (VO_2) rate in hyperthyroid patients compared to euthyroid controls. During exercise, decreased tidal volume at the anaerobic threshold was observed as well as a lowered increment of minute ventilation, VO_2, and oxygen pulse [53].

The studies are equivocal in terms of the effect of treatment with suppressive doses of LT4 on exercise capacity. Some studies revealed similar blood pressure, heart rate, VO_2, VCO_2, and anaerobic threshold response to exercise in LT4-treated patients as in healthy control subjects [158]. Other studies found that ventilation parameters

between patients and controls were comparable only at rest, but the patients treated with suppressive doses of LT4 had a worse response to exercise (i.e., lower maximal workload, lower peak VO_2, and lower anaerobic threshold) [159].

In conclusion, analysis of respiratory gas exchange showed low efficiency of cardiopulmonary function, respiratory muscle weakness, and impaired work capacity in hyperthyroidism which was reversible with restoration of euthyroidism.

Exercise and Thyroid Axis Response

Exercise is a stressful situation that challenges body homeostasis, so that the organism has to reestablish a new dynamic equilibrium in order to minimize cell damage.

One of the systems affected is the hypothalamic–pituitary–thyroid (HPT) axis [160].

Data demonstrate that voluntary exercise adapts the status of the HPT axis, through pathways that are distinct from those observed during food restriction or repeated stress [161]. Lesmana et al. suggested that alteration in TH signaling (increased TRβ1 expression) and TSH reduction observed in vitro after moderate training can contribute to the metabolic adaptation of skeletal muscle to physical activity [162].

Although the belief that a different normal range for thyroid hormones may apply in athletes compared to healthy nonathletes may be considered, data on the effects of exercise on TH metabolism have been inconsistent or even contradictory (see Table 6.4). These divergent results may be due to differences in the intensity of work, duration of exercise, and frequency and design of the training program and to differences in gender, age, and baseline individual physical status of the subjects. In addition, different duration of studies, timing of sampling after exercise, and methodological factors in hormonal assay and data analysis may also be responsible for the discrepancies.

Some studies indicated no major changes in the thyroid axis response to exercise. For example, a study of 26 healthy males, given identical diet and physical activity for a week before the test, revealed an increase in T3, T4, and TSH immediately after exercise. However, it seems that the changes were mainly due to hemoconcentration, since they became insignificant after adjustment for hematocrit (Hct) [163]. Another study in subjects undergoing different exercise endurance showed similar results: no significant change in FT4 and a small increase (partially from hemoconcentration) in serum T3 and rT3 [164].

Interestingly, some studies indicate that TSH increases after exercise with the response dissipating with repetitive testing, which was suggested to indicate a psychological influence on the TSH rise [165]. In another study a fT4 increase of 25% was seen postexercise [166], but the increase may have been confounded by assay interference by an associated rise in FFAs. TSH also rose by 41%, but could not be correlated with T4/T3 levels.

A rise in TSH was seen with both short-duration graded exercise and prolonged exercise, but the latter had a peak of 33% lower than with graded exercise [167]. Another study compared the effect of submaximal and maximal exercise effect on TH levels [168]. Maximal exercise was associated with a decrease in TSH, FT4, and stable rT3 and rises in T3 during activity, whereas submaximal exercise was associated with an increase in TSH, but T3, rT3, and FT4 were unchanged. [168]. Also, when comparing intensive exercise intervals with steady-state endurance exercise, Hackney et al. observed that the change in TH levels was present 12 hours post exercise only in the intensive exercise group implying a longer period necessary for TH to return to normal. In both groups an increase in fT3, fT4, and rT3 was present immediately post exercise with a decrease in fT3 and increase of rT3 12 hours post exercise only in the intensive exercise group [169].TH changes in ultradistance and long-distance runners have also been investigated. Hesse et al. studied the effect of three distances of 75 km, 45 km, and marathon (42.2 km) with the subjects performing the 45-km run being slightly older than the other two groups. T4 increased in the 75 km and marathon group and decreased together with T3 in the 45-km group postrace. rT3, measured only in the marathon and 75-km groups, rose in both groups. The authors

Table 6.4 Reported changes in hypothalamus–pituitary–thyroid axis in association with exercise

Reference	Exercise type	Caloric status	TSH	T4	fT4	T3	fT3	rT3	Comments
[165]	Pre-exercise	NA	↑						Anticipation of exercise
[166]	Ergometry	NA	↑	↑		↑			Normal TRH stim
[173]	Ergometry	NA		NC		↑		↓	Untrained athletes
[173]	Ergometry	NA		↓		NC		↑	Well-trained athletes
[188]	Ergometry	NA		↑		↓		↑	Glucose infusion[a]
[181]	Chronic ergometry	NA	NC						Recreational athletes NC TSH response to TRH
[163]	Maximal treadmill exercise	Sufficient	NC	NC		NC			Transient changes in TH values reflected transcapillary movements of water
[2]	Aerobic exercise	Deficient		↑		↓	↓	↑	Not seen in energy-balanced group
[168]	Running	NA	↓		↓	↑		NC	Maximal exercise
[174]	Running	NA	NC	NC		NC			Endurance athletes[b] versus controls
[168]	Running	NA	↑		NC	NC		NC	Submaximal exercise
[183]	Runners	Deficient	NC	↑		↓			Prevented by caloric increase
[170]	Ultradistance	NA		↑		NC		↑	75 km[c]
[170]	Ultradistance	NA	↓	↓		↓			45 km[c]
[170]	Ultradistance	NA	NC	↑		↓		↑	42.2 km[c]
[169]	Intensive exercise	NA			↑	↑		↑	Post exercise
[169]	Intensive exercise	NA				↓		↑	12 hours post exercise
[169]	Steady state exercise	NA			↑	↑		↑	Post exercise
[169]	Steady state exercise	NA				NC		NC	12 hours post exercise
[164]	Swimming	NA			NC	↑		↑	
[176]	Swimming	NA	↑		↑	NC			20°C[d]
[176]	Swimming	NA	NC		NC	NC			26 °C
[176]	Swimming	NA	↓		↓	NC			32 °C
[197]	Ballet	NA	NC	NC		NC			
[195]	High-intensity resistance training	NA	↓		NC		↓		Leptin levels correlated with TSH levels
[195]	High-intensity endurance training	NA	↑		NC		NC		Same group of rowers underwent 3 weeks of resistance and 3 weeks of endurance training
[180]	Chronic endurance exercise	NA		↓	NC	↓	↓		Identical twins
[183]	Chronic endurance exercise	NA	NC	↓	↓	↓	↓	↓	Amenorrheic[e]
[196]	High-altitude exercise	NA	NC	NC		↓			Increased GH/IGF-1 axis and low T3 syndrome

T4 thyroxine, *T3* triiodothyronine, *fT4* free thyroxine, *fT3* free triiodothyronine, *rT3* reverse triiodothyronine, *TSH* thyrotropin, *TRH* TSH-releasing hormone, *NA* not applicable, *NC* no change, ↑ increase, ↓ decrease

[a]A glucose infusion blunted the exercise-induced changes of rT3, T3, and T4

[b]Endurance athletes had balanced increase in T3 production and disposal rates in comparison to active and sedentary men

[c]TSH, T4, and T3 are lower in older runners, whereas faster runners had higher T4 and TSH in relation to slower runners

[d]High TSH with longer cold water exposure

[e]T4/fT4, T3/fT3, and rT3 were lower in exercising amenorrheic versus sedentary group. The eumenorrheic exercise group only has a slightly lower fT4 level, but T4 and T3 were slightly lower than the eumenorrheic sedentary group

speculated whether the increase in rT3 might be protective against excess glucose metabolism, especially if intracellular glucose deficiency were present [170]. Semple et al. reported on marathon runners revealed no change in TSH, T4, T3, or rT3 levels before and after the marathon [171]. However, another study revealed an increase in TSH and fT4 post-marathon, with a decrease in fT3 and rise in T4 to rT3 conversion, which was still detectable 22 hours following race completion [172].

The level of training of athletes has been shown to affect the TH response to acute exercise. In one investigation, untrained athletes had a rise in T3, a decrease in rT3, and no change in T4, whereas the well-trained athletes were found to have a rise in rT3, no change in T3, and a decrease in T4 levels. It was hypothesized that the rT3 elevation in well-trained athletes might be adaptive to a more efficient cellular oxidation process [173]. Of note in another study, Rone et al. found an increase in T3 production and turnover in well-trained male athletes in comparison to sedentary men [174]. Following a treadmill stress test, TH levels and TRH simulation revealed responses similar in nature among sedentary subjects, regular joggers, and trained marathon runners [175].

Variation in ambient temperature appears to alter the body's TH response to exercise. One study looking at TH differences in swimmers exercising in different water temperatures demonstrated that TSH and fT4 rose in the colder water, were unchanged at 26 °C, and fell at the warmer temperature, but T3 levels were not affected [176]. Cold receptors appear to regulate a rise in TRH and TSH level in cold water, and exposure duration may affect the peak TSH with higher levels owing to longer times in the water [177, 178].

The chronic effects on thyroid hormone parameters have also been studied in endurance-trained athletes. The results of the studies conflict with regard to whether or not baseline TH levels are shifted in well-trained athletes [179]. Identical twins studied during an observed 93 days endurance training period with stable energy intake had an average 5-kg weight loss (primarily fat) and lower baseline fT3, T3 by the end of the exercise period [180]. A shorter study in recreational athletes over 6 weeks revealed no change in TSH or TSH response to TRH stimulation during exercise although the exercise endurance improved [181]. Also no difference was reported in baseline values for T4, T3, and TSH between endurance athletes and sedentary controls over time [174].

Radioactive iodine uptake (RAIU) may be altered secondary to chronic exercise since a lower thyroid uptake of ^{123}I has been found in regular exercising rats and humans in comparison to sedentary subjects [179].

Energy balance plays a role in the body's TH response to exercise. Data on the response of TH to fasting or malnutrition [182] suggest that the T3 decrease and rT3 increase could reflect a regulatory mechanism to regulate catabolism and energy expenditure. Of note, T3 and rT3 return to normal with refeeding. Loucks and Heath [183] found a decrease in T3 (–15%) and fT3 (–18%) along with an increase in rT3 (+24%) in healthy women undergoing aerobic exercise testing with low-caloric intake. However, this "low T3 syndrome" was not seen in individuals receiving a normo-caloric diet in balance with their energy expenditure. Other studies demonstrated that the reduction of energy availability from 45 kcal/kgFFM/day to 10 kcal/kgFFM/day was associated with a decrease in T3 levels in women undergoing 5 days of exercise (see Fig. 6.2a) [184, 185]. Especially in amenorrheic athletes, T3 levels have been found to be lower than in eumenorrheic athletes and sedentary women perhaps suggesting a generalized reduction of the energy-consuming process (see Fig. 6.2b) [185]. Furthermore, the observed correlation between T3 levels and osteocalcin suggests a possible role in collagen formation and matrix mineralization, thus contributing to the athlete triad characterized as a low energy availability or eating disorder, dysmenorrhea, and low bone density [186].

Interestingly, low-caloric diets high in carbohydrate appear to blunt the drop in T3 compared

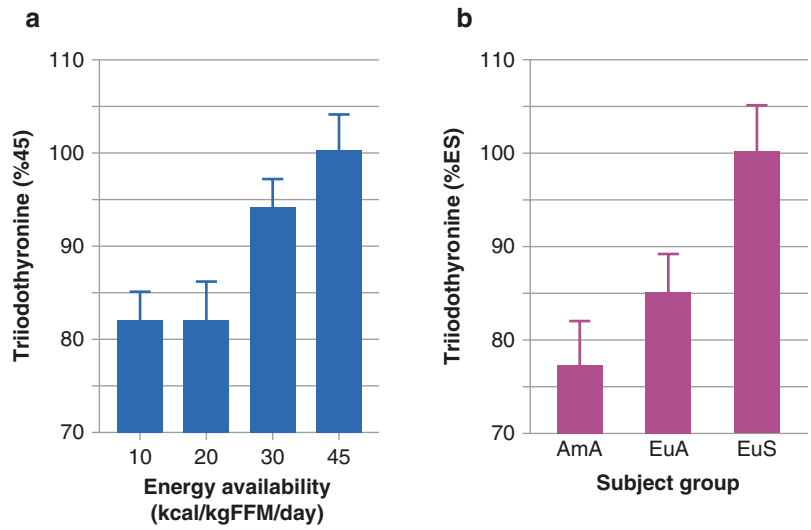

Fig. 6.2 Triiodothyronine (T3) levels (mean ±SE). (**a**) Reduction of T3 levels in exercising women after 5 days at energy availabilities of 45, 30, 20, and 10 kcal/kgFFM/day. (**b**) Amenorrheic athletes (AmA), eumenorrheic athletes (EuA), and sedentary women (EuS). Low T3 levels in the athletes suggest a generalized reduction in the rates of energy consuming processes

to low-carbohydrate intake [187]. Moreover, glucose infusion has been found to diminish the increase in rT3 and T4 along with decrease in T3 [188].

In a military study, rangers were assessed over 4 days of grueling training in conjunction with sleep and caloric deprivation. The training was associated with an initial increase of TH during the first 24 h. After 4 days of training, there was a gradual decrease in T4, fT4, and T3 (65%), whereas rT3 continued to rise. The group that received a higher caloric intake, and therefore less energy deficiency, had a continued increase in T3 and T4. In the energy-deficient groups, TSH decreased during the first day and remained low throughout the training period. The response of TSH to TRH was reduced in all groups, but much less so in the energy-sufficient group [189]. The detected energy deficiency correlated with a decrease in T3 and increase in rT3 in this study [189]. Hackney et al. have demonstrated that these responses to military exercises and their relation to energy deficiency exist in extreme cold as well as hypoxic environments [190, 191].

Higher-altitude exposure has been shown to be associated with an increase in T4 and fT4 [192]. Furthermore, although Stock et al. reported that exercise at elevated altitudes is also notable for a significant increase in T4 and fT4 with even mild activity [193], not all studies entirely agree with these observations [191].

Animal studies revealed an increase in serum T3 immediately after exercise, with a gradual decrease thereafter to significantly lower values than in controls. Concomitantly, T4 levels progressively increased, resulting in the T3/T4 ratio being significantly decreased 60 and 120 min after the exercise, indicating impaired T4-to-T3 conversion [194].

Simsch et al. assessed hypothalamic–thyroid axis and leptin concentrations in six highly trained rowers. After 3 weeks of resistance training, a reduction in TSH, fT3, and leptin was found, while fT4 was unchanged. Interestingly, leptin levels correlated with basal TSH levels. In contrast, after 3 weeks of endurance training, a significant increase of TSH was observed. The authors interpreted these data to indicate that depression of the hypothalamic–thyroid axis and leptin is associated with training intensity [195]. Studies of Benso et al. also support the concept of low T3 syndrome as an adaptive mechanism to intense training as was seen in nine male well-trained climbers studied after climbing Mt. Everest and resulting in a low T3 syndrome with no significant change in ghrelin and leptin despite decrease in body weight [196].

Relative to women, amenorrhea is commonly seen in well-trained female athletes. One study found that amenorrheic subjects had lower T4 and T3 levels than the eumenorrheic groups, but the trained eumenorrheic females had slightly

lower T4 and T3 levels than the eumenorrheic nonathletes as well [197]. Of interest, the amenorrheic athletes tended to eat less fat and eat more carbohydrates with a similar caloric intake in comparison to the two other groups with more normal menstrual function. Also, the amenorrheic exercise group trained more hours and more strenuously than the other two groups. Oxygen uptake (VO_2) was similar in the trained groups, who also weighed less and had lower body fat. As measured by ^{31}phosphorous magnetic resonance spectroscopy (31P-MRS), inorganic phosphate/phosphocreatine (Pi/PCr) was not different at rest or at exercise, and pH did not differ at any activity level. However, PCr recovery was substantially faster in the eumenorrheic endurance-trained group than in the eumenorrheic nonathletes and amenorrheic athletes, and the Pi/PCr recovery was only different between the eumenorrheic-trained athletes and nonathletes [197]. PCr recovery is related to oxidative metabolism, and the fast recovery in trained eumenorrheic athletes indicates a potentially more efficient metabolism. The other parameters examined for exercise metabolism in these subjects were similar. Contrastingly, in another study, levels of TSH, T3, and T4 were not found to be different in oligomenorrheic heavily trained adolescents versus adolescent athletes without "strenuous" exercise with regular menses [198].

Summary

In summary, the thyroid function changes secondary to exercise represent complex physiologic responses, which are difficult to characterize fully. Mitigating factors in the TH response to exercise include age, baseline fitness, nutrition status, ambient temperature, altitude, as well as time, intensity, and type of exercise performed. Another important factor in interpretation of the extant literature is that not all TH blood tests were assessed in every study. Moreover, older studies employed less sensitive assay techniques, whereas various assays have improved over time. The detection of increased FFA in several studies, which may interfere with some TH assays, also cannot be overlooked. However, despite these issues, a review of the literature does reveal certain trends (Table 6.4). One of the more consistent findings is that rT3 tends to increase with exercise especially with associated caloric energy deficiency or ultradistance exercise activities. However, TSH appears to be unaffected by exercise in about 50% of studies with an increase in TSH secondary to cold exposure being a noted exception. T4 was found to increase in 46%, decrease in 26%, and be unchanged in 28% of investigations, although an increase was more typically found with caloric energy deficiency, cold exposure, or ultradistance exercise; fT4 follows a similar pattern to T4. T3 was found to be decreased or be unchanged in 73% of study samples and usually is low with caloric energy deficiency (as in low T3 syndrome); fT3 when measured tended to follow the T3 pattern.

Many of the TH changes seen especially in athletes with negative energy balance appeared to be reversed with either a high-carbohydrate intake or even glucose infusion. Although well-trained athletes may exhibit an increased production and turnover of T4, baseline TH levels do not appear to be affected substantially by chronic exercise (i.e., endurance).

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7 The Male Reproductive System, Exercise, and Training: Endocrine Adaptations

Fabio Lanfranco and Marco Alessandro Minetto

Introduction

Androgens exert strong anabolic effects on skeletal muscle protein synthesis [1, 2], satellite cell number [3], and skeletal muscle growth [4, 5]. Because these changes are of great importance to muscle mass and strength, androgens have been recognized as important hormones that influence sports performance [6]. Exercise-induced changes in testosterone concentrations can moderate or support neuromuscular performance through various short-term mechanisms (e.g., second messengers, lipid/protein pathways, neuronal activity, behavior, cognition, motor system function, muscle properties, and energy metabolism) [7].

On the other hand, the gonadal axis function is strongly affected by physical exercise depending on the intensity and duration of the activity, the fitness level, and the nutritional-metabolic status of the individual [8–10]. Moreover, circulating testosterone and its bioavailable fractions are affected by weight and age. They are also changed by different kinds of stress which may appear as physical stress (i.e., endurance training, sleep deprivation in extreme sports, changes of air pressure in altitude training) or mental stress in relation to sport events and training [9, 10].

The purpose of this chapter is to illustrate the physiologic and pathologic changes that occur in the male gonadal axis secondary to acute exercise and chronic exercise training.

Physiology of the Male Gonadal Axis

The male gonadal axis consists of the testes and the hypothalamus-pituitary unit that controls their function. The testes possess a dual function, i.e., the production of androgens and of the sperm.

Figure 7.1 depicts an outline of the male gonadal axis and of the hormonal regulation of the testicular function.

The pituitary gland is the central structure controlling gonadal function: it releases the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and is regulated by the hypothalamic gonadotropin-releasing hormone (GnRH), which is secreted in a pulsatile fashion with peaks every 90–120 min. GnRH secretion is modulated by a network of excitatory and inhibitory inputs that include either a central control exerted by distinct

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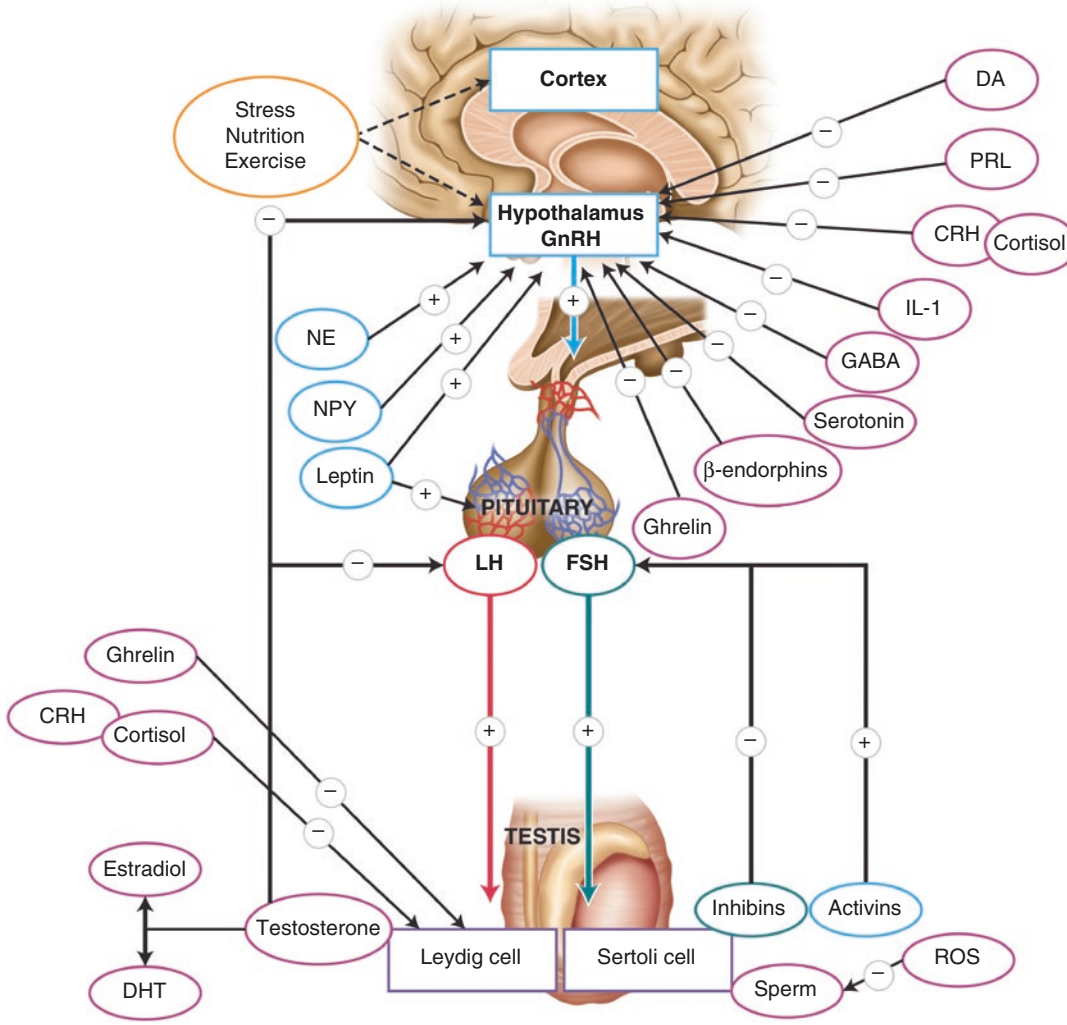

Fig. 7.1 Schematic diagram of the male gonadal axis. CRH corticotropin-releasing hormone, DA dopamine, DHT dihydrotestosterone, FSH follicle-stimulating hormone, GABA gamma-aminobutyric acid, GnRH gonadotropin-releasing hormone, IL-1 interleukin-1, LH luteinizing hormone, NE norepinephrine, NPY neuropeptide Y, PRL prolactin, ROS reactive oxygen species

subgroups of neurons afferent to the GnRH-secreting neurons or the peripheral gonadal steroid feedback [11].

The hypothalamic kisspeptin system exerts a fundamental control in the activation of GnRH-secreting neurons at puberty [11]. In addition, several neurotransmitters and neuromodulators are proposed to influence GnRH secretion: the noradrenergic system and neuropeptide Y (NPY) show stimulatory activity, whereas interleukin-1, opioid peptides, dopamine, serotonine, and gamma-aminobutyric acid (GABA) are inhibitory.

Another important peptide regulating the GnRH secretion is leptin, a peptide hormone secreted by the adipose tissue that helps to regulate the energy balance and mirrors the amount of energy reserve. Leptin purportedly plays a key role either by signaling to the central nervous system the information regarding the amount of available fat stores or by enabling the activation of the gonadal axis through the GnRH secretion

when convenient [11]. Specifically, leptin has been shown to stimulate GnRH and gonadotropin secretions [12]. Additionally, ghrelin, a peptide hormone with growth hormone-releasing action, exerts multiple endocrine and non-endocrine effects including inhibition of the gonadal axis at both the central and peripheral level [13].

Another important mechanism that dynamically controls GnRH synthesis and release is represented by the gonadal steroid feedback. In man, the major hormone controlling GnRH secretion is testosterone, which inhibits gonadotropin secretion via negative feedback both at the hypothalamic and pituitary level. Dihydrotestosterone (DHT) and estradiol also modulate gonadotropin secretion acting at the hypothalamic and/or pituitary level [11, 12, 14, 15]. Noteworthy, the kisspeptin system is implicated in the transmission of both negative and positive feedback of sex steroids on GnRH neurons [11].

Finally, the adverse effect of stress on reproductive function is well known. Several hormonal factors are involved: corticotropin-releasing hormone (CRH) inhibits GnRH secretion, prolactin (PRL) further reduces the GnRH pulse rate [14], and cortisol inhibits both the hypothalamus-pituitary and gonadal functions.

LH and FSH are produced and secreted by the gonadotropic cells of the anterior pituitary. LH regulates testicular androgenesis, whereas FSH, together with locally produced testosterone, is responsible for spermatogenesis. LH binds to specific receptors on the surface of Leydig cells in the testis and regulates the biosynthesis of testosterone. FSH binds to receptors on the Sertoli cells and promotes spermatogenesis: in addition to a number of other proteins, the hormones inhibin B and activins are formed in the Sertoli cells under the influence of FSH. Inhibin B plays an important role in the feedback regulation of FSH secretion, whereas the physiological role of activins has not been conclusively clarified thus far [14, 16].

Testosterone is the most important steroid produced by the testis and is responsible for the development and maintenance of male sex characteristics as well as a number of other anabolic and metabolic effects (e.g., muscle and bone metabolism). Testosterone is produced primarily in the Leydig cells of the testis. It may be further metabolized into a more potent androgen, DHT. Normal testosterone concentrations in adult males range between 12 and 30 nmol/l: testosterone concentrations in blood follow a circadian rhythm with higher levels in the morning hours and about 25% lower levels in the evening [12, 17].

Effects of Physical Exercise on Testicular Steroidogenesis

Short, Intense Exercise Increases Circulating Testosterone

The effects of physical activity on the male gonadal axis vary with the intensity and duration of the activity, the fitness level of the individual, and his nutritional-metabolic status. Relatively short, intense exercise usually increases, while more prolonged exercise usually decreases serum testosterone levels [8, 9, 18, 19]. Increased blood testosterone levels have been reported during relatively strenuous free and treadmill running, weight training, rock climbing, and ergometer cycling [20–22]. Short-term sprints can be seen as strength outburst and are comparable to resistance exercise rather than endurance exercise: in fact, sprint exercise increased blood testosterone concentrations in adolescent boys [23].

The testosterone response increases with increased exercise load [24]. Similar workloads produce similar responses, regardless of whether the load is aerobic or anaerobic [25].

Immediate and 5-min post-exercise measurements showed an increase in testosterone levels both in men and women [26]. Acute exercise-induced testosterone increments are also seen in older men [27]. This acute hormone response was confirmed and described to be markedly stronger in young men compared to old in a study involving ten men with mean age 26.5 years and ten men with mean age 70.0 years [28].

As muscle mass increases with strength training [4] and is correlated with testosterone

levels, it could be expected that the testosterone response to acute exercise is higher in persons constantly involved in strength training. Consistently, a 6-month sprint training program increased plasma testosterone concentrations in response to sprint exercise in adolescent boys [23]. Experienced weight lifters compared to beginners showed similar basal levels of testosterone but were able to evoke a stronger testosterone response during exercise [20]. Contrary to these findings, a long-term training period of 12 weeks involving younger (mean 23 years) and older men (mean 63 years) showed no significant results concerning testosterone levels before or immediately after exercise [29].

Ronnestad et al. [30] investigated the effects of testosterone and growth hormone (GH) transient increase during exercise, indicating that performing leg exercises prior to arm exercises, thereby increasing the levels of testosterone and GH, induced superior strength training adaptations compared to arm training without acute elevation of hormones. It has been found that acute elevation in endogenous testosterone (by strength training) potentiates the androgen receptor (AR) response to a strength training session compared to no acute elevation of endogenous testosterone [31]. It might thus be speculated that the results by Ronnestad et al. are due to an increased AR expression, and through an improved testosterone-receptor interaction, and a subsequent increased protein synthesis, leading to superior strength training adaptations. This hypothesis has also been evaluated by Ahtiainen et al. [32], who have described a correlation of individual pre- to post-training changes in resting AR protein concentration with the changes in cross-sectional area of muscle fibers in a combined group of young and elderly subjects who performed heavy resistance exercise bouts before and after a training period. Collectively, these findings suggested that the individual changes of AR protein concentration in skeletal muscle following resistance training may have a critical impact on training-induced muscular adaptations.

Mechanisms Underlying Increases in Circulating Testosterone Following Short, Intense Exercise

No conclusive and homogeneous evidence about gonadotropin response to an acute exercise bout is available. In fact, LH and FSH levels have been reported to be increased, decreased, or unchanged by short-term strenuous exercise [33–36].

The exercise-associated increment in circulating testosterone is considered not to be mediated by LH, due to the inconsistent LH response and to the evidence that testosterone levels increase more quickly than LH in response to exercise. Possible mechanisms such as hemoconcentration, reduced clearance, and/or increased testosterone synthesis may be involved [34, 36–38]. However, the timing of testosterone response differs from that of other circulating steroids (e.g., androstenedione and dehydroepiandrosterone increase simultaneously with cortisol), thus suggesting that specific testicular mechanisms are involved [36]. These mechanisms may include the activation of the sympathetic system, which stimulates testicular testosterone production during exercise via a direct neural pathway in some species [39]. Catecholamine levels also increase significantly during exercise. Beta-adrenergic blockade inhibits testosterone responses to exercise, whereas L-dopa, phentolamine, and clonidine had no effect [40]. An anticipatory increase in circulating testosterone levels has also been described and seems to be independent of hepatic perfusion or hemoconcentration [33, 36]. Ultimately, the exact mechanisms involved in increasing testosterone concentrations in specific exercise protocols are yet to be delineated.

Prolonged, Submaximal Exercise and Chronic Exercise Training Decrease Circulating Testosterone: From the "Female Athlete Triad" to "Relative Energy Deficiency in Sport (RED-S)"

In contrast to the short-term testosterone increment during and immediately after short, intense

exercise, a suppression of serum testosterone levels occurs during and subsequent to prolonged exercise, in the hours following intense exercise, as well as during chronic exercise training [10].

During the last decades, an increasing number of investigative research studies have pointed to how chronic exposure to endurance exercise training can result in the development of a dysfunction within the reproductive components of the neuroendocrine system. The majority of these studies have concentrated upon women. However, the effects of endurance exercise training on the male reproductive neuroendocrine system have been investigated beginning in the 1980s [41]. Most studies observed athletes during training and competition, giving the impression of generally lowered androgen levels, but lack the comparison with a control group [9].

A controlled study examining the effects of endurance training on the hypothalamus-pituitary-testis axis involved 53 men undergoing endurance training for at least 5 years and a control group of 35 age-matched, sedentary men. Baseline serum testosterone levels of the exercising men were significantly lower than in the control group. Differences in gonadotropins were not seen. Normal regulation would require LH levels to rise with falling testosterone levels, as these have a positive feedback on pituitary gonadotropin release. A suppression in the regulatory axis has been proposed as an explanation of this finding [42].

Contrary to these observations, basal testosterone levels in trained weight lifters were not altered, nor did an increase in the daily training volume change these levels [43]. Similarly, basal testosterone, free testosterone, bioavailable testosterone, and sex hormone-binding globulin concentrations were not significantly different in high top-class athletes (sprinters and jumpers) vs. untrained subjects [22].

Endurance training can be seen as a factor of exposure not only to physical but also to psychological stress. It has been demonstrated in a controlled study that the reactivity patterns of mental/psychological and physical stress response of the hypothalamus-pituitary-adrenal axis are the same in a specific individual. Differential reactivity is rather seen between the so-called high and low responders. Each group has a specific endocrine reactivity pattern concerning the hypothalamus-pituitary-adrenal axis [44]. It seems that the decrease of testosterone levels under the stressful situations of endurance sport is not sufficiently answered by the pituitary. There is no adequate rise in LH levels, which seem to be unaltered or even show a tendency to decrease with the growing amount of stress impact. Nevertheless, age-dependent effects seem to exist in this regard, and the ratio of androgen to estradiol is shifted by physical activity to a more favorable pattern (higher androgen and lower estradiol levels) in older men compared to younger men performing regular mild physical activity [45].

Participation in sports where leanness is considered a competitive advantage, such as running, cycling, wrestling, lightweight rowing, and gymnastics, has been linked to lower body mass index (BMI) [46], eating disorders [47], and low energy availability [48]. Low energy availability in the context of anorexia nervosa has been associated with low testosterone levels in males [49]. Hagmar et al. [50] evaluated athletes from 26 different sports and divided them into those who participated in leanness sports and those who did not. The leanness sport athletes had lower body fat, higher spinal bone mineral density (BMD), lower serum-free testosterone and leptin, and higher IGF-1 binding protein. The authors suggested that the increase in BMD could be because of the increase in mechanical loading in the specific leanness sports, which presumably overcame the effects of lower testosterone and leptin, both of which are bone anabolic hormones.

Fagerberg [51] has recently outlined the negative consequences of low energy availability in male bodybuilding. Bodybuilding is a sport in which athletes compete to show muscular definition, symmetry, and low body fat. The process of contest preparation in bodybuilding includes months of underfeeding, thus increasing the risk of low energy availability and its negative health consequences, including extreme effects on circulating testosterone levels.

In female athletes, low energy availability is a component of the female athlete triad, a term

used to describe the interrelationship of decreased energy availability, subsequent hypothalamus-pituitary-gonadal axis inhibition leading to menstrual irregularity, and decreased bone mineral density [48]. The triad was first described by the American College of Sports Medicine (ACSM) in the 1990s. In 2007, the ACSM published a revised position stand on the female athlete triad describing it more broadly as the harmful effects of low energy availability on menstrual function and bone mineral density [52]. The International Olympic Committee (IOC) has recently proposed an expansion of the concept of the female athlete triad to include males and has coined the term "relative energy deficiency in sport (RED-S)" [53]. The development of the term RED-S had three main purposes: (1) to draw awareness to the fact that energy restriction can have negative consequences in men in addition to women; (2) to highlight other potential negative health and performance consequences of low energy availability in athletes besides bone problems; and (3) to encourage expansive research into the potential myriad effects of low energy availability in various populations, including paralympic athletes [10].

The "Exercise-Hypogonadal Male Condition": Clinical Issues

It has been demonstrated that among subjects engaged in chronic exercise training, a selected group of men develop alterations in their reproductive hormonal profile, i.e., persistently low basal resting testosterone concentrations [54, 55]. In particular, the majority of these men exhibit clinically "normal" testosterone concentrations, but these concentrations are at the low end of normal range or even reach subclinical status. In 2005, Hackney and associates proposed the use of "the exercise-hypogonadal male" as a label for this condition [56].

The health consequences of such hormonal changes are increased risk of abnormal spermatogenesis, male infertility problems, and compromised bone mineralization [54, 55, 57, 58].

Without large-scale epidemiological studies in this area, clear prevalence data is not available [59]. However, several studies show a clear and consistently reduced serum testosterone concentration in highly aerobically trained individuals, suggesting the exercise-hypogonadal male condition (EHMC) can be a common response [42, 59, 60]. It also appears that as the level of athlete increases, so too does the incidence and severity of the condition [61]. In addition, with no long-term data currently available, it is unclear whether the presence of reduced testosterone varies throughout a competitive season and how long it takes for testosterone to return to normal, if at all [59].

The time course for the development of the EHMC or the threshold of exercise training necessary to induce the condition remains unresolved, but preliminary evidence suggest an extended window of time (i.e., years) may be necessary for its development [55] .

EHMC shares similarities with overreaching or overtraining and has also been described in male athletes as a parallel process to the female athlete triad, with hypogonadism replacing functional hypothalamic amenorrhea [62, 63]. The existence of the EHMC fits into the terminology of RED-S as clinical manifestation of it may include sexual dysfunction like infertility and reduced libido as well as reduced BMD with associated increase in risk of bone stress injury [59].

The "Exercise-Hypogonadal Male Condition": Pathophysiological Mechanisms

Exercise-hypogonadal men frequently display a lack of significant elevation in basal LH in correspondence with the reduced testosterone concentration, reflecting hypogonadotrophic-hypogonadism characteristics [41, 54, 64]. These LH abnormalities may involve disparities in luteinizing pulsatility (i.e., pulse frequency and amplitude), although evidence for altered LH pulsatile release is conflicting [65, 66]. Moreover, gonadotropin response to

GnRH has been reported both reduced and increased following prolonged, exhaustive exercise [67, 68].

Exercise-hypogonadal men have been shown to have altered basal PRL [54]. At either excessively low or high circulating levels, PRL can result in suppression of testosterone levels in men [69]. It has been speculated that the absence of PRL at the testicle alters the effectiveness of LH to stimulate testosterone production. This theory is based upon the proposed synergistic effects of PRL upon testicular LH receptors [41]. However, not all investigators reporting low resting testosterone in endurance-trained men have reported the concomitant existence of low resting PRL levels [69]. Some investigations have looked at a potential relationship between high PRL levels and low testosterone, speculating that any "stressful" situation might provoke disproportionate PRL responses in exercise-hypogonadal men and this ultimately promotes a reproductive axis disruption [70].

As previously mentioned, leptin and ghrelin are two hormones associated with appetite regulation which function as metabolic modulators of the gonadotropic axis, as well [13, 71]. Acute and chronic exercise can impact upon resting leptin and ghrelin concentrations, independent of changes in body adiposity [72, 73]. However, to date no research studies have examined whether leptin and/or ghrelin concentrations are altered in exercise-hypogonadal men. Such work would be illuminating on the topic and is needed.

Other research investigations have focused on alterations in testicular ability to produce and secrete testosterone and to respond to exogenous stimuli (i.e., LH or hCG). Whereas animal studies have demonstrated that exercise training compromises testicular enzymatic activity [74], data in exercise-hypogonadal men are contradictory. In fact, some investigations suggest testicular steroidogenesis is normal, while some indicate it is marginally impaired when challenged with exogenous stimuli [54].

Another potential disruptive hormone to the gonadal axis is cortisol. Studies in a wide range of sports (e.g., cycling, marathon running, football, handball, rugby, tennis, swimming, and wrestling) have almost all shown increased cortisol concentrations during exercise [75, 76]. Cortisol secretion increases in response to exercise intensity and duration, as well as to the training level of subjects [77–80], at least in part to mobilize energy stores. An inhibitory effect of the hypothalamus-pituitary-adrenal axis on the reproductive system has been demonstrated in both sexes [81, 82]. In fact, glucocorticoids suppress gonadal axis function at the hypothalamic-pituitary level [81]. Moreover, Inder et al. [83] have demonstrated that dexamethasone administration in humans reduces circulating testosterone and downregulates the muscular expression of the AR. Finally, CRH and its receptors have been identified in the Leydig cells of the testis, where CRH exerts inhibitory actions on testosterone biosynthesis [84].

Interestingly, a sporting event and also training for such represent both a physical and a mental stress [9]. The release of cortisol by activation of the hypothalamic-pituitary-adrenal axis as reaction to mental stress is well documented, especially in competitive situations [44, 85]. Stress responses by the hypothalamic-pituitary-gonadal axis are constantly found as well.

Along this line, anticipatory stress was measured in 50 males before a 1-day experimental stress event (participation in stressful clinical research protocol). Cortisol levels rose significantly, while both testosterone and LH secretion were decreased [86]. Psychological stress markers as measured by scales for anxiety, hostility, and depression were correlated with serum levels of testosterone in a group of males aged 30–55 years. Those classified as highly stressed had significantly lower testosterone levels than their counterparts [87]. A cross-sectional study involving 439 males all aged 51 years showed those with low levels of testosterone (adjusted for body mass index) to exhibit a cluster of psychosocial stress indicators [88]. Nevertheless, other hormonal profile studies reporting the existence of low testosterone in trained men did not show elevated resting cortisol levels suggesting that the hypothalamic-pituitary-adrenal axis is not playing any role in the development of EHMC [41, 59, 60, 89].

However, resting cortisol levels do not necessarily reflect a hyperactivity of the hypothalamus-pituitary-adrenal axis, which can be better defined either by serial blood or salivary sampling [90] or by assay of urinary free cortisol. Thus, at this time the role of cortisol to the changes found in the gonadal axis of trained men is in need of further studies.

Effects of Physical Exercise on Spermatogenesis

Clinical expression of impaired reproductive function in men engaged in chronic exercise training seems uncommon [57, 66, 91]. However, chronic physical exercise may induce a state of oligospermia, a reduction of the total number of motile sperm and an increase in abnormal or immature spermatozoa. Increase in "round cells" has also been reported indicating a possible infectious and/or inflammatory environment [57].

Arce and colleagues [57] were able to retrospectively establish an exercise (i.e., running) volume threshold of 100 km/wk for semen alterations to occur, as they found alterations in sperm density, motility, morphology, and in vitro sperm penetration of standard cervical mucus in endurance-trained runners when compared to resistance athletes or sedentary subjects. Similarly, Safarinejad et al. [68] observed a negative effect of training on sperm parameters in high-intensity training athletes when compared to moderate-intensity ones.

Scientific evidence seems to support the existence of a minimum level of volume for detrimental effects to take place, either hormonal or seminological [54, 57]. As Hackney et al. [54, 56] highlight, alterations may well represent the accumulative effect, more than the acute response, of years of training load.

Some of the latest research has shown that training intensity, and not only volume, is greatly important in this equation as well. In fact, Vaamonde et al. [92] point out that sperm DNA damage and alteration are oxidative stress-related parameters.

High-level athletes have been typically training for many years, making it difficult to establish a potential harmful training threshold (volume and/or intensity) as they normally start training at pre- or peri-pubertal years [93]. Nevertheless, high volume cycling training seems to correlate with sperm morphology anomalies. Wise et al. [94] have examined the association between regular physical activity and semen quality in a large cohort of 2261 men attending an infertility clinic. They found that none of the semen parameters (semen volume, sperm concentration, sperm motility, sperm morphology, and total motile sperm) were materially associated with regular exercise. However, in the subgroup of men who reported bicycling as their primary form of exercise, bicycling at levels of >5 h/wk was associated with low sperm concentration and total motile sperm. These findings generally agree with earlier studies that have shown deleterious effects of bicycling on semen parameters among competitive cyclists [91, 95]. It remains unclear as to whether the changes associated with bicycling are due to mechanical trauma (i.e., caused by compression of scrotum on the bicycle saddle), to a prolonged increase in core scrotal temperature (i.e., related to exercise itself or wearing of constrictive clothing), or some other factors [96].

Oxidative Stress as a Putative Mechanism Underlying Impaired Spermatogenesis in Exercise-Hypogonadal Men

Several mechanisms have been reported to affect the male reproductive function in exercising subjects. Alterations in the hormonal milieu, as discussed in the previous paragraph, may well play a role, since qualitatively and quantitatively normal spermatogenesis is critically dependent on an intact hypothalamus-pituitary-testis axis. On the other hand, it has been reported that endurance exercise is associated with oxidative stress [97]. During endurance exercise, there is a 10- to 20-fold increase in

whole body oxygen (O_2) consumption, and O_2 uptake in the active skeletal muscle increases 100- to 200-fold [98]. This increase in O_2 utilization may result in the production of reactive oxygen species (ROS) at the rates that exceed the body's capacity to detoxify them [99]. An increase in the formation of ROS decreases fertility, as the ROS will attack the membranes of the spermatozoa, decreasing their viability [100]. Vaamonde et al. [101] have reported exercise-related alterations in sperm which may be prevented with antioxidant agents. Vaamonde et al. [102] also reported that, similarly to sperm morphology, cycling volume positively correlates to sperm DNA fragmentation, also observing high correlation between training volume, sperm DNA fragmentation, and percentage of morphological abnormalities [93].

However, an increasing number of studies suggest that exercise training enhances antioxidant capacity [103, 104]. Indeed, the machinery eliminating ROS adapts after regular exercise and actually lowers the amount of ROS that is produced, especially in the major organs (muscles) of oxygen consumption and ROS production. In recent years, the anti-inflammatory and antioxidant properties of regular exercise training have prompted some investigators to explore the effects of different exercise modalities on markers of inflammation and oxidative stress in seminal plasma [105, 106]. Hajizadeh Maleki and colleagues have conducted independent randomized controlled trials looking at the effects of exercise training at different intensity levels on markers of reproductive function and reproductive performance in infertile and fertile men and demonstrated significant improvements in a variety of sperm oxidative stress and inflammation assays as well as semen quality and sperm DNA integrity following 24 weeks of exercise training, suggesting that regular resistance exercise, in particular at a moderate intensity level, positively affects the markers of male reproduction [105, 107–109]. However, how changes in seminal markers of male reproductive function may be connected with reproductive outcomes remains to be determined.

Conclusions

The male gonadal axis function is strongly affected by physical exercise. Relatively short, intense exercise usually increases, while more prolonged exercise usually decreases serum testosterone levels. Restricted energy availability may negatively affect hormone levels both in female and in male endurance athletes as highlighted by the definition of "relative energy deficiency in sport (RED-S)" by the IOC. Reduced or low-normal circulating testosterone concentrations involve health consequences such as an increased risk of abnormal spermatogenesis, infertility problems, and compromised bone mineralization. Thus, awareness must be raised that exercise can represent a potential cause of andrological problems. On the other hand, moderate and low-level exercise has been recently shown to exert positive effects on the male reproductive potential. Ultimately, additional research is needed in this area with proper standardization in assessment tools and study protocols to draw more accurate conclusions about the effects of physical exercise on the male gonadal axis function.

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8 Exercise and the Hypothalamus: Ovulatory Adaptations

Angela Y. Liu, Moira A. Petit, and Jerilynn C. Prior

Introduction

As early as 1939, Hans Selye, who later received the Nobel prize for work on the endocrinology of the adaptation response, reported that muscular exercise was often a cause for "menstrual irregularities" in women [1]. Selye performed controlled animal experiments showing that whether or not exercise suppresses reproduction depends on the abruptness of exercise onset [1]. Forty years later, Shangold et al. [2] published the first prospective observational study documenting gradual shortening of the luteal-phase length with increased running activity in one woman with regular menstrual cycles. Despite these early observations indicating that subtle alterations of ovulatory function occur within cycles of normal length, the exercise science literature has since focused on the absence (amenorrhea) or presence (eumenorrhea) of menstrual flow in women athletes. The purpose of this chapter is to review the subtle but clinically important ovulatory changes in response to exercise.

Hundreds of cross-sectional studies report "athletic amenorrhea,"and inappropriately imply causal relationships between loss of flow and exercise. However, better-designed prospective studies observing normally ovulatory women and closely examining ovulatory function during progressively increasing exercise in reproductively mature women (subsequently termed "exercise training") show only subclinical changes and no amenorrhea when exercise training is the only stressor [3–5]. Prevalent but subtle changes in ovulatory function are the first and most subtle hypothalamic adaptation to exercise training [2, 6]. Failure of hypothalamic adaptation in response to intense stressors such as starvation, psychological distress, illness, or rapidly increasing exercise results in significant disability. Overwhelming stress associated with excessive exercise training (see Chap. 9) and extreme nutritional imbalance (see Chap. 10) are discussed elsewhere in this volume.

In this chapter, we describe the subtle alterations in ovulatory function that occur as a result of hypothalamic adaptation to exercise training and other "stressors." We will also discuss the consequences of ovulatory disturbances, including infertility and a negative bone balance. Before beginning that discussion, however, it is necessary to define both the language and the physiological processes of ovulation.

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The Ovulatory Cycle

The words used to describe the release of an egg and the hormonal characteristics of a cycle in which that occurs need to be defined and described because both are usually obscured by the pervasive, yet imprecise notion that regular, normal-length cycles are always or usually normally ovulatory. We will start by defining the language of reproduction.

Terminology

In the exercise science literature, women are commonly inappropriately classified as "eumenorrheic" (which means "true menstruation!") if their menstrual flow occurs monthly, or oligo/amenorrheic if flow is sporadic or has been absent for 3 or more months [7]. However, cycles of normal length need to also be described by their postovulatory luteal phase or *ovulatory* characteristics. (Note that sometimes "anovulatory" is inaccurately used to mean oligo-amenorrhea). Ovulatory and cycle interval characteristics form a complex continuum (Fig. 8.1). This starts with the most normal cycle type, which is ovulatory with a normal luteal-phase length of 10–16 days (d) and a normal cycle length of 21–35 days [8]. This spectrum ends with the most disturbed ovarian function, which is amenorrhea, defined as the absence of flow for 3 or more months. Between these extremes, cycles that are normal in length may have a short (<10 d) or insufficient (normal length and estradiol levels, but low progesterone) luteal phases, or be anovulatory (normal estradiol but low progesterone levels). This latter is now termed "subclinical ovulatory disturbances." Anovulatory cycles are ones in which cycle intervals may be short, normal, or long in length, but no egg is released and progesterone levels never meet or exceed 9.54 nmol/L (3 ng/mL) [9].

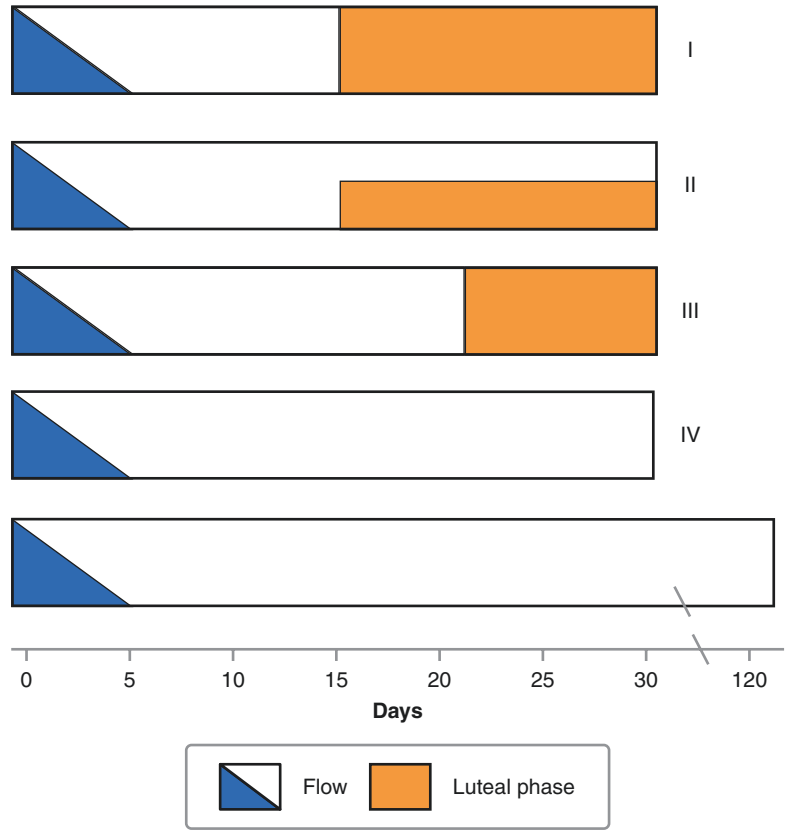

Fig. 8.1 A spectrum of cycle types starting at the top (I) with the most normal, which is of normal length and ovulatory with a normal luteal-phase length. The next cycle (II) is also of normal length and ovulatory, but has an insufficient but normal-length luteal phase. The third cycle (III), also of normal length, illustrates a short luteal-phase cycle. The fourth cycle (IV) is an anovulatory cycle of normal length, and the final cycle (V) is an anovulatory cycle that is longer than normal in cycle length (oligomenorrhea)

Definitions

Given the importance of clarity in science, it is useful to define the terms meant to describe cycle types when ovulatory status is not known. Eumenorrhea implies menstrual cycles that are normal in length, with flow occurring every 21–35 d [10]. When a woman's flow occurs between 36 and <90 d, the term oligomenorrhea is appropriate. For cycle lengths of ≥90 d, women are classified as experiencing amenorrhea. Cycles of varying and abnormal lengths are called "cycle disturbances"; cycles of normal length but short absent or low progesterone levels are called "subclinical ovulatory disturbances."

Cycles are defined as having short luteal-phase lengths if ovulation occurs, but the time from ovulation to the day before the start of flow (luteal phase) is<10 d by quantitative basal temperature analysis [8, 11] or <12 d using the mid-cycle urinary luteinizing hormone (LH) peak as the indicator of luteal-phase onset. An inadequate or insufficient luteal phase means ovulation occurs and luteal-phase length is normal, but peak progesterone levels in the luteal phase are lower than usual (ideally ~45 nmol/L, [14.3 ng/ml]). If ovulation and subsequent corpus luteal formation do not occur, the cycle is anovulatory. Therefore, anovulation refers to cycles in which no eggs are formed (and released). "Subclinical ovulatory disturbances" are cycles that are normal in length, but have either short or inadequate luteal phases or are anovulatory. Cycles that are irregular or abnormal in length and about which ovulatory characteristics are (usually) unknown should be termed "cycle disturbances" and include polymenorrhea (cycles shorter than 21 d) as well as oligomenorrhea and amenorrhea.

Imprecise Language About Reproduction

Two terms are sometimes used that have problems of precision: "eumenorrheic/eumenorrhea" and "anovulatory/anovulation." There are several problems with classifying women as eumenorrheic. The term has been applied to women who "experienced at least 10 menstrual periods per year" [12], even though this would give an average cycle length of 36.5 d (which is abnormally long) and that is oligomenorrhea. Another difficulty with the term, eumenorrheic or eumenorrhea, is that it presumes that all cycles of normal length display the same ovulatory and hormonal characteristics. Data from our 1-year prospective study in ovulatory women of varying exercise habits [4] showed that normal-length cycles could as easily be anovulatory as ovulatory. In that study, all of the anovulatory cycles were normal in length. Therefore, a further erroneous assumption often made in the literature is that only long or short cycles are hormonally abnormal.

The term "anovulatory/anovulation" menstrual cycle is also often misused. Researchers often assume a woman is "anovulatory" if she reports that her cycles are long or irregular—that may or may not be the case. Likewise, the term "anovulation" is commonly used as a synonym for amenorrhea or oligomenorrhea because women whose periods are long or have stopped (unless they have become pregnant) are usually not ovulating.

In short, classifying women only by their cycle intervals implies that the reproductive system works in an on-off or mechanical manner, rather than displaying the broad spectrum of potential responses described above. Classification of women's cycles needs to include the entire range of cycle and ovulatory types, because a distinctly different hormonal profile is present in each case. In addition, the variability and hormonal physiology of cycles, even those of normal length, are important to understand.

Physiology

Just as cycles vary in interval and ovulatory characteristics, so does the cascade of signals from the hypothalamic gonadotrophin-releasing hormone (GnRH) nucleus to the pituitary gonadotrophin-producing cells. Pituitary messages to the ovarian follicle also change, as do hormones from the ovary that give feedback to the pituitary and the hypothalamus. What follows is an effort to clarify the cycle manifestations of the hypothalamic changes described in earlier chapters (i.e., Chaps. 1 and 4).

Ovarian Hormone Levels During the Normal Cycle

An ovulatory menstrual cycle is characterized by systematic and major changes in the levels of estradiol prior to ovulation (follicular phase) and variations of both estradiol and progesterone post-ovulation (luteal phase). Follicular-phase estradiol levels during and just after flow average 60–200 pmol/L (levels that are similar to those in children and men). Estradiol levels subsequently rise over the next 7–18 d to a peak just prior to ovulation that is, on average, 220–250% above the follicular-phase baseline [13]. There is then a decrease to about 100% above baseline for most of the luteal phase before estradiol levels again decrease to low levels just prior to menstruation [13]. In contrast, progesterone levels, which remain low during the entire follicular phase (~0.5–2.0 nmol/L, similar to levels in children and men), increase after ovulation to over 1400% of follicular-phase baseline values. Progesterone levels produced by one corpus luteum remain elevated over 1000% above baseline during the 10–16 d of the luteal phase [13].

The production of estradiol and progesterone is coordinated by, and ultimately dependent on, the timing and magnitude of GnRH pulsatility in the hypothalamus. GnRH stimulates the gonadotrophins, LH, and follicle-stimulating hormone (FSH), to be released from the pituitary. LH peaks at midcycle, and directly triggers follicle rupture and egg release. FSH plays an important role in recruiting intermediate-sized follicles and stimulating the dominant follicle that eventually ovulates. In addition, FSH increases LH receptors on ovarian granulosa cells. GnRH, LH, and FSH are all in feedback regulation by estradiol and progesterone levels. Also, FSH production is actively suppressed by inhibin, a polypeptide hormone that is key in perimenopause [14] but whose potentially important role in reproductive physiology remains poorly understood [15].

Hormonal Profile Changes During Disturbed Cycles

Hormonal characteristics of cycles related to their length will be briefly discussed followed by the hormonal characteristics of cycles that have disturbed ovulatory characteristics. Although few studies have systematically measured estradiol levels in cycles that are short or long, the generalization that shorter cycles have higher estradiol levels is supported by a study in which hormone levels were measured daily during 68 cycles [16]. That study documented that shorter follicular-phase lengths were associated with statistically higher follicular and whole cycle estradiol levels [16]. The logic of this observation is that the more estradiol stimulation of the endometrium, the more likely it is to shed causing bleeding. The opposite is true of long cycles—less estradiol stimulation of the endometrium leads to delayed shedding and flow.

The hormonal characteristics of cycles with disturbed ovulation are less clear. The common feature of all disturbed cycles is the lower amount and/or duration of progesterone production. Estrogen and androgen productions are highly variable in individuals with ovulatory disturbances. Evidence for high estrogen levels with anovulatory cycles is most clearly found in studies of women shortly after puberty [17] and in perimenopausal women [18]. In both instances, estrogen levels exceed the midcycle peak equivalent levels for prolonged periods of time. Androgen excess, which is associated with anovulation, is also associated with high estradiol levels [19], obesity, insulin resistance, and varying degrees of hirsutism.

Evidence that estradiol levels may be normal in anovulatory cycles comes from our observational prospective study [4]. In that group of initially ovulatory women (in whom perimenopause and androgen excess were excluded), the cycles without ovulation were normal in length, and the women who had entirely normal ovulation did not differ in mean estradiol level (measured twice in two cycles a year apart) from the women who experienced anovulation. This flies in the face of the expectation that cycles with disturbed ovulation will have low estradiol levels as had been observed in four women studied by Sherman and Korenman [20]. Sowers et al. also have reported a few days of lower mid-follicular estradiol levels in premenopausal women with disturbed ovulation [21]. However, several other authors in addi-

tion to ourselves have not observed consistently low estradiol levels associated with anovulation [22, 23]. By contrast, the estradiol levels were minimally, although significantly lower in cycles without progesterone levels above 9.54 nmol/L in a single normal-length cycle study in over 3000 women in Norway [9].

In summary, disturbances of cycle interval are often associated with abnormally low or high estradiol levels (inversely related to cycle length), but cycles with ovulatory disturbances may have high, normal, or low estradiol levels and rates of production.

Documentation of Ovulatory Function

This section describes the currently available methods for documenting ovulatory function and the advantages and disadvantages of each. Our primary focus will be to describe the use of "Quantitative Basal Temperature" (QBT), which we have found to be the best available method for continuous, longitudinal monitoring of ovulatory function.

Currently Used Indirect Ovulation Detection Methods

All of the currently available methods for assessing ovulation are indirect, except actual visualization of extrusion of a secondary oocyte from the ovary. The closest to an indirect "gold standard" for ovulation is serial ovarian ultrasounds observing a dominant-sized follicle that "disappears" because it has ruptured and released an egg. Because ovulation requires an LH surge and progesterone levels do not rise if ovulation does not occur, serum or urinary measures of the midcycle LH peak and/or progesterone levels are often used as indicators of ovulation. One method is to perform serial samples of serum or urine daily during the midcycle to detect the LH peak. Alternatively, in the week prior to menses, serum (or plasma) samples showing levels of progesterone of ≥9.54 nmol/L (≥3ng/mL) are indicative of ovulation. The postovulatory increase in progesterone can also be measured in spots of whole blood [24], urine, or saliva [25] or by its effect to increase core temperature or to inhibit the stretch/elastic characteristics of estrogen-stimulated cervical mucus (although this latter effect has not been scientifically evaluated to date).

An estradiol peak is necessary to trigger the midcycle LH peak. Therefore, another indirect assessment of ovulation involves collecting estradiol levels daily with serum samples. Samples must be taken until an estradiol level doubles the preceding level and over 750 pmol/L (by usual assays) is documented. However, midcycle peak estradiol levels may occur and not be followed by an LH peak or by ovulation in premenopausal (as in perimenopausal) women [23, 26, 27]. Therefore, an estradiol peak level is not a specific test of ovulation, nor is the stretch of cervical mucus that estradiol stimulates. To a lesser degree, the same lack of specificity is also true of an LH peak [27].

Limitations of Available Methods for Diagnosis of Ovulation Disturbances

Serial sampling of blood, saliva, or urine is required to adequately document all of the important ovulatory characteristics (including whether ovulation occurred, as well as luteal-phase adequacy and length) of a single cycle. Using these methods to document several consecutive cycles is very labor-intensive, invasive, expensive, and imposes a high degree of burden on participants. Continuous longitudinal documentation of hormone levels in large numbers of women is, therefore, virtually impossible to obtain using these sampling techniques [3, 5, 27]. Similarly, although formerly endometrial biopsy analysis of histological change related to progesterone was considered definitive for luteal-phase adequacy and length, it has a ±2-d SD and is not useful [28]. Finally, serial ultrasound assessments (to show a growing follicular cyst that enlarges to over 18mm and then disappears) are now considered the indirect "gold standard" indicator of the occurrence of ovulation [25], but they lack convenience and reasonable cost for most studies.

The logical question is: why not measure ovulatory characteristics during one cycle and then just monitor cycle intervals over the necessary

period of time? Could you not infer that the subsequent cycles, if they are regular and normal in length, are similar in ovulatory status? That would be an accurate strategy if women's cycles were as stable in ovulatory characteristics as they are in cycle interval. However, ovulatory characteristics are highly variable over time within women [4, 8, 29, 30]. For example, Hinney et al. [29] documented "corpus luteum insufficiency" by a late luteal-phase progesterone level below 25 nmol/L in 109 women of whom only 55, when tested in the following cycle, continued to show corpus luteum insufficiency. Likewise, 5 years after our intensive monitoring of continuous cycles for 1 year in 66 women, cycle lengths (in ≥3 cycles) correlated well with previous ones (r = 0.68, P < 0.05). However, luteal-phase lengths correlated considerably less well (r = 0.39, P = <0.05) [30].

Furthermore, as this chapter will subsequently document, ovulatory disturbances caused by hypothalamic adaptation occur rapidly and as quickly revert to normal ovulation. Thus, studies that measure ovulatory characteristics in only a few cycles or monitor cycles discontinuously (such as every other or every fourth cycle) are not likely to detect ovulatory disturbances (in general) and particularly not likely to document those related to hypothalamic adaptation to exercise. That is especially true if cycle characteristics are documented only in the cycle before exercise intensity is again increased, as has been done in two prospective studies [3, 5].

At least 6 months of continuous sampling, in which both ovulation and luteal-phase lengths are assessed, are necessary to adequately characterize a sedentary, weight-stable woman's menstrual and ovulatory characteristics [31]. In exercising women, it is even more critical to provide a robust baseline from which to examine potential changes associated with exercise training. For all of these reasons, a noninvasive, inexpensive, and "habit-forming" method for documenting ovulatory characteristics is necessary.

Quantitative Basal Temperature (QBT)

Daily basal (meaning first thing after wakening in the morning, fasting, and when metabolism is stable) oral temperatures (often referred to as BBT) potentially allow continuous, longitudinal research into ovulatory characteristics to be conducted. High levels of progesterone during the luteal phase increase the basal temperature. This increase begins to be significant approximately 24–48 h after the LH peak [11]. A monophasic set of basal temperatures during one cycle, in which our least-squares program (Maximina®) detected no significant shift, characterized an anovulatory cycle with progesterone levels that did not rise sufficiently to alter temperatures. A biphasic cycle is indicative of ovulation [8], and the day of the significant temperature shift can be used to define the onset of the luteal phase [11]. In ovulatory cycles, the increased progesterone levels raise the basal temperature during the luteal phase by approximately 0.2–0.3 °C.

However, BBT was a clinical tool before it was a research method. Therefore, the early studies utilizing BBT as a method of detecting ovulation had a number of problems, including that women might take their temperature at different times of day, women had difficulties reading or shaking down the older mercury thermometers, women were expected to plot their own temperatures as a graph (which often resulted in inaccuracies of graphing), and the temperature patterns were evaluated for the presence or absence of ovulation using non-quantitative methods and often "eyeball" or equally nonreproducible methods [32]. Finally, even when more systematic methods of assessing changes in temperatures were described [33], insufficient data relating the temperature shift to hormonal data were available.

In our laboratory, these problems have been resolved by better instruction of women about what the factors in addition to fever that alter the basal temperature (such as awakening earlier or later than usual, or being up in the night) and providing a form on which to record these factors. In addition, we asked women to take their temperature with a digital thermometer reading to two decimal places and to record temperatures in a list, rather than plotting them on a graph. We then devised and applied a

computer program (Maximina®) of least-square analysis to each cycle of temperature data and showed it to be valid against the independently assessed serum LH peak (r = 0.88) [11]. At the same time, we validated the "mean temperature" method of Vollman [8, 11]. This more scientific method we named "Quantitative Basal Temperature", so we could differentiate it from the crude and unscientific BBT methods used in the past.

Thus, we believe we have transformed the previously inaccurate and unreliable BBT method into a scientific tool for documentation of ovulatory characteristics. Furthermore, it is a method that can be easily taught, requires only a relatively inexpensive and durable digital thermometer, and is one that interested women can and will consistently use [4, 34] for lengths of time exceeding a year. Taking of basal oral temperature quickly becomes a habit. However, for this to happen, it does require the interest and commitment of women and of those teaching them.

The major difficulty with widespread use of the QBT method is its lack of accuracy in those whose time of waking and sleeping is variable (e.g., those on shift work, with small children, or students—although it was robust to time of awakening in one study [35]). A simpler method, not dependent on a stable life pattern, and requiring less commitment from women, is needed for documentation of ovulatory characteristics in longitudinal studies and for epidemiology.

Hypothalamic Adaptation and Ovulatory Function

The neuroendocrine physiology of adaptation to exercise and other stressors is complex and not yet completely understood. A review article published more than 10 years ago continues to reflect our current understanding on the subject [36]. It has also been covered by earlier chapters in this volume, and therefore will be reviewed only briefly here. The hypothalamus functions to maintain internal homeostasis in response to internal and external factors. Numerous influences, such as ambient and core temperature changes, energy balance changes, illness (which alters eating and sleeping patterns and may cause elevated temperature levels), and psychological stress, can directly or indirectly alter the pulsatile secretion of GnRH and thus change subsequent reproductive function [36].

The premise of this chapter is that the first and most subtle adaptive responses to exercise training occur during the premenstrual phase of the cycle with a range of ovulatory disturbances (Fig. 8.1) that all result in decreased total exposure to progesterone. As discussed, studies that examine hypothalamic control and the subtle changes that lead to shortening of luteal-phase length with exercise training require long-term, continuous monitoring of ovulatory function.

Ovulatory disturbances in response to exercise training can be viewed as an adaptation to the increased physiological and perhaps psychological stresses of the exercise and are not part of a disease process. The adaptation model suggests these four principles:

1. Ovulatory disturbances are caused by a hypothalamic process that is conservative, e.g., protective of or saving energy for the individual.
2. They are induced by a variety of physical and psychological "stressors," which act through a common mechanism and manifest similar changes.
3. There are gradients of change in response to the severity or intensity of the "stress" or "threat."
4. The adaptive changes reverse to the normal baseline steady state when the "threat" is lessened or eliminated, or the individual has sufficient time and is able to adapt.

Evidence for these points will be described in the following sections. The specific ovulatory adaptations to exercise training, including the gradients of change and reversibility, will be described in the section "Adaptations to Exercise Training" of this chapter.

Hypothalamic Adaptive Processes

Evidence that the subtle alterations that lead to shortening of luteal-phase length are controlled by the hypothalamus is largely circumstantial, because altering hypothalamic function biochemically or with direct nerve cell stimulation is impossible in humans. The strongest evidence that the hypothalamus controls changes in ovulatory function comes from the similar pattern of responses during exposures to a whole range of psychological and physiological stressors.

Corticotrophin-releasing hormone (CRH) discharge increases when any internal or external environmental signal is perceived as stressful (as shown schematically in Fig. 8.2). The increased CRH may either directly or indirectly (via the β-endorphin system) slow the hypothalamic pulsatile release of GnRH [38] and, therefore, decrease pulsatile LH release. Because the pulses of LH stimulate estradiol and androgen secretion, they provide an essential precursor to ovulation.

A systematic review by Hakimi and Cameron [39] on the effect of exercise on ovulation proposed mechanisms by which vigorous exercise disrupted ovulation in women with normal and low body mass index, and by which exercise restores ovulation in overweight and obese women. They describe a U-shaped association of exercise with ovulation: increases in ovulatory disturbances with increased exercise especially in women or normal or lower weight but decreases in (pre-existing) ovulatory disturbances with exercise in overweight women. This is based on ten interventional and four observational cohort studies, which showed that greater than 60 min per day of heavy exercise was associated with an increased risk of anovulation, while vigorous exercise of 30–60 min per day was associated

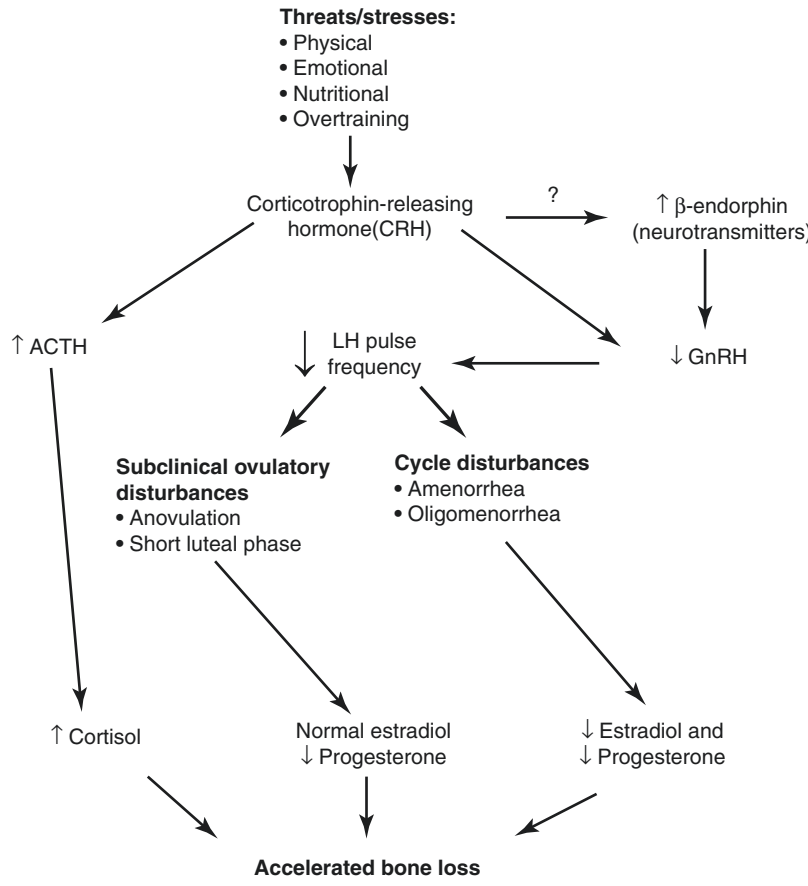

Fig. 8.2 Processes through which physical (including exercise and illness), emotional, or nutritional challenges cause increased release of CRH from the hypothalamus. These factors suppress the reproductive system and stimulate the adrenal axis. *ACTH* corticotrophin; *LH* luteinizing hormone. (Modified from Prior [37])

with a reduced risk of anovulation. Seven studies examining exercise in overweight and obese women with polycystic ovarian syndrome found that exercise was associated with improved ovulatory function. Notably, one limitation in these studies is the lack of assessment of *rate of increase in exercise intensity*. Many of the cross-sectional studies simply observe current exercise duration and intensity. Evidence documents that the rate of progression of exercise training is an important modulator as well [1, 40].

Reproductive and primarily ovulatory changes are "conservative" for the individual, because through multiple pathways, they effectively prevent pregnancy when the woman is unable to physically or emotionally support a healthy process. They are also conservative of energy because less progesterone production, which decreases the otherwise increased core temperature, means women can consume about 300 fewer dietary calories and, like women with normal ovulation, still ensure energy balance [41].

Stress Mechanism

Selye [1] observed about 70 years ago that the adrenal glands were hypertrophied when various kinds of stressors interrupted estrus in rats. He also observed similar patterns of response of the ovaries and the adrenals to excessive exercise, to interference with normal diet, and to emotional stressors. More recently a strong relationship was also documented between social stress and non-ovulation in nonhuman primates. Subordinate female monkeys experienced 16.5% of cycles as non-ovulatory, whereas dominant females over the same time period and in the same conditions experienced only 3.5% of their cycles as anovulatory [42].The subordinate monkeys at autopsy had very enlarged adrenal glands [42]. Cortisol excess, which was similar to levels seen in women under stress, significantly increased the metabolic clearance of progesterone as well as increasing LH pulse amplitude in experimental studies by Kowalski et al. [43]. This research showed that monkeys who were exposed to induced hypercortisolism had lower luteal-phase serum progesterone levels and more ovulatory disturbances [43, 44].

In humans, reversible, modulated suppression of reproduction during illness was documented by lower than normal LH levels in gravely ill, hospitalized postmenopausal women; LH levels recovered as they improved [45]. Similarly, a prospective study in Japanese nursing students showed regular and apparently ovulatory cycles with more frequent ovulatory disturbances during the stressful school year than in the summer break [46].

Weight loss is known to be one of the most powerful physiological hypothalamic stressors [47, 48]. An experimental protocol involving fasting for 3 d in the late follicular phase appears to be more disruptive of follicle development and more likely to suppress LH pulsatility in women who are initially very lean than in those who have normal body weights and fat [49].

Active women with amenorrhea, like overtrained athletic men [50], have increased basal levels of cortisol [51] and blunted cortisol responses to exercise [52, 53]. Berga et al. [52] reported high 24-h cortisol levels in those with hypothalamic forms of oligo-/amenorrhea compared with normally menstruating women. This hypercortisolemia was not observed in women with other reasons for disturbed cycles, such as hyperandrogenism or hyperprolactinemia. A few women initially deemed to have hypothalamic amenorrhea subsequently ovulated during the study and were shown to have concomitantly reduced levels of cortisol [52]. Ding et al. [51] could similarly predict women whose cycle intervals would subsequently become normal because their cortisol excretion was decreased.

High cortisol secretion or urinary excretion has become a useful marker of hypothalamic adaptive responses to stressors including exercise, because all stressors apparently act through the hypothalamic CRH pathway. Therefore, studies in both humans and nonhuman primates demonstrate increased cortisol levels simultaneously with decreased LH pulsatility and/or disturbed ovulatory function during reproductive disturbances coinciding with a variety of stressful situations.

It should be noted that, although hypothalamic disturbances of ovulation characterized by lower

pulsatile release of LH are probably the most common cause for the menstrual cycle disturbances reported in athletes, short luteal-phase cycles or an ovulation associated with androgen excess (and with high, rather than low, LH levels) [54, 55] can also be documented. High androgen and LH levels have been described in swimmers with amenorrhea [54]. In addition, defects of the large corpus luteum cells have been postulated to cause lower luteal-phase progesterone levels, although LH pulsatility and estradiol levels are both normal [29].

Energy Conservation

Reversible cycle disturbances are termed "functional" (that implies psychological) and are not a disease. When discussing ovulatory disturbances as protective against excess energy expenditure, the severity of the disturbance is proportional to the amount of energy conserved. Amenorrhea in women without an extreme eating disorder may be relatively less threatening than anorexia, because compared with menstruating women, it appears to lower BMR only 17% [56]. Anovulatory cycles, which are normal in length, are also less metabolically costly to maintain than ovulatory cycles and prevent the risk of pregnancy with its high-energy demands. The basal temperature increase during the luteal phase raises metabolic rate. Barr et al. [41] documented that women's dietary intake was increased approximately 300 kcal/d during the hormonally confirmed luteal phase of cycles compared with anovulatory cycles in the same group of women; all were without exercise or weight changes during the six-cycle study [41].

A shortened luteal-phase length (in contrast to anovulation) occurs in response to the least threatening intensity or kind of stressor. Energy demands are higher when the luteal-phase length is shortened than they are in anovulatory cycles, due to up to 9 d of progesterone-related temperature elevation in the former. We believe that shortening of luteal-phase lengths is the most common adaptive response to stressors, such as weight loss, emotional stress, illness, or exercise training. It is of importance that, despite the minimal alteration of ovarian physiology, fertilization and implantation of the egg are still prevented by subclinical short luteal phase and luteal insufficient cycles.

Synergism or Interactions Among Factors Influencing Ovulatory Function

The concept of adaptation with a common hypothalamic change caused by many different stressors implies that the response to one, such as exercise, would depend on the current state of other factors, such as energy balance or emotional stress. Therefore, it is important to consider those factors that are known to influence ovulatory function and to acknowledge that individuals may respond differently to any given stress depending on the presence of many personal variables. The adaptive response is altered by such factors as the individual's current energy balance, underlying characteristics of the individual (i.e., levels of reproductive maturation, weight, and emotional well-being), intensity of the threat, and the rapidity with which it is introduced. Multiple emotional and psychological stressors, weight loss or restrictive eating, and the need to feel "in control" all are often perceived as stressful by the hypothalamus and influence reproductive function (Fig. 8.3). These stressors all appear to act through the common hypothalamic CRH pathway.

Energy Balance

It is likely that exercise and other stressors affect LH pulsatility through their influence on energy balance [57]. Other chapters in this volume discuss this (see Chaps. 11 and 17). We postulated in 1982 that hypothalamic insulin receptors might provide a common signal [6]. Those who are ill or over-exercising would have decreases in their insulin levels as a consequence of negative caloric balance. It is well accepted that severe weight loss or an extreme energy deficit, such as with anorexia nervosa, suppresses reproductive function. In such extreme cases, CRH

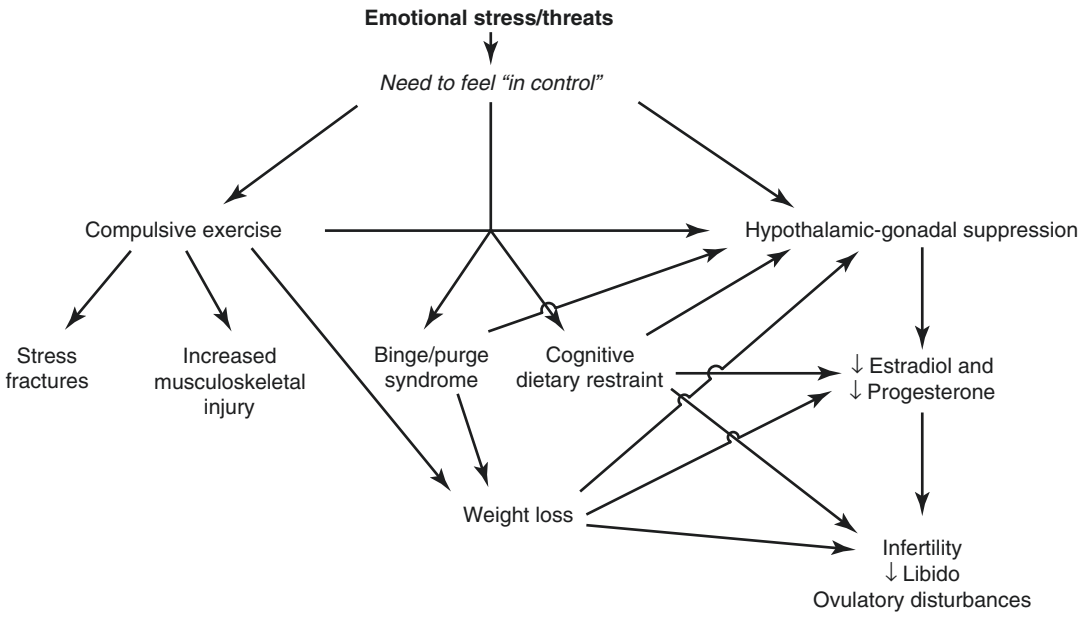

Fig. 8.3 Interrelationships among multiple factors (stress, compulsive exercise (associated with an increased risk for relative energy deficiency), and cognitive dietary restraint) that appear to be causally related to the development of ovulatory disturbances

levels are high [58] and amenorrhea will likely result. More subtle reproductive disturbances often occur when the relative threat is less intense, but the conditions that facilitate pregnancy are still not optimal. For example, ovulatory disturbances may occur with healthy weight loss or dieting [59], as well as when recreational exercise or emotional stress increase. In each case, the greater the need for energy conservation, the more severe the ovulatory or cycle length disturbance [48, 60].

In 2014, Mountjoy et al. as part of an International Olympic Committee Expert Group presented the concept of relative energy deficiency in sport (RED-S) (Fig. 8.4). This is a more physiological and comprehensive alternative to what was called the "female athlete triad" that refereed only to athletic women [61]. RED-S is a syndrome characterized by impaired physiological function in areas such as metabolic rate, reproductive function (that, for women means menstrual cycle and ovulatory changes), bone health, immunity, protein synthesis, and cardiovascular health. It is due to an imbalance between dietary energy intakes relative to energy expenditures; when expenditure exceeds intake, this results in a relative energy deficiency. The syndrome of RED-S is applicable to both women and men and takes a physiological rather than a disease approach to the multitude of changes related to exercise training.

The magnitude of energy deficit affected the frequency of menstrual cycle and ovulatory disturbances in healthy women [63]. The average percentage of energy deficit, in a study of untrained, regularly cycling and ovulating women aged 18–30 y followed over four menstrual cycles, was the major predictor of the frequency of menstrual cycle/ovulatory disturbances even when adjusted for weight loss. Luteal-phase disturbances, although they only vaguely defined their documentation, were the most frequently observed reproductive changes. However, there were no differences by the degree of relative energy insufficiency in the development of anovulatory cycles or oligomenorrhea [63]. When these authors defined energy availability as energy intake minus

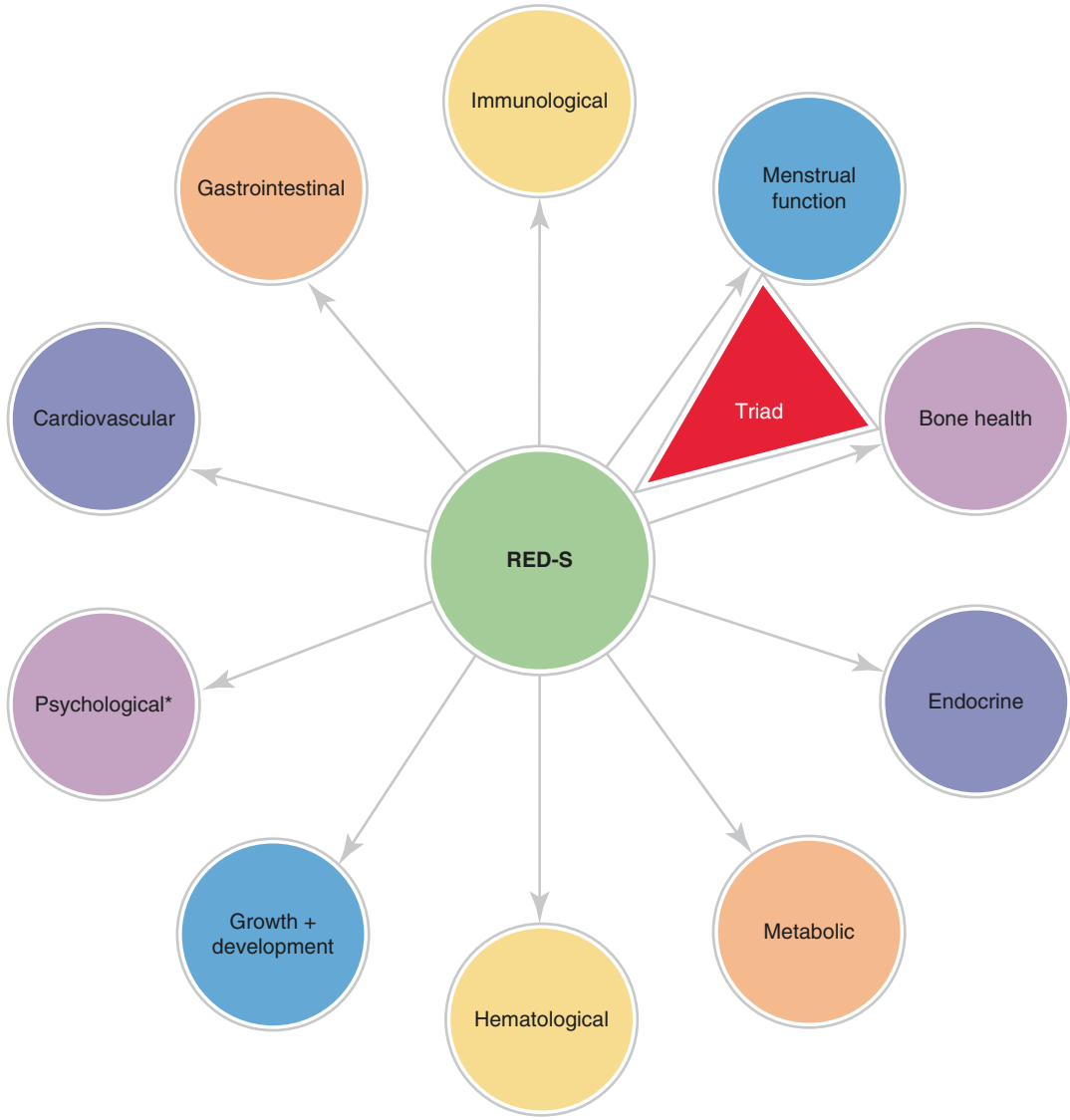

Fig. 8.4 Potential health effects of relative energy deficiency in sport (RED-S). This is applicable to both men and women athletes. Note that ∗Psychological consequences may precede or result from RED-S. (Modified from [61] and the concept adapted from the original idea of N. Constantini [62])

exercise energy expenditure divided by kilograms of lean body mass and looked for associations with ovulatory disturbances in 91 exercising women, they found it discriminated clinical menstrual status (e.g., amenorrhea vs. regular menstrual cycles) but not subclinical ovulatory disturbances [64]. This, again, highlights the distinction between menstrual cycle length changes that are clinically obvious and the silent and more prevalent ovulatory disturbances.

Cognitive Dietary Restraint

Subtle ovulatory disturbances also occur with cognitive dietary restraint (also called "eating restraint"), a psychological attitude in which women feel they must limit food intake to avoid obesity. Women who are classified as highly restrained (based on the Three Factor Eating Questionnaire [65]) are very conscious of their food intake, but they do not necessarily consume

fewer calories than weight- and age-matched controls who are not restrained [66, 67]. Because maintaining or achieving their desired weight is so important to their emotional well-being, eating is associated with psychological stress for women with cognitive dietary restraint. A very early study using the Eating Restraint Scale of the Three Factor Eating Questionnaire showed that women with higher scores were more likely to have short luteal-phase cycles [68]. Three studies from our laboratory also examined ovulatory function and eating restraint in normal weight, regularly cycling, and ovulatory women who varied in their usual activity levels [69] and in regularly cycling vegetarian and non-vegetarian women [70]. A more recent study in young adult women (most of whom were post-secondary students) showed that those with higher eating restraint scores had more ovulatory disturbances and higher 24-hour urine-free cortisol levels [71]. The frequency of subclinical ovulatory disturbances and the degree of cognitive dietary restraint were associated with less positive changes in bone mineral density, although cortisol did not appear to modulate that relationship [71]. In all of these studies, the Restraint Scale of the Three Factor Eating Questionnaire [65] was administered initially, and menstrual cycle characteristics were documented prospectively over three or six cycles or 2 years, respectively. In all studies, women in the highest versus lowest tertile of restraint were significantly more likely to experience a short luteal phase or anovulatory cycle. These findings could not be attributed to differences in energy intakes, exercise levels, or body mass index (BMI is weight in kg divided by height in m^2) levels. Women with eating restraint did not differ in BMI, weight, energy intake, activity levels, or cycle lengths from the less restrained women in each respective population [69–71]. Since cycle intervals were unaltered, none of these women would have known their ovulation was disturbed.

Changes in metabolic hormone levels have been documented related to resting energy expenditure. Metabolic alterations include growth hormone resistance and reduced IGF-1 concentrations; increased cortisol, ghrelin, peptide YY, and adiponectin; and decreased triiodothyronine and kisspeptin [72]. Progesterone therapy in an RCT in menopausal women caused a small but significant increase in the level of free T4 [73]. Collectively, these changes appear to suppress the hypothalamic–pituitary–ovarian axis in an adaptive, graded manner [72].

It is probable that the effect of cognitive dietary restraint on ovulatory function is mediated through hypothalamic adaptation pathways. The evidence that subtle ovulatory disturbances are more common among those with greater cognitive dietary restraint, despite similar energy intakes and expenditures, emphasizes that hypothalamic ovulatory disturbances may result from relatively minor psychological as well as physiological stressors.

Hypothalamic Reproductive "Maturation"

Another variable influencing the ability of the hypothalamic/pituitary/ovarian system to respond to stressors is its relative maturity. For example, the majority of menstrual cycles are anovulatory in the first year after menarche [8]. However, on average, women do not develop the highest rate of ovulatory cycles until they are approximately 12 years after menarche [8] (or gynecologic age 12). This implies that some are still gynecologically immature. It fits with the adaptation hypothesis that those whose hypothalamic–reproductive axis has not yet become sturdily and regularly ovulatory are more likely than those with mature reproductive patterns to respond to stress with altered cycle lengths as well as with ovulatory disturbances [46].

One of the first studies documenting the reproductive hormonal characteristics of young athletes showed that both swimmers and controls had short luteal-phase cycles, but in swimmers, the luteal phase was even shorter than in sedentary controls [74]. Although participant numbers were small, these data confirm the more extensive data of Vollman [8] that teenagers are susceptible to subtle disturbances of ovulation. Young runners (gynecologic age <10 year, mean chronologic

age 20 year) are also more likely to have disturbed folliculogenesis and decreased estradiol, progesterone, gonadotrophins, and testosterone levels than are gynecologically mature women (gynecologic age >15 year, mean chronological age 31 year) [75]. Therefore, data suggest that the combination of more intense training and an immature hypothalamus are potentially additives in suppressing reproduction in young women.

Mature gynecological aged women who begin exercise or intensify training only experience ovulatory and not cycle-length changes. However, evidence suggests, although we do not yet have appropriate experiments to document it conclusively, that a woman in her 20s who is initially only intermittently ovulatory and begins to exercise or intensifies exercise training may well develop cycle as well as ovulatory disturbances. This young woman, with weight loss or emotional distress added to exercise training, would likely develop oligomenorrhea or amenorrhea.

Evidence says that age at menarche is influenced by the energy imbalance related to intense exercise training [76–78]. Although genetic factors also have a strong influence on menarcheal age [79], dancers and gymnasts who experience lower energy availability are more likely to have delayed menarche compared with their sedentary sisters even though they are genetically very similar. Puberty involves maturation of axillary and pubic hair as well as breast enlargement and areolar/nipple maturation. Interestingly, when young athletes are forced (often because of injury) to interrupt their gymnastics or dance training, rapid development through one or more of the Tanner breast stages commonly occurs [78, 80].

Anorexia nervosa commonly occurs in women and during puberty. Weight loss and young age may make them more vulnerable to anorexia. In a similar manner, they will likely be more prone to exercise effects on ovulatory function, especially if exercise is combined with restricted energy intake or psychological performance pressure from coaches and parents. It is also probable that women experiencing reproductive and ovulatory disturbances in response to stress when younger will be more susceptible to exaggerated stress responses throughout life [81].

The pubertal maturation of the breast is primarily dependent on ovarian hormones, with little or no influence of adrenal steroids. By contrast, pubic hair maturation can proceed with the normal adolescent increases in adrenal androgen secretion, without significant increases in ovarian hormones. Discrepancy in the degree of Tanner stage breast compared with pubic hair maturation is probably a clue to hypothalamic adaptive changes related to exercise training and/or other stressors. Warren et al. [78] reported that pubic hair development occurred at a normal age in young women dancers, but there was a trend to delays in breast development and age at menarche. Clinical data from ovarian hormone treatment of male-to-female transgender individuals [82] and observations during a prospective study of puberty [83] both suggest that normal breast development to the fully mature Tanner stage V breast will not be reached without adequate exposure to ovulation and thus to high progesterone levels.

Stress Intensity and the Rate of Increase in Stress Intensity

Whether ovulation becomes disturbed partially depends on the intensity of the stress and partly on the rate of introduction of that stress. For example, in one study all rats responded to "inescapable" shock by suppressed gonadotrophin secretion [84], whereas only some rats were susceptible to the relatively less threatening stress of gradually increased endurance exercise [1].

Hans Selye coined the term "general adaptation syndrome" and published early controlled trials of exercise and energy restriction stress on rats [1]. Selye's experiments showed a dramatically different response to gradually increasing exercise compared with rapid imposition of exercise training (or caloric restriction) (Fig. 8.5). Animals which started running at 3.5 km/d developed anestrus (the rat equivalent of amenorrhea) with interstitial atrophy, few mature follicles, and increased weight of their adrenal glands. A second group of rats gradually increased exercise intensity to reach 3.5 km/d over 4 weeks (Fig. 8.5). Even though

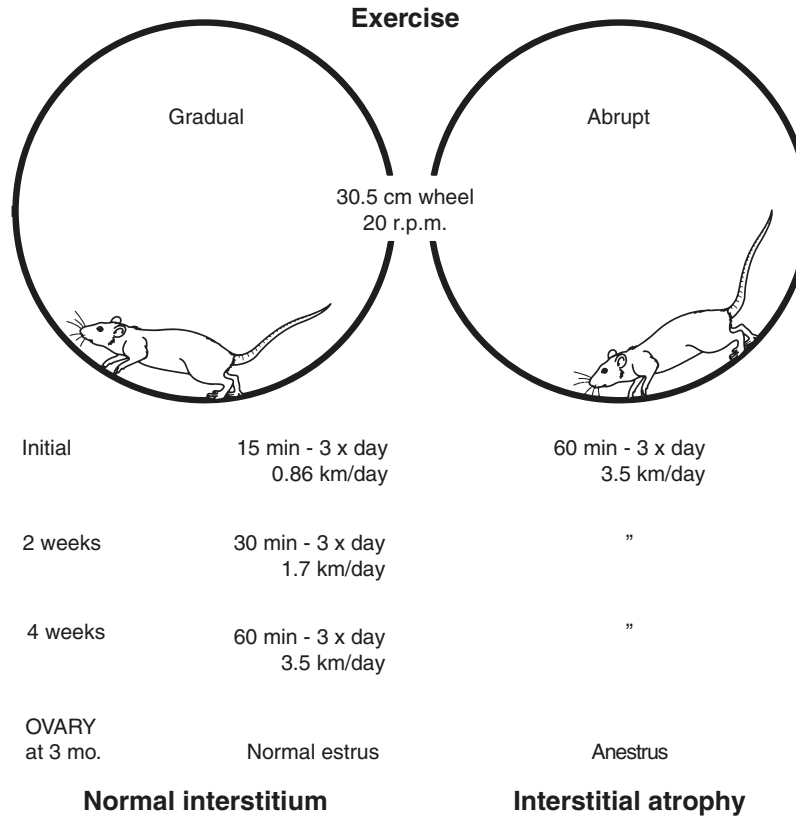

Fig. 8.5 Illustration of the concept of the "general adaptation syndrome" developed by Hans Selye. Exercise was introduced abruptly or gradually in rats randomized to one or the other group. The photomicrographs show ovulatory adaptation by normal interstitum and follicular development in rats with gradual increase in exercise. Abrupt introduction of exercise led to anestrus (the rat equivalent of amenorrhea), interstitial atrophy, and development of only a few mature follicles. (Data redrawn from Ref. [1])

the rats in the second group maintained the same level of exercise intensity as the first group for 2 of the 3 months, reproductive function remained normal, and ovarian follicle development was appropriate. Similar differences in response were observed in rats treated with rapid "semi-starvation" compared with gradual decreases in caloric intake [1]. Selye subsequently showed a similar pattern of reproductive response in restrained rats as those separated from their cage-mates or siblings. These data suggest that similar mechanisms of hypothalamic adaptation on the reproductive system occur in response to exercise training, weight loss, and psychological stress as well as to illness [45].

In Selye's day, before immunoassays for hormones were available, the level of stress was best indicated by adrenal gland weights. Because reproductive disturbances occurred in parallel, they were also assessed as "adaptive" and related to a generalized stress response. These observations are consistent with current data showing elevated cortisol levels in women with hypothalamic disturbances of ovulation, oligomenorrhea, and amenorrhea [85].

These classical animal stress experiments are only now being reproduced in humans. However, as will be discussed in more detail below, the data available in mature women suggest that a high training intensity and volume is well tolerated if adequate nutrition and a suitable time for adaptation to that exercise are allowed.

Finally, although interactions among "treat" or "stress" variables had been postulated in women [6], it has not been experimentally documented. One very important prospective, controlled experimental study in female cynomolgus monkeys has shown that there are synergistic or added reproductive system effects of psychological "stresses" on top of metabolic or energy-related threats [86].

Adaptations to Exercise Training

Exercise Training Studies in Reproductively Mature Women

Only a few studies have prospectively documented changes in cycle and ovulatory characteristics as with exercise training in mature women. The first prospective documentation, in only one woman, used the elasticity of cervical mucus as a marker of the midcycle estradiol peak to show shortening of the luteal phase associated with an increase in weekly running distance [2]. Other early studies showed an increased prevalence of short luteal phase or anovulatory cycles associated with increasing intensity or volume of exercise training [87, 88]. In a group of 14 reproductively mature women (gynecologic age >15 year, mean chronologic age 35 years) who had been training for a marathon, only one-third of a total of 48 cycles prior to a marathon (three cycles/woman) were ovulatory with normal luteal-phase lengths [88]. The only difference between nonovulatory and ovulatory cycles appeared to be the length of the usual training run from approximately 2–5 miles [88].

A study of longer duration (14–15 month) in women not initially proven to be ovulatory showed a decrease in the volume of menstrual blood and lower estradiol levels with marathon training [89]. Running activity increased from 24 to 100 km/week over the study period. Ovulatory characteristics were not examined, however, and the inclusion of participants from ages 24 to 57 years old [89] confounds these outcomes. Nevertheless, in that study, and in none of the others to be subsequently described, did the women develop amenorrhea, despite rapid increases in running activity/intensity mandated by some of the protocols.

In Table 8.1 we compare three important prospective studies of exercise and reproduction. These studies have all sought to establish an influence of exercise training on the reproductive hormonal characteristics of both the follicular and the luteal phases of the menstrual cycle as well as ovulatory changes during exercise training: Bullen [27, 90], Bonen [3], and Rogol et al. [5]. Because of their importance to this discussion, each study is described in detail below.

Bullen and colleagues [27, 90] monitored 28 college-aged women residing at a summer camp by measuring hormonal characteristics for 2 cycles using analysis of daily overnight urines and evening temperatures. These women (whose mean age was 20 years) were confirmed to be ovulatory prior to entry into the study and were also randomly assigned to either weight-loss or weight-maintenance groups. Running activity increased from 4.5 to 10 miles/d by week 5 of the 8-week camp. In addition to running 10 miles/d, women also participated in 3 h/d of varied recreational activities. Bullen and colleagues documented that none of the women in the study developed amenorrhea despite their young age and that they were exposed to several stressors, including change of residence, intense and rapidly increasing exercise training, and caloric restriction (in the weight-loss group). Ovulatory disturbances and shortened luteal-phase cycles were common, however, and only 8 of the 28 women ovulated normally in both cycles. The addition of weight loss to the exercise training caused a further significant increase in ovulation disturbances as well as oligomenorrhea in a few women [27, 90].

Bonen and colleagues [3] set out to determine whether a dose–response between running mileage/week and reproductive function was operative. In particular, by observing sedentary, mature women who ran at varying exercise loads, they tried to determine whether or not a threshold of exercise intensity was present above which luteal-phase disturbances would begin. Bonen [3] monitored mature women over 2–4 month who were variously training at <16, 16–32, or 32–48 km/week. These investigators showed that although there were trends toward shortening of the luteal phase in the first cycle measured after training began, no consistent luteal-phase length changes were documented, nor were there any differences in ovulatory characteristics between women in different intensity groups [3].

A study by Rogol et al. [5] was similar to Bonen's, but used VO_2max testing to document the anaerobic threshold or when lactate began to

Table 8.1 Published prospective studies of exercise training on menstrual cycle and luteal phase lengths

Author	Bullen et al. [27]			Bonen [3]		Rogol et al. [5]	
Total (*n*)[a]	28			57		23	
Chronologic age	22 (0.6)			30.0 (1.3)		31.4 (1.3)	
Gynecologic age	10 (0.6)			17.1 (1.4)		17.8 (0.9)	
Mean (SE) Study groups	Exercise + weight maintenance (A) Exercise + weight loss (B) (max of −0.45 kg/week)			<10 miles/week for 2 months (A) <10 miles/week for 4 months (B) 10–20 miles/week for 2 month (C) 10–20 miles/week for 4 months (D) 20–30 miles/week for 2 months (E) 20–30 miles/week for 4 months (F)		Train at lactate threshold (n = 9) Train above lactate threshold (n = 8)	
Duration of exercise training	2 months			2–4 months		1 year	
Exercise schedule	Running 4 miles/d progressing to 10 miles/d by week 5, plus 3.5 h of cycling, tennis, or volleyball			As described above		Start: 6.25 miles/week Weeks 1–20: add 1.25 miles every second week Weeks 20–39: hold at 24 miles/week Weeks 40–end: add 1.25 miles every second week (max of 40 or 65 miles/week)	
Exercise intensity	70–80% of max aerobic capacity (adjusted each month)					Not reported 6 d/week ran at lactate threshold 3 d/week ran at lactate threshold and 3 d/week ran above lactate threshold	
Sampling method	Daily BBT and daily urinary sampling (overnight)			Daily blood samples		Daily blood samples day 9 through end of cycle	
Sampling intervals	Continuous			Every second cycle		Every fourth cycle	
Luteal length (LL) Mean (SE)	Mean LL not available cycle types during training			Control cycle	Mean LL 14.2 (1.5)	Cycle 1	Mean LL 13.9 (0.6)
	Study group	A	B	Run cycle 1	12.6 (1.0)	Cycle 4	13.4 (0.7)
	%Ovulatory	25	6	Run cycle 3 (only includes groups B, D, and F)	14.2 (1.5)	Cycle 8	13.8 (0.7)
	%Short luteal phase	66	63			Cycle 12	12.8 (0.7)
	%Anovulatory	42	81	Detrain, cycle 3 or 5	12.1 (1.3)		
Additional stressors	Young gynecologic age Weight loss Away from home Intense exercise training			NA		NA	

[a]Number of participants who completed the study

be produced. This assessment was used to gradually increase the exercise intensity to maintain physical activity just below or above the "lactate threshold." This allowed investigators to more accurately document the exercise load, which was gradually increasing over 1 year. Participants' hormone levels were intensively sampled every 4 months before the next increase in exercise intensity. Rogol et al. [5] also reported that neither running intensity nor duration affected ovulatory function in women training for 1 year at increasing intensities that were maintained either above or below their own adjusted lactate threshold.

Several differences exist between the studies of Bullen and those performed by Bonen and Rogol, which at least partially explain their discrepant outcomes. The rapid introduction of a high volume of training and the addition of weight loss in Bullen's protocol provides a greater stress load and would thus be more likely to lead to ovulatory disturbances than an exercise program alone in older women who remained in their own homes and communities [3, 5]. In addition, the women in Bullen et al.'s [27] study were significantly younger in both chronological and gynecological ages. Another important difference is in design—Bullen and colleagues increased exercise intensity rapidly, whereas the other two studies were more gradual in exercise intensification. Finally, these studies differ in the methods and time course of monitoring. Bullen et al. [27] monitored cycles consecutively and inclusively. In contrast, Bonen and Rogol et al. assessed ovulatory characteristics intermittently every two or every four cycles, respectively. Shortened luteal-phase length or anovulatory cycles may have been missed because monitoring occurred after one or three cycles of probable adaptation to a new exercise load. Any ovulatory disturbances would have likely occurred in the first cycle following the increase in training volume. By the second or fourth cycle after the increase in intensity/duration of training, adaptation would have occurred, homeostatic balance would be achieved, and normal ovulatory function would have returned.

We, like Bullen et al. [27], have monitored luteal length and ovulation continuously, but over 1 year in 66 community-dwelling women of varying self-chosen activity levels [4]. As described earlier, all women were confirmed to be normally ovulatory on two consecutive cycles prior to study entry. Despite that, over 80% of the women experienced at least one short luteal phase or anovulatory cycle during the year of study. When the average cycle, luteal phase, and two cycles of hormone levels were used, no differences were found by exercise habit in the number or severity of ovulatory disturbances, or in estradiol and progesterone levels. That was true regardless of whether the women were completing <1 h of aerobic exercise/week (normally active controls), running more than 1 h/week, but not training for a specific event (consistent runners), or runners increasing training in preparation for a marathon that 19 women completed during the study year [4]. The reason for the subclinical ovulatory disturbances that *did* occur was not initially understood. However, we have subsequently found them to be more prevalent in women scoring high on the Restraint Scale, suggesting they are related to cognitive dietary restraint [69–71].

The same study was recently used to compare the characteristics of the pre-marathon cycle in the marathon-training women with a season-matched cycle in the consistent runners. Exercise training without weight loss can be shown to cause shortening of the luteal phase. The luteal-phase characteristics of the cycle before the marathon were compared in marathon-training women with their own initial and final cycles and the pre-marathon cycle with a season-matched middle cycle from the consistent runners. Compared to both their own cycles during less intense training and all of the cycles in the consistent runners, significant shortening of the luteal-phase length before the marathon occurred in the marathon-training women (Petit & Prior, Personal communication, 2010).

Hypothalamic adaptation to the runners' baseline exercise probably had occurred before they passed the screening for two consecutive ovulatory cycles and became qualified to enter the study. However, the intensified training before

the marathon appeared to cause shortening of the luteal phase in the cycle prior to the marathon when their training mileage was the greatest. The detailed dietary, weight, body fat, and hormonal characteristics also monitored before the marathon are being studied for explanations other than exercise training to explain the luteal-phase shortening that was documented. These data all suggest that adaptation to increased exercise, even as intense as training for a marathon, normally occurs with only shortening of the luteal phase in well-nourished, reproductively mature women who have no major emotional distress [91]. In addition, as discussed below, adaptation allows a woman's reproductive system to show rapid shortening of the luteal phase and equally rapid reversion to normal.

Observable Changes Prior to Ovulation Disturbances: Molimina

Prior to shortening of the luteal-phase length, which is the first objective change in reproductive function, other observable but even more subtle changes are commonly reported by mature women who are beginning exercise training. The earliest change with moderate, recreational levels of exercise is a decrease in molimina [92] as recorded by the daily Menstrual Cycle Diary© [37] (available at www.cemcor.ca). "Molimina," whose Greek etymology means "the work of bringing on flow," includes the set of physical and emotional, but not troublesome, indicators of the coming menstrual flow. Although premenstrual symptoms may occur in both ovulatory and non-ovulatory cycles [93], we previously believed that molimina indicated that ovulation had occurred. However, a recent large study in over 400 unselected women could not confirm the molimina/ovulation association. However, it *did* show, in the few women who observed it, a highly ovulatory cycle-specific development of *axillary* breast tenderness during the week before flow [94].

An additional indicator of an ovulatory cycle is the disappearance of elastic or stretchy cervical mucus after the midcycle estrogen surge. Because progesterone inhibits cervical production of elastic mucus, this time pattern of the presence and then the disappearance of mucus is also a potential indicator that ovulation has occurred.

We asked whether exercise would decrease premenstrual experiences by studying a group of proven ovulatory women runners who were increasing their exercise training over 6 months. Exercise training was associated with decreased fluid symptoms and decreased feelings of depression despite no changes in weight or cycle characteristics [92]. Age and weight matched non-exercising and ovulatory women studied in parallel experienced no significant changes in premenstrual experiences over the same study period [92].

Time Course of Ovulatory Adaptation

With the addition of more strenuous training, endocrine changes progress to a shortened luteal phase. The next and more disturbed cycle is anovulatory. This sometimes occurs a straining workload increases [6]. The sedentary woman whose training and cycle characteristics are shown in Fig. 8.6 developed severe back pain during the 12th cycle and did not ovulate. It is likely that she developed anovulation because she not only had to deal with the stress of the pain but also what for her was an important worry that she would be unable to compete in and finish the marathon for which she had trained so hard.

In a woman with well-established normally ovulatory cycles (probably after gynecological age 12), exercise-training adaptive changes do not normally progress to anovulation. However, if an additional stressor is added, such as illness, insufficient energy intake, weight loss, and/or emotional stress (see sections "Exercise Training Studies in Reproductively Mature Women" and "Reversibility/Adaptation" in this chapter), anovulation may develop. Amenorrhea will usually not develop unless the woman is of young gynecologic age, is not yet sturdily ovulatory, and has stresses in addition to exercise training, such as eating restraint or psychological stress, energy imbalance, rapid induction of exercise, or rapid weight loss.

Reversibility/Adaptation

A few within-person studies are useful to illustrate further the progression and reversibility of ovulatory adaptation. Figures 8.6 and 8.7 show luteal-phase lengths as documented by QBT [91] for 1 year of consecutive cycles in two mature, normal-weight women. One of these women, as discussed above, was a sedentary woman who trained for and ran a marathon during the year of observation (Fig. 8.6). The other was a rather lean and compulsive runner who wanted to become pregnant (Fig. 8.7) [91]. The first woman's prospective record indicated alternating cycles showing short luteal-phases (<10 d) and normal luteal-phase lengths with anovulation during the cycle before and of the marathon race. As mentioned above, the pain and worry of an injury as well as exercise training likely accounted for anovulatory cycles. A normal luteal-phase length cycle returned when both her emotional stress and her training workload decreased immediately after her successful marathon.

Figure 8.7 shows prospective documentation of ovulatory characteristics over 1 year in another woman who was running regularly, but was quite lean and stressed. She showed consistently short luteal-phase cycles early in the year. In an effort to reverse her secondary infertility, she decreased running for one cycle, but this was emotionally stressful. Her secondary infertility was due to inadequate or insufficient luteal-phase characteristics documented by endometrial biopsy. When she stopped running for approximately 6 weeks, she became pregnant.

These detailed case histories of two women who monitored their individual exercise and ovulatory characteristics over an extended period

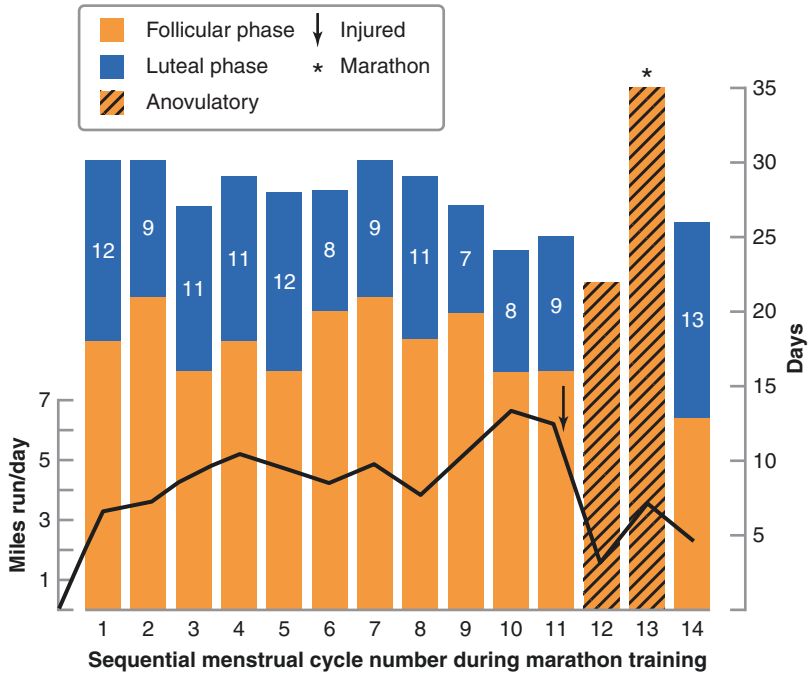

Fig. 8.6 This *bar graph*, illustrating cycle lengths as *bars* and luteal-phase lengths as blue areas within those bars, shows sequential menstrual cycles and ovulation during 1 year of marathon training in a previously sedentary woman. Note the alternating short and normal luteal phases and progression to anovulatory cycles (in cycles #12 and #13) just after the most intense and highest mileage of training just before and in the marathon cycle. The arrow shows injury which caused her to decrease training and major emotional stress that likely contributed to the anovulatory cycle. When she decreased her training following the marathon, ovulation and her luteal-phase lengths were restored to normal. (Modified from Ref. [91])

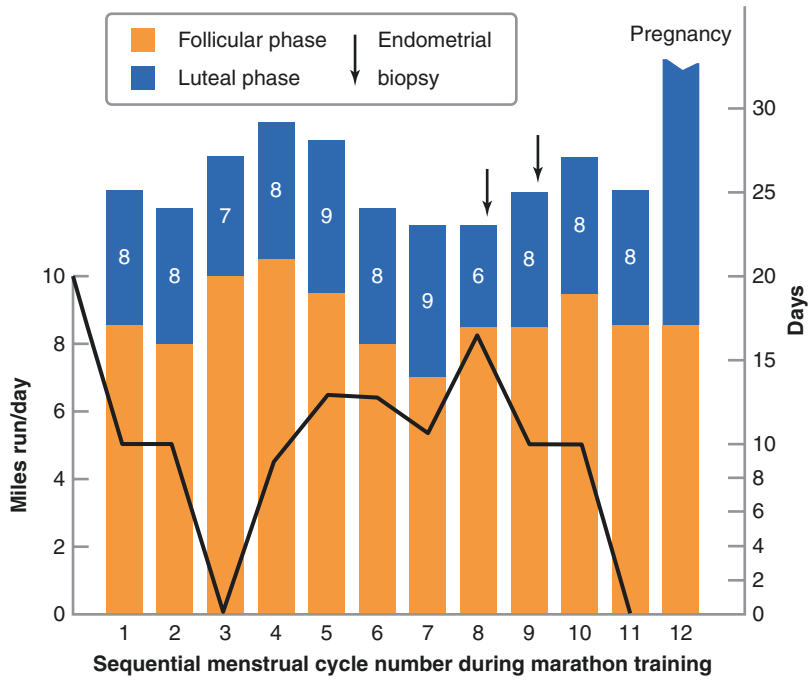

Fig. 8.7 This bar graph is similar in format to Fig. 8.6 and shows the sequential menstrual cycle and ovulatory characteristics in a woman who was training intensely and compulsively. Even with decreased running during the first few cycles, she continued having short luteal-phase cycles. Endometrial biopsies (*arrows*) were consistent with luteal-phase deficiency. In the middle of cycle 11, she stopped running and became pregnant before a normal luteal phase could be documented. She carried the child to term and delivered a healthy baby. (Modified from Ref. [91])

of time indicate the rapid hypothalamic adaptation and reversibility of ovulatory disturbances related to exercise training [91]. These data have since been supported in larger samples of women runners [4, 88].

Very few data clearly document adaptation to exercise over longer than a year. As an example, it is useful to observe the second marathon-training year in the woman whose cycles before and after her first marathon were documented in Fig. 8.6. Her cycles and ovulatory characteristics before her second marathon a year after her first are shown in Fig. 8.8. During the first marathon, she had shown short luteal-phase cycles progressing to anovulatory cycles the month prior to (M-1) and of (M) the marathon. In her second marathon, 1 year later, luteal length remained normal throughout her training, although her training was similar in volume and intensity to her earlier marathon. It appears possible that by the second year, she had adapted to the marathon training, which allowed her cycles to maintain normal ovulation. Key in each of these stories is the fact that the woman was basically emotionally healthy and maintained normal body weights, and good energy intakes avoiding relative energy deficiency (Fig. 8.4).

In mature women, adaptation to exercise training with reversal to normally ovulatory, normal-length cycles commonly occurs within one cycle. These adaptive changes of luteal-phase length with increasing exercise training are modeled in Fig. 8.9. Note that, as in the woman described above who trained for her first marathon, by the end of the year, the model suggests that a level of exercise intensity that had provoked ovulatory

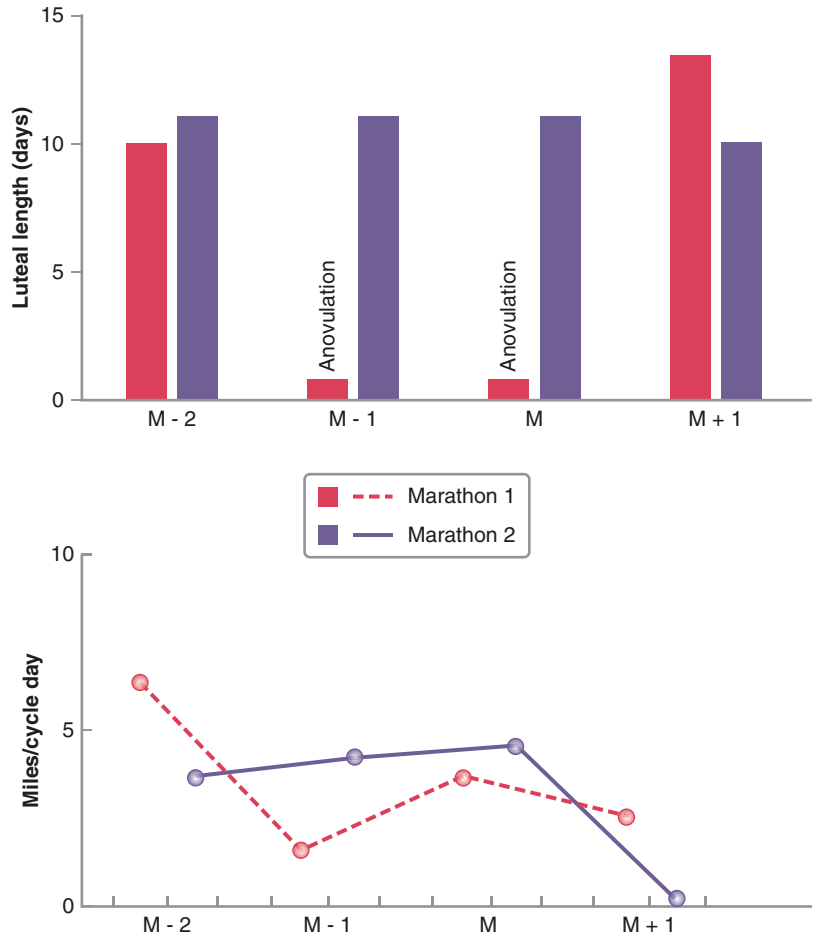

Fig. 8.8 Luteal-phase lengths during the cycles before and just after a marathon in the first marathon training and race (as shown in Fig. 8.6) and in subsequent marathon a year later. During the second marathon, despite similar or increased mileage, there were no luteal-phase disturbances documented. This illustrates reproductive adaptation to the levels of exercise this woman was now performing. (Modified from Prior et al. [95])

disturbances now no longer causes a change from a normal ovulatory cycle.

Bullen's study [27] also demonstrated rapid reversibility when training ceased. Although a few women developed oligomenorrhea as well as disturbances of ovulation, all of the women regained both normal cycle intervals and normal ovulatory function within a few months after the end of the summer training camp. Furthermore, it is common for athletic women to become pregnant within months of decreasing their training (and loss of competitive stress), even though they may have had several years of anovulatory cycles or amenorrhea [91, 96, 97]. However, in exercising younger women, in whom the hypothalamus has not fully matured, the return to or achievement of normal ovulatory cycles will often take longer.

Although the majority of the data just presented were collected using QBT analyses many years ago, no studies since have closely examined ovulatory characteristics continuously during several months of exercise training. The development of new methods of monitoring ovulation and luteal-phase length, for example, with salivary progesterone [25], should soon allow the nuances of cycle adaptation to be more specifically characterized and mechanisms and modulating factors more carefully delineated.

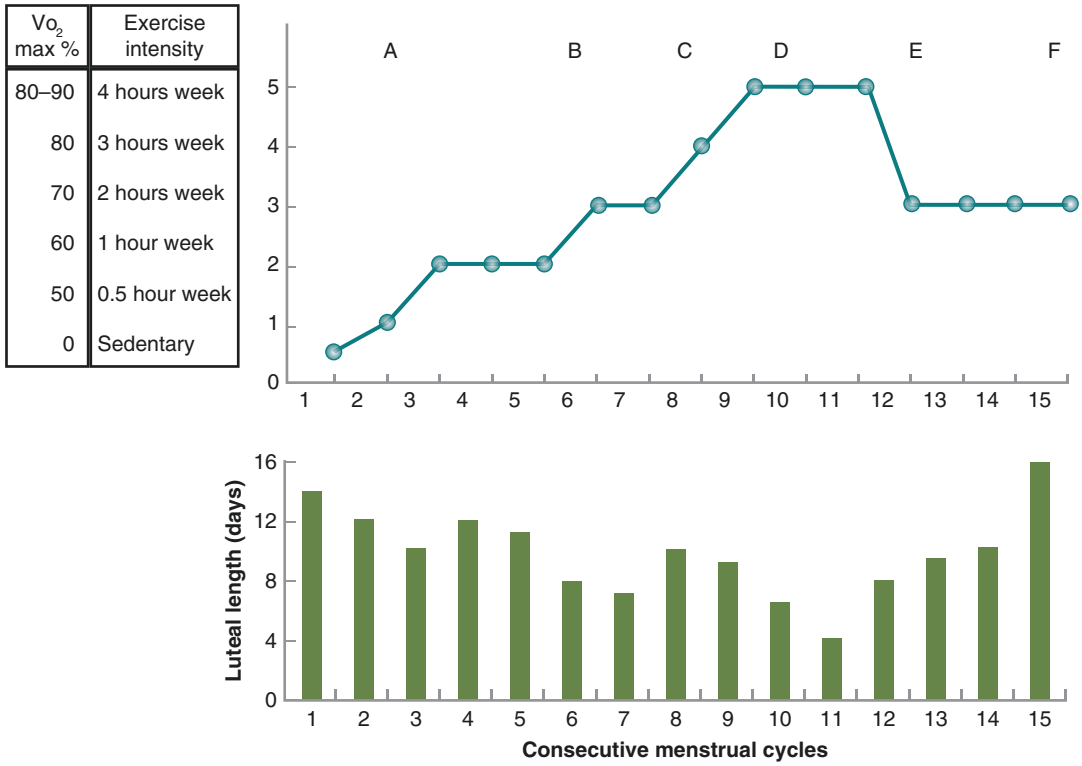

Fig. 8.9 Theoretical model of the luteal-phase changes that occur over time with increasing exercise in an ovulatory woman who is undergoing exercise training. Note that at the end of the year's sequence of cycles, despite a considerable exercise load, luteal-phase length and ovulation are normal. (Modified from Prior et al. [95])

Clinical Applications/Treatment

The practical and clinical implications of ovulatory adaptation to exercise training are not the purpose of this chapter. However, it is important that the clinician and coach be alert to document persistent disturbances of luteal-phase length or any anovulatory cycles because we now know they are associated with significant bone loss [98]. These ovulatory disturbances, if observed, are very useful indicators that the exercise training load is excessive for that woman's level of hypothalamic reproductive maturation and/or when combined with other potentially present stressors, e.g., competitive anxiety, insufficient caloric intake, moving away from home, weight loss, eating restraint, or even illness.

If ovulatory disturbances are documented, it is very easy to provide physiological treatment. Evidence suggests and the Centre for Menstrual Cycle and Ovulation Research recommends that persistent ovulatory disturbances should be treated by prescribing either cyclic oral micronized progesterone (300 mg at bedtime) or medroxyprogesterone (10 mg) on days 14–27 of the woman's own cycle [34, 99] (http://www.cemcor.ca/resources/cyclic-progesterone-therapy).

Although this "treatment" does not directly correct the hypothalamic stressor(s) or energy insufficiency that led to the disturbance in the first place, feedback to the hypothalamus by progesterone may aid in reproductive maturation and ovulatory recovery. The most useful function of cyclic progesterone is to provide physiological levels of progesterone, which will cause regular

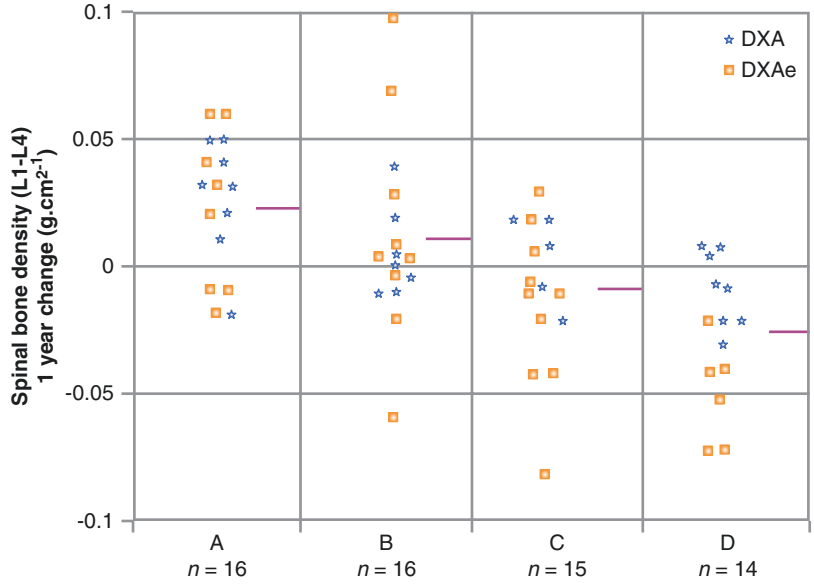

Fig. 8.10 This dot-plot figure shows individual rates of 1-year spinal bone mineral density change by dual-energy X-ray absorptiometry (DXA) in 61 active, healthy, normal-weight women ages 20–35 with amenorrhea, oligomenorrhea, subclinical anovulation, or subclinical short luteal phases stratified by reproductive status and randomized to receive medroxyprogesterone acetate (that acts through the osteoblast progesterone receptor) cyclically for 10 d/cycle (10 mg/d of MPA) with or without active/calcium therapy (1000 mg/day) or placebo. Women in A were taking cyclic MPA plus calcium; B cyclic MPA plus placebo; C placebo MPA plus calcium; and D double placebos. The effects of cyclic MPA were highly significant (P = 0.0001), and calcium was borderline (P = 0.07). There was a significant 2.0% loss of bone in the double-placebo (D) control group [34]

menstrual flow if estradiol levels are normal and, acting through the osteoblast progesterone receptor (PR), will increase bone mineral density (BMD) [100]. Cyclic medroxyprogesterone (acting through the osteoblast PR) in a randomized, placebo-controlled 1-year trial caused a significant 2% increase in spinal areal BMD in mild-moderately active women with hypothalamic disturbances of cycles or ovulation (Fig. 8.10) [34]. Although combined hormonal contraceptives (CHC) are the usual therapy for "functional" hypothalamic amenorrhea or oligomenorrhea, in *adolescent* women, CHC may cause skeletal [101] and reproductive [102] harm. CHC therapy for treatment of hypothalamic amenorrhea has also been associated with significantly lower rates of recovery (42%) and slower recovery than in women who declined any therapy [103].

The most important reason for the clinician or coach to know about ovulatory disturbances is to recognize them as adaptive and reversible and to teach each woman to observe and understand the menstrual cycle and ovulatory changes she may experience. In this era of "self-help medicine," keeping the Menstrual Cycle Diary©, QBT (both free at www.cemcor.ca), and training records will increase self-knowledge and thus well-being for health-conscious women.

Conclusions

This chapter has reviewed the subtle adaptations of women's reproductive system to gradually and appropriately increasing exercise training. Evidence suggests that decreases in luteal-phase progesterone production and duration (subclinical ovulatory disturbances) are the first and the major adaptive responses of the hypothalamic–pituitary–ovarian system in mature women to increasing exercise intensity. More obvious changes in cycle lengths may occur if the relative energy insufficiency of sport is also present or if

the exercise-training woman is within 5–10 years of menarche. If no additional stressor other than the exercise is present, the subclinical ovulatory disturbances will reverse to normal within the next cycle, even though the exercise training level is maintained.

These physiological and psychological changes during exercise training are protective for the individual, are reversible, and cause no long-term harm. However, if subclinical ovulatory disturbances persist (any anovulatory and $\geq$ 2 short luteal-phase cycles/year) [4], despite clinically normal cycles, bone loss occurs [4, 98]. In addition, fertility is impaired by silent ovulatory disturbances. Persistence of these ovulatory disturbances may be commonly related to the psychological stress caused by cognitive dietary restraint [71] and psychosocial stressors related to women's inferior cultural status [86]. The benefits of exercise for cardiovascular [104], skeletal [105], and emotional health [106] are well supported by scientific evidence, yet the concept persists that exercise causes women to develop amenorrhea which is an important negative reproductive event.

In this chapter, and as described in the concept of relative energy deficiency in sport [61], we have attempted to erase that perception by viewing women's responses to exercise training as adaptive. When increasing levels of exercise are introduced gradually and energy balance is maintained, adaptation can occur, and the result is a minimal change. Ovulatory disturbances occur normally when initiating a more intense training program or increasing exercise load, but will reverse rapidly to normal once adaptation has occurred. When taken to an extreme or combined with other psychological (including cognitive dietary restraint) or physiological stressors, exercise can cause definite negative reproductive changes. In that circumstance, persistent ovulatory disturbances occur, which, depending on the age, nutritional state, and emotional support of the woman, may progress to oligomenorrhea or amenorrhea.

Amenorrhea, although it is uncommonly associated with exercise in mature, ovulatory women, may occur in the face of exercise combined with a negative energy balance or when several stressors coexist, especially in women who have not established regularly ovulatory cycles. Gynecological immaturity is a significant factor impairing the ability of women to appropriately adapt to exercise stress. This implies that caution should be taken in the progression and sequence of increasing exercise intensity in young athletes.

In summary, although the concept of adaptation to exercise training has been known for almost 70 years [1] and has been applied to women's reproduction since the 1980s, few well-controlled studies have documented the subtlest evidence of this adaptation: subclinical ovulatory disturbances.

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Adrenergic Regulation of Energy Metabolism

9

Michael Kjær and Kai Lange

Introduction

During exercise, energy turnover increases and adrenergic mechanisms play an important role in this regulation. In addition, increased adrenergic activity during exercise also results in an increased heart rate and in an enhanced force of myocardial contraction as well as in vasoconstriction in the splanchnic circulation, in the kidneys, and in noncontracting muscles. These circulatory changes favor a redistribution of blood flow to exercising muscle as well as an increased cardiac output [1]. Furthermore, the adrenergic activity stimulates sweat glands and thereby influences thermoregulation, and it causes an increased contractility of skeletal muscle as well as influences exercise-induced suppression of components of the human immune system. In the present chapter, it is demonstrated how adrenergic activity can influence substrate mobilization and utilization both directly and indirectly via secretion of hormones.

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Adrenergic Responses to Acute Exercise

Adrenergic activity can be assessed both by direct measurements of electrical activity in superficial sympathetic nerves and by measurement of circulating norepinephrine and epinephrine in the blood. The direct recording of sympathetic activity can be performed to resting muscle only, but during exercise of, e.g., the arms, sympathetic activity to the resting leg muscle has been shown to increase with progressively increasing intensity of arm exercise [2]. In addition to these measurements, a correlation has been found between sympathetic nerve activity and plasma levels of norepinephrine [3]. Although a correlation between circulating norepinephrine and direct recordings of sympathetic nerve activity from the peroneal nerve has been demonstrated during exercise, the increase in sympathetic outflow to the various regions of the body differs somewhat during exercise. During exercise, using methods to measure norepinephrine spillover, it has been demonstrated that the increase in sympathetic activity during exercise is dominated by an increased sympathetic activity directed toward active muscle. During two-legged exercise, approx. 50% of all circulating norepinephrine is released from sympathetic nerve endings in active muscle. Furthermore, when arm exercise is added to leg exercise, the norepinephrine

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spillover from active leg muscle also increases despite unchanged work output and unchanged blood flow to the leg muscles [4].

In addition to norepinephrine released from sympathetic nerve endings, epinephrine is released from the adrenal medulla in response to sympathetic neural activity during exercise. The circulating epinephrine is responsible for the major adrenergic effect on energy metabolism during exercise compared with norepinephrine. In the present chapter, the adrenergic effect on carbohydrate and fat metabolism will be discussed, but epinephrine per se has been shown also to increase protein metabolism in isolated electrically stimulated rat muscle [5].

The levels of circulating free norepinephrine and epinephrine increase with exercise intensity expressed by the percentage of maximal individual performance (%VO_2 max). This holds true both during prolonged exercise and in response to short-term intermittent exercise and to intense weight training. The increase in plasma norepinephrine and epinephrine occurs rapidly in arterial blood, and it has been calculated that the half-life of epinephrine is around 2–3 min during exercise. Circulating levels of catecholamines can only be considered as overall markers of sympathoadrenergic activity and are influenced not only by secretion but also by clearance of the hormone. Whereas clearance of norepinephrine is difficult to determine on a whole-body level owing to the fact that it is extracted at two levels in series, namely, both the lung and the systemic organs [6], the turnover of epinephrine can be studied in humans, using a radio-labeled tracer. It has been shown that whole-body clearance of epinephrine increases by 15% at low exercise intensities and decreases around 20% below basal levels after more intense exercise [7]. However, since the increase in plasma epinephrine seen during dynamic exercise in humans is five- to tenfold, these changes are caused by increases in secretion from the adrenal medulla rather than by changes in clearance. Among the major contributors to epinephrine clearance are the hepatosplanchnic area and the kidneys.

Motor Control and Reflex Influence on Adrenergic Response

In experiments using partial neuromuscular blockade to weaken the muscle force and thereby increase the motor center activity needed to produce a certain force output, it was found that exercise-induced increases in levels of circulating catecholamines were augmented compared to control experiments with saline infusion [8]. These findings are supported by experiments in paralyzed cats where direct stimulation of the subthalamic locomotor areas in the brain resulted in adrenergic hormonal responses similar to the ones seen during voluntary exercise [9]. Together, these experiments support the view that motor center activity can directly stimulate sympathoadrenergic activity during exercise directly and independently of feedback from contracting muscle. That central factors linked to exercise intensity are not sufficient to elicit a maximal adrenergic response can be demonstrated in different ways. When exercising a small muscle group (e.g., one knee extensor) even at maximal intensity, only a small catecholamine response can be observed [4]. Furthermore, when maximal work output was reduced by more than 60% with a neuromuscular blockade (tubocurarine), despite subjects working at the highest possible effort, adrenergic responses were far from maximal [10]. In addition to central factors, peripheral neural feedback can be demonstrated using lumbar epidural anesthesia in doses sufficiently high to block impulses in thin afferent nerves but preserving motor nerves and the ability to perform exercise to the highest possible degree. During static exercise, but not during dynamic exercise, catecholamine responses were inhibited when afferent responses were absent [11, 12]. Interestingly, both ACTH and β-endorphin responses during submaximal exercise were abolished during epidural anesthesia [11, 13]. In support of a role of afferent nerves in adrenergic responses, plasma catecholamines increased in response to direct stimulation of these nerve fibers in cats [14]. An alternative model to study feedback mechanisms during exercise is to use

patients with metabolic deficiencies. Both in myophosphorylase (McArdle's disease) and phosphofructokinase deficiency and in mitochondrial myopathy, an excessive neuroendocrine response and exaggerated mobilization of extramuscular substrate (glucose and free fatty acid (FFA)) were found, most likely a coupling toward the oxidative demands of the muscle cell rather than to the oxidative capacity of the working muscle [15–17].

Adrenergic Activity After Physical Training

Vigorous endurance training will reduce the catecholamine response to a given absolute workload [18], whereas neither sympathetic nerve activity nor norepinephrine levels at maximal workloads differ between individuals with different training status [19]. This supports the view that physical training does not alter the capacity of the sympathetic nervous system, but that responses to submaximal exercise are linked closely to the relative rather than to the absolute workload [20]. Surprisingly, however, it has in a 24-h study been found that highly trained individuals had a higher catecholamine release over the day compared with sedentary individuals [21]. Epinephrine response in trained individuals vs. sedentary has been shown to be enlarged when stimulated by a variety of stimuli, such as hypoglycemia, caffeine, glucagon, hypoxia, and hypercapnia [20, 22–25]. This indicates that the capacity to secrete epinephrine from the adrenal medulla improves with training. In rats that underwent 10 weeks of intense swim training, the adrenal medullary volume and the adrenal content of epinephrine were larger in trained rats compared with controls who were either weight matched, sham-trained, or cold-stressed [26]. Although these findings indicate that the improved secretion capacity of epinephrine is a result of training, this will most likely require several years of training. In well-trained athletes who underwent hypoglycemia before and 4–5 weeks after an injury that resulted in inactivity, epinephrine responses did not change with this short-lasting alteration in activity level [27]. However, still it is interesting that endocrine glands apparently are able to adapt to physical training and alter their secretion capacity, similar to other tissues like muscle and heart.

Hepato-splanchnic Glucose Production and Adrenergic Activity

During intense exercise the rise in hepatic glucose production was parallel with a rise in plasma catecholamine levels [28–30]. In addition, in models where electrically induced cycling was used in spinal cord-injured individuals with impaired sympathoadrenergic activity, hepatic glucose production was abolished [31].

In swimming rats, the removal of the adrenal medulla reduced the hepatic glycogenolysis [32], as well as the exercise-induced increase in hepatic glucose production in running rats [33]. However, most studies have been unable to demonstrate any effect of epinephrine on liver glycogen breakdown during exercise [34–37]. In running dogs, evidence has been provided that epinephrine may play a minor role in liver glucose output late during exercise [38] probably owing to an increased gluconeogenic precursor level. Furthermore, adrenalectomized individuals maintain a normal rise in hepatic glucose production during exercise [39], and only when epinephrine is infused in these patients, hepatic glucose production was augmented during the early stages of exercise (unpublished observation).

Direct stimulation of liver nerves caused an increase in hepatic glycogenolysis, and the hypothesis has been put forward that liver nerves are important for the exercise-induced rise in liver glucose output. In contrast to this, surgical or chemical denervation of the liver in various species did not reduce the exercise-induced increment in hepatic glucose production [32, 33, 40, 41], which indicates that sympathetic liver nerves are not essential during exercise. In humans, the role of liver nerves and epinephrine has been

studied with application of local anesthesia around the sympathetic celiac ganglion innervating the liver, pancreas, and adrenal medulla [42]. Pancreatic hormones were standardized by infusion of somatostatin, glucagon, and insulin. During blockade, the exercise-induced epinephrine response was inhibited by up to 90%, and presumably liver nerves were also blocked, but this did not diminish the glucose production response to exercise. This indicates that sympathoadrenergic activity is not responsible for an exercise-induced rise in splanchnic glucose output. In further support of this hypothesis, the exercise-induced increase in liver glucose production was identical in liver-transplanted patients compared to healthy control subjects as well as in kidney-transplanted patients who received a similar hormonal and immunosuppressive drug treatment as liver-transplanted patients [43]. Liver-transplanted patients were investigated approx. 8 months after surgery, and no sign of reinnervation occurred in any of the patients as judged by the content of norepinephrine in liver biopsies [44]. Finally, in recent experiments in exercising dogs that underwent a selective blockade of hepatic α- and β-receptors, it was demonstrated that circulating norepinephrine and epinephrine do not participate in the stimulation of glucose production during intense exercise [45, 46]. Taken together, sympathetic liver nerves or circulating norepinephrine play no role in glucose mobilization from the liver during exercise, and circulating epinephrine only plays a minor role during intense exercise and late during prolonged exercise.

Adrenergic Effect on Skeletal Muscle Carbohydrate Metabolism

Muscle contractions per se increase glucose uptake, and humoral factors can modify this [47]. Insulin and contractions have a synergistic effect on glucose uptake with contractions [48], whereas epinephrine has been demonstrated to decrease glucose clearance in running dogs [49]. In addition to this, femoral arterial infusion of epinephrine into an exercising leg in humans caused a reduction in the normal exercise-induced glucose uptake [50]. More recently, it has been shown that in adrenalectomized individuals performing leg cycling for 45 min at 50% VO_2 max followed by 15 min at 85% VO_2 max, the rise in glucose uptake during exercise was reduced when epinephrine was infused to substitute plasma epinephrine levels normally observed during exercise (unpublished observation). The mechanism behind this is at present unknown but could be related to an enhanced glycogenolysis, increased intramuscular glucose concentration, or altered uptake of FFA, all changes that can influence glucose uptake.

It has been shown that adrenergic activity can enhance the glycogen breakdown in muscle during contraction both in exercising animals [51] and in humans [50, 52]. However, those studies often used supraphysiological doses of epinephrine, and later studies in humans using lower doses have only been able to demonstrate a higher activation of phosphorylase, but could not demonstrate any marked increase in glycogen breakdown [53]. Noradrenergic activity probably does not play any role in muscle glycogenolysis, since unilateral hind limb sympathectomy did not diminish glycogen breakdown in swimming rats [54].

Sympathoadrenergic Activity and Fat Metabolism

Lipolysis in fat tissue is enhanced by β-adrenergic activity, and catecholamine responsiveness of β-adrenergic receptors in adipose tissue is increased after acute exercise [55]. By the use of microdialysis of subcutaneous abdominal tissue, it was demonstrated that nonselective β-adrenoceptor blockade inhibited the exercise-induced increase in dialysate levels of glycerol [56]. Although this indicates a role for adrenergic activity in fat metabolism during exercise, the relative role between sympathetic nerve activity and circulating norepinephrine/epinephrine is currently not known. Intravenous infusion of epinephrine in resting humans caused an increase in lipolytic activity as determined by microdialysis

of subcutaneous adipose tissue, an effect that was desensitized by repeated epinephrine infusions [57]. The direct role of sympathetic nerve activity on adipose tissue has recently been addressed using microdialysis, and it was found that during handgrip exercise, the increase in umbilical glycerol release was attenuated in spinal cord-injured individuals with impaired sympathetic nerve activity when compared with healthy control individuals [58]. It should be noted that this very moderate type of stress was not able to document any increase in lipolysis in the clavicular region. Furthermore, in a recent study, glycerol output in subcutaneous abdominal adipose tissue was found to be lower during prolonged arm-cranking in spinal cord-injured individuals compared with controls performing a similar relative workload (unpublished observation). Taken together, indices are provided that sympathetic nerves to adipose tissue stimulate lipolysis directly during exercise. If regional differences (visceral vs. subcutaneous fat) exist in responsiveness of the adipose tissue toward increased sympathetic activity, this could play an important role in the treatment of adipositas.

Not only adipose tissue but also intramuscular fat can be stimulated by catecholamines, and both lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) play important roles in this regulation [59]. HSL might be under control by both contractions and epinephrine, and it has recently been shown that activation of HSL and glycogen phosphorylase occurs in parallel in adrenalectomized individuals who receive infusion with epinephrine during exercise (unpublished observation). This could indicate that mobilization of intramuscular triglyceride and glycogen occurs simultaneously, stimulated by adrenergic activity, and that choice of substrate for energy production takes place at another level.

Summary

Physical exercise causes an increase in adrenergic activity that can be determined both by changes in plasma catecholamines and in intraneural sympathetic activity. Release of norepinephrine from contracting muscles and release of epinephrine from the adrenal medulla are major contributors to high levels of plasma catecholamines. Both feed-forward stimulation from motor centers in the brain and afferent impulses from working muscles stimulate sympathoadrenergic activity, and a coupling to oxidative demands of the working muscle is likely. Long-term physical training increases the size and secretory capacity of the adrenal medulla, which may improve exercise capacity. Sympathoadrenergic activity only plays a minor role in regulation of hepatic glucose release, but via depressing insulin secretion and influencing target tissue, adrenergic activity improves glycogen and fatty acid mobilization.

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10 Sex Differences in Energy Balance and Weight Control

Kristin S. Ondrak

Introduction: What Is Energy Balance and What Factors Influence It?

Before understanding how men and women differ with regard to the storage and utilization of energy, it is important to define the concept of energy balance. Historically, physiologists described energy balance as the difference between the amount of kcal ingested through food vs. the amount of kcal expended through physical activity and basal metabolic processes. The resulting value is one's body mass. With this balance in mind, when caloric intake is similar to caloric expenditure, a state of neutral energy balance occurs and body mass remains stable. However, when intake exceeds expenditure, positive energy balance ensues and the body stores the excess energy, thereby increasing body mass. The opposite results when expenditure exceeds intake, and this is termed negative energy balance or energy deficit [38]. While these relationships are well supported in research, it is also important to note that a variety of factors impact this relationship, and one cannot simply compare caloric deficits or excesses over the short term and expect body mass to change accordingly [25]. Some of these factors include hormones, age, acute bouts of exercise, chronic exercise training, and changes in body composition, namely, gains or losses in lean mass. These factors will be discussed throughout this chapter, as well as how they differ between men and women.

In addition to recognizing that energy balance is more complex than it may appear, it is important to keep in mind that intake and expenditure should be compared over the long term. When even small deficits or excesses in daily energy balance occur day after day, substantial changes in body mass can result. For example, in a 4-year cohort analysis study of women, researchers found that the addition of daily sugar-sweetened beverages to one's diet resulted in substantial increases in body mass and increased their risk for type II diabetes [41]. These researchers found that by increasing the consumption of sugar-sweetened beverages from less than one per week to one or more per day, these women consumed an extra 358 kcal/day and gained ~4.5 kg on average [41]. Thus, seemingly small additions to one's daily diet can quickly add up to substantial changes in body mass and energy balance. Along the same lines, small changes in daily habits may result in large increases in calories expended. For example, standing still, as in an elevator, is associated with a caloric expenditure of 1.3 METS, while taking the stairs expends 4.0–8.8 METS depending on the speed [15].

Another component to discuss is the expenditure of calories and how that impacts energy balance. When individuals are compared, there is a

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substantial amount of variation in the number of calories burned in a given day. This daily energy expenditure (EE) is comprised of three main components: resting metabolic rate (RMR) which comprises 60–75% of the total calories expended, diet-induced thermogenesis which accounts for 10–15% of the total, and activity thermogenesis, which is subdivided into exercise and non-exercise components [17]. The largest component, RMR, is closely related to the amount of fat-free mass (i.e., lean mass) an individual has, and it is generally higher in men compared to women [37]. That is, men tend to burn more calories even at rest compared to women, and this is largely attributed to their higher amounts of metabolically active lean mass. Another complicating factor is age; research has shown that basal metabolic rate decreases as we get older [24]. For example, BMR was 4.6% lower in older participants, compared to younger, in a study of individuals ranging from 15 to 64 years of age [28]. Much of what we know about age-related declines in BMR stems from cross-sectional studies, though, with most of the difference being attributed to declines in lean mass. Additional longitudinal research on age-related declines in BMR is necessary [24].

In addition to its role in elevating RMR, lean body mass is also related to diet-induced thermogenesis. That is, greater amounts of lean mass are associated with a greater number of calories being burned following consumption of food. Other factors that influence diet-induced thermogenesis include age, sex, fitness level, and menstrual cycle phase. Interestingly, some studies have shown that body composition and physical activity levels were more closely related to EE than were age and sex [28]. In fact, fat-free mass had the strongest relationship with 24-h EE, and the values were similar between women and men (r^2 = 0.79 for females and 0.76 for males) [28]. As might be expected, there is wide variation in the number of kilocalories an individual burns each day via physical activity. Daily physical activity levels and related caloric expenditure from activity thermogenesis generally decline with age in both men and women [17]. Research on sex differences in physical activity levels is mixed, however; some researchers have reported lower levels of physical activity EE in women compared to men [37], while others have reported no difference [17]. Similarly, sex differences in measures of EE disappear after taking body composition into account (EE per kg fat-free mass). In summary, these sex differences in RMR, diet-induced thermogenesis, and activity thermogenesis begin to explain how men and women differ with regard to overall energy balance and weight control.

After reviewing the components of daily EE and related energy balance, one can see that there are many factors involved in energy balance and the propensity to change or maintain body mass. This chapter will examine the relationship between these variables with an emphasis on the roles of hormones in weight control and how they differ in males and females. It is important to note that this chapter focuses on healthy, normal-weight adults as the norm, as these processes differ in adults who are overweight or obese as well as in children.

Hormones That Impact Energy Balance, Distribution of Fat, and Weight Control

Numerous hormones influence EE and body mass in humans. Some of the same hormones influence the distribution of body fat, and not surprisingly, the patterns of storage differ between women and men. For simplicity in this chapter, the hormones are grouped according to their primary functions as follows: metabolic (leptin, insulin, ghrelin, anorexigens, and orexigens), sex (estrogen and androgens), and stress (catecholamines and cortisol) hormones. Their roles and impact on body mass and weight control are explained in each respective subsection.

Metabolic Hormones

Several hormones with metabolic functions impact energy balance in humans, namely, leptin, insulin, and ghrelin. *Leptin*, the most recently discovered hormone, is catabolic in nature and provides satiety signals to the brain [16, 19, 42,

50]. It is released by adipose tissue, and its circulating levels are closely related to the amount of fat mass in adults. Leptin is released in greater quantities from subcutaneous fat stores compared to visceral locations [13]. Some researchers suggest that leptin is more closely related to total body fat levels in females compared to males [51]. It follows that these authors reported that females are more sensitive to the actions of leptin than are males. Leptin plays an important role in regulating long-term energy balance rather than the acute fluctuations that occur after each meal [21]. Once released, leptin, along with insulin, acts at the level of the hypothalamus where it induces feelings of fullness, signaling for the person to stop eating. However, leptin's role in energy balance is complex as it is influenced by numerous other hormones including thyroid hormones (T_4 and T_3), cortisol, insulin, and growth hormone (GH) [35].

Insulin is another metabolic hormone that influences energy balance. It is secreted from pancreatic β-cells in response to increases in blood glucose. Insulin levels are indicative of visceral fat levels in humans [8, 12]. The correlation between body fat and insulin is particularly strong in males, and some authors suggest that men are more sensitive to insulin than females [51]. Thus, these sex-related differences in insulin and leptin sensitivity provide a possible mechanism explaining the metabolic differences in weight control among men and women.

Similar to leptin, insulin *reduces* appetite over the long term [16, 19, 21]. As a result, these hormones are often classified as anorexigenic (i.e., appetite suppressants). Ironically, individuals with excess body mass (i.e., overweight or obese) often display resistance to leptin and/or insulin [16, 19, 33]. This suggests that being in a state of chronic positive energy balance alters the body's ability to respond to satiety cues and regulate blood glucose levels. These changes also explain why overweight and obese men and women are at an increased risk for developing impaired glucose tolerance and subsequently type II diabetes.

Ghrelin is another metabolic hormone impacting energy balance. It stimulates hunger in the short term and, not surprisingly, is released in great amounts by the stomach [21, 29, 30, 53]. Ghrelin levels fall after meals in a manner proportional to the energy load of the meal consumed, suggesting that this hormone plays a role in inducing satiety and regulating energy balance [21, 29]. Interestingly, ghrelin's actions are opposite of insulin, although ghrelin plays a role in its release [21]. Insulin and ghrelin are negatively correlated; individuals with high insulin levels tend to have low ghrelin levels. It is not surprising that this hormonal profile of elevated insulin and low ghrelin is common in overweight and obese individuals in particular, as ghrelin levels and body mass index (BMI) are inversely correlated [21, 34].

Ghrelin's regulation of acute energy balance in the short term is due to its effect on the hypothalamus as it stimulates the release of numerous signals that increase hunger. These orexigens (appetite stimulants) include neuropeptide Y (NPY), agouti-related protein (AgRP), and melanocyte-stimulating hormone (α-MSH) [21, 29]. In one of the first studies of ghrelin during exercise, plasma acylated ghrelin levels and related ratings of hunger declined during and following an acute running bout in young men [9]. This highlights ghrelin's role in stimulating appetite and is intuitive that ghrelin levels are suppressed during exercise. Notably, in a well-designed study of male and female twin pairs, resting plasma ghrelin levels were significantly higher in women compared to men [34]. Taken together, these sex differences in anorexigens and orexigens suggest that the signals controlling hunger and satiety differ among men and women. Additional anorexigens including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY) are discussed in the next subsection along with the influence of sex hormones on each. These differences are another important consideration for understanding sex-related differences in energy balance and weight control.

Sex Hormones, Orexigens, and Anorexigens

Estrogens and *androgens* play a large role in body weight regulation, fat distribution, and energy balance in humans and rodent models. Of these sex

hormones, women tend to have higher levels of circulating estrogen (technically estrogens – estradiol-β17, estrone, and estriol), while men display greater levels of androgens. Estrogen is related to decreased levels of visceral fat in men and women; alternatively, androgens are related to lower levels of visceral fat in males but higher levels of visceral fat in females [3, 5, 8].

In addition to estrogen's role guiding the development of secondary sex characteristics and bone mass, estrogen also has important metabolic roles including the reduction of appetite and body mass [1]. Additionally, estrogen interacts with the metabolic hormones leptin and insulin to influence body fat distribution and overall energy balance.

The release of estrogen impacts appetite by also decreasing the action and/or effectiveness of several orexigens (i.e., appetite stimulants) including ghrelin, neuropeptide Y (NPY), and melanin-concentrating hormone (MCH) [36, 47]. This data supports estrogen's role in decreasing food intake through its influence on orexigens. Estrogen also leads to a reduction in food intake through its effects on anorexigenic hormones including insulin, leptin, serotonin, and cholecystokinin (CCK) [11, 14]. It is important to recognize the importance of other appetite suppressants such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY) [44]. Along with leptin, insulin, and ghrelin, these factors have been shown to decrease hunger signals at hypothalamus [44].

Hormonal Interactions

Researchers have developed a potential model to explain the relationship between estrogen, fat distribution, and leptin and insulin [43], as shown in Fig. 10.1. They theorize that in premenopausal women, estrogen reduces visceral fat through enhanced lipolysis and decreased lipogenesis. Estrogen also retains subcutaneous fat and is related to increases in resting leptin and reductions in resting insulin levels. However, in men and postmenopausal women, these researchers propose that the lower levels of estrogen and lowered activity of estrogen receptor alpha are related to increases in visceral fat and reductions in subcutaneous fat and leptin, while concomitantly insulin levels are increased [31, 43]. Some of this may be explained by the direct relationship between leptin and subcutaneous fat as the latter secretes leptin. These sex-related differences in hormone levels and fat distribution have been supported by other researchers as well [10].

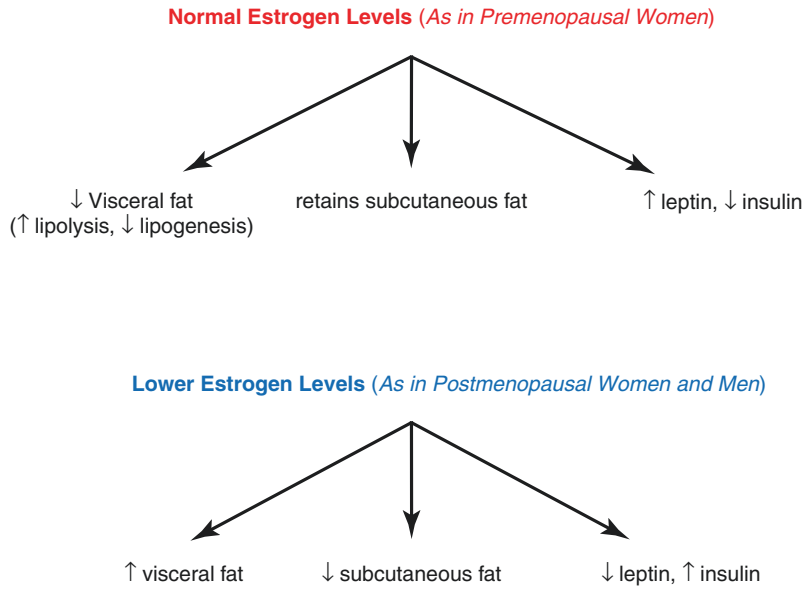

Fig. 10.1 Theoretical model of estrogen's relationship with fat, leptin, and insulin

Androgens such as testosterone and dehydroepiandrosterone (DHEA; and its sulfated form, DHEA-S) also play an important role in energy balance, specifically in influencing where males and females store their body fat. Women tend to deposit and retain more adipose tissue around their hips, buttocks, and thighs, known as a "gynoid" or "pear" body shape. On the other hand, men tend to store more fat around their waist and midsection, known as an "android" or "apple" body shape [5]. The underlying cause of the gynoid shape in women and android shape in men may be due to differences in adipogenesis and the environment (e.g., hormonal milieu) within developing adipose cells. For example, research has shown that women have greater levels of early-differentiated adipocytes compared to men (measured in abdominal and femoral fat depots) [45]. These authors also speculated that sex differences in regional fat distribution may be due to differences in the microenvironment of the cells and related apoptosis, innervation, blood supply, and responsiveness to hormones [45].

In addition to the aforementioned sex differences in body fat distribution, men tend to have higher levels of visceral fat, while women generally store more fat subcutaneously. Androgen levels may play a role in these relationships as greater amounts of visceral fat have been associated with lower androgen levels in men and excess androgen levels in women [5]. Unfortunately for men, visceral fat carries an increased cardiovascular disease risk compared to subcutaneous fat. This often puts men at an increased risk for cardiovascular disease [3–5]. Not surprisingly, inverse correlations have been reported between body fat and EE from physical activity in men ($r = -0.34$, $p < 0.03$) [37]. Therefore, sex differences in estrogen and androgens are related to body fat and its distribution; these differences in turn influence EE and balance in men and women.

Stress Hormones

Stress hormones such as catecholamines and cortisol are another group of hormones that have a large impact on energy balance. To further complicate the matter, stress hormones also interact with sex hormones, thus altering their actions [32]. Catecholamines such as epinephrine and norepinephrine are released in response to sympathetic nervous system stimulation when a stressor occurs, whether real or perceived; a common example is exercise. In response to catecholamine release, appetite centers in the hypothalamus are suppressed, and related food intake declines.

The primary function of catecholamines and the stress hormone cortisol is to provide energy for the body to face the stressor. Rather than stimulating appetite, these hormones cause the body to break down stored energy, and one example is by stimulating lipolysis. This process is also enhanced by thyroid hormones, cortisol, growth hormone, and estrogen [35]. Thus, there are numerous hormonal signals triggering fat breakdown throughout the body. These hormones and their related lipolytic actions are extremely important during exercise, especially at low to moderate intensities. Catecholamines also increase available energy by increasing glycogenolysis in both the liver and the muscle [54]. The data concerning sex differences in catecholamines at rest and during exercise is conflicting; some studies have shown no difference in men and women, while others have reported slightly higher levels of epinephrine and norepinephrine in men [54]. Likewise, research has shown that men and women have similar levels of both blood and salivary cortisol measures at rest [27]. However, these authors identified sex differences in salivary cortisol in response to stress, such that women in the luteal phase of their menstrual cycle had similar responses to men and both were greater than women in the follicular phase of their menstrual cycle or women on oral contraceptives [27]. This suggests that both sex and the menstrual cycle phase of women should be considered when evaluating cortisol levels and their impact on energy balance.

While the functions of numerous metabolic, sex, and stress hormones that impact energy balance were discussed, the following sections will describe how these hormones are affected by physical activity. The related changes in appetite,

energy intake, and energy balance will also be discussed, and sex-related differences in these relationships will be discussed when possible.

How Does Physical Activity Influence Appetite, Satiety, and Energy Balance?

It is commonly believed that increased level of physical activity and exercise leads to stimulation of appetite. However, research in this area has reported mixed results. For example, researchers have shown that in general, physical activity does *not* have a large influence on the balance between intake and expenditure [7]. That is, increases in physical activity do not necessarily stimulate appetite, just as reductions in physical activity do not lead to substantial decreases in appetite. Blundell [6] has built upon the work of previous researchers and proposed two zones to describe how changes in physical activity relate to changes in appetite and food intake, a regulated zone and a non-regulated zone. In the regulated zone, increases in physical activity are related to increased drive to eat; however in the non-regulated zone, reductions in physical activity, or becoming sedentary, are not indicative of reductions in food intake. Thus, lack of regulation shows that caloric expenditure is not always related to hunger signals. This author also suggested that increasing one's level of physical activity should help move them into the regulated zone where these variables are more tightly connected, hence regulated. These trends between physical activity level and energy intake were supported in a recent review of cross-sectional research [2]. These authors reported that low levels of habitual physical activity were associated with higher levels of energy intake compared to those with medium and even high levels of physical activity; however, individuals reporting very high levels of physical activity had the highest level of energy intake as one would expect [2]. Hormonal changes also exist in relation to low energy intake. For example, ghrelin, cortisol, and NPY have been shown to increase, while leptin and PYY, among others, decrease in women experiencing chronic negative energy balance [20].

The relationship between an acute bout of exercise and appetite was summarized recently in a comprehensive review by Dorling et al. [18]. Following acute bouts of aerobic exercise, studies have shown a slight suppression of appetite, especially when the bout was ≥60% of VO_2 peak, and no consistent sex differences have been shown [18]. This suppression is likely related to reductions in acylated ghrelin which is reduced following exercise of this intensity [18]. These alterations do not last long, though, with hormonal levels and related appetite returning to normal levels within hours of the cessation of exercise. The effect of chronic exercise training on appetite and food intake is not as clear, as studies have shown increases, no change, and even reductions in appetite [18].

The next question, then, is whether sex differences exist in the relationship between physical activity and appetite. The literature in this area is mixed. In a recent review article, Thackray et al. [46] concluded that there was little to no evidence showing sex differences in the relationship between these variables. However, other researchers have reported that women exhibit a greater tendency to either increase energy intake following physical activity or have a more difficult time achieving negative caloric balance and weight loss via physical activity, compared to men [7]. This conclusion has been supported by other researchers as well [23, 48] and by the greater prevalence of obesity in women worldwide compared to men (15% of women vs. 11% of men) [52]. Similar results were reported in a review of 290 participants from 22 studies as physical activity was inversely related to percent body fat in males (partial $r = 0.35$, $p < 0.001$) but not in females (partial $r = 0.16$, $p > 0.05$), after accounting for age [48]. While the mechanisms behind these differences were beyond the scope of the reviews, authors have hypothesized that sex differences in fat may be attributable to women's need for sufficient fat stores for successful reproduction [7, 23].

In another study of exercise and appetite, a group of 12 normal-weight men and women

exercised for 14 days, and the resulting changes in energy balance were examined [49]. Participants took part in periods of no additional exercise as well as moderate- and high-intensity exercise, with the order counterbalanced, and were fed ad libitum. The authors reported that the additional EE from the exercise did not elicit equal increases in energy intake; rather the average caloric compensation was only ~30%. This yielded average negative energy balances ranging from −0.9 to −3.8 MJ/day in women and −1.6 to −4.7 MJ/day in men [49]. The authors acknowledge that while tightly controlled, this study only represents the initial compensation to exercise-induced energy deficits and longer studies are needed to elucidate the chronic relationships between these variables. Such studies as these provide additional data that contributes to our understanding of sex-related differences in weight control and long-term energy balance. However, much more work in this area of research is necessary.

Sex Differences in Exercise-Induced Hormonal Changes

Energy expenditure from physical activity is influenced by the metabolic-, stress-, and sex-related hormones described earlier in this chapter. In turn, hormone release is altered in response to physical activity and exercise. Acute bouts of exercise are related to increases in catecholamines, growth hormone, cortisol, thyroid hormones, estrogen, and androgens, while insulin and leptin tend to decrease [35]. These patterns of hormonal release differ somewhat in response to *chronic* exercise training, as many are decreased in response, but some remain unchanged.

To further complicate these relationships, some authors have found that hormonal changes in response to exercise may differ between men and women. For example, GH levels have been shown to increase to a greater degree in women, compared to men, during exercise [39]. Additionally, in a study examining the hormonal changes following exercise performed in several energy states, researchers noted reductions in resting leptin and insulin and increases in acylated ghrelin in response to exercise performed in a state of negative energy balance, and women had higher acylated ghrelin and lower insulin following the bout, compared to men [23]. This supports the notion that following physical activity, women's appetite is stimulated to a greater degree than men's [23]. These researchers proposed a model to help explain some trends they observed. Specifically, they proposed that in men, physical activity reduces appetite but does not change metabolic hormones such as ghrelin, insulin, and leptin substantially. Therefore, there would be no compensatory changes in energy intake for men, and the energy deficit caused by the increased expenditure would result in reduced body fat. Conversely, in women, physical activity may have no effect on appetite yet cause large hormonal changes. These changes, along with the maintenance of appetite in women, may result in a state of positive energy balance which may preserve or even increase their levels of body fat [23]. This model may explain why women tend to maintain or even gain body mass or fat in response to physical activity, whereas men typically do not. As previously described, the common theory explaining these sex differences is that the hormonal differences exist to protect women's fat mass to a greater extent than men's in order to ensure successful reproduction. However, not all studies have supported these sex differences in the hormones regulating appetite or their response to exercise [46]. Nonetheless, the theoretical model of Hagobian and Braun is an interesting hypothesis that future research needs to examine more closely.

Researchers have also examined sex differences in substrate utilization during exercise. In a study of seven men and seven women endurance-trained cyclists matched by peak oxygen uptake (VO_2 peak) per kg lean body mass, there were no sex differences in respiratory exchange ratio (RER) during moderate-intensity exercise, indicating similar contributions from fat and carbohydrates [40]. Likewise, there were no sex differences in insulin, epinephrine, or norepinephrine concentrations during exercise. However, the sources of fat differed between the sexes as men

derived less energy from myocellular triacylglycerols compared to females, and males also had a larger greater proportion of energy that was unaccounted for in fat and carbohydrates sources. Other researchers have reported conflicting results regarding sex differences in fuel metabolism during cycling. Some researchers found that women rely more heavily on fats during exercise (51% vs. 44% for women and men, respectively), while men obtain more energy from carbohydrates (53% and 46%, respectively) when cycling for 2 hours at 40% of their maximal oxygen uptake (VO_2 max) [26]. Furthermore, these exercise responses are affected in women by the phase of the menstrual cycle and associated estrogen hormonal changes [22]. These differences are likely attributable to the higher concentrations of epinephrine and norepinephrine seen in men compared to women. It is important to recognize that while conflicting results are often reported, readers must consider the intensity and duration of the exercise within studies as they have a large influence on substrate use and the related hormone response. In summary, collectively these studies provide additional data supporting sex-related differences in energy usage, energy balance, and ultimately weight control.

Summary

This chapter examined the influence of several hormones and their effect on energy balance and weight control in men and women. Sex differences were explored and include the following: females generally store more fat subcutaneously, while men store more fat in visceral locations; females are more sensitive to the actions of leptin, while men are more sensitive to insulin; and each of these hormones is closely related to one's level of adiposity. These sex differences often place men at an increased risk for the development of cardiovascular disease due to its association with visceral fat. In addition, the hormonal profile of women favors conservation of fat mass, most likely for reproduction, and some data show that women respond differently to physical activity compared to men. These differences suggest that sex-specific strategies may be necessary for maintaining or altering energy balance and body mass in men and women.

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11 Exercise Training in the Normal Female: Effects of Low Energy Availability on Reproductive Function

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Abbreviations

ACSM	American College of Sports Medicine
BMI	Body mass index
BW	Body weight
EA	Energy availability
EI	Energy intake
FFM	Fat-free mass
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
HPG	Hypothalamic-pituitary-gonadal
IGFBP	IGF-binding protein
IGF-I	Insulin-like growth factor-I
kcal	Kilocalories
LBM	Lean body mass
LH	Luteinizing hormone
NEB	Negative energy balance
NEEE	Non-exercise energy expenditure
PYY	Peptide YY
RM	Resting metabolism
T3	Tri-iodothyronine
TEEE	Total energy expended during exercise
WEE	Waking energy expenditure

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Introduction: The Female Athlete Triad

This chapter summarizes the studies in our laboratory and others that identified low energy availability as the key factor causing the Female Athlete Triad and identifies four distinct origins of low energy availability among female athletes. In 2007, the American College of Sports Medicine (ACSM) published a revised position stand on the Female Athlete Triad [1], which replaced its earlier position stand on the same subject [2]. The revised position stand corrected the former misunderstanding of the Triad as a narrow syndrome consisting of disordered eating, amenorrhea, and osteoporosis by describing the Triad more broadly as the harmful effects of low energy availability on menstrual function and bone mineral density. The revised position stand emphasized that energy availability can be severely reduced by exercise energy expenditure alone without clinical eating disorders, disordered eating, or even dietary restriction. It also explained that low energy availability induces more menstrual disorders than amenorrhea and that these functional hypothalamic menstrual disorders must be carefully distinguished by differential diagnosis from other kinds of menstrual disorders not caused by low energy availability that are, therefore, unrelated to the Triad. The revised position stand also explained that bone mineral density in young athletes must be quantified in terms of Z-scores

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instead of T-scores and that during adolescence low energy availability can cause Z-scores to decline as T-scores increase. Subsequently, treatment and return to play guidelines for the Triad were published in 2014 by the Female Athlete Triad Coalition [3, 4]. In 2014, an International Olympic Committee consensus statement introduced the term Relative Energy Deficiency in Sport to extend the concept of the Female Athlete Triad to include effects of energy deficiency beyond the reproductive and skeletal systems in men as well as women [5]. This chapter focuses on effects of low energy availability on reproductive function, specifically in women.

Hypothetical Mechanisms of Functional Hypothalamic Menstrual Disorders in Exercising Women

As in other fields of research, competing schools of thought developed to explain the high prevalence of menstrual disorders observed in exercising women. Of the several early mechanisms proposed, three were most widely held.

Body Composition

In 1974, body composition was offered as an explanation for the amenorrhea observed in anorexia nervosa patients [6]. This idea was a refinement of an earlier hypothesis about body weight accounting for the timing of menarche [7]. The body composition hypothesis held that menarche occurs in girls when the amount of energy stored in their bodies as fat rises to a critical 17% of their body weight, and that menstrual function is lost later when their body fat declines to less than a critical 22% of body weight [6].

The body composition hypothesis was the most widely publicized explanation for menstrual disorders in athletes in the lay community and the most widely embraced by the clinical community, even though it was the least widely accepted within the scientific community. The hypothesis was based entirely on correlations without any supporting experimental evidence [8]. Actually, observations of athletes did not consistently verify an association of menstrual status with body composition (e.g., Ref. [9]) and did not display the correct temporal relationship between changes in body composition and menstrual function (for reviews, see [10–13]). Rather, eumenorrheic and amenorrheic athletes were found to span a common range of body composition [14] leaner than that of eumenorrheic sedentary women. In addition, after the growth and sexual development of prepubertal animals had been blocked by dietary restriction, normal luteinizing hormone (LH) pulsatility resumed only a few hours after ad libitum feeding was permitted, before any change in body weight or composition could occur [15]. Moreover, when surgical reduction of the stomachs of severely obese women (body weight ~130 kg; body mass index [BMI] ~47) reduced the amount of food that they could eat, rapid weight loss and amenorrhea occurred while the patients were still obese (body weight ~97 kg; BMI ~35) [16].

Despite such criticisms, scientific interest in the body composition hypothesis was renewed with the discovery in 1994 of the adipocyte hormone leptin [17], with the observation of statistically significant correlations between leptin levels and body fatness in rodents and humans (e.g., Ref. [18]) and with the discovery of leptin receptors on hypothalamic neurons. Since then, an abundance of experimental evidence from rodents and human has demonstrated that a minimal level of leptin is permissive (i.e., necessary but not sufficient) for sexual development and function [19]. This permissive effect occurs indirectly via receptors on hypothalamic kisspeptin neurons that communicate with the hypothalamic gonadotropin-releasing hormone (GnRH) neurons that regulate LH pulsatility [20].

A 9-month double-blind, randomized, clinical trial administered pharmacological doses of leptin to women with functional hypothalamic amenorrhea whose BMI was in the range 18–25 kg/m^2 [21]. Prior to treatment, their leptin levels (mean ± SD = 4.6 ± 2.0 ng/ml) were within the lower portion of the range (7.4 ± 3.7 ng/ml) cited by the leptin assay manufacturer (Millipore Corp.) for women in this range of BMI [22]. Leptin levels comparable to those reported by the manufacturer

have been found in other women with similar ranges of BMI [23–29]. The leptin dosages administered to the women with functional hypothalamic amenorrhea in this experiment raised their leptin levels more than tenfold (mean ± SD = 59 ± 37 ng/ml). Yet menstrual cycles occurred only intermittently, with the number of menstruating women fluctuating from month to month between 3 of 10 (30%) and 4 of 7 (57%).

By contrast, nutritional counseling has restored spontaneous menstrual cycles in 75% of women with functional hypothalamic amenorrhea within 5 months [30]. Although leptin was originally thought to communicate information about fat stores, it was later found to vary profoundly in response to fasting, dietary restriction, refeeding after dietary restriction, and overfeeding before any changes in adiposity occurred [31–34]. This led to the hypothesis that leptin also signals information about dietary intake and specifically carbohydrate intake after leptin synthesis was found to be regulated by the tiny flux of glucose through the hexosamine biosynthesis pathway in both muscle and adipose tissue [35]. In eumenorrheic and amenorrheic athletes, leptin was found to differ not in its average concentration, but rather in the presence and absence, respectively, of a diurnal rhythm [23], and the diurnal rhythm was found to depend not on energy intake but rather on energy availability or more specifically on carbohydrate availability [27]. Thus, if leptin does participate in the functional regulation of the GnRH pulse generator in exercising women, it seems more likely to do so as a signal of low energy or carbohydrate availability than as a signal of low energy stores.

Energy Availability

In 1980, Warren was the first to suggest that menstrual function in dancers might be disrupted by an "energy drain" [36], but an empirically testable energy availability hypothesis was first clearly stated in terms of brain energy availability by Winterer, Cutler, and Loriaux in 1984 [37]. They hypothesized that failure to provide sufficient metabolic fuels to meet the energy requirements of the brain causes an alteration in brain function that disrupts the GnRH pulse generator, although the mechanism of this alteration was unknown.

At the organismal level, the energy availability hypothesis recognizes that mammals partition energy among several major metabolic activities, including cellular maintenance, immunity, thermoregulation, locomotion, growth, and reproduction [38] and that the expenditure of energy in one of these functions, such as locomotion, makes it unavailable for others, such as reproduction. Considerable observational data from biological field trials supports this idea and indicated that the dependence of reproductive function on energy availability operates principally in females (For reviews, see [38–42]. Experiments had induced anestrus in Syrian hamsters by food restriction, by the administration of pharmacological blockers of carbohydrate and fat metabolism, by insulin administration (which shunts metabolic fuels into storage), and by cold exposure (which consumes metabolic fuels in thermogenesis) [38]. Disruptions of reproductive function were independent of body size and composition.

The energy availability hypothesis was also supported by endocrine observations of athletes. Amenorrheic athletes displayed low blood glucose levels during the feeding phase of the day [43], low insulin and high IGF binding protein-1 (IGFBP) during the fasting phase [43], loss of the leptin diurnal rhythm [23], high fasting acylated ghrelin [44], high peptide YY (PYY) [45], and low tri-iodothyronine (T_3) levels in the morning [46, 47]. All of these abnormalities in metabolic substrates and hormones are signs of energy deficiency. T_3 regulates basal metabolic rate, and low T_3 occurs in numerous conditions, from fasting to cancer, in which dietary energy intake is insufficient to meet metabolic demands. In addition, eumenorrheic and amenorrheic athletes both displayed low insulin and high IGFBP-1 levels during the feeding phase of the day, as well as low leptin [23] and elevated growth hormone (GH) levels over 24 hours [43]. Indeed, eumenorrheic and amenorrheic athletes were found to be distinguished not by different 24-hour mean concentrations of leptin but rather by different amplitudes in the diurnal rhythm of leptin [23].

Amenorrheic and eumenorrheic athletes reported similar stable body weights, despite dietary energy intakes similar to those of sedentary women [46, 48–52]. That is, they reported their dietary energy intakes to be much less than would be expected for an athlete's level of physical activity. This apparent discrepancy between stable body weight and unexpectedly low dietary energy intake was controversial. Since energy intake and expenditure are very difficult to measure accurately, the apparent discrepancy might have been attributable to methodological errors. Some investigators attributed the apparent discrepancy between energy intake and expenditure in athletic women to underreporting of dietary intake [53, 54], because such underreporting is common in all populations [55], but underreporting did not account for the abnormalities in metabolic substrates and hormones observed in athletes. Furthermore, behavior modification and endocrine-mediated alterations of resting metabolic rate operate to stabilize body weight despite dietary energy excess and deficiency [56].

Exercise Stress

The exercise stress hypothesis held that exercise disrupts the GnRH pulse generator by activating the hypothalamic–pituitary–adrenal axis. In order for the stress hypothesis to be meaningfully independent of the energy availability hypothesis, however, the adrenal axis must be activated independently of the energy cost of the exercise.

Certainly, there are central and peripheral mechanisms by which the adrenal axis can disrupt the ovarian axis [57], and prolonged aerobic exercise without glucose supplementation does activate the adrenal axis. Selye first induced anestrus and ovarian atrophy in rats by abruptly forcing them to run strenuously for prolonged periods [58]. Later, others also induced anestrus by forced swimming [59, 60], by forced running [61], and by requiring animals to run farther and farther for smaller and smaller food rewards [62, 63]. The elevated cortisol levels induced in such experiments were interpreted as signs of stress, and the resulting disruptions of the hypothalamic-pituitary-gonadal (HPG) axis were widely interpreted as evidence that "exercise stress" has a counter-regulatory influence on the female reproductive system.

Amenorrheic athletes also display mildly elevated cortisol levels [43, 48, 64–66]. This observation was the basis for attributing their amenorrhea to stress. Mild hypercortisolism is also associated with amenorrhea in patients with functional hypothalamic amenorrhea [67] and anorexia nervosa [68]. This interpretation overlooked the glucoregulatory functions of cortisol, which inhibit skeletal muscle glucose uptake and promote skeletal muscle proteolysis for hepatic gluconeogenesis in response to low blood glucose levels [69]. Thus, it was possible that the mild hypercortisolism observed in amenorrheic athletes might have reflected a chronic energy deficiency rather than exercise stress.

At the time, it was not known whether the adrenal cortical axis mechanisms that disrupt the HPG axis in forced exercise experiments on animals also operate in voluntarily exercising women. Indeed, up to that time, all animal experiments investigating the influence of the "activity stress paradigm" on reproductive function had confounded the stress of exercise with the stress of the method used to force animals to exercise. These experiments had also been confounded by the energy cost of the exercise performed, and glucose supplementation during exercise was found to blunt the usual rise in cortisol in both rats [70] and men [71]. As a result, in 1990 the literature on stress contained only ambiguous evidence that the stress of exercise disrupts the HPG axis in either animals or humans.

Prospective Clinical Experiments

Experiments Confounding Exercise Stress and Energy Availability

Several investigators attempted to induce menstrual disorders through chronic exercise training, but most [72–75] applied only a moderate volume of exercise, or the volume of exercise was increased gradually over several months, and

diet was uncontrolled or unquantified. One study [75] selected physically trained subjects who appeared to have been luteally suppressed before the study even began [76].

Only one experiment had successfully induced menstrual disorders in regularly menstruating women [77]. Modeled on Selye's early animal experiments [58], this single successful experiment imposed a high volume of aerobic exercise abruptly, thereby suppressing follicular development, the LH surge, and luteal function in a large proportion of the subjects in the first month and in an even larger proportion in the second. Both proportions were greater in a subgroup fed a controlled weight loss diet than in another subgroup fed for weight maintenance, but even the weight maintenance subgroup may have been underfed, since behavior modification and endocrine-mediated alterations of resting metabolic rate operate to stabilize body weight despite dietary energy excess and deficiency [56].

Such experiments, in which outcome variables are properties of the menstrual cycle, require sustained observations over a period of several weeks. Such prolonged experimental protocols suffer from practical problems with subject retention and compliance with experimental treatments. To avoid these difficulties, short-term experimental protocols were developed in which LH pulsatility was chosen as the outcome variable, because ovarian function is critically dependent on LH pulsatility. Of course, short-term effects on LH pulsatility are not proof of chronic effects on ovarian function, but hypotheses about mechanisms regulating LH pulsatility could be tested in highly controlled short-term experiments, and then chronic effects could be confirmed in prolonged experiments later.

One such short-term experimental protocol found that a combination of increased exercise and dietary restriction disrupts LH pulsatility during the early follicular phase [78]. LH pulse frequency during 12 waking hours was lower in four habitually physically active women when their exercise training regimen was increased during a few days of dietary restriction than during dietary supplementation. However, this experiment did not determine whether LH pulse frequency could be suppressed by exercise without dietary restriction or whether the stress of exercise had a suppressive effect on LH pulsatility beyond the impact of the energy cost of exercise on energy availability.

Experiments Distinguishing the Independent Effects of Exercise Stress and Energy Availability

For several years, we focused our efforts on a series of studies that we called the "Excalibur" experiments that were designed to determine the independent effects of exercise stress and energy availability on the HPG axis [28, 29, 79–83]. For these experiments, we defined energy availability operationally as dietary energy intake minus exercise energy expenditure. Conceptually, this corresponds to the amount of dietary energy remaining after exercise training for all other physiological functions. Although not the actual physiological quantity hypothetically affecting the HPG axis at the cellular level, our operational definition in behavioral terms had the advantage of being readily measurable and controllable. We controlled the dietary energy intake of our subjects by feeding them diets of known amount and composition as their only food during the experiments. We also required them to exercise under supervision in our laboratory on a treadmill while we measured and controlled their energy expenditure until they had expended a predetermined amount of energy. In the absence of any empirically operational definition of stress [84], we defined exercise stress independently as everything associated with exercise except its energy cost.

Through careful subject selection, we took steps to minimize the influence of potentially confounding factors. Healthy, regularly menstruating, habitually sedentary, nonobese, nonsmoking women 18–34 years of age at least 5 years past menarche, with no recent history of dieting, weight loss, or aerobic training were recruited. Before being admitted to the study, these volunteers underwent an extensive screening procedure, including written medical, men-

strual, dietary, and athletic histories, a physical examination, a 12-lead resting electrocardiogram, a 7-day prospective dietary record, determination of body composition by hydrostatic weighing or whole body air-displacement plethysmography, and a treadmill test to determine their aerobic capacity. Volunteers were admitted into experiments only if they presented no current use of medications including oral contraceptives and no history of heart, liver, or renal disease, diabetes, and menstrual or thyroid disorders. They must also have had documented prospective records of menstrual cycles 26–32 days in length for at least the previous 3 months. They were required to be 18–30% body fat, with habitual energy intakes between 35 and 55 kcal/kg lean body mass (LBM)/day based on their 7-day diet records, with maximal aerobic capacities less than 42 ml O_2/kg body weight (BW)/min, and they must have been performing less than 60 minutes of habitual aerobic activity per week for the previous 3 months.

The narrow range of our subjects' menstrual cycle lengths implied that we restricted our subject pool to the central 60% of menstrual cycle lengths in the population and that from this pool we chose women whose menstrual cycle lengths were in the least variable 20% of the population [85]. Thus, if anything, our subjects' reproductive systems were robust against disturbance by commonly occurring environmental and behavioral influences. We could be confident, therefore, that if our treatments disrupted the reproductive systems of these women, they would disrupt the reproductive systems of other women, too. We could also be confident that our subjects' metabolism had not been disturbed by any confounding medical conditions or dietary or exercise habits before our treatments were applied.

Excalibur I

Excalibur I [79] was designed to investigate whether exercise stress had any suppressive effect on T_3 levels independent of the impact of the energy cost of exercise on energy availability. We were interested in T_3 because it regulates the rate of energy expenditure at rest and because it was known to be suppressed in amenorrheic athletes. We reasoned that if the energy cost of exercise necessitates such major metabolic adjustments as the suppression of reproductive function, then these metabolic adjustments might be mediated in part by suppressing T_3.

Over the course of the Excalibur experiments, our insight into how to correctly quantify energy availability (EA) for subjects of various body sizes gradually matured. At the time of Excalibur I, we normalized energy intake (EI) and exercise energy expenditure to body weight (BW). We also measured exercise energy expenditure as the total energy expended during exercise (TEEE), as measured by an ergometer.

$$\mathrm{EA} = \left(\mathrm{EI} - \mathrm{TEEE}\right) / \mathrm{BW}$$

In Excalibur I, we found that severely low energy availability (8 kilocalories per kilogram of body weight per day, kcal/kgBW/day) suppressed T_3 levels by 15%, while exercise stress had no effect on T_3. T_3 levels were suppressed similarly regardless of whether energy availability was reduced by dietary energy restriction or by exercise energy expenditure. Furthermore, the suppression of T_3 in exercising women was prevented by supplementing their diet in compensation for the energy cost of their exercise. These findings were consistent with the energy availability hypothesis and inconsistent with the exercise stress hypothesis.

Excalibur II

Excalibur II [80] was designed to reveal whether T_3 levels in exercising women vary in linear proportion to energy availability or are suppressed abruptly at a particular threshold of energy availability. By this time we had realized that very little energy expenditure occurs in body fat. Accordingly, we changed our normalization of energy intake and expenditure to lean body mass (LBM) which would exclude body fat.

$$\mathrm{EA} = \left(\mathrm{EI} - \mathrm{TEEE}\right) / \mathrm{LBM}$$

We administered various levels of energy availability to exercising women and found that the suppression of T_3 by low energy availability occurred

abruptly at a threshold of energy availability near 25 kcal/kgLBM/day. For our women of average body size (59 kg) and composition (24.5% body fat), that threshold was about 1000 kcal/day.

Excalibur III

Normal ovarian function depends not on some stable concentration of LH but rather on the occurrence of pulsatile surges of LH concentrations in the blood at regular intervals. These pulses correspond to regular secretory bursts of LH from the pituitary gland in response to similar secretory bursts of GnRH from the hypothalamus. The frequency (at intervals of 70–180 minutes) and amplitude of these pulses vary around the menstrual cycle. In sedentary women in the early follicular phase, the pulsatile pattern is characterized as high frequency and low amplitude. In regularly menstruating athletes, the pulses occur less often and are larger in amplitude but still at regular intervals. In amenorrheic athletes, LH pulses occur even less often and irregularly [48].

Therefore, in Excalibur III [81, 82], we investigated whether exercise has any suppressive effect on LH pulsatility beyond the impact of its energy cost on energy availability. The design of Excalibur III is illustrated in Fig. 11.1. For 4 days in the mid-follicular phase of two menstrual cycles, we controlled the energy availability of two groups of women. During one cycle, we administered a balanced energy availability of 45 kcal/kgLBM/day, and during the other cycle, we administered a low energy availability of 10 kcal/kgLBM/day. One group of subjects performed no exercise during the two treatment periods. A second group performed the same large volume of high-intensity exercise that we had utilized in Excalibur I (30 kcal/kgLBM/day at 70% VO_2max; maximal aerobic capacity]). We imposed balanced and low energy availabilities on the non-exercising group by feeding them 45 and 10 kcal/kgLBM/day, respectively. We imposed the same balanced and low energy availabilities on the group performing 30 kcal/kgLBM/day of exercise by feeding them 75 and 40 kcal/kgLBM/day, respectively.

Between Excalibur II and III, we had had another insight into the proper quantification of energy availability. Prior to Excalibur III [81, 82],

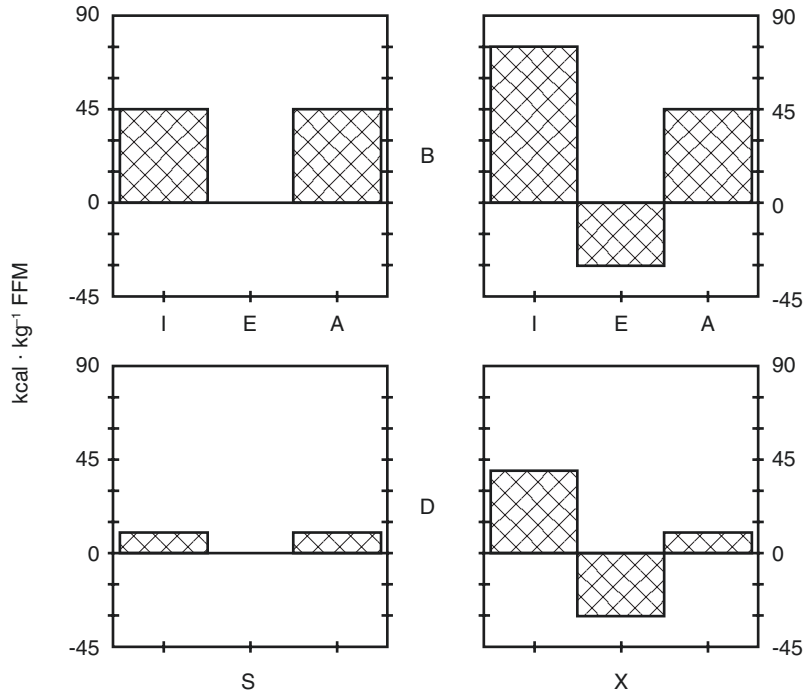

Fig. 11.1 Experimental design of Excalibur III. Dietary energy intake (I) and exercise energy expenditure (E) were controlled to achieve balanced (B = 45 kcal/kgLBM/day) and deprived (D = 10 kcal/kgLBM/day) energy availability (A = I-E) treatments. Deprived energy availability was achieved by dietary restriction alone in sedentary women (S) and by exercise energy expenditure alone in exercising women (X) (1 kcal = 4.18 kJ). (Reproduced with permission from [82])

we had calculated energy availability by subtracting total energy expenditure during exercise (TEEE) from dietary energy intake. While we were designing Excalibur III, however, we recognized that if our exercising subjects had not been exercising, their other routine activities during the same hours would have resulted in some non-exercise energy expenditure (NEEE). Therefore, the actual energy expenditure due to exercise itself (EEE) was less than the total energy expenditure measured during exercise (EEE = TEEE – NEEE). This adjustment would be especially important for Excalibur III, in which some subjects exercised and others did not. So, in Excalibur III and our later experiments, we changed again the way we calculated energy availability by subtracting from dietary energy intake only the portion of total energy expenditure during exercise that was directly attributable to the exercise itself.

$$EA = (EI - EEE) / LBM$$

We achieved this by using an activity monitor to measure our subjects' energy expenditure in their normal daily activities during the same hours of the day when they would be exercising in our experiment. We then subtracted this non-exercise energy expenditure in routine activities from their total energy expenditure during exercise to obtain the amount of energy expenditure during exercise that was specifically attributable to the exercise. In retrospect, our subjects' energy expenditure in routine activities on a non-exercising day during the same 3 hours when they exercised in Excalibur II had amounted to 5 kcal/kgLBM/day, and our calculations of energy availability had been underestimated by the same amount.

At the end of each of the 4-day treatments in Excalibur III, we admitted the women to a general clinical research center and drew blood samples from them at 10-minute intervals for 24 hours. Later, we measured the amount of LH in each sample and used a special statistical computer program to detect and to calculate the frequency and amplitude of their LH pulses. We determined the effects of energy availability on these frequencies and amplitudes by contrasting data taken while performing the same exercise at different energy availabilities, and we determined the independent effect of exercise stress by contrasting groups exercising differently at the same energy availabilities.

We found that low energy availability reduced LH pulse frequency and increased LH pulse amplitude, while exercise stress had no suppressive effect on LH pulsatility beyond the impact of the energy cost of exercise on energy availability (Fig. 11.2). LH pulsatility was disrupted by extreme energy restriction alone and by extreme

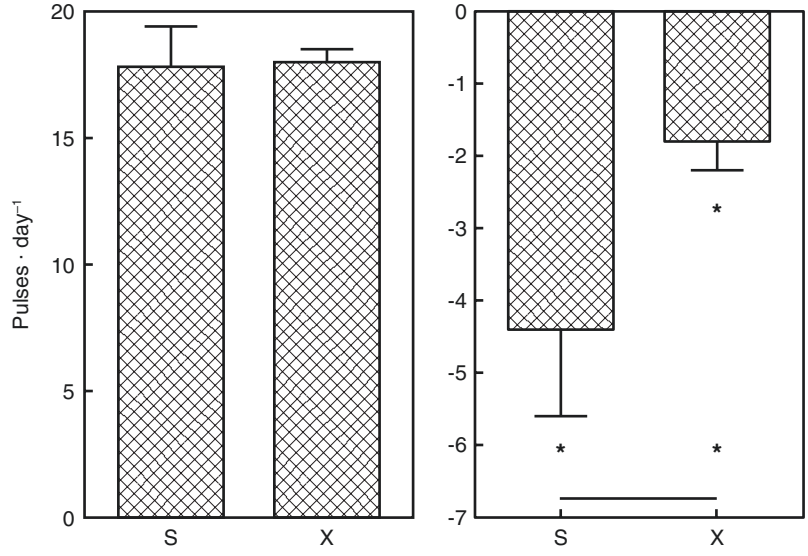

Fig. 11.2 Effects of low energy availability on LH pulsatility in Excalibur III. Left: Luteinizing hormone (LH) pulse frequency in sedentary (S) and exercising (X) women with the same balanced energy availability (45 kcal/kgLBM/day). Right: Reduction in LH pulse frequency caused by low energy availability (10 kcal/kgLBM/day) in sedentary (S) and exercising (X) women. *$p < 0.01$. (Adapted from [81, 82])

exercise energy expenditure alone. Dietary supplementation prevented the suppression of LH pulsatility by exercise energy expenditure. Others have shown that short-term fasting also reduces LH pulse frequency in sedentary women during the early follicular phase [86, 87] and that in lean women, ovarian function is also impaired during the ensuing menstrual cycle [87].

In Excalibur III, low energy availability also suppressed plasma glucose, insulin, insulin-like growth factor-I (IGF-I), leptin, and T_3 while raising growth hormone (GH) and cortisol levels. All these effects are reminiscent of abnormalities observed in amenorrheic athletes [43, 46–48, 64–66].

This contradiction of the exercise stress hypothesis has been confirmed by more prolonged experiments on animals. Amenorrhea was induced in monkeys by training them to run voluntarily on a motorized treadmill for longer and longer periods, while their food intake remained constant [88]. Then their menstrual cycles were restored by supplementing their diets without any moderation of their exercise regimen [89]. The exercise stress hypothesis was also contradicted in a novel animal model of the entire Female Athlete Triad [90]. In this modified activity stress paradigm, rats were habituated to voluntary wheel running for 90 days and then randomized to control and restricted diets for the next 90 days. Although both groups ran similar distances and expended similar amounts of energy in exercise, estradiol was suppressed, estrous cycling ceased, ovaries were atrophied, and the bone mineral content of the femur and tibia were reduced only in the underfed rats.

The suppression of LH pulse frequency by low energy availability in Excalibur III was actually *smaller* in exercising women than in non-exercising women with the same low energy availability [82]. This result was unexpected, and it suggested that LH pulsatility might actually depend on a more specific metabolic factor that is easily confused with energy availability, but which is less compromised by exercise energy expenditure than by dietary energy restriction.

Research in other mammals suggests that GnRH neuron activity and LH pulsatility are actually regulated by brain glucose availability [38, 41]. The adult female human brain oxidizes about 80 g of glucose each day at a continuous rate. This must be provided daily by dietary carbohydrate, because the brain's rate of energy expenditure can deplete liver glycogen stores in less than a day [91]. To that end, moderate exercise oxidizes as much glucose in an hour.

In the non-exercising women in Excalibur III, low energy availability due to dietary energy restriction reduced carbohydrate intake by 77%. This reduction in carbohydrate intake was similar to the 73% increase in carbohydrate oxidation revealed by respiratory gas analysis in the exercising women during the balanced energy availability treatment. By contrast, carbohydrate oxidation increased only 49% in the exercising women under low energy availability conditions. This alteration in fuel selection conserved almost 70% of the brain's daily glucose requirement. Thus, exercise may compromise brain glucose availability less than dietary energy restriction, and this may account for the smaller disruption of LH pulsatility that we observed in exercising women than in dietary-restricted women. Thus, LH pulsatility may depend specifically on carbohydrate availability rather than energy availability in women, just as it does in other mammals.

Excalibur IV

Excalibur IV [83] was designed to reveal whether refeeding reverses the suppression of LH pulsatility in women as quickly as it does in other mammalian species. In food-restricted female rats [15, 92] and ewes [93], and in fasted heifers [94] and male rhesus monkeys [95], a single ad libitum meal stimulates LH pulses within 2 hours. Such observations have been interpreted to imply that the physiological signals produced by a single large meal are sufficient to activate the hypothalamic GnRH neurons that control LH pulsatility [96].

We suspected that the restoration of LH pulsatility by refeeding might be considerably slower in energetically disrupted women than in other mammals, because the human brain requires so much more energy than does the brain of any other mammal. The brain competes against all

other tissues of the body for energy, and the adult human brain requires 20% of basal metabolic energy, compared to only 2% for most species and 8% for nonhuman primates [97]. Therefore, we suspected that a single meal might not provide enough energy to activate GnRH neurons in energetically disrupted women.

To stringently test this hypothesis, we assayed LH in blood samples drawn from women at 10 minute intervals for 48 hours during the mid-follicular phase, first during 24 hours on the fifth day of low energy availability treatments and then during 24 hours of aggressive refeeding. A combination of moderate dietary energy restriction (25 kcal/kgLBM/day) and moderate exercise energy expenditure (15 kcal/kgLBM/day) was administered to impose a low energy availability of 10 kcal/kgLBM/day. The aggressive refeeding regimen was comprised of 15 meals providing a total of 85 kcal/kgLBM/day. Combined with the same exercise treatment, the energy availability during the 24 hours of aggressive refeeding was 70 kcal/kgLBM/day.

Compared to measurements of LH pulsatility in 18 other women studied previously in our laboratory under balanced energy availability conditions and at the same phase of the menstrual cycle, low energy availability suppressed LH pulsatility unambiguously in five of the eight subjects treated in this experiment. Their LH pulse frequency was reduced 57% to 8.2 ± 1.5 pulses/24 hours, well below the 5th percentile of LH pulse frequencies in energy balanced women (14.6 pulses/24 hours), while their LH pulse amplitude was increased 94% to 3.1 ± 0.3 IU/L, well above the 95th percentile of LH pulse amplitudes in energy balanced women (2.5 IU/L).

Amongst these women, aggressive refeeding raised LH pulse frequency by only 2.4 ± 1.0 pulses/24 hours, still far below the 5th percentile of LH pulse frequency in energy balanced women. Meanwhile, the unambiguously elevated LH pulse amplitude was completely unaffected (Δ = 0.0 ± 0.4 IU/L) by aggressive refeeding. Results were similar when all eight subjects were included in the analysis. Aggressive refeeding pushed the group as a whole to, but not past, the 5th and 95th percentiles of LH pulse frequency and amplitude, respectively. Thus, as we had suspected, 24 hours of a refeeding protocol much more aggressive than the ad libitum refeeding protocols commonly employed in animal experiments had very little restorative effect on LH pulsatility in our energetically suppressed women.

Excalibur V

In an experimental protocol similar to that of Excalibur II, Excalibur V determined the dose-response effects of low energy availability on LH pulsatility in habitually sedentary, regularly menstruating young women [28]. To do this, we administered balanced and one of three low energy availabilities (45 and either 10, 20, or 30 kcal/kgLBM/day) to healthy, habitually sedentary, regularly menstruating women for 5 days. The design is illustrated in Fig. 11.3.

We found that LH pulsatility was disrupted within 5 days below a threshold of energy availability at ~30 kcal/kgLBM/day (Fig. 11.4). This was, in fact, the same actual energy availability that we had reported as 25 kcal/kgLBM/day in Excalibur II [80], because we had underestimated energy availability by 5 kcal/kgLBM/day in Excalibur II, as described in the discussion of Excalibur III above.

The disruption of LH pulsatility below 30 kcal/kgLBM/day in Excalibur V was consistent with many observational studies of amenorrheic runners, all of which indicated energy availabilities less than 30 kcal/kgLBM/day [98]. It was also consistent with the only prospective study of the refeeding of amenorrheic athletes, in which menstrual cycles had been restored in runners by increasing their energy availability from 25 to 31 kcal/kgLBM/day [99]. Energy availabilities below 30 kcal/kgLBM/day have also been reported in eumenorrheic athletes [98], 80% of whom display subclinical ovarian disorders in which the suppression of progesterone may also impair fertility [100].

In the same experiment, we also determined the dose-response effects of low energy availability on several metabolic substrates and hormones. Down to an energy availability of 30 kcal/kgLBM/day, the responses of insulin,

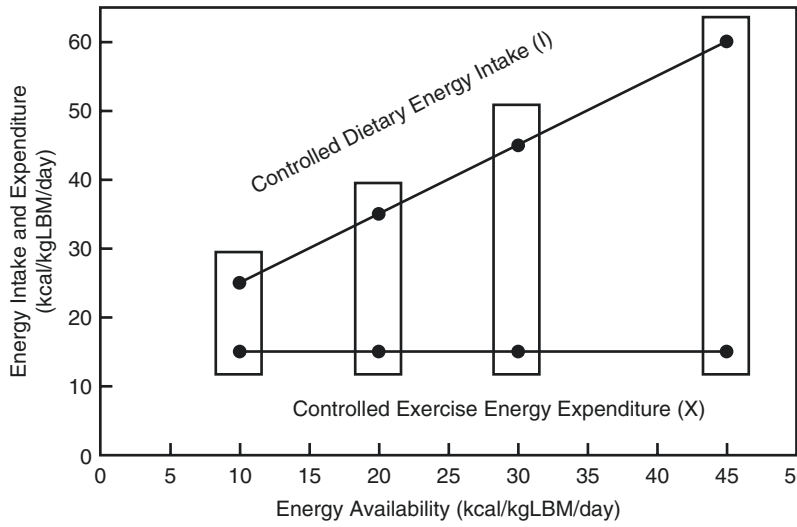

Fig. 11.3 Experimental design of Excalibur V. Women were assigned to contrasting energy availability treatments of 45 and 10, 45 and 20, and 45 and 30 kcal/kgLBM/day. All subjects performed a controlled exercise energy expenditure of 15 kcal/kgLBM/day in aerobic exercise at 70% VO_2 max under supervision, while their dietary energy intake was controlled to achieve the intended energy availabilities. (Reproduced with permission from [28], *Copyright 2003, The Endocrine Society*)

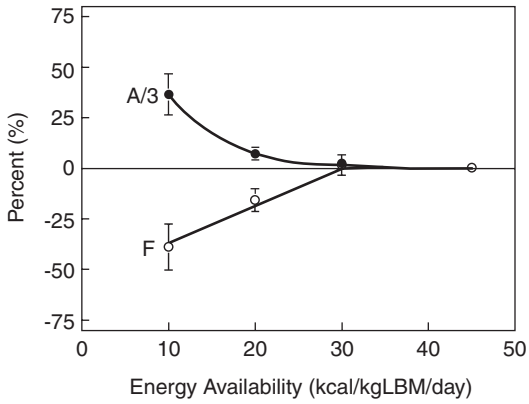

Fig. 11.4 Incremental effects of energy availability on LH pulse amplitude (A/3) and LH pulse frequency (F) in Excalibur V. Effects are expressed relative to values at 45 kcal/kgLBM/day. Effects on LH pulse amplitude have been divided by three for graphical symmetry. As energy availability declines from energy balance at approximately 45 kcal/kgLBM/day, effects begin at a threshold at approximately 30 kcal/kgLBM/day and become more extreme as energy availability is further reduced below 20 kcal/kgLBM/day. (Reproduced with permission from [28], *Copyright 2003, The Endocrine Society*)

cortisol, insulin-like growth factor (IGF)-I/IGF-binding protein (IGFBP)-1, IGF-I/IGFBP-3, leptin, and T_3 maintained plasma glucose levels to within 3% of normal values. Below that threshold, however, plasma glucose levels fell despite further increases in the responses of the metabolic hormones, and effects on LH pulsatility appeared.

Excalibur V also revealed the dose-response effects of low energy availability on biochemical markers of bone turnover [101]. Urinary concentrations of N-telopeptide of type I collagen, a marker of the rate of whole body bone resorption, rose as estradiol concentrations declined, when energy availability was lowered to 10 kcal/kgLBM/d. By comparison, markers of bone formation declined at higher energy availabilities. Concentrations of serum carboxy-terminal propeptide of type I procollagen, a marker of bone type I collagen synthesis, and insulin declined linearly with energy availability. By contrast, concentrations of osteocalcin, a marker of bone mineralization, declined abruptly below 30 kcal/kgLBM/day together with IGF-I and T_3, which modulates the hepatic synthesis of IGF-I in response to GH stimulation. Such uncoupling of bone turnover, with increased resorption and reduced formation, can lead to irreversible reductions in bone mineral density [102].

Excalibur VI

The prevalence of amenorrhea has been reported to decline from 67% in marathon runners younger than 15 years of gynecological age to only 9% in those who were older [103]. Meanwhile, in the general population, the incidence of menstrual disorders declines during the decade after menarche as fertility increases [104]. Excalibur VI investigated whether these two observations might both be explained by a declining sensitivity of LH pulsatility to low energy availability as the energy cost of growth decreases [29]. Calcium balance, which is an index of growth, does not decline to zero until 14 years of gynecological age [105].

In Excalibur VI, contrasting balanced and low energy availabilities (45 and 10 kcal/kgFFM/day) were administered to healthy, habitually sedentary, regularly menstruating, older adolescent women (5–8 years of gynecological age, ~20 years of calendar age) and young adult women (14–18 years of gynecological age, ~29 years of calendar age) for 5 days. Low energy availability suppressed LH pulsatility in the adolescents but not in the adults, even though metabolic and endocrine signals of energy deficiency (i.e., plasma glucose, β-hydroxybutyrate, insulin, cortisol, T_3, leptin, IGF-1, and GH) were altered as much or more in the adults as in the adolescents [29].

This insensitivity of LH pulsatility to energy deficiency in adult women was subsequently confirmed by a corresponding insensitivity of ovarian function to energy deficiency [106]. In that experiment, the energy availability of women 25–40 years of age was reduced to ~25 kcal/kgFFM/day for 4 months by a combination of dietary restriction (~600 kcal/day) and exercise (~200 kcal/day). This subthreshold energy deficiency reduced the body fatness of these reproductively mature women from 32% to 27% but caused no more than a mild suppression of luteal function.

An adult reproductive system that is more robust against insults of energy deficiency may be explained by a greater availability of glucose to the brain in adults than in adolescents at the same energy availabilities. This might occur if peripheral tissues in full-grown adults do not compete as aggressively against the brain for available energy or carbohydrate. Alternatively, the sensitivity of sensors in the central nervous system to signals of energy deficiency may decline during adolescence. These possibilities remain to be investigated.

Other Efforts to Manipulate Energy Availability in Habitually Sedentary Women

A recent study by Lieberman et al. [107] investigated the effects of energy availability on menstrual function by reanalyzing data collected in an earlier experiment that had attempted to administer controlled negative energy balance (NEB) treatments of −15%, −30%, and −60% to separate groups of habitually sedentary regularly menstruating women for 3 months [108]. Thirty-five women with 5–15 years of gynecological age and ovulatory cycles as long as 35 days were studied, even though cycles of 36 days were to be classified as a clinical menstrual disturbance (oligomenorrhea) and the average within-person annual standard deviation of cycle length at the subjects' age is 4 days [85].

In practice, NEB turned out to be less negative and more widely dispersed than intended (mean ± 2SD, −8 ± 10%, −22 ± 21%, and −42 ± 9%). Moreover, metabolic hormone indicators of energy deficiency did not display dose-response effects of group differences in NEB. Assuming the underlying diet and exercise data were correct, Lieberman et al. calculated energy availability values in each menstrual cycle and found a continuum of energy availability treatments from 18 to 51 kcal/kgFFM/day.

Unfortunately, Lieberman et al. did not report the effects on metabolic hormones. They found no dose-response effects of energy availability on ovarian steroids. Altogether, they found that 36% of 105 menstrual cycles across the range of energy availability displayed menstrual disturbances and 85% of these were subclinical (luteal phase deficiency and anovulation). Collectively, only one menstrual cycle was missed.

Lieberman et al. concluded that their results "do not support that a threshold energy availability exists below which menstrual disturbances are induced," thereby appearing to confirm the

Female Athlete Triad as a continuum of interrelated disorders. However, given the 10–15% incidence of oligomenorrhea and the 15–65% incidence of subclinical menstrual disturbances in free-living women of the same gynecological age [109, 110], the observations of Lieberman et al. are better interpreted as what would be expected without any intervention. Moreover, without a crossover design and without dose-response effects of energy availability on any physiological indicator of energy deficiency, Lieberman et al. simply lack evidence that the disturbances they observed were caused by the treatments administered.

Reversal of Amenorrhea in Amenorrheic Athletes

Cialdella-Kam et al. [111] administered a carbohydrate-protein dietary supplement of 360 kcal/day to athletes with clinical menstrual disorders (7 amenorrheic and 1 oligomenorrheic). After 6 months, the eight athletes had resumed menses with seven of them resuming ovulation. However, it should be noted that the investigators calculated EA without subtracting non-exercise energy expenditure NEEE. Therefore, as they acknowledged in another paper [112], their pre and post EA values probably underestimated actual EA values by 1–2 kcal/kgFFM/day. Prior to this study, there had been pilot studies published of amenorrheic athletes who increased caloric intake for several months, and changes in menstrual status were observed [99, 113].

Pre and post EA values depended, of course, on the definition of exercise. When exercise was defined as activity when energy expenditure was greater than 4.0 METS, the dietary supplement increased energy availability from 37 to 45 kcal/kgFFM/day ($p = 0.10$). However, when exercise was defined more broadly to include all planned exercise plus bicycle commuting and all walking, energy availabilities were lower with the dietary supplement increasing them from 28 when amenorrheic to 39 kcal/kgFFM/day after restoration of menses ($p = 0.09$) [112].

More prospective research is needed to determine successful behavioral strategies that amenorrheic athletes with low energy availability can use to resume menstrual status. For example, research on appetite suppression by exercise and dietary restriction suggests that it may be important for athletes to consume planned amounts of energy at planned times, by discipline instead of appetite [114].

Conclusions About the Hypothetical Mechanisms of Functional Hypothalamic Amenorrhea in Female Athletes

We are unaware of any experiments that have determined the independent effect of body composition on the HPG axis. From the available experimental data, however, it would appear to be more likely that a lean body composition and disruption of the HPG axis are both effects of low energy availability than that a lean body composition disrupts the HPG axis. Our short-term experiments on women have demonstrated that exercise stress has no suppressive effect on LH pulsatility beyond the impact of the energy cost of the exercise on energy availability. These short-term 4–5-day experiments investigating the independent effects of exercise stress and low energy availability on LH pulsatility predicted and, as we expected, were later confirmed by long-term experiments investigating the independent effects of exercise stress and low energy availability on estrus and menstrual cycles. Prospective controlled experiments on both humans and animal models have demonstrated that the factor disrupting the HPG axis in physically active women is low energy availability. These experiments suggest that women may be able to prevent or to reverse menstrual disorders by dietary reform alone without moderating their exercise regimen. As long as dietary energy intake is managed to keep energy availability above 30 kcal/kgLBM/day, there may be no need to interfere with endurance, strength, and skill training. Finally, the susceptibility of women to the disruption of reproductive function by energy deficiency appears to be substantially greater in those younger than 15 years of gynecological age.

Causes of Low Energy Availability in Female Athletes

Effective treatment of low energy availability in athletes requires that the origin of the low energy availability be identified. Low energy availability behaviors appear to derive from four different origins [1, 114]. Some athletes intentionally reduce energy availability in a rational, but misguided, pursuit of the body size, body composition, and mix of metabolic fuel stores that are thought to optimize performance in their particular sport. Complex objectives may include reducing fat mass while increasing muscle mass and maximizing glycogen stores. For such athletes who reduce energy availability excessively, nutrition education and guidance regarding appropriate, individualized intermediate and ultimate goals, schedules, and methods may be sufficient to modify their diet and exercise behavior.

In other athletes, low energy availability originates in an eating disorder. Eating disorders are clinical mental illnesses that are often accompanied by other mental illnesses [115, 116]. Therefore, eating disorders require psychiatric treatment, often inpatient treatment, as well as nutritional counseling. Because the mortality of eating disorders is so high, sports organizations need to develop institutional methods for distinguishing undernourished athletes with eating disorders from those who do not have eating disorders. This distinction may not be obvious, since undernourished athletes who are only trying to optimize performance may practice many of the same disordered eating behaviors (e.g., skipping meals, vomiting, using laxatives, etc.) as athletes with eating disorders. Athletes with eating disorders are distinctive in their resistance to the efforts of coaches, trainers, nutritionists, and physicians to modify their behavior.

The third origin of low energy availability in athletes is the suppression of appetite by prolonged exercise. This effect is compounded by the appetite-suppressing effect of diets containing high percentages of carbohydrates, which are commonly recommended to athletes in endurance sports. Even though many studies on this subject have been published over the past 20 years [114, 117], appetite remains a largely neglected topic in the field of sports nutrition. Indeed, the word "appetite" appears only twice, in the recently revised joint position stand of the American Dietetic Association, the Dietitians of Canada, and the American College of Sports Medicine on nutrition and athletic performance [118].

Briefly, food deprivation increases hunger, but the same energy deficit produced by exercise energy expenditure does not [119]. The appetite-suppressing effect of prolonged exercise has been demonstrated in controlled experiments with protocols ranging from a few hours to 12 weeks [114]. The effect is mediated by the orexigenic hormone ghrelin, which induces us to begin eating, and by several anorexigenic hormones (including peptide YY, glucagon-like peptide 1, and pancreatic polypeptide) that induce us to stop eating. Exercise does not stimulate an increase in ghrelin concentrations but does stimulate increases in the concentrations of anorexigenic hormones (see associated Chaps. 12 and 30). As a result, "there is no strong biological imperative to match energy intake to activity-induced energy expenditure" [120].

Meanwhile, the appetite-suppressing effect of diets containing high percentages of carbohydrates has been demonstrated in experimental protocols ranging from a week [121] to a month [122, 123]. As the percentage of carbohydrates in the diet was reduced, ad libitum energy intake spontaneously increased. As a result, the actual amount of carbohydrate consumed was preserved even though the percentage of carbohydrates in the diet decreased from 67% to 55%. The mechanism of this effect has not yet been identified but may involve the greater bulk and fiber content of carbohydrate-rich foods.

Importantly, the large effects of these two factors are additive [121] so that together they can reduce energy availability below 30 kcal/kgFFM/day in endurance athletes. To avoid inadvertent low energy availability, therefore, athletes in endurance sports need to be trained to eat by discipline (i.e., planned amounts of selected foods at scheduled times) instead of appetite.

The fourth apparent origin of low energy availability among female athletes is that young

women under-eat for social reasons unrelated to sport. Around the world, about twice as many young women as young men at every decile of body mass index perceive themselves to be overweight, and the numbers actively trying to lose weight are even more disproportionate [124]. Alarmingly, the disproportion even *increases* as BMI declines, so that almost 9 times as many lean women as lean men are actively trying to lose weight! Indeed, more young female athletes report improvement of appearance than improvement of performance as a reason for dieting [125]. As a result, social issues unrelated to sport may need to be addressed to persuade female athletes to eat by discipline *beyond* their appetites.

Sources of Error in the Estimation and Control of EA

In publications of the Excalibur experiments, the portion of body composition apart from fat mass is termed LBM. It is better termed fat-free mass (FFM). Then, as currently understood, energy availability (EA) is quantified by measuring dietary energy intake (EI), exercise energy expenditure (EEE), and fat-free mass (FFM). EA is then calculated as:

$$EA = (EI - EEE) / FFM$$

A common source of error (by us in Excalibur I and II and by others) in studies of EA in athletes has derived from the misunderstanding of EEE as the total energy expenditure that would be measured by an ergometer during exercise. *This misunderstanding has led to underestimations of EA, misinterpretations of data, and unwarranted criticisms of the concept.*

As described in the discussion of Excalibur III above, EEE is defined as the *extra* energy expended *beyond* the energy that would have been expended if no exercise had been performed (see Fig. 11.5). Defining EEE in this way enables EA to be fairly compared between different groups of subjects who do and do not exercise and between repeated observations of the same subjects when they do and do not exercise. Because energy expenditure varies with routine activities during the day, to calculate EA consistently with the Excalibur experiments, non-exercise energy expenditure (NEEE) must be measured on another non-exercising day during the same waking hours when exercise is performed. Then EEE is calculated as the difference between total energy expenditure during exercise (TEEE) and NEEE on the other day:

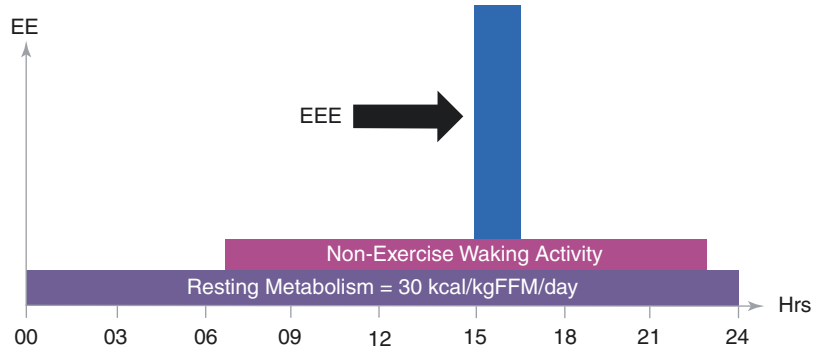

Fig. 11.5 Calculation of exercise energy expenditure (EEE). (A. Top) EEE is the amount of energy that a woman expends because she is an athlete and does not include the energy she expends in resting metabolism and other waking activities. (B. Middle) Ergometers measure total energy expenditure during exercise (TEEE), which overestimates EEE by ~2 kcal/kgFFM/d per hour of exercise. For high-intensity exercise of short duration, the resulting error in calculating energy availability as EA = (EI – TEEE)/FFM is negligibly small for clinical purposes. (C. Bottom) For low-intensity exercise of long duration, however, the error in EA = (EI – TEEE)/FFM is very large and will lead to unwarranted changes in diet and exercise behavior. (Adapted from [126])

$$EEE = TEEE - NEEE$$

In an example described in a previous review [126], the resting metabolism (RM) of an athlete in energy balance on a non-exercising day is assumed to be 2/3 of her EI. For EI = 2100 kcal/day (8.8 MJ/day), RM = 1400 kcal/day (5.8 MJ/day) or 58 kcal/hour (244 kJ/h). If she sleeps 8 hours, her routine activities in waking energy expenditure (WEE) would expend the rest of her EI. Ignoring for simplicity other sources of diurnal variation in energy expenditure, her average rate of WEE would be 700 kcal/16 hours = 44 kcal/h (182 kJ/h). If her fat-free mass (FFM) is 45 kg, then her rate of non-exercise energy expenditure (NEEE) during exercise would be:

$$NEEE = (RM + WEE)/FFM = (58 + 44)/45 = 2.3\,\text{kcal/kgFFM/h}\,(9.5\,\text{kJ/h})$$

If the athlete's total energy expenditure during a 40-minute run is TEEE = 500 kcal, then:

$$EEE = TEEE - NEEE = 500/45 - (2/3) * 2.3 = 11.1 - 1.5 = 9.6\,\text{kcal/kgFFM}$$

For such brief, high-intensity exercise, NEEE (1.5 kcal/kgFFM) is too small to cause an error in judgment about the adequacy of EA. However, if the same TEEE had been expended in 4 hours of gymnastics training, NEEE (9.2 kcal/kgFFM) would be too large to ignore:

$$EEE = TEEE - NEEE = 500/45 - 4 * 2.3 = 11.1 - 9.2 = 1.9\,\text{kcal/kgFFM}$$

If this gymnast were to restrict her dietary intake to EI = 1575 kcal/day, ignoring NEEE would lead to excessive concern about her EA and unwarranted demands for behavior modifications:

With NEEE:

$$EA = (EI - EEE)/FFM = 1575/45 - 1.9 = 33.1\,\text{kcal/kgFFM/day}\,(138\,\text{kJ/kgFFM/day})$$

Ignoring NEEE:

$$(EA = EI - TEEE)/FFM = 1575/45 - 9.6 = 25.4\,\text{kcal/kgFFM/day}\,(106\,\text{kJ/kgFFM/day})$$

Other sources of error in the calculation of EA derive from errors in the estimation of EI, EEE, and FFM. As pointed out in another review [126], a few simple calculations with realistic values quickly reveal that the greatest efforts should be made to record EI accurately. Consider an athlete with body mass = 60 kg, %Fat = 25%, EEE = 500 kcal/day, and EI = 2100 kcal/day (8.8 MJ/day). Her FFM is (1–0.25) × 60 = 45 kg and her EA is

$$EA = (EI - EEE)/FFM = (2100 - 500)/45 = 35.6\,\text{kcal/kgFFM/day}\,(149\,\text{kJ/kgFFM/day})$$

A 2% error rate in %Fat determinations is not uncommon with body composition analyzers. Subsequently, a 2% overestimate of %Fat (i.e., 27% in the above example) leads to an underestimate of FFM (43.8 kg) and a negligible error in EA:

$$EA = (2100 - 500)/43.8 = 36.5\,\text{kcal/kgFFM/day}\,(153\,\text{kJ/kgFFM/day})$$

A 10% error in EEE would correspond to a runner erring by half a mile in the length of a 5-mile run. A 10% underestimation of EEE leads to a similarly negligible error in EA:

$$EA = (2100 - 450)/45 = 36.7\,\text{kcal/kgFFM/day}\,(153\,\text{kJ/kgFFM/day})$$

Underestimations of EI as big as 20% have been suspected by some dietitians. A 20% underestimation of EI would lead to a large error in EA:

$$EA = (0.8 \times 2100 - 500)/45 = 26.2\,\text{kcal/kgFFM/day}\,(110\,\text{kJ/kgFFM/day})$$

Even a 10% underestimation of EI would lead to a substantial error in EA:

$$EA = (0.9 \times 2100 - 500)/45 = 30.9\,\text{kcal/kgFFM/day}\,(129\,\text{kJ/kgFFM/day})$$

A 10% error in EI (210 kcal) is similar to the energy content of 2–3 slices of bread. If EI is underestimated by 10–20%, then these substantial errors in EA will lead to misinterpretations of experimental data and mismanagement of athletes. Therefore, accurate estimations of EA depend most importantly on complete dietary records. In the Excalibur experiments, the accuracy of EI treatments was achieved by administering and supervising known meals. Difficult as that is for investigators and participants alike, quantifying EI in observational studies of free-living athletes is even more challenging.

Conclusion: Needed Research

More short-term experiments are needed to resolve the ambiguity about whether LH pulsatility depends on energy in general or on specific macronutrients in particular. Clinical trials are needed to verify that women can prevent or reverse functional hypothalamic amenorrhea by dietary reform alone without moderating the exercise regimen and to develop effective interventions that may be sport-specific. In addition, more animal experiments using the new modified activity stress paradigm ([90]) are needed to explore the physiological and neuroendocrine mechanisms of the Female Athlete Triad in more detail. Finally, more experiments like Excalibur III are needed to determine whether other stressors besides exercise have any suppressive effect on LH pulsatility beyond the impact of their energy cost on energy availability. Long-term experiments like Excalibur V are needed to look at EA threshold effects on other aspects of reproductive function besides LH pulsatility, but the expense and controls needed to properly conduct such studies make them challenging to conduct.

Conflict of Interest Anne Loucks is a founder and shareholder of AEIOU Scientific, LLC.

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12 Ghrelin Responses to Acute Exercise and Training

Jaak Jürimäe

Introduction

The importance of physical exercise to influence energy balance and body mass is widely recognized [1]. A complex neuroendocrine system is involved in the regulation of energy homeostasis including central and peripheral tissues [2, 3]. Important to this regulatory system is the existence of several appetite hormones, including adipose and gut tissue hormones that communicate the status of body energy stores to the hypothalamus [2]. Energy intake is an integral to energy balance and is regulated via neuronal circuits interacting with gut hormones, key among these being ghrelin and peptide YY [4, 5]. It appears that peptide YY functions as a negative feedback signal and is responsible for inducing satiety and cessation of eating after food intake [5]. In contrast, ghrelin is a hormone well known for its acute orexigenic properties stimulating food consumption [6, 7]. Changes in these circulating appetite hormones influence the physiological drive to eat, weight gain and also reproductive function [4]. Furthermore, ghrelin may also be involved in pubertal development, where rapid growth and development need careful coordination of energy balance and appetite regulatory signals [4]. Finally, circulating ghrelin concentrations may vary dramatically depending on specific body composition, physical activity and physical fitness parameters [2]. This chapter focuses on the available information about the effects of acute exercise and chronic exercise training on the secretion of ghrelin.

Ghrelin, a peptide secreted by distinct endocrine cells of the stomach, was first described as an endogenous ligand for the growth hormone secretagogue receptor [8]. However, ghrelin role in body mass regulation is more prominent than its role in growth hormone secretion [9]. Ghrelin promotes positive energy balance by increasing appetite and food intake [10, 11]. Specifically, the rise in circulating ghrelin concentration before a meal is a physiological signal for hunger and the body's cue for meal initiation [12]. Therefore, the rise in ghrelin levels and hunger occurs independent of food and time of day cues [12]. Meal responses of ghrelin are related to acute caloric intake over a typical day of eating in normal-weight subjects [13]. Furthermore, ghrelin levels have been demonstrated to be negatively correlated with 24-h caloric intake [14], and ghrelin concentrations decrease after caloric intake and increase while fasting [2]. The decrease in ghrelin release is related to the specific amount of calories ingested [15]. Accordingly, ghrelin is responsive to diet- and exercise-induced changes in body mass [16].

In addition to total ghrelin, acylated and desacylated forms of ghrelin have been described

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[17]. The acylated form of ghrelin is thought to be essential for ghrelin biological activity [18], whereas unacylated ghrelin has been suggested to be biologically inactive [19]. Specifically, acylated ghrelin has been reported to be associated with the regulation of growth hormone secretion, cardiac performance, cell proliferation and adipogenesis and affects appetite, food intake and energy balance [8, 20, 21]. There are also some studies suggesting that unacylated ghrelin is related to insulin resistance [22–24]. It has also been demonstrated that total ghrelin and acylated ghrelin are positively correlated [25–27] and both forms of the ghrelin potentially play a role in energy balance [28]. Based on these results, it could be suggested that acylated and desacylated forms of ghrelin change similar to changes in energy balance, and total ghrelin concentration can be used as a biomarker in energy balance studies [4, 29, 30]. Future studies, nonetheless, are needed to better clarify the responses of total ghrelin and its specific forms in various conditions of energy balance.

Ghrelin During Growth and Maturation in Children

Ghrelin is a hormone that could influence somatic growth [4] and sexual maturation [31]. Specifically, a negative association of circulating ghrelin level with age [4, 31] and pubertal development [32] has been found. It has been hypothesized that ghrelin provides a link between energy homeostasis, body composition and pubertal development through actions on the hypothalamus [33], where ghrelin stimulates the secretion of gonadotropin-releasing hormone, which in turn stimulates the secretion of the gonadotropins required for pubertal onset [16]. It has been found that the initiation of puberty substantially decreases ghrelin concentrations in both sexes [4, 31]. A negative correlation between ghrelin and testosterone has been found in boys entering puberty [32]. In contrast, a recent study demonstrated no effect of testosterone and estradiol on ghrelin decrease during pubertal growth in boys and girls, respectively [4]. It was found that a drop in circulating total ghrelin to its lowest levels occurred during peak pubertal growth [4]. Furthermore, Cheng et al. [4] suggested that adolescent ghrelin concentrations may be more strongly associated with markers of somatic growth than sexual maturation. Specifically, circulating ghrelin levels were inversely correlated with insulin-like growth factor-1 concentrations and with annual height and weight velocity in both sexes [4]. Accordingly, the decrease in circulating ghrelin levels at the onset of puberty is apparent [4, 34, 35], despite the fact that puberty is characterized by increased appetite and food intake [31] and ghrelin is known to stimulate appetite [28, 36]. Research suggests that there could be an increased sensitivity for appetite stimulation by ghrelin over puberty [31] and/or low ghrelin concentrations signal adequate nutritional status to support rapid somatic growth and development of reproductive capacity [4] to sustain growth in this period. In addition, elevated energy expenditure and, therefore, also an increased energy intake in physically active children during pubertal maturation are linked to higher circulating ghrelin levels in these children compared with physically inactive children [36]. Accordingly, it could be argued that regular physical activity still causes higher ghrelin levels during puberty to stimulate appetite and food intake to cover higher energy homeostasis [36]. This is supported by the finding that there is a negative correlation of cardiorespiratory fitness as measured by peak oxygen consumption with total ghrelin [37] and acylated and desacylated forms of ghrelin [38] in boys during puberty. However, different forms of ghrelin were not associated with directly measured physical activity intensities in pubertal boys with differing body composition [38]. Collectively, these results demonstrate that somatic growth and maturation are associated with ghrelin, which concentrations decrease with advancing age and puberty. However, further longitudinal studies throughout puberty in children with various physical activity and body composition levels are needed to better understand how physical fitness and activity may influence circulating ghrelin concentrations dur-

ing puberty in children with different body composition values before any definitive conclusions can be drawn.

In longitudinal investigations with growing and maturing athletes, total ghrelin levels have been studied in female gymnasts [35, 39] and male and female swimmers [40, 41]. It could be argued that regular sport training increases ghrelin levels to stimulate appetite and food intake to cover higher energy homeostasis in these young athletes [2, 42]. Ghrelin may act as a hormone signalling a need for energy conservation, and ghrelin secretion is triggered to counter a further deficit in energy storage to help to maintain body mass [2, 43]. Accordingly, higher basal ghrelin concentrations have been found in prepubertal and adolescent athletes when compared with untrained controls [34, 35, 44]. However, basal ghrelin levels decreased in both prepubertal rhythmic gymnasts and age-matched lean untrained controls over a 12-month study period [39], showing that an increasing age decreases ghrelin concentrations similarly in both groups despite large differences in daily energy expenditure [2, 36]. Therefore, ghrelin concentrations were still significantly higher in the rhythmic gymnasts when compared with untrained controls at both measurement times during prepuberty [39]. However, when rhythmic gymnasts and untrained controls reached puberty, ghrelin levels were decreased in both groups and were not different between groups with different energy expenditure levels [35]. Similarly, a significant decrease in basal ghrelin levels was observed in male swimmers after the evolution of puberty [41], while basal ghrelin levels were not changed in pubertal female swimmers with advancing pubertal maturation over a 2-year study period [40]. It can be suggested that basal ghrelin levels are higher in prepubertal children who participate in sport training in comparison with age-matched untrained controls, while basal ghrelin levels decrease when young athletes reach puberty even in the presence of chronically elevated energy expenditure [2, 36]. Furthermore, pubertal maturation appears to reduce circulating ghrelin concentrations in growing athletes of both sexes, despite heavy athletic activity [2, 36].

Ghrelin Relationships with Adiposity and Energy Availability

Ghrelin levels are significantly lower in obese individuals [45–47] and substantially elevated in patients with anorexia nervosa [12, 48, 49], proposed as a likely adaptive mechanism response [12, 50]. Accordingly with these patterns, there is a negative association of ghrelin concentration with body mass [32, 51], body mass index [32, 52], total body fat mass [34, 51], visceral fat mass [53, 54] and total body lean mass [32, 55]. It has also been suggested that circulating ghrelin level could be regarded as a signal of decreased total body lean mass in healthy elderly females [56]. In addition, there are also studies to show an inverse correlation between ghrelin concentration and body height [32, 57, 58] and body height velocity [4] during growth in children.

Diet-induced weight loss in obese individuals has been accompanied by increases in circulating total ghrelin concentrations [59]. For example, plasma ghrelin levels increased by 17% in overweight women who reduced their body mass by 4.5% after 10-week body weight loss intervention programme [60], while a 6-month supervised weight loss programme that caused 17.4% body weight loss induced 24% increase in ghrelin levels [61]. In addition, short-term diet-induced body weight loss in obese subjects resulted in higher total ghrelin concentrations, which remained elevated also over weight maintenance periods of 6 and 12 months [59]. Similarly, long-term exercise intervention together with diet-control investigations has demonstrated that total ghrelin levels increase in response to exercise-induced body weight loss in obese subjects and not because of food restriction per se, acting via a negative feedback loop that regulates body mass [7, 62]. It has been suggested that changes in total ghrelin concentrations appear to be most sensitive to changes in body mass resulting from overall energy deficit, independent of specific effects of nutritional intake and/or physical exercise [7, 62]. There are studies to demonstrate that manipulations in food intake and exercise energy expenditure show a close relationship between circulating ghrelin

and energy availability [63, 64]. For example, Scheid et al. [64] measured total ghrelin, energy balance and body composition parameters before and after 3-month intervention period in exercising women and found that circulating ghrelin does not play a role in the adaptive changes associated with exercise training when exercise occurs in the absence of body weight loss. However, fasting ghrelin level increased when body mass is lost and may respond to even smaller changes in energy availability [64]. In addition, the change in total ghrelin level was inversely correlated with the change in body mass, body mass index, lean body mass and energy availability after diet- and exercise-associated weight loss [64]. In contrast, no impact of aerobic training on acylated ghrelin levels was observed in overweight and obese men [65]. It has been suggested that differences in body fat mass loss, exercise volume and duration, and gender may influence possible differences in ghrelin responses to weight reduction [59]. In addition, King et al. [66] showed that equivalent energy deficits induced by food restriction or physical exercise have markedly different effects on appetite, energy intake and acylated ghrelin concentrations. While food restriction elicited a rapid increase in appetite and energy intake and these responses appear to be related to postprandial suppression of acylated ghrelin, acute energy deficits induced by vigorous intensity exercise session did not alter appetite or energy intake and may be related to the failure of acute exercise to induce compensatory acylated ghrelin responses [66]. These results together suggest that changes in body mass are needed before any changes in circulating ghrelin levels could be seen in untrained individuals.

Ghrelin Responses to Acute Exercise

There are a number of studies including athletes that have investigated the influence of acute bout of exercise on total ghrelin [37, 67–82] and on acylated ghrelin [66, 83–98] concentrations. Different investigations with healthy untrained individuals [37, 67, 68, 78] and also well-trained endurance athletes [71, 74, 82] would suggest that exercise-induced acute negative energy balance may not be sufficient to alter total ghrelin response. Conversely, however, there are studies demonstrating that total ghrelin level increased [69, 70, 72, 73] or decreased [75–77, 80, 81] as a result of short-term exercise session. In addition, studies with acylated ghrelin have mostly reported significant suppression [83, 84, 86, 89, 90, 94–98] or no change [66, 85, 92] in measured acylated ghrelin concentration after acute exercise. However, there are also studies that have observed significant postexercise increase in acylated ghrelin concentration [91, 93]. Accordingly, acute exercise studies have demonstrated different responses of different ghrelin forms to the acute exercise in subjects with different body composition and physical activity levels.

A study by Dall et al. [68] reported no change in total ghrelin concentration after acute cycling exercise for 45 min at the intensity of anaerobic threshold in healthy middle-aged men. Similarly, total ghrelin levels remained unchanged after acute submaximal running workloads (50%, 70% and 90% of maximal oxygen consumption [VO_{2MAX}]) [78] and also after a single bout of treadmill running for 60 min [67] in healthy physically fit male individuals. In well-trained endurance athletes, a progressively intense intermittent exercise trial on treadmill at different exercise intensities (10 min at 60%, 10 min at 75%, 5 min at 90% and 2 min at 100% of VO_{2MAX} [74] and 30 min on-water sculling exercise performed either below or above the intensity of individual anaerobic threshold [71] did not change total ghrelin concentration. It could be argued that acute exercise energy expenditure was not sufficient to alter total ghrelin response in these studies [1]. Accordingly, significant postexercise increases in total ghrelin concentration after prolonged 2-h endurance rowing at the intensity of 80% of individual anaerobic threshold [34] and after 3-h endurance cycling at the intensity of 50% of maximal aerobic power [69] have been observed in endurance-trained athletes. Assuming that the energy balance drives the ghrelin response to prolonged rowing

exercise with the estimated energy expenditure of 1200–1500 kcal, it was conceivable to see that the increased postexercise total ghrelin concentration was associated with the amount of work performed ($r = 0.75$; $p < 0.05$) in rowers [34]. Furthermore, it was argued that the reduced resting levels of total ghrelin may have influenced the significant exercise-induced increase in ghrelin concentration in rowers [34]. The results of these studies [34, 69] would suggest that a certain threshold reduction in energy availability should be reached before any significant postexercise increases in total ghrelin concentration occur and that the amplitude of the total ghrelin increase could be linked to the energetic status induced by acute exercise stress and the resting levels of ghrelin in athletes [1]. However, to what extent exercise intensity may influence total ghrelin response to acute exercise has not yet been determined, although it has been suggested that low- rather than high-intensity exercise with longer duration stimulates total ghrelin levels [70]. Specifically, Erdmann et al. [70] investigated the effect of exercise intensity and duration on total ghrelin release, hunger and food intake in normal-weight untrained healthy individuals. Total ghrelin concentrations were increased by 50–70 pg/ml as a result of prolonged low-intensity bicycling exercise with a duration of up to 2 h, while no changes in total ghrelin were observed during higher intensity exercise [70]. In addition, only 2-h prolonged aerobic exercise at the intensity of 50 W with an exercise energy expenditure of 340 kcal lead to an increase in food intake without having an effect on hunger sensations [70]. An increase in plasma ghrelin concentration during exercise without alterations of hunger sensations under similar conditions of low-intensity exercise and energy expenditure was also found in another study [79]. Nonetheless, the stimulation of food intake during prolonged exercise was most likely not due to changes in circulating total ghrelin levels [70]. These results together demonstrate that total ghrelin concentrations can be increased as a result of a low-intensity prolonged exercise session when the exercise energy expenditure is high enough also in untrained subjects.

There are studies to suggest that acute exercise stress could also result in a decrease of total ghrelin concentration [75, 77, 80, 81]. These studies have used more intensive exercise bouts including resistance exercise protocols [75, 77, 80, 81], and it has been suggested that glucoregulatory stress from the acute intense exercise could result in a suppression of circulating ghrelin during the recovery period from the exercise [74, 75]. Indeed, studies that have utilized more intensive exercise bouts have demonstrated that maximal exercise-induced large increases in insulin [74, 75] and growth hormone [75, 81] levels may suppress total ghrelin concentration during the recovery period. However, there are also investigations that contradict the results of these studies as exercise-induced increases in both total ghrelin and growth hormone values have been observed after prolonged low-intensity exercise in endurance-trained males [69] and also in overweight postmenopausal women [79]. Others have argued that postexercise ghrelin responses may be independent of changes in energy balance [6] and that acute exercise stress increases energy intake only some time postexercise [6, 83]. To this end, Broom et al. [83] investigated the effects of 1 h running at 72% of VO_{2MAX} on total and acylated ghrelin concentrations. They found that total ghrelin was not changed, while acylated ghrelin was decreased as a result of exercise [83]. Accordingly, it has been argued that although there is a close relationship between total and acylated ghrelin concentrations [25–27], it cannot be excluded that after acute exercise this relationship may be somewhat different [42, 70, 83].

Different studies have demonstrated that relatively high-intensity exercise sessions (≥70% VO_{2MAX}) may suppress acylated ghrelin concentrations [99, 100]. Typically, this hormonal decrease coincides with a transient reduction in appetite during and immediately after the exercise [87, 88], while there are also studies that have found no changes in appetite as a result of acute exercise [89, 92, 101]. It is possible that the lack of commonly observed appetite suppression may be due to a difference in training status or fitness of studied subjects [89]. In accordance, there is an evidence to suggest that highly trained

individuals are more accustomed to exercise stress and therefore do not have as great hormonal, including acylated ghrelin, response to acute exercise as in untrained individuals [88, 99]. For example, Broom et al. [84] found that plasma acylated ghrelin and hunger ratings fell and remained suppressed for 1.5 h after 90 min running at the intensity of 70% of VO_{2MAX} (≈70% decrease in acylated ghrelin) in healthy men. In other studies with endurance-trained men, circulating acylated ghrelin concentrations were decreased after 45 min of cycling at the intensity of ≈76% of VO_{2MAX} (≈23% decrease in acylated ghrelin) [89] and after 20 km run (≈14% decrease in acylated ghrelin) [90]. Therefore, the suppression of acylated ghrelin in endurance-trained athletes was transient, with concentrations not different from baseline already after 30 [90] and 40 [89] min postexercise. A recent study by Mattin et al. [92] observed no significant changes in acylated ghrelin and appetite scores as a result of 60 min cycling at the intensities of 40% and 70% of VO_{2MAX} in healthy men. Therefore, although not statistically significant, acylated ghrelin responded differently to exercise intensity, as serum levels decreased by ≈27% at the intensity of 70% of VO_{2MAX} and increased by ≈12% at the intensity of 40% of VO_{2MAX} [92]. Larson-Meyer et al. [91] also found a significant increase in acylated ghrelin immediately after 60 min running at the intensity of 70% of VO_{2MAX} in female runners. Therefore, appetite was not affected by running exercise, and postexercise acylated ghrelin was not associated with appetite scores [91]. It was argued that the energy cost of the running exercise may promote increased acylated ghrelin secretion after exercise in these athletes [91]. The results also suggested that acylated ghrelin is not a major contributor to postexercise food intake, perhaps because the signal is dampened by increases in different anorexigenic peptides at the same time [91, 102]. In accordance, other studies have also argued that it is possible that the transient suppression of circulating acylated ghrelin that can be observed during acute exercise may be entirely unrelated to appetite regulation [50, 85]. These results together suggest that acylated ghrelin is responsive to different conditions and modes of endurance exercise, duration and intensity, but the direction of the hormone response can be varied [95]. The differences in acylated ghrelin responses to acute exercise can also be attributed to subject physical fitness, pre-exercise meal consumption and timing as well as the timing of the hormone measurements and possible environmental factors such as temperature and altitude [103]. There is a need for further investigations to elucidate the exact mechanisms regulating ghrelin synthesis and clearance during and after acute exercise.

Chronic Exercise Training and Ghrelin Responses

Chronic exercise training perturbs energy balance and can potentially alter body mass and composition. There are a number of studies that have reported an increase in circulating ghrelin concentrations after long-term exercise interventions in previously untrained individuals [13, 43, 62, 104–108], while other studies have not found any changes in ghrelin concentrations as a result of prolonged exercise training [51, 109–111]. It appears that circulating ghrelin levels increase with body weight loss [62, 105, 107, 108] and decrease with body weight gain [12, 112]. Accordingly, data on ghrelin responses to prolonged exercise training are mainly available from obese individuals (i.e. individuals involved in weight loss programme) [62, 106, 107, 109, 113], whereas only limited data are provided for athletes [29, 30, 114–116]. Most of the previous investigations have studied total ghrelin response to prolonged exercise training [13, 29, 30, 43, 62, 108, 109, 114, 115], while relatively few intervention studies have measured acylated [111, 113, 116] or unacylated [23, 24] ghrelin concentrations separately. Currently, there appears to be only one published study that has investigated the response of acylated ghrelin to prolonged training period in athletes [116].

Previous investigations have mostly found that total ghrelin concentrations increase during situations of body weight loss and suggest that weight loss is the most potential factor influencing ghre-

lin response to exercise training [13, 43, 62, 107, 108, 117]. In an earlier study, Leidy et al. [108] found that fasting ghrelin concentration was increased twofold in a group of normal-weight women who experienced weight loss (>1.5 kg) as a result of a 3-month energy deficit-imposing diet and 5-days-a-week exercise training intervention programme [108]. Therefore, body mass, body fat mass and resting metabolic rate significantly decreased before the increase in fasting ghrelin occurred [108]. It was suggested that circulating total ghrelin responds in a compensatory manner to changes in energy homeostasis in healthy young women and that ghrelin exhibits particular sensitivity to changes in body mass [108]. In another study, Foster-Schubert et al. [62] reported that total ghrelin levels increased by 18% in sedentary overweight postmenopausal women who lost more than 3 kg body mass after 1-year aerobic exercise training programme. Another 1-year moderate-to-vigorous intensity aerobic exercise for 45 min 5 days a week demonstrated that greater weight loss was associated with larger increases in total ghrelin concentrations in overweight and obese postmenopausal women [107]. Similarly, moderate-intensity aerobic exercise training 5 days a week for 12 weeks increased circulating acylated ghrelin concentrations in overweight and obese men and women [113]. In contrast to these findings, fasting acylated ghrelin concentrations decreased after a moderate dose (14 kcal/kg body mass weekly) but did not change after a low-dose (8 kcal/kg body mass weekly) moderate-intensity aerobic exercise training lasting 4 months in healthy nonobese older women [111]. It was argued that exercise training dose can have specific effects on acylated ghrelin that are not dependent on body weight or body fat mass loss [111]. However, there was a lack of acylated ghrelin level change in those participants who lost body weight or body fat mass as a result of 4-month training period [111]. In another study, Ravussin et al. [51] observed that neither positive energy balance caused by overfeeding nor negative energy balance induced by exercise training had a significant effect on total ghrelin concentration over a 100-day study period. The impact of negative energy balance on total ghrelin levels at the end of the investigation was smaller, due to the possible effect of accustomization [51]. Another study with a group of morbidly obese men and women demonstrated that fasting circulating total ghrelin levels remained unchanged despite 5% body weight loss induced by a 3-week integrated body weight reduction programme with exercise training [109]. The amplitude of ghrelin response to negative energy balance in these studies could be linked to the energetic status of studied individuals, which is attributable to specific body fat mass and exercise training characteristics. Accordingly, data regarding the influence of exercise training programme on circulating ghrelin in previously untrained individuals suggests that exercise training per se has no impact on circulating ghrelin levels and changes in ghrelin concentrations that are seen as a result of exercise training intervention take place as secondary changes to body weight loss [117].

Evidence suggests that the degree of negative energy balance and/or body weight loss threshold to increase circulating ghrelin concentrations has not yet been determined [1, 17]. In heavily exercising females, menstrual disturbances have been linked to an energy deficiency, where caloric intake is inadequate for exercise energy expenditure [12, 118]. These menstrual disturbances, together with an energy deficiency, are largely attributable to athletic events, where the emphasis is on the achievement of thin and lean physiques, which may require low body mass and body fat percent such as in gymnastics, figure skating and long-distance running [12]. Accordingly, higher ghrelin levels have been observed in amenorrheic athletes than in normally ovulating women who train [17, 119]. In fact, there are data to suggest that young female athletes with varying severities of menstrual disturbances can be distinguished from each other based on their circulating ghrelin levels [12, 48, 120, 121]. To this end, as energy deficiency increases in severity across the continuum of menstrual cycle disturbances, physically active women with amenorrhea have the lowest resting energy expenditure relative to lean body mass, together with the increased ghrelin levels [48, 121]. In contrast, physically active women with

subtle menstrual disturbances and nonathletic controls present higher resting energy expenditure relative to lean body mass and lower ghrelin concentrations [48, 121]. Increased ghrelin levels in young female athletes with amenorrhea may have a role in reproductive system [12, 119, 120]. An inverse relationship between acylated ghrelin concentration and gonadal steroids was observed in athletes [48], and acylated ghrelin levels may differentiate between athletes who will or will not develop functional hypothalamic amenorrhea during heavy training [48, 119]. Accordingly, it is likely that high circulating ghrelin concentrations contribute to functional hypothalamic amenorrhea by altering gonadotropin-releasing hormone and luteinizing hormone pulsatility [119, 120]. Therefore, body fat mass has an important negative influence on basal ghrelin levels in amenorrheic athletes [48, 120]. An increase in energy intake in amenorrheic athletes induces a decrease in basal ghrelin concentrations, which is paralleled by increases in body mass and resumption of menses [119]. Accordingly, it appears that circulating ghrelin is a biomarker of energy imbalance across the menstrual cycle in female athletes [36, 122]. Since ghrelin levels are consistently elevated in energy deficiency such as functional hypothalamic amenorrhea, ghrelin could be an important marker of energy deficiency and chronic undernutrition [12] and should be measured to monitor the health of female athletes.

The mechanisms by which changes in energy balance and/or body mass impact on circulating ghrelin levels are not fully understood [2, 36, 117]. It has been proposed that leptin, which levels directly correlate with body fat mass, may have an influence on circulating ghrelin concentrations [117]. Specifically, a negative association between circulating leptin and ghrelin concentrations has been reported [34], and an increase in circulating ghrelin levels in response to body weight loss may therefore occur as a result of a decrease in circulating leptin concentrations [117]. Therefore, alterations in ghrelin levels as a result of changes in body fat mass may therefore be secondary to changes in leptin [117]. In addition, insulin may also mediate some of the effects of body adiposity on circulating ghrelin [117] as circulating ghrelin concentrations are inversely correlated with insulin and insulin resistance values [123]. It has been suggested that relatively low ghrelin concentrations observed in obese individuals may be a result of insulin resistance that is a characteristic in obesity and which has an inhibitory effect on ghrelin concentrations, rather than excess body mass by itself [123]. Collectively, this may represent one mechanism by which insulin is implicated in the homeostatic regulation of energy balance [117].

Only few studies have investigated ghrelin response to different exercise training periods in adult male [30, 114–116] and female [29] athletes. Specifically, in male athletes, ghrelin responses to a weight reduction period before competitions in bodybuilders [30], an intensive training camp in football players [116] and a high-volume low-intensity endurance [114] and a high-volume low-intensity concurrent endurance and resistance [115] training periods in competitive rowers have been studied. In addition, ghrelin responses to intensified training period were also studied in female synchronized swimmers [29]. While studies with national-level male bodybuilders [30] and international-level female synchronized swimmers [29] demonstrated that total ghrelin levels increased together with a body weight reduction as a result of negative energy balance, no differences in total ghrelin concentrations together with no changes in body mass values were observed in competitive male rowers as a result of increased training volume [114, 115]. Accordingly, it can be speculated that body weight loss is also important to reduce total ghrelin concentrations in studied athletes. In contrast, circulating acylated ghrelin concentrations were significantly lowered during the 9-day intensive training camp, which tripled the training volume in male college-level footballers [116]. Therefore, no changes in body mass values were observed, and an increase in physiological stress was associated with a decrease in appetite [116]. It was suggested that an early-phase physiological stress response may decrease the acylated ghrelin concentrations in male athletes during an intensive training camp [116]. The reason for different results between this study with

other studies in athletes is not clear. It is possible that these discrepancies are due to factors related to the different modes of exercise, energy availability and competitive level of athletes. However, it is also likely that differences in dietary control, sample collection and assay procedures may also be implicated [117]. Clearly, further studies with elite athletes with different training programmes are needed before any definitive conclusions can be drawn.

Relative to women athletes, a national team of female synchronized swimmers performed a 4-week intensified training period, where a baseline training load of about 22 h was increased by a 20.5% across the intensified training period, which caused a significant decrease in body fat percent from 17.3% to 16.4% in these elite female athletes [29]. In addition, a decrease in energy availability was observed, which was accompanied by an increase in ghrelin and decrease in leptin, reflecting a decrease in energy stores across the investigation period [29]. The results of the Schaal et al.'s [29] study demonstrate that a state of an increased fatigue and rather low energy availability in these elite female athletes was characterized by a significant increase in ghrelin levels shortly before the season's target competitions. Accordingly, it may be suggested that an increased ghrelin concentrations can be used as a marker of increased training stress and inadequate energy availability in elite female athletes.

In a study with male bodybuilders, 14 athletes were divided into seven competitors and seven control athletes, who were followed for 11 weeks before the national championships [30]. Competitors were able to significantly decrease their mean body mass by 4.1 kg during the 11-week period, whereas no changes in body composition or ghrelin values were observed in the control athletes [30]. In competitors' group, the energy deficit at about 536 kcal/day after the first 5-week period was already sufficient to cause a significant increase in total ghrelin concentrations, whereas no further increase in ghrelin levels was observed with the energy deficit reaching 978 kcal/day after 11-week preparatory period [30]. The athletes in the present investigation were competitive bodybuilders with a mean body fat percent of 9.6% at the beginning of the study and 6.5% at the end of the study [30]. It was argued that ghrelin secretion might have reached its limits at some point, and the negative energy balance of more than 900 kcal/day and a significant body weight loss of 2.4 kg in the second 5-week training period (between weeks 6 and 11) were not sufficient to further the significant total ghrelin increase in these athletes [30]. It was concluded that circulating ghrelin levels increase in well-trained bodybuilders with relatively low body fat percent but reach a plateau beyond which there is no further increase in total ghrelin levels, despite continuing negative energy balance and body weight loss [30].

In studies with male rowers, total ghrelin concentrations were measured after a reference week with usual training volume, after 2 weeks of high-volume training and after a recovery week with reduced training volume [114, 115]. In the first study, 90% of the trainings (rowing, running or cycling) were aerobic type of exercise and only 10% resistance type of exercise [114], while in the second study about 50% of the trainings were low-intensity resistance exercise and 50% aerobic type of exercise [115]. It appeared that fasting ghrelin concentrations were not increased as a result of the 2-week period of extended training volume in both studies [114, 115], while a decrease in fasting ghrelin was observed after a recovery week [115]. Although energy intake and energy expenditure increased significantly, the negative energy balance after the 2-week period of high-volume training and energy restriction was about 455 and 408 kcal/day in endurance [114] and concurrent resistance and endurance [115] training studies, respectively. It could be argued that during specific metabolic conditions resulting from the preceding high-volume training period with high energy expenditure, negative energy balance, temporarily restricted caloric condition in fasting state and probably relatively low body energy stores (i.e. low body fat percent) may all contribute to further exercise-induced effects on energy expenditure that leads to downregulation of ghrelin concentration in male rowers [114, 115].

Conclusions and Future Directions

Energy homeostasis is regulated by a neuroendocrine system that also includes different appetite hormones including ghrelin. Ghrelin concentrations decrease during growth and pubertal maturation and are linked to nutritional status, with lower levels in obese and higher levels in underweight individuals. Therefore, basal ghrelin levels are elevated in growing athletes, while pubertal onset decreases ghrelin levels even in the presence of chronically elevated energy expenditure in young athletes. Since increased participation of children in competitive sport is evident, more research on the exercise-induced modification of the appetite hormones including ghrelin is warranted. It has to be considered that in those sport disciplines where heavy training with large energy expenditure starts at a relatively young age, there is a greater risk for developing the female athletic triad already during adolescent period. It can be suggested that growing and maturing athletes should be monitored at short intervals to better understand the influence of high athletic activity on hormonal markers including ghrelin that are involved in overall growth and energy homeostasis. Ghrelin can be used as an indicator of energy imbalance across the menstrual cycle in female athletes. Elevated ghrelin concentrations have been observed in female athletes with chronic energy deficiency, and ghrelin may differentiate between athletes who will or will not develop functional hypothalamic amenorrhea and be at risk for Relative Energy Deficiency Syndrome in Sport (RED-S). In addition, most of the investigations have studied the role of ghrelin in energy availability in different groups of obese individuals, while less studies have been done with athletes to investigate the possibility to use circulating ghrelin as a possible marker of training stress.

The current available information regarding the role of different forms of ghrelin concentrations in energy balance during acute exercise and prolonged training stress is not entirely clear. Acute exercise studies have demonstrated varied responses of different ghrelin forms to the acute exercise in individuals with different body composition and physical activity levels. Various investigations with healthy untrained individuals and also well-trained endurance athletes would suggest that exercise-induced acute negative energy balance may not be sufficient to alter total ghrelin and/or acylated ghrelin response. There are also studies that have argued that a certain threshold reduction in energy availability should be reached before any significant postexercise increases in total and/or acylated ghrelin levels occur and that the amplitude of the ghrelin increase could be linked to the energetic status induced by acute exercise stress and the resting levels of ghrelin in athletes. However, to what extent exercise intensity may influence circulating ghrelin response to acute exercise has not yet exactly been determined, although it has been suggested that low- rather than high-intensity exercise with longer duration stimulates ghrelin response. In contrast, different studies with acylated ghrelin have mostly reported significant suppression in measured acylated ghrelin concentration when performed at higher intensities. Therefore, the transient suppression of circulating acylated ghrelin that can be observed during acute exercise may be entirely unrelated to appetite regulation. The differences in ghrelin responses to acute exercise can be attributed to subject physical fitness, pre-exercise meal consumption and timing, the timing of the hormone measurements as well as sampling processing and assay protocols. Additional research is needed to elucidate the exact mechanisms regulating ghrelin synthesis and clearance during and after acute exercise.

Results regarding the influence of exercise training programme on circulating ghrelin are more consistent and mainly suggest that exercise training per se has no impact on circulating ghrelin levels, and changes in ghrelin concentrations as a result of exercise training intervention take place as secondary to body weight loss. Therefore, the majority of training studies have investigated the responses of total ghrelin concentrations, with relatively less studies measuring acylated ghrelin separately. Typically, circulating ghrelin levels increase with body weight loss and decrease with body weight gain. Data on ghrelin responses to prolonged exercise training are

mainly available from obese individuals, whereas only limited data are provided for athletes. It has been suggested that there is a negative energy balance and/or body weight loss threshold to increase circulating ghrelin concentrations that has not yet been exactly determined. It appears that basal and postexercise ghrelin responses without altering body mass are not sensitive enough to represent changes in training volume and energy availability in athletes. There is also some evidence to suggest that although ghrelin increases together with body weight loss in highly trained athletes with already relatively low body fat mass, there may be a plateau beyond which there is no further increase in circulating ghrelin concentrations despite continuing negative energy balance and body weight loss. Further investigations are needed to describe the exact role of ghrelin at different training conditions in athletes representing different sport events. Collectively, additional research including longitudinal studies in different populations with various body composition and physical activity patterns is warranted to better describe the role of ghrelin and its specific forms in conditions of energy deficiency, surplus and balance.

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13 Hormonal Regulation of Fluid and Electrolyte Homeostasis During Exercise

Charles E. Wade

Introduction

In response to exercise, there are numerous alterations in fluid and electrolyte homeostasis. These perturbations occur immediately upon initiation of exercise and can persist for hours or even days after completion of exercise. The endocrine system plays an important role in the regulation of fluid and electrolyte homeostasis that must occur with exercise. Dysregulation of the endocrine system may limit exercise activity and, in some incidences, result in debilitating morbidities or death. This chapter emphasizes responses to exercise and reviews the importance and factors involved in the maintenance of fluid and electrolyte balance. Previous reviews will be used to address the basics of effected systems; however, emphasis is placed on new data and the current discussions about performance of work and exercise.

The term exercise is an ambiguous term covering a broad range of physical activities. The term is employed to define activities such as running and cycling but is also used to cover the activities of daily living and work. Thus, when discussing responses to exercise, it is important to clarify the type of activity, the level at which it is performed, and the duration. In defining the responses to exercise, it is essential to understand the definitions of workload. The absolute workload is the level of exercise being performed, such as running on a treadmill at a defined speed. For individual subjects, this would produce a variable response depending on their level of fitness/training. Therefore, to compare exercise responses between subjects, relative workload is often employed as a normalization technique [1–3]. Relative workload is expressed as a percentage of the maximum capability of the individual to perform that specific exercise and is often further standardized to the heart rate or oxygen consumption of the subject.

Physiologic Responses to Exercise

A variety of conditions results from alterations in fluid and electrolytes and affects the performance of exercise and work. The disruption of the balance of fluids and electrolytes correlates with limitation of work capacity; however, the range of changes tolerated may be extended with training and repeated exposures. In general the body can undergo one of several responses to exercise: dehydration, dysnatremia, hypovolemia, or hypervolemia. The following text will review each.

Dehydration is defined as a reduction in total body water (TBW) and an increase in plasma

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electrolyte concentrations. Heavy exercise and extreme heat are two of the most prevalent causes of dehydration, as both are associated with exercise and subsequent loss of fluid volume due to sweating and inadequate fluid intake [4–7]. Current evidence suggest that dehydration resulting in a decrease of greater than 2% body mass will adversely affect exercise performance [6–8]. However, decrements in self-paced exercise may not occur until a 4% loss in body weight [9]. Regardless of the level of dehydration, a loss of TBW and an increase in plasma electrolyte concentrations are associated with limited work performance and, in extreme cases, death.

Dysnatremia covers the occurrence of both increases and decreases in plasma sodium observed with exercise [10–14]. Siegel et al. noted an incidence rate of dysnatremia of 32.5% in 1319 collapsed marathon runners [12]. Of these, 85% were hypernatremic and 15% hyponatremic. Both of these conditions have been associated with deaths in competitive runners.

Hypovolemia is a decrease in blood volume in the absence of changes in plasma electrolyte concentrations. This can occur with exercise or hemorrhage [15] and follows periods of water submersion to the neck or the administration of diuretics commonly used in the treatment of hypertension [16, 17]. Hypovolemia necessitates an increase in heart rate at submaximal workloads and a more rapid increase in body temperature, both indicative of limited work performance [7].

In contrast, hypervolemia is the expansion of blood volume. There is extensive literature on the expansion of blood volume by increasing the red cell mass; however, within the scope of this chapter, this term refers to expansion of the plasma volume. Plasma volume is expanded by exercise training and by acute excessive ingestion of fluids, hyperhydration [7, 18–20]. Warburton et al. reviewed the literature on the effect of acute expansion of plasma volume and found minimal increases in maximum oxygen consumption, but there were negligible changes in exercise endurance [20].

Modulation of Hormones in Responses to Exercise

Workload Intensity

The response of hormones to exercise is closely related to the amount of relative work performed. There are three basic patterns of hormones during exercise. The first is an increase proportional to the increase in relative workload. For example, with each increase in workload, there is a constant increase in the plasma hormone concentration of atrial natriuretic peptide (ANP) (Fig. 13.1a). The second pattern is a logarithmic/exponential increase such as that reported for plasma renin activity (PRA) (Fig. 13.1b). With increasing workloads, the level of hormone increases at an exponentially faster rate. The third pattern is related to an onset of an increase at a given threshold; this is observed for vasopressin (Fig. 13.1c). A threshold response for exercise is usually associated with the onset of anaerobic metabolism and a relative workload of about 70%. This has also been associated with the increase in stress-related hormones such as cortisol and adrenocorticotropic hormone (ACTH). These patterns of increased hormone concentrations are consistently observed in studies of acute exercise when the response is expressed relative to the workload of the task performed.

Exercise Duration

The duration of exercise is also a confounding factor in the response of hormones to exercise. Extended time, rather than intensity, may have a greater influence on the levels of hormones during exercise. This is especially true of hormones involved in the regulation of fluid and electrolyte homeostasis. As exercise progresses, there is an increased metabolic heat necessitating sweating and therefore the loss of water and electrolytes. The increase in aldosterone, which regulates sodium balance, is increased twofold with acute maximal exercise (i.e., running on a treadmill) and returns to baseline levels within an hour.

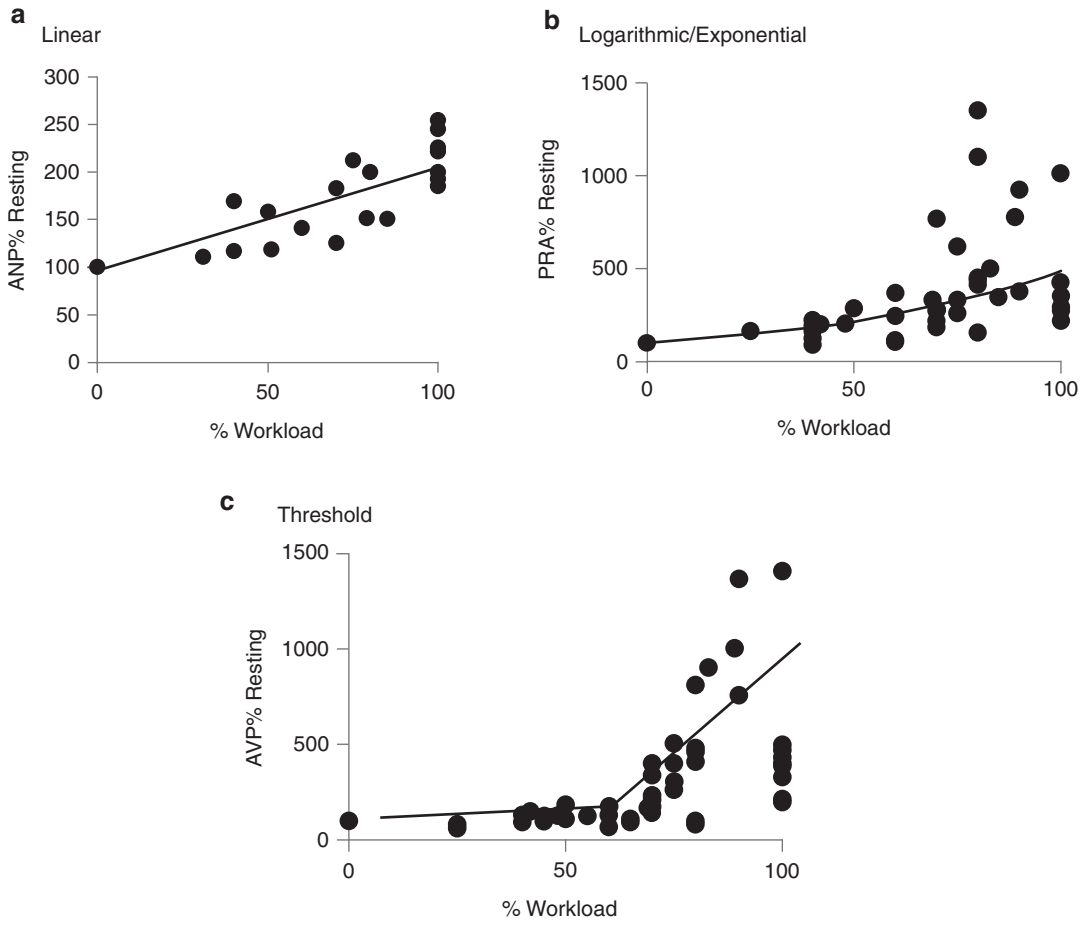

Fig. 13.1 The various patterns of response of hormones to exercise. The individual *dots* represent the response from independent studies of exercise on a cycle ergometer with varying workloads. The variance represents differences in how the exercise was performed, state of hydration of the subjects, and difference in assay techniques. With these confounders the patterns in response to exercise are still present. The linear example is demonstrated by the response of atrial natriuretic peptide (ANP; (**a**) 11 studies), the logarithmic/exponential increase by plasma renin activity (PRA; (**b**) 20 studies), and the threshold response by vasopressin (AVP; (**c**) 23 studies)

Extended exercise times elicit similar changes in plasma volume and sodium concentrations, but aldosterone concentration increases three to four times the basal levels and remains elevated for over 24 h [21]. The greater and enduring response to exercise of longer duration is postulated to be due to additional regulators associated with the "stress" of exercise [10, 22]. Of note, hormone concentrations may vary over time with exercise of long duration, such as during a marathon or ultra-endurance events. For example, ANP is increased by a factor of ten during the first 10 km of a marathon but subsequently decreased to levels only fivefold greater than baseline [23]. In addition, the conditions under which recovery is conducted, access to fluids or cool down exercise, are influential in the postexercise responses and need to be clarified [24, 25]. Recently, Hew-Butler et al. compared the hormonal responses to maximal exercise with a mean duration of 10–60 min of exercise at a treadmill speed equivalent to 60% of the maximum [26]. With maximal acute exercise, significant increases were reported for vasopressin (5-fold) and aldosterone

(2-fold), while at submaximal effort, only aldosterone was increased (3.3-fold). Thus, both the length of time and intensity of the workload must be considered when studying the regulation and function of hormones in response to exercise.

Training

The level of training of a subject may influence the hormonal response to exercise [27, 28]. While much of the variance between subjects at absolute workloads may be due to differences in the relative workload being performed, there are still aspects of training that change the response. Individuals undergoing persistent heavy bouts of exercise training may have alterations in resting levels. In subjects doing daily long-distance runs, plasma aldosterone concentrations are elevated compared to controls [29]. However, for the majority of hormones regulating fluid and electrolyte homeostasis, training does not appear to be as an important of a factor as the intensity and duration of exercise in the response of these hormones.

Hydration Status

The initial hydration status of a subject may influence subsequent responses to exercise. Fluid intake during the performance of exercise is also an influencing factor. Dehydration or hyperhydration alters initial hormone levels; however, the subsequent response to exercise appears independent. Geelen et al. found that following dehydration, ingestion of fluid caused a rapid and pronounced reduction in vasopressin and an increase in norepinephrine that was independent of changes in plasma osmolality and volume [30]. No changes were noted in epinephrine, aldosterone, PRA, or ANP. Additional investigations reported that the greater the volume consumed, the more pronounced the decrease in vasopressin and increase in norepinephrine [24, 31–33]. This suggests an oropharyngeal reflex may be present and mediated by the sympathetic nervous system.

Khamnei et al. evaluated the effect of the combination of exercise and postexercise fluid intake on vasopressin [24, 32]. Subjects exercised at 50% of their maximum oxygen uptake for 30 min. Exercise resulted in a 45% increase in vasopressin which was sustained after exercise in the absence of fluid intake. In contrast, when a large volume of fluid was ingested after exercise, control levels of vasopressin were obtained within 3 min. These findings suggest fluid intake may have a profound effect on hormonal responses during exercise, independent of changes in plasma volume and osmolality. Hew-Butler has put forth the hypothesis that inappropriate increases in vasopressin during prolonged exercise in the presence of adequate fluid intake may be a contributing factor to hyponatremia and subsequent morbidity [10, 22]. This line of research awaits additional well-controlled prospective studies to fully identify underlying mechanisms.

Sex

Sex of the subject is another factor with demonstrated differences. In women, the phase of the menstrual cycle in which exercise is performed may alter the hormonal responses. Resting aldosterone levels are increased during the mid-luteal phase of the cycle, and the response to exercise is amplified [34]. Further work by Stachenfeld and coworkers has demonstrated the effect of progesterone and estrogen on the levels and responses of hormones that are important in fluid and electrolyte homeostasis [35, 36]. In patients with coronary heart disease, basal levels of vasopressin were elevated in men; however, in responses to a 6 min walk test that increased vasopressin, ANP, norepinephrine, and epinephrine, there were no differences between males and females [37]. Following exercise in well-trained subjects to decrease body mass by 3%, women had a lower PRA and faster recovery of aldosterone and slower recovery of vasopressin compared to men [38]. Overall, there are minimal differences reported between male and females in resting hormone levels, and differences in response to exercise are not fully delineated [39].

Health Status

The initial health of the subject is an influencing factor in the hormonal responses to exercise and offers insights to the pathophysiology of various disease processes and in some cases a means of diagnosis and/or rehabilitation. The presence of disease represents a shift in homeostasis that requires alteration in the responsiveness of hormones important in fluid and electrolyte homeostasis. In age-matched subjects, Shim and coworkers reported that subjects with an exaggerated blood pressure response to exercise, which is indicative of a greater risk for hypertension and prevalence of cardiac hypertrophy, had elevated levels of angiotensin II at rest and an augmented increase in response to exercise [40]. However, there were no significant differences in norepinephrine, epinephrine, PRA, or aldosterone at the end of exercise. Kjaer et al. studied patients with congestive heart failure (CHF) and compared them with healthy subjects at 50 and 75% of their maximum workloads on a cycle ergometer [41]. Basal levels of ANP, brain natriuretic peptide (BNP), vasopressin, and PRA were elevated in patients with CHF. In response to exercise, ANP, arginine vasopressin (AVP), norepinephrine, and epinephrine were all increased in both groups. Even though higher absolute levels were observed in subjects with CHF, when expressed as a percent of basal concentrations group, differences were negated. BNP was increased with exercise only in patients with CHF.

Coiro et al. assessed the response of vasopressin to exercise to exhaustion on a bicycle ergometer in subjects with diabetes and controls and further segregated the groups as smokers and nonsmokers [42]. Baseline vasopressin concentrations at rest (2.1–2.6 pg/mL) were not different between groups. In all groups, there was a significant increase in vasopressin in response to exercise. While smoking was not identified as a contributing factor, there was a greater increase in vasopressin in subjects with diabetes (12–13 pg/mL) than controls (7–8 pg/mL). The difference between diabetic and normal subjects could not be attributed to cardiovascular or respiratory responses.

Other Influencing Factors

Other confounders, such as position of exercise and age, have been identified to influence hormonal responses to exertion. Wolf et al. compared supine and upright exercise on a cycle ergometer at a relative workload of 40–50% for 20 min. With supine exercise, the response of PRA and aldosterone to exercise was increased by 90% and 49%, respectively, in contrast to upright exercise [43]. These differences occurred in the absence of difference between the types of exercise in plasma osmolality or blood pressure. Perrault et al. found ANP concentrations to be increased and vasopressin, PRA, and norepinephrine to be reduced, during supine exercise on a cycle ergometer in comparison to exercise in an upright position [44]. During the performance of a marathon, subjects with a mean age of 47 years had an increase in ANP to 104 pg/mL compared to 43 pg/mL in younger subjects with a mean age of 28 years [23]. In addition, differences in hormone concentrations reported in response to exercise may be in part explained by the differing methods of measurement. The presence of such confounders in the comparison of the hormonal responses to exercise has not been systematically addressed, partially limiting our interpretation of the role of hormones in fluid and electrolyte homeostasis during exercise.

Hormone Responses to Exercise

The hormones of consequence to fluid and electrolyte balance in exercising humans are those involved in the regulation of thirst and function of the kidneys and sweat glands. The essential hormones are the catecholamines, vasopressin, the renin-angiotensin-aldosterone system, and natriuretic peptides. While these hormones have a variety of functions, the focus of the present review will be on their responses to exercise and impact on fluid and electrolyte homeostasis during and following exercise. Circulating levels of these hormones are altered during exercise as a function of changes in secretion,

metabolism, and volume of distribution. The most common measurement of these hormones in association with exercise is the circulating concentrations, which will be the focus of the present effort.

Catecholamines

Catecholamines, specifically norepinephrine and epinephrine, are derived from increases in sympathetic nervous system activity and the adrenal glands [45, 46]. The kidneys are also suggested as a source of norepinephrine [47]. Levels of circulating catecholamines respond rapidly upon the onset of exercise in order to redistribute blood flow to meet metabolic demands [2, 48, 49]. In response to exercise, there is a progressive increase in circulating norepinephrine levels from 1.3 to 3.0 nmol/L at rest to 12.0 nmol/L following maximal exercise [45, 50–52]. The increase in epinephrine occurs later in the course of exercise and can rise from resting levels of 380–655 pmol/L to concentrations over 3000 pmol/L. The increase in the ratio of norepinephrine to epinephrine demonstrates activation of the sympathetic nervous system and is attributed to active spillover from the muscles during exercise [45, 52–54]. With continued exercise, there is an attenuation of the increase in the ratio of norepinephrine to epinephrine, which is indicative of an increase in the release of epinephrine predominately from the adrenal medulla under the control of hypothalamic mediation in addition to the sympathetic nervous system. Following exercise, plasma levels of catecholamines return to resting levels in a matter of minutes, as they have a short half-life due to degradation and reuptake by the sympathetic nervous system. Recent studies that inhibited the reuptake of norepinephrine have demonstrated an increase in the time necessary to complete work equal to 30 min of exercise at 75% of maximal workload [55, 56]. These studies suggest clearance from the circulation of norepinephrine plays a role in fatigue.

Vasopressin

AVP is also known as vasopressin or antidiuretic hormone (ADH). It is a neurohypophysial hormone synthesized in the hypothalamus and stored in the posterior pituitary [57, 58]. Vasopressin is a pressor that alters peripheral resistance, but its greatest effect is on the reabsorption of water in the collecting tubules of the kidneys. Secretion of vasopressin is regulated by alterations in plasma osmolality and blood pressure. Circulating concentrations of vasopressin in humans are 1–4 pg/mL [57, 59–62]. With maximal exercise, vasopressin concentrations of 4–24 pg/mL are reported. Maximum conservation of water by the kidneys is observed at vasopressin levels of 10–20 pg/mL. With progressive increases in exercise, elevation of vasopressin is not observed until 70% of maximum workload is attained, i.e., the anaerobic threshold (Fig. 13.1c). Animal experiments have demonstrated an increase in activation of hypothalamic neurons that is indicative of increased vasopressin content (production) and of performing above the anaerobic threshold [63]. Thus, the response of vasopressin appears to be associated with the onset of anaerobic metabolism, which is also related to increases in "stress hormones" such as cortisol and ACTH. An increase in vasopressin may persist for over 60 min after exercise or longer if access to fluids is restricted. Of note, at low workloads of about 25% of the anaerobic threshold, vasopressin decreases have been reported.

A variety of factors have been demonstrated to mediate the increase in vasopressin with exercise, including the increase in osmolality and reduction in intravascular volume; however, the increase in plasma osmolality appears to be the primary mediator (Fig. 13.2) [59, 64, 65]. In subjects exercising at 65% of maximum while running on a treadmill, there was a progressive increase in vasopressin with progressive workloads [66]. In subsequent tests which involved dehydration that decreased body weight by 3 and 5%, resting vasopressin levels were increased in association with the decrease in blood volume; however, in response to exercise, further increases

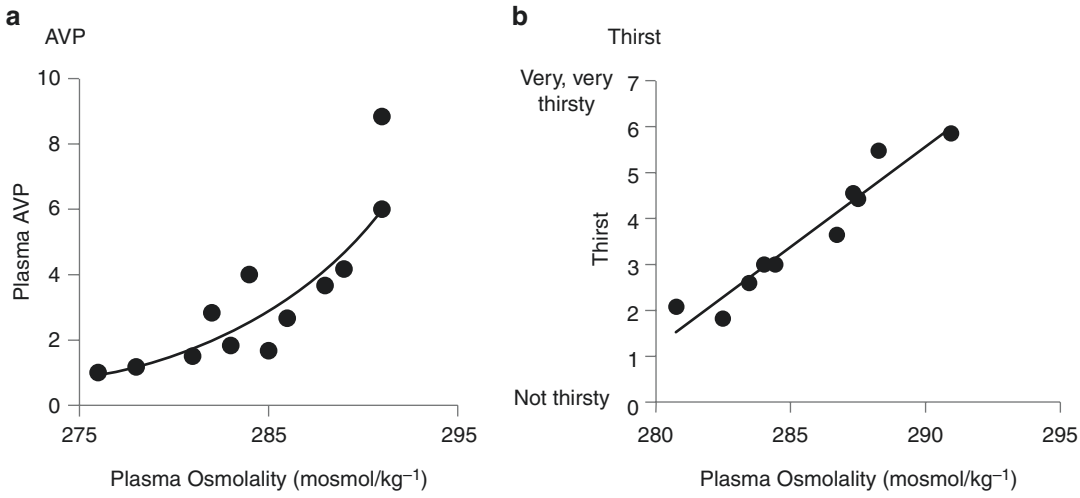

Fig. 13.2 (**a**) Levels of vasopressin and (**b**) subjective assessment of thirst in association to plasma changes in osmolality during moderate exercise. Measurements were from subjects with different levels of fitness, under various levels of hydration. (Redrawn from Merry et al. [27])

in vasopressin were related to the magnitude of the increase in osmolality. Brandenberger et al. evaluated rehydration during exercise giving subjects no fluids, water, or an isotonic solution. Intake of water reduced osmolality but did not alter plasma volume [67]. Consumption of the isotonic solution did not change osmolality but increased plasma volume. Both methods of rehydration decreased the rise in vasopressin levels with exercise, as well as those of PRA and cortisol. Others have reported similar findings [32, 44, 68]. The independence of the increase in osmolality and blood volume, and the regulation of vasopressin in response to exercise, is similar to that reported with dehydration. Coiro and colleagues have demonstrated that the increase in vasopressin during exercise to exhaustion may be attenuated by blockade of *5-HT3* serotonergic receptors and administration of somatostatin, supporting another means of mediating the increase in vasopressin during exercise [69]. Recently, Hew-Butler et al. have questioned the relationship of vasopressin and plasma osmolality during exercise. In subjects participating in an ultramarathon, they observed 3.9-fold increase in plasma vasopressin, no significant change in plasma sodium, and a significant decrease in plasma volume [10, 22]. They also evaluated cyclists during a 109 km race and observed nearly identical changes [70]. In subjects participating in an ultramarathon, they observed a 3.9-fold increase in plasma vasopressin in the absence of a significant change in plasma sodium though plasma volume was significantly decreased. These authors and others hypothesize that under conditions of prolonged exercise, the osmotic regulation of vasopressin is overshadowed by non-osmotic stimuli, of which, the reduction in blood volume plays a minor role [14, 71, 72]. The increase in AVP was associated with elevations in cortisol, oxytocin, and BNP, which underscores the relationship of AVP release with "exercise stress." Irrespective of the means, vasopressin is elevated by more than fourfold during acute exercise to exhaustion or intense prolonged exercise.

Renin-Angiotensin-Aldosterone

The renin-angiotensin-aldosterone systems are closely coupled and increased in response to exercise. Renin is released from the kidney in response to sympathetic nerve stimulation, as well as norepinephrine spillover, resulting in

increased plasma concentrations [17, 45, 52, 73–76]. Renin then converts angiotensinogen to angiotensin I, which is subsequently transformed to angiotensin II in the lung. Angiotensin II promotes the release of aldosterone from the adrenal gland.

With exercise, all aspects of this system are increased and play a variety of roles in the regulation of fluid and electrolyte homeostasis [3, 45, 60, 77, 78]. At rest, PRA has levels in the order of 0.15–0.55 ng angiotensin I/mL/h and with maximal exercise increases to levels of 1.11–1.67 ng angiotensin I/mL/h. There is an exponential increase in renin activity with increasing workloads; significant differences are reported at levels of 60–70% of maximum (Fig. 13.1b). The increase in PRA with exercise is positively associated with the increase in angiotensin II. Basal levels of angiotensin II are 15–25 ng/L, with values of 130–160 ng/L achieved with maximal exercise. Aldosterone release is regulated by angiotensin II, as well as ACTH and the plasma levels of sodium and potassium. Aldosterone concentration increases from resting levels of 80–830 pmol/L to concentrations of 250–3330 pmol/L with maximal exercise. Blockade of the conversion of angiotensin I to angiotensin II does not attenuate the response of aldosterone to maximal exercise, which supports the theory that other pertinent regulatory factors are involved [79, 80]. The elevation of aldosterone may persist for days after exercise, and levels are dependent upon the sodium and water intake [21]. In the postexercise period, the increase in aldosterone may be the product of increased water intake, which reduces the plasma sodium concentration or the persistent elevation of aldosterone—which is due to activation of the ACTH. Irrespective of the cause, the increase in aldosterone due to exercise plays a role in the conservation of sodium in the sweat glands and kidneys (Fig. 13.3).

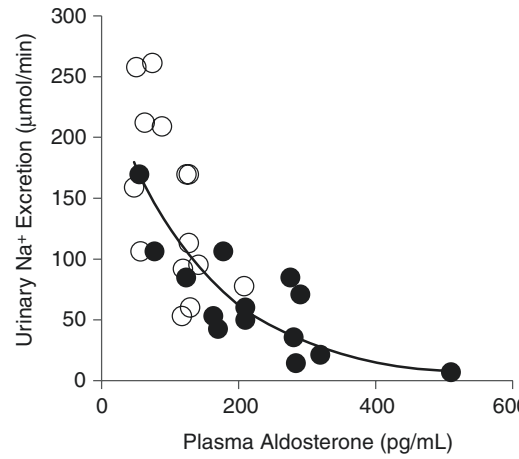

Fig. 13.3 Plasma aldosterone concentrations were compared to the urinary excretion of sodium at the end of a 2 h run (*closed circles*) and following 48 h of recovery with food and water ad libitum (*open circles*). With exercise, there was an increase in aldosterone, and over the recovery period, there was a decrease. (Adapted from Wade et al. [29]))

Natriuretic Peptides

Peptides demonstrated to elicit a natriuresis have been deemed natriuretic peptides. These include ANP, BNP, urodilatin, and adrenomedullin. These peptides appear to participate in the regulation of fluid homeostasis by protecting against volume and pressure overloads. Though these peptides have been extensively studied over the past 30 years in patients with disease such as heart failure, pulmonary hypertension, and chronic renal disease, their response to and role during exercise are not well defined. Additionally, well-designed studies in control subjects or during competitive events have yet to be undertaken.

Atrial Natriuretic Peptide

ANP is increased with exercise in a linear response (Fig. 13.1a). Resting plasma levels of 10–49 pg/mL are increased to over 100 pg/mL with acute maximal exercise [22, 25, 39, 44, 81, 82]. In response to long-duration exercise, there is initially a pronounced increase, a subsequent fall, and then a re-elevation of levels, persisting until completion of the exercise [23]. Resting levels are obtained within hours of cessation of the activity [77]. The primary stimulus for the increase in ANPwith acute exercise is an increase in atrial stretch due to an increase in venous return [62]. However as exercise progresses, atrial pressure decreases as blood flow is

redistributed (cardiovascular drift) to meet the metabolic demand of active tissues and to dissipate the thermal load [48, 49]. The response of ANP to extended exercise may be increased if water is ingested, suggesting a fluid volume change directly on the heart mediating release [83, 84]. Recently, pronounced increases in ANP with exercise have been associated with increases in cardiac troponin levels, suggesting myocardial damage during heavy exercise could be a contributing factor to increases in ANP [85]. In cardiac transplant patients, ANP levels are elevated, and the response to exercise is accentuated. This suggests that in normal subjects with naturally innervated hearts, there may be neural inhibition of ANP release [44, 86, 87]. Support for this hypothesis is the observation in patients with hypertension that chronic beta-blockade substantially increases the ANP response to exercise [88]. Sodium intake appears to also affect the ANP response to exercise [89, 90]. During submaximal cycle ergometer exercise when subjects were on a low-sodium diet, ANP increased from 42 to 59 pg/mL, in contrast to a high-sodium diet where the increase was from 72 to 119 pg/mL. Thus, the increase in ANP with exercise appears to be related to a number of factors: stretch of the atrium due to volume changes, neurological inputs, and sodium intake.

Brain Natriuretic Peptide

BNP, as its name implies, was first identified in the brain and subsequently identified in other tissues, specifically in the heart [91, 92]. BNP is collocated with ANP in the heart and appears to have similar paths of regulation and actions. BNP is not consistently altered in normal subjects in response to acute exercise [41, 83, 93–95]. However, with long-duration exercise, such as a 100 km ultramarathon, BNP levels were increased from resting values of 3.3–18.8 fmol/mL at the end of the race. The response of BNP to exercise is altered by a number of conditions [96]. When subjects performed submaximal exercise on a low-sodium diet, an increase in BNP was not noted; however, on a high-sodium diet, a significant increase was seen. A similar finding was reported with the presence or absence of fluid intake in the course of exercise [83]. If subjects did not ingest water, there was no response to exercise, but if fluid was provided, BNP was increased with exercise. In hypertensive subjects, the increase in BNP with exercise was the same with or without beta-blockade, in contrast to the greater increase in ANP with beta-blockade [88]. This suggests that while similar mechanisms, such as atrial stretch, fluid intake, and sodium status, modify the response of both BNP and ANP to exercise, the neurological component present in the regulation of ANP is not an important factor for BNP.

Urodilatin

Urodilatin, a natriuretic hormone derived in the kidneys, has been suggested to play a role in the renal handling of sodium [97, 98]. Schmidt et al. assessed the response of urodilatin and ANP during bicycle ergometer exercise at 60% of maximum for 1 h [99]. Plasma ANP concentrations increased, and the excretion of urodilatin decreased; i.e., the hormones had a negative correlation. The decrease in urodilatin was associated with a reduction in the percent of the filtered sodium load excreted. As urodilatin increased, the amount of sodium lost also increased. These findings suggest a possible role in the regulation of sodium homeostasis during exercise that needs to be investigated further.

Adrenomedullin

Adrenomedullin is reported to have natriuretic and diuretic effects. Adrenomedullin is produced in the vascular endothelium and in smooth muscle cells. In humans, plasma concentrations are responsive to changes in blood volume [100, 101]. Furthermore, changes in adrenomedullin are correlated with changes in ANP and BNP in patients. In normotensive subjects, adrenomedullin concentrations in response to submaximal exercise of short duration were not altered, even though ANP and BNP levels were increased. In contrast, during maximal exercise, Tanaka and colleagues found adrenomedullin to be increased by 45% compared to at rest and to be negatively associated with systolic blood pressure [102]. Piquard et al. also reported that with acute maximal

exercise, adrenomedullin increased from resting levels of 15–29 pmol/L at the end of exercise [103]. Yet others have found adrenomedullin to be increased with submaximal exercise and decreased with maximal exercise [104, 105]. Therefore, further investigation is warranted to elucidate the responses and actions for adrenomedullin during exercise.

Fluid and Electrolyte Regulation

The management of fluids and electrolytes is a careful balance between loss of salts and water through sweat, shifts between body compartments, and conservation by the kidneys and replenishment through ingestion [106]. While some losses are tolerated during exercise, once critical levels are exceeded, there are decrements in performance. In order to avoid these reductions, a series of compensatory mechanisms are activated that have to work in concert to maintain the milieu, to optimize performance, and to avoid subsequent morbidities and mortality.

Total Body Water

During exercise there is a loss of TBW, predominately via sweating and in part from increased respiratory loss. The reduction of TBW is tolerated until a critical level is attained. The loss of TBW during exercise is equivalent to the reduction in total body mass over the period of exercise performance. Though this assumption has been questioned, there is still a strong relationship between the decrease in TBW and body mass [107–109]. During long-duration exercise, the reduction in TBW may exceed 5% of body mass. In a 70 kg person, this would equate to fluid loss of 3000–4000 mL [6, 8, 110]. In laboratory experiments, a reduction of more than 2% body mass has been shown to decrease performance [110]. In contrast during competitive endurance events, a reduction of greater than 4% body mass was demonstrated to have a decrement in performance [9]. Of note, even with free access to water, a loss in TBW during exercise is observed. This water loss, in the presence of fluids to ingest, is referred to as voluntary dehydration [18, 111]. Voluntary dehydration represents about 20–30% of the total loss of body water during an activity, as 70–80% is replaced by supplemental intake over the period of exercise. During a marathon the average body mass loss was 2.3% even though fluids were available. Interestingly, in subjects finishing under 3 h, the loss was 3.1%, from 3 to 4 h 2.5%, and over 4 h 1.8% [112]. The ability to tolerate a greater decrease in TBW was inversely associated with finish time. These observations suggest that individuals who are successful in these events are able to tolerate a greater TBW loss and still perform at a high level. The loss of fluids sustained in the course of exercise is usually replaced in the subsequent 24 h [21, 29, 60, 113]. Irrespective of the TBW loss tolerance, at some point the loss of TBW will impact the performance of an individual.

The loss of TBW during exercise is not equally distributed throughout the body or between body fluid compartments. Over the course of exercise, there is a redistribution of fluids among the various compartments of the body, with a pronounced reduction in plasma volume [59, 61, 65, 114]. The reduction in plasma volume during maximal acute exercise is 8–12%, resulting in a 5–7% decrease in blood volume. This shift of fluids from the vascular space to the extravascular space has been attributed in part to increases in endothelial permeability, which could possibly be modified within specific tissues by angiotensin II, vasopressin, and norepinephrine [115–117]. The decrease in blood volume is compensated for by an increase in cardiac output and a redistribution of blood flow [48, 49, 118, 119]. During the performance of exercise, the redistribution of fluids within the vascular compartment is required to meet the metabolic demands of active tissues and to dissipate the thermal load resulting from the increase in metabolism. This redistribution of flow is the result of increases in local vascular resistance, which is in part due to hormonal regulation, predominately by catecholamines, angiotensin II, and vasopressin.

Sweating

The principal means of fluid and electrolyte loss during exercise is in sweat. Sweating is essential to dissipate the increased thermal load incurred by the elevation of metabolism with exercise [120]. The density of sweat pores is highly variable among subjects, as is the magnitude of sweat produced due to the subjects' level of training and prior adaptation and acclimation to a hot environment [90, 120–123]. The rate of fluid loss by sweating can be as high as 1500 mL/h [6, 18, 108, 124]. The magnitude of fluid loss in sweat is hormonally mediated by vasopressin [125, 126]. Circulating levels of vasopressin are positively associated with the rate and composition of sweat during exercise. The rate of sweating during exercise is coupled with the changes in plasma osmolality and volume, the primary mediators of vasopressin; thus, it has been difficult to separate cause and effect [118, 119, 127, 128]. However, local subcutaneous injection of vasopressin alters the rate and composition of sweat from glands exposed to an increase in local skin temperature [129]. Plasma vasopressin concentrations have been associated with sweat sodium concentration and osmolality, suggesting vasopressin promotes water conservation in the sweat gland [125, 130]. In addition, studies involving a possible role of catecholamines on sweat rate have resulted in conflicting findings [131, 132]. However, in a study of the effect of fluid intake, it was shown that the ingestion of a large volume, >3 L, was associated with an increase in sweating, reduction in plasma concentration of norepinephrine, and an increase in skin blood flow. In contrast, the opposite effects were seen with ingestion of a small volume, >0.5 L, during long-duration submaximal exercise in the heat. Therefore, an increase in catecholamines appears to be associated with a decrease in skin blood flow that results in a decrease in sweating.

Sweat is composed of a significant amount of electrolytes [90, 120, 121, 133, 134]. Thus, during exercise the predominate means of the loss of electrolytes is through sweat. The concentration of sodium in sweat ranges from 20 to 135 mmol/L, potassium from 3 to 35 mmol/L, and chloride from 10 to 100 mmol/L, in contrast to "normal" plasma concentrations (sodium 135–145 mmol/L, potassium 3.5–5.0 mmol/L, and chloride 96–106 mmol/L) [135]. While the levels of electrolytes in sweat are lower than in plasma, the losses are significant. At a sweat rate of 1.5 L/h at a sodium concentration of 60 mmol/L, a total of 90 mmol would be lost or 3% of total body sodium. As noted above, however, the concentrations of electrolytes in sweat are highly variable. Electrolyte concentrations of sweat are decreased as a result of training and heat acclimation [65, 90, 121]. The lower concentrations reduce the tonicity of the sweat and therefore facilitate evaporation and cooling. In a comparison of 10 min of acute maximal exercise to 60 min of submaximal exercise (60% of maximum workload), minimal differences in the electrolyte concentrations were noted: sodium 70 vs. 77 mmol/L, potassium 7.7 vs. 4.8 mmol/L, and osmolality 171 vs. 172 mOSM/L for maximal and submaximal exercise, respectively. The reductions in the sodium concentration of sweat appear to be in part mediated by aldosterone [121, 136].

Fluid and Electrolyte Intake

Consumption is the primary means of replacing the fluid and electrolytes losses incurred during the course of exercise [18, 137, 138]. In the performance of long-duration exercise, 80% of the fluid lost in sweat is replaced by voluntary ingestion if free access to fluids is provided [108, 137]. The extent to which volume losses are replaced is dependent upon the composition of the ingested fluid [137–141]. In humans during extended exercise, the volume of fluid replacement appears to be closely regulated. In contrast, the replacement of electrolytes does not appear to be as closely titrated and is a by-product of normal nutrient intake. Takamata et al. suggested that 6–24 h after heavy exercise, salt appetite is increased in association with a decrease in plasma osmolality and sodium concentrations resulting from fluid intake [113]. Leshem et al.

monitored salt intake after exercise and found a voluntary increase of 50% in the amount of salt added to food [142]. Passe et al. assessed the acceptance of hypertonic saline fluids during exercise and reported an increase in palatability of a 60 mmol/lL sodium solution, suggesting a relationship between sensory reception, hedonic response, and drink composition in the replacement of electrolytes post exercise [143]. Replacement of electrolytes may be coupled with hunger and increase in salt appetite. In animal models salt appetite is strongly associated with angiotensin II; however, this proposed relationship has yet to be definitively demonstrated in humans [11, 144, 145].

As previously noted, the replacement of fluids is closely controlled over the course of exercise and thus readily adjusted for following exercise. This tight regulation is modulated by thirst, the subjective sensation to seek and drink fluids [144–147]. The subjective sensation of thirst can persist for hours after exercise [113]. As described earlier there is a level of voluntary dehydration that can be tolerated in the performance of long-duration exercise, but the majority, about 80%, of the fluid loss is replaced by drinking. The residual loss associated with the level of voluntary dehydration is usually replaced within 24 h [21, 29, 113]. This process is associated with a variety of factors, such as the increase in plasma osmolality and reduction in blood volume, both of which are closely tied to the regulation of numerous hormones. Immediately after exercise Takamata et al. found the subjective evaluation of thirst to be immediately reduced upon ingestion of fluids yet increased hours later in spite of plasma osmolality being reduced [113]. This increase in thirst was associated with an elevation of aldosterone and presumably angiotensin II [91, 147]. If the replacement fluid is water, plasma osmolality and sodium concentration can be decreased before blood volume loss is corrected, thus presenting conflicting regulatory mechanisms resulting in a reduction in thirst [148, 149]. Merry et al. reported the subjective sensation of thirst to be associated with an increase in osmolality during moderate exercise under various levels of hydration in subject with different levels of fitness (Fig. 13.2) [27]. Osmolality was also related to an increase in vasopressin, suggesting a possible association between vasopressin and thirst. Keneflick et al. assessed the response of thirst during 1 h of walking at 50% of maximum on a treadmill in temperate (27 °C) or cold (4 °C) environments [150]. In the cold environment, the sensation of thirst was reduced by 40% and associated with lower levels of vasopressin, even though plasma osmolality was increased. The authors speculated that peripheral vasoconstriction increased central blood volume that was sensed as an actual increase in blood volume. This hypothesis is supported in part by the observation that immersion and dehydration, which increase and decrease central blood volume, respectively, alter thirst via volume-induced stimulation of the cardiopulmonary baroreceptors. Stimulation of these baroreceptors by an increase in volume results in decreased vasopressin and PRA and increased ANP [151]. In contrast dehydration causing a reduction in volume elicits the opposite responses [33]. The specific roles of these hormones in the regulation of thirst during and following exercise have yet to be clearly defined.

The ingestion of fluid during the performance of exercise has been advocated to sustain performance [4, 6, 110]. To determine fluid replacement by water ingestion during exercise, Robinson et al. had subjects perform two bouts of exercise, one with and another without fluids, on a cycle ergometer for 1 h at 85% of their maximum oxygen uptake [133]. The subjects ingested 1.5 L of water to replace the fluid loss due to sweating, which resulted in a 60% decrease in the loss of body mass. The ingestion of fluid did not alter sweat rate, the increase in body temperature, or perceived exertion. Though plasma osmolality and sodium concentrations had a greater increase in the absence of water intake, no differences in vasopressin or angiotensin II were reported. These findings were confirmed by McConell et al. who stated that ingestion of fluids had little benefit on exercise of 1 h [152]. However, others have consistently

shown hypohydration to impair performance. There is an absence of data as to whether someone exercising should drink "as much as tolerable," "to replace the weight lost during exercise," or "ad libitum"; thus, Noakes et al. had also questioned the effects of fluid hydration during exercise [153]. The role of hormones in this debate is even more difficult to evaluate. Rehydration is shown to attenuate the response of atrial natriuretic hormone, vasopressin, and PRA to exercise [24, 32, 66]. Furthermore, the role of these hormones in the modulation of thirst during exercise is confounded. At present the data supports maintenance of an adequate hydration status to avoid the adverse effects of dehydration. The means of achieving this, and the levels needed, have yet to be defined.

In light of the present state of data in this area, an understanding of the function of hormones in the regulation of thirst is essential. Hew-Butler has reviewed the role of vasopressin in fluid balance and its possible role in dysnatremia, specifically exercise-associated hyponatremia [10]. Hyponatremia with exercise may result from water retention associated with excess fluid intake, sodium loss predominately via sweat, or more likely a combination of these factors. Put forth is the hypothesis that non-osmotic-mediated AVP release from the pituitary increases circulating levels of vasopressin leading to retention of water, even if fluid intake does not exceed recommended guidelines. This inappropriate fluid retention/overload could be a contributing factor of hyponatremia and its subsequent sequelae. The efforts from this group, in the lab and in the field, provide insights as to the contribution of vasopressin and other hormones to the regulation of fluid and electrolyte homeostasis [10, 22, 26, 70, 71].

Renal Function

The action of hormones in the regulation of kidney function is well defined due to their role in the pathophysiology of hypertension. While extensive studies have been directed at the study of hormones on kidney function during exercise, the contribution of the kidneys to fluid and electrolyte balance is limited [59, 60, 154–157]. Zambraski described the limited contribution of the kidney noting that in a normal individual, the kidneys produce about 1 mL of urine a minute or 60 mL/h [53]. This is in comparison to the loss of fluid from sweat on the order of 1000–1500 mL/h, during moderate to heavy exercise. Zambraski estimated that during exercise the renal conservation of water would only account for 4% of the loss of water and about 8% for the sodium [53]. Thus, the conservation of fluid by the kidney is hampered by the limited amounts of water and electrolyte excreted in the basal state. Nevertheless, the hormonal influences on the kidney provide insights into their role in the overall maintenance of fluid and electrolyte homeostasis during and following exercise [53, 60, 158].

Renal Blood Flow

At rest the kidney receives about 20% or approximately 1000 mL/min of the overall cardiac output. During exercise renal blood flow is reduced in relation to the intensity and duration of exercise. With mild to moderate exercise (50–70% of maximum workload), there are negligible changes, but with maximal exercise flow is decreased by 40–60% from the normal [45, 48, 53, 158–160]. The reduction in renal blood flow persists for over 1 h after completion of the exercise. This reduction is caused by vasoconstriction of afferent arterioles, associated with an increase in sympathetic nerve activity and circulating levels of norepinephrine derived from spillover from the kidney [45, 47, 53, 159, 161]. In animal models upon initiation of exercise, there is an immediate reduction in renal blood flow which increases over time to a steady state associated with the level of exercise [162]. This immediate decrease suggests the predominance of the neural regulatory component in the initial phase of exercise. The reduction in renal blood flow decreases the volume of fluid and electrolytes delivered to the glomeruli of the kidney and in turn contributes to regional shifts in renal blood flow within the kidneys.

Glomerular Filtration Rate

The amount of fluid moving across the membrane of the glomeruli of the kidney is termed the glomerular filtration rate. The movement of fluid is the product of the drive pressure across the membrane and oncotic pressure of the plasma. As noted above there is an increase in afferent arteriole resistance with exercise; however, this is accompanied by an increase in efferent arteriole resistance facilitating filtration. The increase in efferent arteriole resistance is controlled by angiotensin II. Changes in the rate of glomerular filtration are related to the intensity and duration of exercise and may persist for up to 24 h after exercise [163, 164]. Minimal changes in filtration are observed with exercise of less than 50% of maximum. With acute maximal exercise or long-duration exercise above 70% of maximum, the rate of filtration may be decreased by 50–70%. With heavy exercise there is also an increase in the permeability of the glomerular membrane as demonstrated by the occurrence of an increase in protein excretion [53, 165]. This alteration of permeability is suggested to be in part mediated by norepinephrine, vasopressin, and angiotensin II and results in an increase in the excretion of protein [53, 163, 166, 167].

Urine Flow Rate

Urine flow rate is the product of the amount of fluid filtered (glomerular filtration rate) and the net reabsorption of fluid in the tubules. With exercise of low intensity, there is either no change or a slight increase in urine flow rate [39, 155]. With acute maximal exercise or long-duration exercise eliciting voluntary dehydration, urine flow rates are decreased by 20–60% of the normal basal levels of 0.8–1.2 mL/min [53, 59, 60]. This minimal decrease results in the conservation of water in light of the losses due to sweating. The decrease in the amount of filtered water is predominately due to vasoconstriction of the afferent arterioles caused by norepinephrine [45, 48, 131, 161]. Exercise also causes an increase in the osmolality of urine, indicative of an increase in the reabsorption of water [57–59]. However, decreases have been reported in urinary osmolality indicative of an increase in free water clearance during heavy exercise [53, 59]. Therefore the role of vasopressin in the control of water reabsorption in the collecting tubule during exercise has been questioned. There may be inhibition of vasopressin or the possibility of a "washout" of the osmotic gradient in the medullary area of the kidney due to the redistribution of blood flow associated with the actions of angiotensin II. After exercise the reduction in urine flow persists and may contribute to the rectification of fluid loss along with increased drinking [21, 113].

Renal Handling of Electrolytes

At the normal rate of glomerular filtration, the amount of fluid equivalent to the TBW is filtered in 5–6 h. The filtrate contains electrolyte concentrations equivalent to those of plasma. Over the course of traversing through the kidneys, 80–99% of the filtered load of electrolytes is reabsorbed. This reabsorption is hormonally mediated for sodium and establishes an electrochemical gradient for the handling of other electrolytes and an osmotic gradient for the handling of other solutes. With acute exercise, the decrease in the excretion of electrolytes is predominately due to the reduction in glomerular filtration rate [21, 113, 156]. During and following long-duration exercise, the reabsorption of sodium is regulated by aldosterone [21, 113]. With daily heavy exercise, there is a persistent increase in aldosterone, which is strongly associated with an increase in the reabsorption of sodium (Fig. 13.3) [21].

In summary, with exercise, kidney function changes and is regulated by a number of hormonal systems. The major alterations effecting fluid and electrolyte homeostasis are a decrease in renal blood flow and an increase in the reabsorption of sodium. There are several fallacies as to the contribution of these changes in kidney function to the net maintenance of fluids and electrolytes. The primary misunderstanding is the quantitative contribution of the kidney to fluid balance and the roles of hormones in these changes.

Summary

Exercise elicits increases in a number of hormones important in the regulation of fluid and electrolyte homeostasis. The action of these hormones may persist for hours and days after completion of the exercise. While increases in hormone levels are noted, the regulation and actions of these hormones are often not well defined, specifically in relation to the changes in fluid and electrolyte balance during exercise. There are issues as to the influence by the type and duration of exercise on hormonal responses that are not often accounted for. Recent efforts employing multifactorial analysis are just beginning to define some of these factors. In addition, the role of hormones in the etiology of the detrimental effects of exercise, such as dehydration and dysnatremia, is beginning to be addressed. Finally, evidence is mounting to show that exercise plays a vital role in fluid and electrolyte homeostasis. Observations of the hormonal responses to exercise will lead to a better understanding of both exercise physiology and related disease processes.

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14

Hormonal Regulation of the Positive and Negative Effects of Exercise on Bone

Whitney R.D. Duff and Philip D. Chilibeck

Introduction

Adequate levels of physical activity are important for bone health. Proper exercise training increases bone mineral density and prevents osteoporosis and fractures [1]. Too much exercise without adequate energy replacement may however negatively affect hormone status (especially reproductive hormones), and this may have a negative impact on bone health. The intent of this chapter is to cover this spectrum of effects of exercise on bone physiology and health.

The "Negative Effects of Exercise on Hormonal Regulation of Bone" section of this chapter covers the negative effects of high levels of exercise, especially without adequate dietary energy or calcium intake, on sex hormone (i.e., estrogen, testosterone) and calciotropic hormone (i.e., parathyroid hormone, calcitonin, and vitamin D) levels and how this can negatively impact bone health.

The "Positive Effects of Exercise on Hormonal Regulation of Bone" section of this chapter covers how proper exercise training may be complimentary with sex hormones or may enhance anabolic and calciotropic hormones to improve bone health. Exercise may have additive or synergistic effects for increasing bone density when combined with estrogen replacement therapy in postmenopausal women. Although studies are mixed, exercising may induce acute increases in release of anabolic hormones (i.e., testosterone, growth hormone, insulin-like growth factor-1) or may alter release of calciotropic hormones (i.e., decrease parathyroid hormone, increase calcitonin and vitamin D). This may lead to changes in basal levels of these hormones with chronic training and improvement in bone health.

Negative Effects of Exercise on Hormonal Regulation of Bone

Negative Effects of Exercise on Reproductive Hormone Status

Estrogen and Progesterone

Estrogen and progesterone are important sex hormones for maintenance of bone health in women. Estrogen increases intestinal absorption of calcium [2] and decreases activity of bone-resorbing cells (osteoclasts) [3], whereas progesterone may positively affect cells involved in bone formation (osteoblasts) [4]. Excessive exercise without adequate energy replacement may have a negative impact on these hormones, resulting in amenorrhea, and negative impacts on bone health [5].

Athletic Amenorrhea Athletic amenorrhea (i.e., cessation of menses in premenopausal women) is most common in sports that require lower body mass [2–4], including long-distance

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running [5] and rowing [6], and activities that involve subjective judgment [7], such as gymnastics [8], figure skating [9], and ballet [10]. Athletes in these sports may develop disordered eating in an attempt to maintain the lower body mass required. The "female athlete triad" was proposed as a diagnosis when disordered eating was present with amenorrhea and osteoporosis [7, 11]. Now considered a spectrum ranging from normal to varying degrees of pathology of the three components, the female athlete triad can be diagnosed when low energy availability (with or without disordered eating) is present along with a dysfunction in menstrual function and/or low bone mineral density [7, 12]. The modifications to the diagnoses guidelines increased overall prevalence of the disorder, although total prevalence is still unknown [4, 7].

Amenorrheic athletes have lower bone density than not only their eumenorrheic counterparts but also sedentary controls and have a two- to four-fold higher stress fracture rate [13, 14]. For some athletes however, high intensity of exercise, especially activities that involve high-impact forces or high strains on bone due to muscle pull, may offset some of the negative effects of amenorrhea. This may be the case in gymnasts [8, 15–17] and rowers [6]. Amenorrheic dancers may [18] or may not [10] have an elevated bone density at weight-bearing sites, but have an increased incidence of stress fractures [19]. Longer durations of menstrual dysfunction in dancers equate to larger deficits in bone density at the spine [14, 20, 21], with scoliosis forming in some [19]. Amenorrheic runners or endurance athletes generally have decreased bone density, even at weight-bearing sites [22–26], and this is associated with higher prevalence of stress fracture [27–31]. Weight-bearing exercise may therefore be of sufficient impact to be protective in gymnasts, but not in dancers and runners when normal menstruation is not maintained [26, 29].

Etiology of Athletic Amenorrhea Athletic amenorrhea has been attributed to increased cortisol levels due to chronic exercise stress or an imbalance between energy expenditure and energy intake (i.e., decreased energy availability), leading to reduced gonadotropin-releasing hormone pulse generation at the hypothalamus, subsequent decreased release of follicle-stimulating and luteinizing hormone from the anterior pituitary during the follicular phase, and reduction in the production of estrogen and progesterone in the luteal phase [2, 7]. Considering those on the spectrum with less severe menstrual dysfunction (i.e., not complete amenorrhea), a blunted release of these hormones still occur due to luteal phase defects [32]. The evidence for both hypotheses has previously been outlined by Loucks et al. [33] (see Chap. 11 in this book). The "stress hormone" hypothesis is supported by findings that high growth hormone levels and hypercortisolemia are often reported in amenorrheic athletes indicating alterations in the hypothalamic-pituitary-adrenal axis [21, 34–39] and centrally driven increases in corticotropin-releasing factors that negatively affect gonadotropin secretion in animals and humans [35, 36, 39–42].

The "low energy availability" hypothesis is strongly supported by the literature. Firstly, amenorrheic and eumenorrheic athletes have lower dietary energy intakes relative to energy expenditure [43, 44] and have endocrine profiles (i.e., reduced levels of thyroid hormones; Fig. 14.1) [43, 45, 46] that occur during chronic energy deficiency [47]. Further, increased levels of the *starvation hormone* adiponectin, signaling "low energy availability" [39, 48], have been found in gymnasts and ballet dancers [39, 49]. Leptin, with physiological levels proportional to fat mass, could be considered the *mediator hormone* between energy availability and reproduction [50]. Low fat mass results in decreased production of leptin and increased production of ghrelin in amenorrheic athletes [2, 37, 39, 51–55]; this suppresses the hypothalamic-pituitary-gonadal (HPG) axis leading to decreased estrogen levels [2, 39, 56]. Finally, short-term induction of menstrual cycle changes with exercise can be prevented by adequate dietary compensation [33] or administration of leptin [57]. "Low energy availability" (i.e., negative energy balance) and

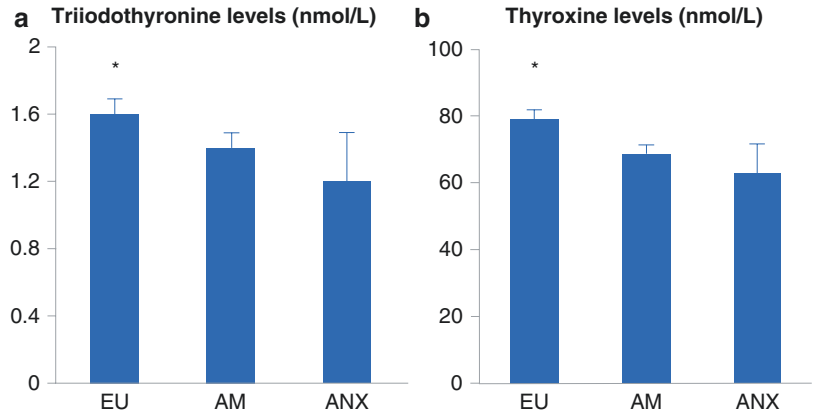

Fig. 14.1 *EU* eumenorrheic athletes; *AM* amenorrheic athletes; *ANX* anorexics. Amenorrheic athletes have endocrine profiles (i.e., decreased thyroid hormones) similar to anorexics with chronic energy deficiency. (Data taken from Refs. [43, 47]). ∗Eumenorrheic means are significantly different from amenorrheic and anorexic means ($p < 0.05$)

the effect on the hypothalamus-pituitary-ovarian axis are described in detail by Stafford et al. [2] (summarized in Fig. 14.2). The decrease in bone mineral density with athletic amenorrhea is thought to be attributable to low energy availability affecting bone turnover, with resorption favored over formation [50]. It is therefore suggested that athletes may be able to reverse menstrual disorders and prevent bone loss without decreasing their energy expenditure (i.e., physical activity levels) by increasing their dietary caloric intake [33]. This is supported by studies where energy availability is decreased either by energy restriction or by increased exercise. Low energy availability induced by energy restriction or increased exercise reduced leptin and insulin-like growth factor-1 (IGF-1) levels, but only energy restriction decreased markers of bone formation [58].

Prevention and Treatment of Athletic Amenorrhea Previous pharmacologic strategies to improve bone health in hypothalamic amenorrhea have included estrogen therapy and calcium supplementation. Estrogen therapy has shown mixed results, with some studies showing improvements or maintenance of bone density and with several others showing inconclusive results [13]. A recent systematic review and meta-analysis of controlled and noncontrolled studies showed estrogen therapy to induce an overall statistically significant increase of 3.3% at the lumbar spine site only [59]. Confounding factors such as spontaneous resumption of menses and weight gain may influence the changes in bone density versus estrogen per se. Estrogen likely has no effect on metabolic factors that impair bone formation, but rather controls bone resorption, which may not necessarily be elevated in amenorrheic athletes [13, 60, 61]. As such, amenorrheic dancers and runners who resumed irregular menses and/or gained weight had larger gains in spine bone density (6.3–17%) over 15–24 months compared to those who achieved neither [62–64]. Further, weight gain was shown to independently predict bone density gains in oligo- and amenorrheic runners, although dietary calcium intake did as well [65]. Considering that clinical findings of estrogen therapy do not strongly support use for improving bone health in amenorrheic athletes [13, 50, 59], clinicians tend to recommend calcium and vitamin D supplementation [66]; however, the latter also has insufficient evidence in amenorrheic athletes, and there is no consensus on appropriate dosage in this population [13]. Thus, the "cornerstone" of treatment has been identified as improving energy availability by increasing caloric intake while maintaining energy expenditure, resulting in weight gain and

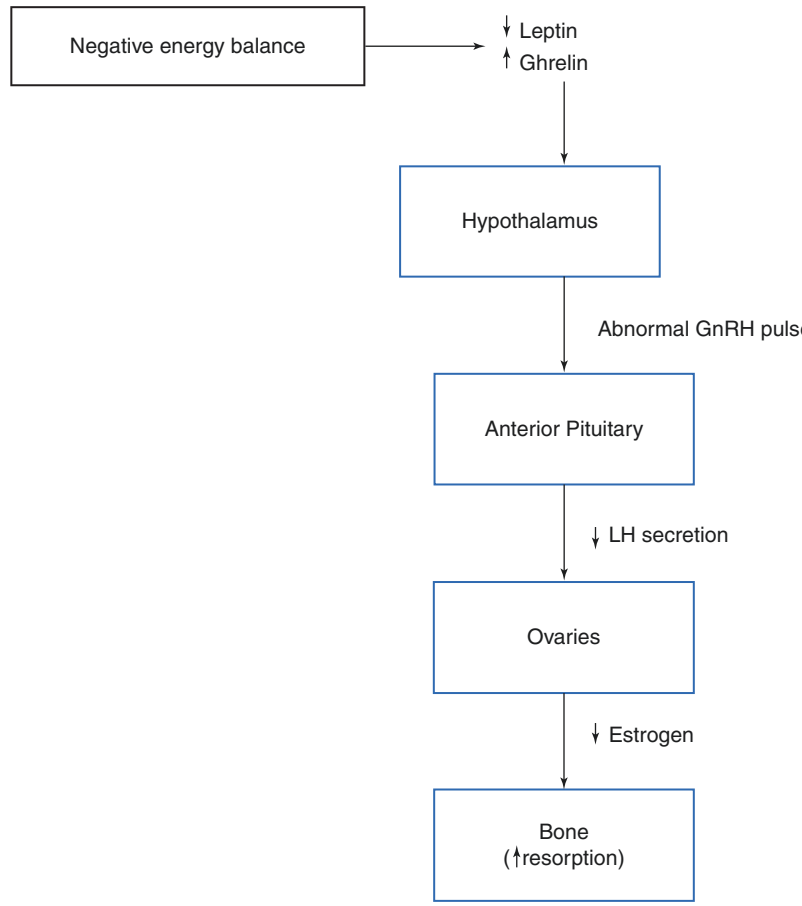

Fig. 14.2 The hypothalamic-pituitary-ovarian axis. Energy imbalance causes hypoestrogenism and amenorrhea. Decreases in leptin and increases in ghrelin may influence gonadotropin-releasing hormone (GnRH) secretion causing subsequent decreases in luteinizing hormone (LH) and estrogen production. (Adapted from Stafford et al. [2])

hopefully resumption of regular menses [7, 12, 13, 33, 50, 67, 68]. Despite improvements with these treatments, bone density often still remains lower than control levels in many formerly amenorrheic athletes [2, 32, 62, 63, 69, 70]. This suggests adolescent athletes with amenorrhea-induced low bone mineral content may experience difficulty with "catch-up" accrual [22, 71] and may develop premenopausal osteopenia and be at higher risk of osteoporotic fractures later in life [2, 13]. Even elite amenorrheic gymnasts showed compromised skeletal health after retirement [72, 73]. Prevention of irreversible bone loss due to reproductive, stress, and metabolic hormone dysregulation as a result of energy deficiency is prioritized over treatment [39, 70, 74]. Treatment is difficult as athletes will be resistant [46], but regardless should consider multiple individualized factors [4], and utilize a comprehensive assessment by a multidisciplinary team that includes a physician, dietitian, and psychologist [2, 75, 76].

Testosterone

Excessive exercise without adequate energy replacement may also affect sex hormone status in men [77–80]. Adequate testosterone levels are important for proper calcium absorption [81] and stimulation of osteoblasts and therefore bone formation [82]. Reduced concentrations of free and total testosterone in response to chronic endurance exercise have been deemed the "exercise-hypogonadal male condition" [79]. More recently, a parallel to the female athlete triad has been suggested in males engaged in sports that emphasize leanness or weight control, with the triad including low energy availability (with or without disordered eating), hypogonadotropic

hypogonadism, and low bone mineral density [83]. Disruption in the hypothalamic-pituitary-testicular axis with excessive exercise in males [79] may have similar etiology as dysregulations seen in females, owing to decreased energy availability [78] or production of stress hormones [84–87]. It is suggested however that the HPG axis may be less sensitive to physical stress and more sensitive to disordered eating in males [88]. A few studies, but not all, have successfully linked low energy availability to the suppression of the HPG axis in men [31, 89–91]. Previous evidence supports this link, such that a shift in caloric balance following a season in wrestlers allowed for an increase in body weight, returning testosterone levels to normal [78]. However, the available research on this topic in men is extremely limited compared to that which has studied women.

Simple measures of hormone levels, such as testosterone, that influence reproduction have been relied upon to determine hypogonadotropic hypogonadism in males, since clinical determination would require complicated techniques such as sperm and fertility analyses, rather than simply a lack of menses to determine the female alternative of amenorrhea [83]. Cross-sectional studies have shown testosterone levels are lower in endurance- and resistance-trained men compared to controls [77, 78, 89, 90, 92–96], while studies also demonstrate testosterone suppression after periods of high volume training [97–103]. Notably, some studies showed a lack of elevation of luteinizing hormone corresponding to suppressed testosterone [77, 93, 103] which may be attributed to deficiency of gonadotropin-releasing hormone, as seen in female athletes [104, 105]. Further, male athletes who participate in low- or no-impact and weight-class sports are at higher risk of impaired bone health, although a representative prevalence remains unknown [83]. These observations may imply a connection between training, suppressed testosterone, and impaired bone health. This is evident in a couple of studies where reduced testosterone in male cyclists [106] or runners [31], attributable to low energy availability, was associated with lower lumbar spine bone mineral density or increased fractures. However, the association between testosterone levels and bone health is less clear in other studies. Some studies show that while male runners [88, 107–111] and cyclists [112–114] have reduced bone mass, primarily at the lumbar spine, testosterone levels are normal [88, 107, 110, 112]. To add to these observations in runners, in one study a negative association between training volume and bone density with no difference in testosterone levels was shown [110], while another study showed a negative association between training volume and testosterone levels with bone density unaffected [115]. One useful study that assessed testosterone, luteinizing hormone, and bone density demonstrated that after 5 months of training, triathletes (classified as endurance athletes) had higher bone density despite lower testosterone levels without elevated luteinizing hormone than controls [103]. Finally, a very recent study noted that resistance-trained runners had higher bone density at all sites than nonresistance-trained runners and controls, with no differences in testosterone or any bone biomarkers except vitamin D (which was higher); these authors concluded the benefits for bone were therefore attributable to chronic loading of the bone and not physiologically modulated by low testosterone [104].

Reduced levels of androgens in males have rarely been linked to corresponding reductions in bone mass, possibly because testosterone levels remain within the normal range, albeit usually at the extreme low end (unlike estrogen in females, which fall below the normal range) [61, 79, 88]. In one case study, a male with hypogonadism, reduced bone mass, and skeletal fragility had testosterone levels return to normal after treatment with clomiphene citrate which stimulates gonadotropin secretion [116]. In a 4-year exercise intervention in middle-aged men, Remes et al. [117] reported significant associations between estradiol and testosterone and bone turnover markers at baseline, although only associations between estradiol and bone formation were significant at the 1-year and post-intervention mark. More recently it was shown that estradiol levels in male athletes, rather than testosterone, predicted bone mineral density, although, notably, testosterone

predicted estradiol levels [88]. Further research is needed to confirm which factors of the triad affects the hypothalamic-pituitary-testicular axis the greatest. Such research should focus on alterations in *estrogens* rather than *androgens* in males [83] and include metabolic hormones leptin and ghrelin [79].

Negative Effect of Exercise on Calciotropic Hormones

The calciotropic hormones (parathyroid hormone, calcitonin, and to an extent, 1,25-dihydroxyvitamin D_3 [vitamin D]) are involved in calcium homeostasis and bone metabolism [118]. Parathyroid hormone is released from the parathyroid gland in response to low blood calcium levels. Parathyroid hormone stimulates osteoclasts to resorb bone so that blood calcium levels can be restored [119]. Calcitonin has a less powerful but opposite effect. Calcitonin is released from the thyroid gland when blood calcium levels are high and inhibits bone resorption. Vitamin D stimulates active intestinal calcium absorption. High levels of exercise may alter secretion of these calciotropic hormones, negatively affecting bone mineral status.

Negative Effects of Exercise on Parathyroid Hormone

Although parathyroid hormone is mainly recognized for its role in bone resorption, it has also been known to have a role in bone formation [120]. Whether parathyroid hormone has catabolic or anabolic effects depends on the mode of administration (when given as a pharmaceutical), signaling mechanism, and duration of exposure [121, 122], with continuous infusion stimulating bone resorption and intermittent exposure stimulating bone formation [123, 124]. Exercise is another important mediator of parathyroid hormone that is dependent on exercise duration and intensity [123]. Thus, studies of the effect of exercise on parathyroid hormone and bone responses are mixed. The studies demonstrating negative or no effects are discussed below, with studies demonstrating positive effects discussed later in the chapter.

A study in mice utilizing an acute exercise bout (30-minute running) led to a twofold increase in systemic parathyroid hormone [125]. The response of parathyroid hormone to acute exercise bouts in humans is quite variable with studies reporting an increase in release [121, 126–132], a decrease in release [133], and no change [134–136]. One study in particular compared acute bouts performed at 15% above or below ventilatory threshold; parathyroid hormone was only increased after the higher intensity bout, suggesting a stimulation threshold [137]. Thus, intensity as well as duration, type of exercise, and recovery may influence parathyroid response, accounting for the discrepancies between studies [123]. Acute bouts of higher-intensity exercise are thus likely to increase release, and in this case, parathyroid may promote an anabolic effect on bone by increasing osteoblast response to mechanical loading [118, 128, 132, 138]. In response to an exhaustive acute exercise session, there were no differences between trained endurance athletes and recreationally active athletes, with both groups showing a postexercise increase in parathyroid hormone, as well as markers of bone turnover; thus, training status does not appear to influence the response to acute exercise [121, 139]. Aside from exercise variables, parathyroid response to acute exercise bouts can also be affected by calcium concentrations, acidosis, catecholamines, and training [123]. Acute exercise bouts cause decreases in serum ionized calcium concentrations, possibly through dermal losses via sweating or increased urinary calcium [140]. This acute decrease in ionized calcium may stimulate release of parathyroid hormone and an increase in bone resorption [140]. This implies acute release of parathyroid hormone is deleterious, in contrast to the studies mentioned above. Consuming calcium before exercise sessions, or injection of calcium during exercise sessions, may attenuate this effect [140, 141].

Two short-term (6–8 weeks) training studies have shown no changes in parathyroid hormone, with no substantial changes in bone resorption

[142, 143]. However, the response of bone formation was different between these studies, with the study in young women showing increases [143] and the study in older men showing decreases [142]. These differences could be attributed to the population study (age, sex), as well as the training type. One longitudinal study also showed no changes in parathyroid hormone or bone resorption after a 7-month triathlon season, with increases in bone formation and subsequent gains in lumbar spine bone density [144]. On the other hand, excessive chronic high-intensity exercise training may cause an increase in the continuous release of parathyroid hormone [126] and a deleterious effect on bone. This may be related to increases in stress hormones, such as catecholamines. Parathyroid hormone release is stimulated by catecholamines in animal models [145]; this correlates with the intensity [146] or volume [126] of exercise. Two longitudinal exercise training studies indicated elevated basal levels of parathyroid hormone, which were associated with increased bone turnover and reduced bone mineral [147, 148]. This effect is not consistent however, as further training resulted in a decrease in parathyroid hormone and an increase in bone mineral [148], and in another study, training resulted in elevated parathyroid hormone levels and an increase in bone mineral [149]. There may be an interaction between different hormone systems that affect the set point at which parathyroid hormone is released, with a decrease in estrogen (as seen with chronic overtraining) resulting in increased parathyroid hormone release [134]. Low estrogen levels in young overtrained athletes may amplify the effects of parathyroid hormone on bone turnover, similar to what is seen in postmenopausal women [150].

Negative Effects of Exercise on Calcitonin and Vitamin D

Excessive levels of exercise may negatively impact calcitonin and vitamin D levels. Female runners with low bone mass had decreased calcitonin release in response to elevated blood calcium levels following exercise, whereas runners with normal bone mass had increased calcitonin release [134]. This may avert the beneficial effect of calcitonin on preventing bone resorption in the subset of runners with low bone mass.

Studies assessing vitamin D levels in athletes demonstrate that concentrations vary greatly [151] and can be influenced by the time of year and sunlight exposure, as well as diet and other lifestyle choices [152]. Vitamin D levels may be lower in runners with low bone mass (i.e., amenorrheic athletes) in comparison to eumenorrheic athletes and controls [27], although levels were still within a normal range. Male cyclists (i.e., non-weight bearing) have also been shown to have low bone density coincident with low vitamin D status [106, 112, 153]; this may be related to low energy availability [106]. The majority of recent studies show that athletes are deficient or insufficient in vitamin D, with deficiencies tending to exist in winter months; therefore, effect of season may have a greater influence on vitamin D status that is compounded by excessive exercise with inadequate dietary intake [154–162]. Similar to what occurs in hypoestrogenism, the stimulus of loading the bone may override the negative effect of vitamin D deficiency [151]. Indeed, a large cross-sectional study examined male athletes of differing sports, ages, and ethnicities and found no association between bone density and vitamin D deficiency [163], and a study in female synchronized swimmers showed that although vitamin D and insulin-like growth factor-1 (IGF-1) levels were suppressed, bone ultrasound measurements and markers of bone turnover were not different compared to controls [164].

Positive Effects of Exercise on Hormonal Regulation of Bone

Interactions Between Exercise and Estrogen for Increasing Bone Mass

Exercise and estrogen replacement may be complimentary therapies for increasing bone mass in postmenopausal women. When the two are combined, their effects on some bone sites may

be synergistic (i.e., greater than the addition of each therapy alone). Animal studies have demonstrated either additive [165] or synergistic [166, 167] effects with the two therapies in postmenopausal models. The majority of studies in postmenopausal women have shown exercise and estrogen replacement therapy to have an additive [168, 169] or synergistic [170] effect on bone mass of the spine and a synergistic effect on whole-body bone mass [168, 170]. One study found a synergistic effect on bone density at all sites measured (hip, spine, and total body) [171]. Another study found individual benefits on bone density at the spine, but no synergistic effects [172]. However, a recent meta-analysis showed hormone replacement therapy in combination with exercise training had greater benefits for bone density at the femoral neck and lumbar spine than exercise alone, seemingly confirming a synergistic effect [173]. Despite this, women have become hesitant to utilize hormone replacement therapy for bone health due to safety concerns [174]; thus, interest in phytoestrogens as alternative therapy has grown. Animal studies using postmenopausal models have shown cooperative effects of exercise and isoflavone (a plant-based phytoestrogen) on bone density and properties at the hip, lumbar spine, and total body [175–177]. However, a recent study in postmenopausal women contradicted animal study findings, showing that either therapy alone maintained bone density of the total hip, but when the therapies were combined, there was a negative interaction that resulted in a decrease at the same site [178]. The differences between animal and human studies may reflect the signaling mechanism, with lower doses in humans activating primarily estrogen receptor-β which downregulates the detection of exercise loads, while higher doses in animals also activate estrogen receptor-α which increases proliferation of osteoblasts in response to loads [178]. Thus, this implies that estrogen may augment the response of bone to loading (or vice versa) with exercise and estrogen synergistically increasing bone mass when estrogen receptor-α is preferentially activated [179].

Effects of Exercise on Anabolic Hormones

Anabolic hormones, such as testosterone, growth hormone, and IGF-1 increase following acute exercise sessions, and basal levels of these hormones may also increase in response to chronic training. Synthesis of IGF-1 may be in conjunction with growth hormone, as its synthesis in the liver or other sites, such as muscle or bone, may be mediated by growth hormone [180]. Each of these anabolic hormones activates osteoblasts and therefore stimulates bone formation [82, 181, 182]. This section covers the effects of acute and chronic exercise on release of anabolic hormones and their potential for positively affecting the bone.

Acute Effects of Exercise on Anabolic Hormones and Bone Metabolism

Acute exercise sessions may stimulate increases in blood levels of anabolic hormones in both men and women. In men, a single bout of exercise has been shown to result in increases in growth hormone [183–186], IGF-1 [186, 187], and testosterone levels [80, 183, 184, 188, 189]. In women, growth hormone [190–192], IGF-1 [193], and testosterone [191, 194] levels also increased in response to acute exercise. Thus, similar responses to acute exercise occur in women, particularly regarding growth hormone [195–197], although the response seems to be attenuated with aging in both sexes [185, 198, 199]. The latter is likely due to insufficient exercise stimulus in older adults [200]. The exercise-induced response of growth hormone is well recognized and may be due to neural input, lactate, or nitric oxide, with pulsatile release amplified when "threshold" is attained [195]. It is presumed that hepatic secretion of IGF-1 is stimulated by the elevations in growth hormone [196]. However, increased production of IGF-1 has not always followed the same pattern as growth hormone changes [186, 187, 191, 192]; therefore, their release may be independent. The adrenal cortex releases testosterone, and this may be the mechanism by which testosterone levels in females are increased with exercise [194].

Several studies have related the increases in anabolic hormones with acute exercise to changes in markers of bone turnover. Repeated one-leg, knee-extension exercise resulted in an increase in serum growth hormone, with an exercise-induced uptake of growth hormone over the thigh and a release of IGF-1, in men and women with a simultaneous increase in markers of bone turnover [135]. A 30-minute cycling session in trained males increased serum growth hormone and IGF-1 [201], while biomarkers of bone turnover also increased [202]. Finally, high-force eccentric contractions in males induced increases in IGF-1 and makers of bone turnover [203]. This release of anabolic hormones and increase of bone turnover may result in increased bone formation with training, which may translate to enhanced bone mineral with long-term training.

Effects of Exercise Training on Anabolic Hormones and Bone Mass

A high bone mass in some athletic groups may be associated with high basal levels of anabolic hormones. For example, young women involved with resistance training [204] or gymnastics training [205] have higher bone mass along with higher levels of IGF-1 compared to aerobically trained women and sedentary controls. In aerobically trained females, testosterone levels are significantly associated with bone density [206]. Endurance-trained postmenopausal women have higher bone density, IGF-1 levels, and a trend toward higher growth hormone levels than sedentary controls [207]. Alternatively, amenorrheic adolescent endurance athletes have lower bone mass and lower levels of IGF-1 levels than sedentary controls, with IGF-1 levels acting as an independent predictor of apparent lumbar bone density [25]. These cross-sectional studies suggest that exercise training may enhance basal anabolic hormone levels and stimulate bone formation. For premenopausal women, this may hold true as long as regular menses are maintained. Higher bone mass in athletes, however, is not always associated with increased anabolic hormone levels. Male masters athletes involved in speed-power events had greater bone mineral density than endurance athletes and controls, but with no differences in testosterone or IGF-1 levels [208]. Also, male runners who participated in resistance training had higher bone mineral density than runners not participating in resistance training, but with no differences in testosterone [104].

Longitudinal training studies relating increases in anabolic hormones to increases in bone mineral density are mixed in their findings. Following a 7-month triathlon season, male triathletes had significant gains in lumbar spine bone density, with corresponding increases in IGF-1, although testosterone levels did not change [144]. Six months of aquatic exercise in postmenopausal women increased IGF-1 and growth hormone levels, along with enhancements in bone properties of the calcaneus, as assessed by ultrasound [209]. Twelve months of resistance or jump training in middle-aged men with low bone mass increased lumbar spine bone mineral density, bone formation markers (relative to resorption markers), and IGF-1 levels [210]. Other studies in humans assessing changes in anabolic hormones and bone health have demonstrated that beneficial effects of training on bone can be realized without changes in anabolic hormones. Eight weeks of resistance training in older men and women reduced markers of bone resorption without changes in IGF-1 levels [211]. Sixteen to 24 weeks of resistance training of middle-aged or older men increased femoral neck or lumbar spine bone mineral density without changes in levels of testosterone, growth hormone, and IGF-1 [212, 213]. Likewise, gymnastics training produced significant increases in lumbar spine bone mineral density in young women [214] and calcaneus mechanical competence in pre- and peri-pubertal males [215] without a change in serum IGF-1 levels.

Positive Effects of Exercise on Calciotropic Hormones

Parathyroid Hormone

Parathyroid hormone stimulates bone resorption to maintain homeostasis when blood calcium levels are low [119] although several studies

have shown the parathyroid response is independent of calcium [127, 146, 216, 217]. With chronic exercise training, parathyroid hormone levels may be lowered [130, 207, 209]. This has been associated with higher bone mineral values: In cross-sectional studies, male and female endurance-trained athletes have been found to have lower serum parathyroid hormone levels associated with higher bone mineral density when compared to inactive controls [130, 207]. Six months of aquatic exercise training in postmenopausal women reduced parathyroid hormone levels while enhancing bone structural properties at the calcaneus [209]. Rats endurance-trained by treadmill exercise also have lower parathyroid hormone levels and higher bone mass compared to untrained rats [218]. It is suggested that endurance training induces a new set point of parathyroid hormone release regulated by calcium, or permanently suppresses its release [118], since corresponding higher calcium concentrations were found with low parathyroid levels [130, 207]. More recently, mice were put through a short-term (21 days) training program, while parathyroid hormone was inhibited or increased. Parathyroid inhibition attenuated the structural-level mechanical property increases seen in placebo-treated mice, while parathyroid enhancement increased trabecular and cortical bone volume with no effect on tissue- and structural-level mechanical properties as seen in placebo-treated mice [125] .

In contrast to the above studies, an increase in basal levels of parathyroid hormone has been found following a resistance training program that increased bone mineral density in postmenopausal women [149]. As mentioned in the "Negative Effects of Exercise on Parathyroid Hormone" section, parathyroid hormone may have anabolic effects on bone through stimulation of osteoblasts, if released in an intermittent fashion [124]. Further research is needed to determine the exact direction of changes in basal parathyroid hormone levels in response to different training protocols and whether these changes can be considered beneficial or detrimental to bone.

Calcitonin and Vitamin D

Few studies have looked at the effects of exercise on calcitonin levels. In response to an acute exercise bout, calcitonin levels have been shown to increase [133]. Limited studies have determined the effects of exercise training, and those that did have shown inconsistent results. Short-term exercise training was shown to have no effect on serum calcitonin levels in one study [207], while other studies showed increased calcitonin levels [209, 219, 220]; this could prevent bone resorption.

Cross-sectional studies indicate that vitamin D levels may be elevated in endurance-trained [207] and resistance-trained [104, 221] individuals, as well as decathletes [222, 223]. This is associated with a higher bone mass in some of these individuals compared to inactive controls [104, 207, 221, 222]. Rats trained by treadmill exercise have an increase in vitamin D levels, increased calcium balance, increased intestinal calcium absorption efficiency, and increased bone mass compared to untrained rats [218, 224]. Increases in growth hormone release with exercise training [135, 207] may simulate the production of the active form of vitamin D [225], resulting in increased intestinal calcium absorption [223] and increased bone mass [118]. Male triathletes showed increased vitamin D levels after a 7-month season, with gains in lumbar spine bone density [144]. While growth hormone was not measured in this study, IGF-1 increased post-season; this may also affect vitamin D production.

Directions for Future Research

Extreme exercise training negatively impacts bone owing to increased stress and changes in metabolic hormones (i.e., increased cortisol and ghrelin, reduced leptin), eventually suppressing the HPG axis and decreasing estrogen (in females) or testosterone (in males) production. This suppression manifests as athletic amenorrhea, in conjunction with the female athlete triad, in premenopausal women and has been researched a great deal. However, research on

hypogonadotropic hypogonadism and the athletic triad in males is still lacking. Such future research in male athletes should focus on alterations in estrogens [83] and metabolic hormones [79] and include longitudinal follow-ups to determine if males also experience difficulty in "catch-up" accrual of bone mineral. Further, longer-term studies determining the efficacy of treatment plans for the *athletic triad* that incorporate individualized factors and are delivered by a multidisciplinary team should be studied [2, 4, 75, 76]. Such treatment plans should focus on determining if improving energy availability can prevent reductions in reproductive hormones that may occur with chronic exercise.

Research has consistently shown that acute exercise results in an increased anabolic hormone response in both men and women with corresponding changes in bone turnover. Further, cross-sectional data shows athletes have high basal anabolic hormone levels and bone mass. However, more research is required to understand the effects of exercise training on anabolic hormones and bone density. Such research should focus on the development of exercise prescriptions for optimal enhancement of long-term hormone profiles that result in bone formation.

Evidence regarding vitamin D in relation to bone in athletes is quite consistent. Research on the other calciotropic hormones, calcitonin and parathyroid hormone, is lacking or inconsistent. Studies determining the effects of acute exercise and exercise training on calcitonin levels and bone are needed. Further research is needed to determine the response of parathyroid hormone to different training protocols and whether these changes can be considered beneficial or detrimental to bone.

Summary

Chronic exercise training without adequate energy replacement induces release of stress and metabolic hormones, which in turn suppress the hypothalamic-pituitary-gonadal axis and downregulate production of reproductive hormones. These hormonal changes ultimately lead to low bone mineral density, which presents with low energy availability and a dysfunction in menstrual function (females) or hypogonadotropic hypogonadism (males) in the athletic triad [83]. In some cases, particularly in gymnasts, overloading the bone negates the deleterious effects of hypoestrogenism. Regardless, prevention strategies in males and premenopausal females should focus on early identification of those at risk of developing the athletic triad. Prevention is particularly pertinent because evidence suggests bone loss experienced in previously amenorrheic athletes is irreversible. If it is too late for prevention, the "cornerstone" of treatment is improving energy availability. Postmenopausal women can counteract deleterious effects of hypoestrogenism via hormone replacement therapy and exercise training, which has a synergistic effect for bone density at clinically relevant sites (i.e., femoral neck and lumbar spine).

Athletes tend to be deficient in the calciotropic hormone vitamin D, particularly in winter months, with this effect compounded by low energy availability. However, the stimulus of bone loading may again override the negative effects of the deficiency. The effect of exercise on other calciotropic hormones (e.g., parathyroid hormone) is highly dependent on exercise variables. Due to a stimulation threshold, acute bouts of high-intensity exercise increase release of parathyroid hormone that results in an anabolic effect on bone, if calcium levels are adequate prior to exercise. Alternatively, chronic exercise training may decrease parathyroid hormone levels, which has been associated with higher bone mineral values. However, the set point at which parathyroid hormone is released is altered with changing estrogen levels and calcium concentrations.

Anabolic hormones (i.e., testosterone, growth hormone, and IGF-1) increase in response to acute bouts of exercise in both men and women, although the response is attenuated with age likely due to insufficient exercise stimulus (see Chap. 23 in this book). Trained individuals have high basal levels of anabolic

hormones that may be associated with high bone mass, suggesting the changes in bone turnover with acute exercise may translate to improved bone health with long-term training.

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15 Interrelations Between Acute and Chronic Exercise Stress and the Immune and Endocrine Systems

Jonathan Peake

Introduction

Interaction between the endocrine and immune system is necessary to regulate our health. However, under some conditions, stress hormones can overstimulate or suppress the immune system, resulting in harmful consequences [1]. Stress is often considered negative, yet it is an intrinsic part of everyday life. Stress is not clearly defined; it is context-specific and depends on the nature of factors that challenge our body. Internal stimuli will elicit different stress reactions compared with external stimuli [1]. Similarly, some stressors will induce responses that may benefit survival, whereas others will cause disturbances that may endanger our health. Stress also depends on how our bodies perceive and respond to stressful stimuli [1].

Several important factors determine whether stress hormones stimulate or inhibit the immune system. These factors include [1]:

- The effects of stress on the distribution of immune cells in the body
- The duration of stress
- Hormone concentrations
- The timing of stress hormone exposure relative to the activation status of immune cells (i.e., naïve vs. activated, early vs. late activation)

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Exercise is a reproducible and quantifiable model of stress and is useful for studying the interactions between the endocrine and immune systems. Exercise stimulates the secretion of a variety of stress hormones, but catecholamines, cortisol and growth hormone are most closely linked with exercise-induced changes in immune function. Research on the interactions between endocrine and immune systems following acute exercise and chronic training is important. Regular exposure to mild short-term stress can potentially enhance immune function and lead to various health benefits. Conversely, prolonged exposure to the chronic stress of intense training may inhibit certain immune functions that are required for health maintenance. This chapter describes the regulatory roles of stress hormones on immune cell counts and activity during acute exercise and following chronic exercise training. Figure 15.1 summarises the immunoendocrine interactions during exercise and their potential functional significance.

Mechanisms of Interaction: In Vitro Evidence

Stress hormones modulate immune function directly by binding to cognate receptors on immune cells and indirectly by modulating the production of cytokines (e.g., IFN-γ, IL-1β, IL-6, TNF-α) [2]. Glucocorticoid receptors are

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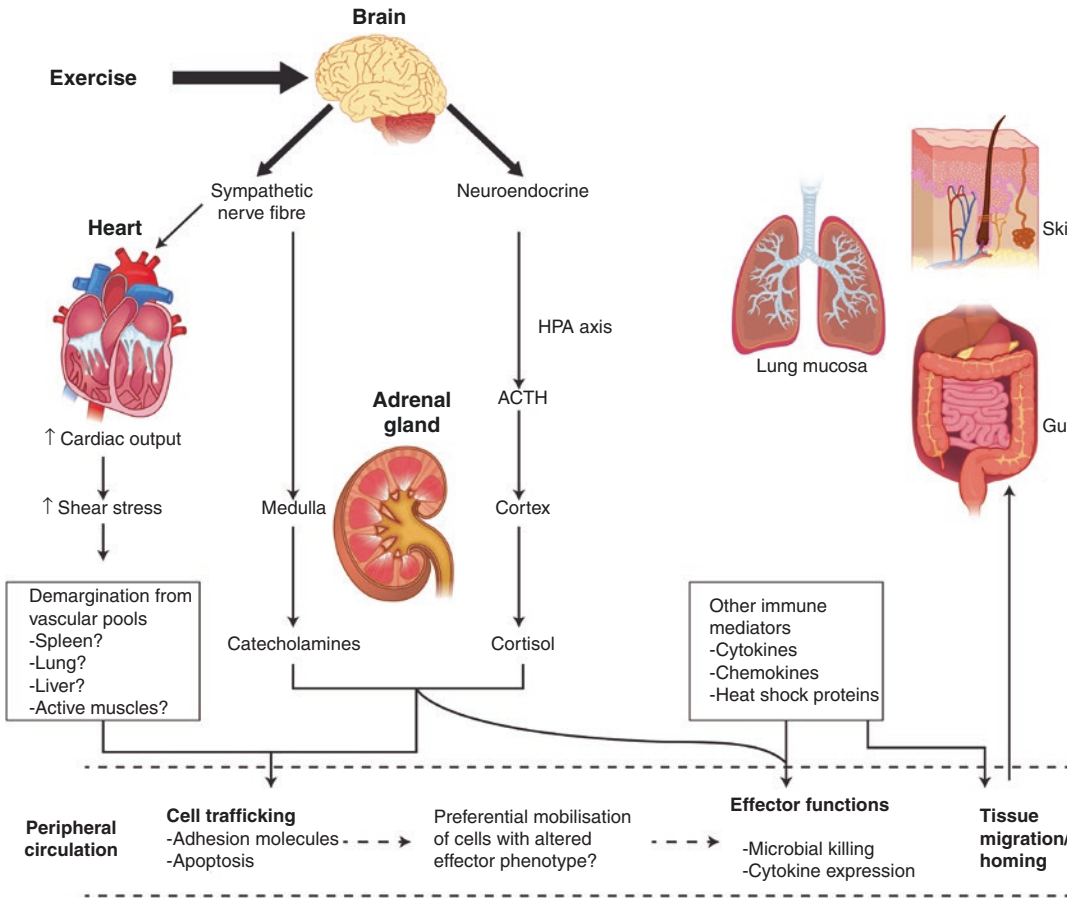

Fig. 15.1 Potential mechanisms by which stress hormone interacts with the immune system during exercise. (Modified from TOM-Systemdruck GmbH, Walsh et al. [170])

expressed on monocytes and B lymphocytes, whereas glucocorticoid receptor expression is much lower on $CD3^+$ T cells and neutrophils [3, 4]. β_2-adrenoreceptors for catecholamines are expressed on (in descending order) natural killer (NK) cells, monocytes, B lymphocytes and T suppressor lymphocytes [5]. Macrophages [6] and neutrophils [7] also express β_2-adrenoreceptors. Within T lymphocyte subpopulations, β_2-adrenoreceptors are mainly expressed in naïve $CD4^+$ T cells and T helper 1 and T helper 2 cells [8–10]. mRNA for α-adrenoreceptors is expressed by activated T cells [11] and in peripheral blood mononuclear cells of patients with juvenile rheumatoid arthritis but not healthy individuals [12]. Although B lymphocytes, monocytes and neutrophils all express growth hormone receptors [13–15], growth hormone most likely exerts its effects on the immune system by binding to prolactin receptors, which are expressed on monocytes and B and T cells [16]. Immune cells also express receptors for other stress hormones, including substance P [17], neuropeptide Y [18], corticotrophin-releasing hormone [19] and serotonin [20].

Glucocorticoids regulate the activity of immune cells by binding to glucocorticoid receptors, which in turn suppresses the transcription factors activator protein 1 (AP-1) and nuclear factor κ B (NFκB) [21]. Glucocorticoids inhibit AP-1 transcriptional activity by preventing the oncoproteins c-Fos and c-Jun from binding to the AP-1 consensus binding site in DNA [22]. Glucocorticoids inhibit NFκB transcriptional activity through two mechanisms.

Firstly, glucocorticoids can induce expression of the inhibitory protein IκB, which then prevents NFκB from translocating to the nucleus where it initiates transcription [23]. Secondly, physical interaction or cross-talk between glucocorticoid receptors and NFκB can suppress transcription [24, 25]. By suppressing the transcriptional activity of AP-1 and NFκB, glucocorticoids regulate various immune functions, including cytokine production [21]. In particular, glucocorticoids inhibit monocyte production of type 1 cytokines IL-12 and IFN-γ, which in turn favours the production of type 2 cytokines IL-4 and IL-10 by $CD4^+$ lymphocytes and peripheral blood mononuclear cells [26–29]. Type 1 cytokines regulate the activity T cytotoxic cells, NK cells and macrophages which defend against intracellular pathogens. Type 2 cytokines regulate the activity of B lymphocytes, eosinophils and mast cells, which defend against extracellular pathogens [30]. The type 1/type 2 cytokine balance determines the balance between cell-mediated vs. humoral immunity and the risk of various immune-related disorders [31]. For information on the effects of glucocorticoids on other aspects of immune function, readers are referred to other more comprehensive reviews [21, 32].

Binding of catecholamines to β_2-adrenoreceptors can inhibit IL-2 and IFN-γ and stimulate IL-4 and IL-10 production by T cells and peripheral blood mononuclear cells [26, 33, 34]. Similar to glucocorticoids, catecholamines can therefore induce a shift towards type 2 cytokine production. The combined effects of glucocorticoids and catecholamines on IFN-γ, IL-4 and IL-10 production by peripheral blood mononuclear cells are in fact additive [26]. However, there are some inconsistencies in the literature concerning the effects of β-agonists on cytokine production. Some studies report that T helper 2 lymphocytes do not respond to β-agonist stimulation [9, 35], but more recent data indicate that activated T cells do produce cytokines following β-agonist stimulation [10]. The effects of β-agonists on cytokine production may also be dose-dependent. Low concentrations of β-agonists (i.e., 1–10 nM) stimulate cytokine production, whereas high concentrations (i.e., 100 nM to 10 μM) inhibit cytokine production by T cells [10]. Downstream from cyclic AMP, β-agonists inhibit cytokine production by T cells by blocking the calcium-/calmodulin-dependent protein phosphatase calcineurin and p38 mitogen-activated protein kinase, but not NFκB [10, 36]. For information on the effects of catecholamines on other aspects of immune function, readers are referred to other more comprehensive reviews [21, 31, 37].

In comparison with glucocorticoids and catecholamines, less is known about the effects of growth hormone and prolactin on the immune system. The actions of growth hormone and insulin-like growth factor-1 (IGF-1) do not overlap entirely, but growth hormone exerts many of its actions through IGF-1. Neither growth hormone nor IGF-1 is essential for immune function, but growth hormone influences various aspects of immune cell development and activity [38]. Growth hormone inhibits apoptosis of $CD4^+$ T cells following treatment with dexamethasone [39]. Growth hormone, through binding to its receptor on the surface of T cells, may activate phosphatidylinositol 3 kinase (which regulates cell proliferation) and NFκB (which controls apoptosis through the anti-apoptosis protein Bcl2) [40]. IGF-1 also stimulates macrophages to produce reactive oxygen species [41] and increases NK cell activity [42]. Prolactin is also not essential to normal immune function [38], but it can promote lymphocyte proliferation [43] and haematopoiesis [44].

Interactions between the neuroendocrine and immune systems are bidirectional. Pro-inflammatory cytokines released from immune cells (e.g., IL-1β, IL-6 and TNF-α) mediate communication between the immune system and the central nervous system. Cytokines can alter activity of the central nervous system through humoral, neural and cellular pathways [45]. Cytokines can pass the blood–brain barrier directly [46]. Alternatively, immune cells can pass across the blood–brain barrier and release cytokines into the central nervous system [47]. Cells comprising the blood–brain barrier also secrete various cytokines [48]. Cytokines may signal the central nervous system by stimulating afferent nerves, although this concept remains

somewhat controversial [49]. One theory proposes that cytokines target the blood–brain barrier during systemic inflammation, whereas they target afferent nerves during localised inflammation [49]. Cytokines can pass back across the blood–brain barrier into the circulation following intracerebroventricular injection of lipopolysaccharide (LPS) [50]. Cytokines interact with components of the central nervous system, resulting in behavioural changes. Specifically, cytokines alter neurotransmitter function, neuroendocrine activity, neural plasticity and neural circuitry. These actions can induce fever, changes in appetite, fatigue and depression [45].

Stress Hormones and Leukocyte Mobilisation In Vivo

A number of studies have investigated the effects of stress hormones on circulating leukocyte numbers by infusing variable doses of stress hormones in healthy humans over 30 min up to 5 h. Cortisol raises the number of circulating neutrophils, whereas it suppresses the number of lymphocytes, and does not alter the number of Leu^+ NK cells [51, 52]. By contrast, adrenaline increases the number of circulating total lymphocytes and NK cells [51, 53–55]. The number of circulating monocytes also rises 1–2 h following infusion of adrenaline [53, 55, 56].

In contrast with NK cells, the effects of adrenaline and the β-agonist isoproterenol on circulating T lymphocyte subpopulations are somewhat variable. In response to these agents, the number/percentage of circulating $CD4^+$ T helper cells decreases [54, 56, 57] or increases [53, 58], whereas the number/percentage of circulating $CD8^+$ T cytotoxic cells increases [53, 54, 58], decreases [57] or remains unchanged [56, 59]. The number/percentage of circulating B lymphocytes decreases [53] or remains unchanged following infusion of adrenaline or isoproterenol [54, 56, 59]. More recent research indicates that adrenaline increases the number of circulating $CCR7{-}CD45RA^+CD8^+$ effector T cells, $CD4{-}CD8{-}$ γ/δ T cells, $CD3^+CD56^+$ NK T-like cells, $CD16^+CD56^{dim}$ cytotoxic NK cells and $CD14^{dim}CD16^+$ pro-inflammatory monocytes. These cells most likely originate from marginated pools on the endothelial surface of blood vessels [60]. In addition to these findings, γ/δ T cells and T cells expressing chemokine receptors (CXCR2, CXCR3 and CCR5) are mobilised into the circulation following psychological stress. These responses correlate with cardiac activation [61, 62].

The effects of noradrenaline on circulating leukocytes are also variable. One study has reported that noradrenaline raised the number of circulating neutrophils, monocyte, lymphocytes and $CD16^+$ NK cells [58, 63]. Another study found no changes in the numbers of these cell types or T lymphocyte subpopulations following treatment with noradrenaline [54]. These inconsistent findings may be due to differences between these studies in noradrenaline dose and in the duration of hormone infusion and blood sampling times relative to the period of infusion.

Combined treatment with cortisol and adrenaline increases the number of circulating neutrophils for up to 12 h [52]. Growth hormone infusion in humans (2 IU) increases neutrophil number, but does not alter blood mononuclear cell subpopulations [64].

Stress Hormones and Leukocyte Function In Vivo

Several of the studies described above have also examined changes in immune cell function following infusion of stress hormones in healthy humans. Cortisol does not alter Leu^+ NK cell activity [51] or neutrophil chemotaxis or production of reactive oxygen species [65]. By contrast, adrenaline increases the activity of $CD16^+$ NK cells [53, 55]. Similarly, noradrenaline infusion in humans (16 μg/min for 1 h) also increases $CD16^+$ NK cell activity [63]. The effects of catecholamines and isoproterenol on lymphocyte proliferation vary. Isoproterenol reduces lymphocyte proliferation [54], whereas adrenaline and noradrenaline have no effect [54, 57]. This disparity may be due to variable changes in lymphocyte subpopulations in response to these agents. Adrenaline increases the number of T

cells that express IFN-γ, IL-2, IL-4 and TNF-α [53]. Adrenaline and noradrenaline infusions also raise the plasma concentrations of IL-6 and IL-1 receptor antagonist (IL-1ra) under normal resting conditions [66–68]. In contrast, adrenaline infusion prior to experimental endotoxemia reduces subsequent changes in the plasma concentrations of IL-6, IL-8 and TNF-α [69]. Hydrocortisone treatment immediately prior to experimental endotoxemia does not alter subsequent changes in plasma IL-6 concentration but attenuates plasma TNF-α concentration and increases plasma IL-10 concentration endotoxemia [70, 71]. Conversely, IL-6 and IFN-γ increase the plasma concentrations of cortisol and ACTH cortisol [72, 73], while infusion of LPS increases the plasma concentrations of adrenaline and cortisol [59].

To summarise, glucocorticoids, catecholamines and growth hormone bind to specific receptors on the surface of immune cells. This hormone-receptor binding mediates leukocyte trafficking and functional activity. In vitro, glucocorticoids and catecholamines induce a shift in the balance of type 1/type 2 cytokines towards greater production of type 2 cytokines. Growth hormone regulates immune cell activity through IGF-1 and can inhibit apoptosis of T lymphocytes. In vivo, cortisol mobilises neutrophils but reduces the number of circulating lymphocytes and does not alter circulating natural killer cell numbers. Catecholamines increase the total number of circulating lymphocytes, monocytes and natural killer cells. They also stimulate natural killer cell activity. By contrast, the effects of catecholamines on circulating lymphocyte subpopulations and lymphocyte activity are more variable. By crossing the blood–brain barrier, immune cells and cytokines can alter the function of the central nervous system.

Immunoendocrine Responses to Acute Exercise

Exercise immunologists have used various approaches to investigate the interaction between the endocrine and immune systems during exercise. On a basic level, some research has assessed the correlation between changes in stress hormones and immunological variables following exercise. Other research has examined the interactions between the endocrine and immune systems by using different exercise workloads, carbohydrate and caffeine supplementation, thermal stress or drugs. A small number of studies have also investigated how exercise-induced immune changes alter the activity of the central nervous system.

Correlations Between Stress Hormones and Immunological Variables

McCarthy et al. [74] first provided evidence that following brief, intense exercise, the number of circulating lymphocytes correlated positively with the plasma concentrations of adrenaline ($\rho = 0.67$, $p < 0.05$) and noradrenaline ($\rho = 0.68$, $p < 0.05$). Plasma adrenaline concentration also correlates positively with the number of circulating neutrophils after short, intense exercise [74, 75] and endurance exercise [76]. Rhind et al. investigated the relationships between stress hormones and immune cells following exercise. Stepwise multiple linear regression indicated that plasma adrenaline concentration accounted for some of the variation in $CD3^+$ T cells, $CD4^+$ T helper cells, $CD8^+$ T cytotoxic cells and $CD3-/CD16^+/CD56^+$ NK cells [77]. Plasma noradrenaline concentration also explained some of the variation in $CD3-/CD16^+/CD56^+$ NK cells and $CD19^+$ B cells [77]. Steensberg et al. [78] discovered that following 2.5 h running at 75% VO_{2max} (maximal oxygen uptake), the number of T helper 2 cells that produce IL-2 and IFN-γ decreases below pre-exercise values, and this response is inversely correlated with plasma adrenaline concentration. Brenner et al. [79] used stepwise multiple linear regression to examine stress hormones and immune cells following cold exposure. Plasma noradrenaline concentration accounted for some of the variation in $CD3^+$ T cells, $CD8^+$ T cytotoxic cells and $CD19^+$ B cells, whereas plasma adrenaline concentration was only linked with changes in $CD19^+$ B cells [79].

The relationship between plasma cortisol concentration and the number of circulating immune cells is more variable. Some studies report no relationship [74, 80] or an inverse relationship [81] between plasma cortisol concentration and the number of circulating neutrophils after exercise. Other studies suggest that cortisol does mediate neutrophil mobilisation following exercise [76, 77, 82, 83]. The association between plasma cortisol concentration and the number of circulating monocytes following exercise is also inconsistent [77, 81]. It does seem, however, that plasma cortisol concentration accounts for some of the variation in $CD4^+$ T helper cells and $CD19^+$ B cells following exercise [77]. These inconsistent findings may be due to variation in blood sampling points used to examine the association between plasma cortisol concentration and the number of circulating immune cells. In contrast with adrenaline, cortisol mobilises neutrophils into the circulation in a more delayed and prolonged fashion [51, 52]. Recent evidence indicates that plasma cortisol concentration correlates strongly with lymphocyte apoptosis after resistance exercise [84]. Although growth hormone can mobilise neutrophils at rest [64], there is no clear evidence to indicate that growth hormone regulates the number of circulating neutrophils following exercise [81].

Several studies suggest that stress hormones also regulate cytokine responses to exercise. The plasma concentrations of adrenaline, noradrenaline, cortisol and growth hormone correlate with the plasma concentrations of IL-6, IL-1ra, IL-12 and TNF-α following exercise in both thermoneutral and hot conditions [85–87]. The plasma concentrations of noradrenaline and cortisol also correlate with plasma IL-6 concentration following cold exposure [79, 88]. It is unclear whether hormones or cytokines are the driving factor behind these relationships. Stress hormones and cytokines regulate body temperature during exercise, albeit through distinct mechanisms [89]. Adrenaline may stimulate a small rise in plasma IL-6 concentration during exercise [68]. Alternatively, the correlation between plasma adrenaline and IL-6 concentrations following exercise may be purely coincidental, because both adrenaline and IL-6 regulate muscle glycogen depletion during exercise [90, 91]. IL-6 release from skeletal muscle during exercise correlates with arterial IL-6 concentration [92]. Treatment with the glucocorticoids hydrocortisone and dexamethasone reduces plasma IL-6 concentration during exercise [85]. However, IL-6 stimulates cortisol release at rest [72]. Further research is required to clarify the interactions between IL-6 and cortisol during exercise.

Exercise Workload, Stress Hormones and Immunological Variables

Stress hormones are released into the circulation as the intensity of exercise increases. Plasma adrenaline, noradrenaline and growth hormone concentrations rise in an exponential manner with increasing intensity [93–95]. By contrast, plasma cortisol concentration only increases above exercise intensities of >60% VO_{2max} [76, 96, 97]. Based on these hormone responses, a number of studies have compared immunological responses to exercise of variable intensity and duration.

Foster et al. [93] first provided evidence that catecholamines influence leukocyte mobilisation as a function of exercise intensity. The number of circulating granulocytes and lymphocytes increased with workload. Using the β-antagonist propranolol, they demonstrated that during exercise, catecholamines regulate changes in lymphocytes, but not granulocytes [93]. Compared with moderate-intensity exercise, the number of circulating monocytes is similar, while $CD4^+$ T helper cells, $CD8^+$ T cytotoxic cells and T cell proliferation decrease below pre-exercise values after high-intensity exercise [82, 97, 98]. Conversely, the number of $CD19^+$ B cells is higher after high- vs. moderate-intensity exercise [82]. The number of circulating NK cells and NK cell activity is similar immediately after moderate- and high-intensity exercise, while NK cells and activity decrease below pre-exercise values 2 h after high-intensity exercise [98]. These studies did not evaluate the relationship between stress hormones and these intensity-dependent

immune changes. However, it seems likely that stress hormones play a more dominant role in mediating immune changes during high-intensity exercise. The plasma concentrations of IL-6, IL-1ra and IL-10 are also higher following high- vs. moderate-intensity exercise [76, 92, 99, 100]. As discussed above, adrenaline may stimulate a minor rise in plasma IL-6 and IL-1ra concentration during exercise [66, 68], but it is more likely that IL-6 stimulates IL-1ra and IL-10 late in exercise [72].

Carbohydrate Supplementation, Stress Hormones and Immunological Variables

Cortisol and adrenaline play key roles in mediating metabolism during exercise [90, 101]. Many studies have used carbohydrate supplementation to manipulate stress hormone responses and examine the mechanisms of exercise-induced changes in immune cell counts and activity.

With the exception of a few studies [102–104], carbohydrate consumption during endurance exercise generally reduces the plasma concentrations of adrenaline, cortisol and growth hormone [105–112]. This decrease in the release of stress hormones most likely accounts for the decline in the number of circulating neutrophils and monocytes following carbohydrate ingestion during exercise [102, 103, 107, 109–111, 113]. By contrast, although carbohydrate supplementation attenuates plasma cortisol concentration, in general, it does not prevent the post-exercise decline in the number of circulating lymphocytes, lymphocyte subsets or NK cells [110, 114–118].

The effects of carbohydrate supplementation on other exercise-induced changes in immune cell function are variable. Despite changes in stress hormones, not all studies demonstrate that carbohydrate consumption maintains or increases neutrophil and monocyte function [102, 103, 107, 109, 113, 119]. Most research indicates that carbohydrate supplementation does not prevent the post-exercise decrease in lymphocyte proliferation [114, 118, 120]. However, Lancaster et al. [115] found that consuming carbohydrate reduces plasma cortisol concentration and helps to maintain the number of IFN-γ^+ $CD4^+$ and $CD8^+$ T cells and IFN-γ production by these cells during exercise. The metabolic stress of low muscle glycogen appears to increase plasma cortisol concentration and the number of circulating leukocytes, but does not alter lymphocyte proliferation during exercise [121, 122]. Carbohydrate supplementation increases IL-2- and IFN-γ-stimulated NK cell activity, but not IL-4- and IL-12-stimulated NK cell activity [116, 117]. These effects on NK cell activity are independent of changes in plasma cortisol concentration [116, 117]. Nieman et al. [123] discovered that carbohydrate ingestion during exercise reduced plasma cortisol concentration but did not alter salivary immunoglobulin A concentration (when adjusted for saliva protein concentration and secretion rate). However, changes in salivary immunoglobulin A concentration were negatively correlated with plasma cortisol concentration, and this relationship predicted the incidence of upper respiratory illness in the 2 weeks after exercise [123].

With a few exceptions [103, 106, 112], most research shows that carbohydrate attenuates the rise in plasma concentrations of IL-6, IL-10 and IL-1ra (but not IL-8 or TNF-α) following exercise [105, 108–111]. These cytokine responses to consuming carbohydrate during exercise may be partly linked to changes in catecholamine release. Carbohydrate supplementation does not influence leukocyte mRNA expression of IL-6, IL-8, IL-10 and IL-1ra or monocyte intracellular production of IL-6 and TNF-α following exercise [105, 106]. Carbohydrate ingestion attenuates the release of IL-6 from the skeletal muscle during exercise, but the effects of carbohydrate on mRNA expression of IL-6 and IL-8 in the skeletal muscle following exercise are variable [110, 111, 124, 125].

Caffeine Supplementation, Stress Hormones and Immunological Variables

Although caffeine is a well-known stimulant of the central nervous system, only a small number of studies have focused on its effects on stress

hormones and immune responses to exercise. Ingesting 6 mg caffeine 1 h before endurance exercise consistently raises plasma adrenaline concentration [126–130]. Compared with a placebo treatment, caffeine supplementation does not alter the number of circulating neutrophils following exercise or neutrophil production of reactive oxygen species [129, 130]. The number of circulating CD3−/CD56$^+$ NK cells is greater compared with a placebo treatment, whereas changes in the number of activated NK cells expressing CD69 are variable after exercise and caffeine ingestion [131, 132]. Changes in the total number of circulating lymphocytes after exercise and caffeine intake are also variable [129, 130]. The numbers of circulating CD4$^+$ T helper cells and CD8$^+$ T cytotoxic cells are lower, while the numbers of these cells that express the activation marker CD69 are greater after exercise and caffeine intake compared with a placebo treatment [126]. Caffeine supplementation also increases the concentration and secretion rate of salivary immunoglobulin A and the plasma concentration of heat shock protein 72 after exercise compared with a placebo treatment [127, 128]. This variation in the effects of caffeine on exercise-induced immune changes may be due to differences in exercise protocol, blood sampling times and the habitual caffeine intake of the study participants.

Thermal Stress, Stress Hormones and Immunological Variables

Some researchers have compared changes in stress hormones and immunological variables following exercise in hot vs. cold/thermoneutral conditions. Several studies have examined responses to exercise in hot vs. cold water. This approach appears to be more effective than comparing responses to exercise in hot vs. cold/thermoneutral ambient conditions, because water is a more effective conductor of heat than air. For detailed discussion on the effects of thermal stress on the endocrine and immune systems, interested readers should consult the comprehensive review by Walsh and Whitham [89].

Plasma stress hormone concentrations are higher following exercise in hot vs. cold water, and these responses most likely account for the higher numbers of circulating neutrophils and lymphocytes following exercise in hot water [77, 81, 133–135]. However, not all research supports a link between stress hormones and the number of circulating leukocytes following exercise in hot conditions [136, 137]. This relationship may vary depending on the demands of exercise. Within the lymphocyte subsets, CD3$^+$ T cells, CD34$^+$ T helper cells, CD8$^+$ T cytotoxic cells and CD3−/CD16$^+$/CD56$^+$ NK cells (but not CD19$^+$ B cells) are higher at the end of exercise in hot vs. cold/thermoneutral conditions [77, 122]. By contrast, the number of circulating CD3$^+$ T cells is lower 2 h after exercise in hot vs. thermoneutral conditions [122].

The effects of thermal stress on neutrophil function following exercise are also variable, with reports of an increase [138], a decrease [137] or no change [122, 134]. Thermal stress during exercise increases lymphocyte proliferation per cell (despite higher plasma cortisol concentration) [122], whereas it does not alter NK cell activity per cell [122, 139]. The plasma concentrations of IL-10, IL-1ra, IL-12 and TNF-α are consistently higher after exercise in hot vs. cold/thermoneutral conditions, whereas changes in the plasma concentrations of IL-6, IL-8 and granulocyte-colony-stimulating factor (G-CSF) are less consistent [86, 134, 136–138].

Drugs, Stress Hormones and Immunological Variables

Several studies have used drugs to manipulate stress hormone responses to exercise and examine the resultant immunological responses. The findings of these studies are equivocal, possibly because of variation in the exercise protocols, treatment periods and drugs used in these studies.

As described previously, Foster et al. [93] treated men with a single dose of the non-selective β_1-/β_2-antagonist propranolol 10 min before incremental exercise. They discovered

that during exercise, propranolol reduced the rise in the number of circulating lymphocytes, but not neutrophils or plasma catecholamine concentrations. This finding suggests that catecholamines may not regulate leukocyte mobilisation directly during incremental exercise. Instead, catecholamines may work indirectly by increasing blood flow, which strips leukocytes from the endothelial surface of blood vessels in marginal pools such as the lungs. Murray et al. [140] conducted a follow-up study in which they treated men and women with propranolol or the selective β_1-antagonist metoprolol for 1 week prior to an incremental exercise test. Neither drug reduced post-exercise plasma catecholamine concentrations compared with the control trial. However, compared with the control trial, propranolol (but not metoprolol) reduced the total number of circulating lymphocytes, numbers of $CD4^+$ T helper cells and $CD8^+$ T cytotoxic cells and NK cell numbers and activity and reduced the post-exercise decline in lymphocyte proliferation [140]. These findings suggest that circulating catecholamines may not mobilise lymphocytes into the circulation. Instead, these cells may be mobilised from the spleen in response to direct activation of β_1-/β_2-adrenergic receptors in the spleen [141].

Starkie et al. [142] treated men with the selective α_1-antagonist prazosin and the non-selective β-antagonist timolol or placebo 2 h prior to 20 min cycling at ~78% VO_{2max}. Plasma catecholamine concentrations were higher, whereas plasma cortisol concentration was lower after exercise in the drug trial compared with the placebo trial. Starkie et al. [142] attributed the greater catecholamine response in the drug trial to reduced clearance of catecholamines by β-receptors. The numbers of circulating lymphocytes and monocytes increased during exercise in both trials but were lower immediately after exercise in the drug trial compared with the placebo trial—despite the higher plasma catecholamine concentrations. This finding conflicts with other research showing that infusion of adrenaline or isoproterenol *raises* the number of circulating lymphocytes [51, 53, 54]. One possible explanation for this difference is that the drugs used in the study by Starkie et al. [142] may target different adrenergic receptors on lymphocyte compared with adrenaline or isoproterenol. The numbers of circulating IFN-γ^+ $CD3^+$ T cells, IL-2^+ $CD3^+$ T cells and IFN-γ^+ CD3−/$CD56^+$ NK cells increased during exercise in both trials. However, the numbers of these cells were lower after exercise in the drug trial compared with the placebo trial. IL-2 production by $CD3^+$ T cells and IFN-γ production by both IFN-γ^+ $CD3^+$ T cells and IFN-γ^+ CD3−/$CD56^+$ NK cells decreased during exercise similarly in both trials. These findings suggest that α- and/or β-adrenergic receptor stimulation does not regulate cytokine production by T cells and NK cells during exercise.

Mazzeo et al. [143] treated women with prazosin or placebo for 3 days before cycling for 50 min at 50% VO_{2max}. Prazosin reduced plasma IL-6 concentration after exercise compared with the placebo. Papanicolaou et al. [85] treated men with hydrocortisone, dexamethasone or a placebo 4 h before 25 min running at 78% VO_{2max}. Both hydrocortisone and dexamethasone attenuated plasma IL-6 concentration after exercise compared with the placebo.

Evidence for Interactions Between the Central Nervous and Immune Systems

As outlined above, considerable attention has focused on how stress hormones regulate immune responses to exercise. The immune system is also capable of altering the function of the central nervous system. Several studies have examined this issue, and it is likely that more research will be conducted in this area in the future. In mice, exercise-induced muscle damage stimulates macrophages residing in the brain to secrete IL-1β into the surrounding tissue [144, 145]. This response appears to increase perceptions of fatigue, reduce voluntary activity and delay recovery from exercise [146]. In humans, Steensberg et al. [147] observed that at rest, the concentrations of IL-6 and the cellular chaperone heat shock protein 72 (HSP72) are two to three

times higher in cerebrospinal fluid compared with plasma. Although exercise stimulates the systemic release of IL-6 and heat shock protein 72, their concentrations remain stable in cerebral spinal fluid, which indicates that they do not cross the blood–brain barrier [147]. The brain releases small amounts of IL-6 into the systemic circulation during exercise, and this is independent of hyperthermia [148]. The functional significance of this response is not certain. It may provide a signal to the liver to increase glucose output, or it may be a more general indication of increased neural activity during exercise [148].

Chronic Interactions Between the Endocrine and Immune Systems

Compared with the amount of research on acute exercise, fewer studies have examined interactions between stress hormones and immunological variables following chronic training. Most studies have simply documented the effects of intensified training on simultaneous changes in stress hormones and immune cell counts at rest and/or in response to acute exercise. Very few studies have specifically examined the relationship between changes in stress hormones and immune cell counts and function.

Several studies report no changes in resting plasma and urinary cortisol concentrations, immune cell counts or serum cytokine concentrations after intensified training [149–153]. Robson-Ansley et al. [154] discovered no changes in resting plasma cortisol or the number of circulating neutrophil counts but did find that resting plasma IL-6 concentration was persistently elevated following 4 weeks of intense training. Fry et al. [155] observed that resting plasma cortisol concentration decreased, while the numbers of circulating neutrophils, monocytes and lymphocytes did not change after 10 days of intense interval training. The number of circulating $CD3^+$, $CD4^+$ and $CD8^+$ T cells and $CD20^+$ B cells also remained unchanged, whereas the number of circulating $CD56^+$ NK cells decreased and $CD25^+$ T cells increased following 10 days of training [155]. It is unlikely, however, that these changes in $CD56^+$ NK cells and $CD25^+$ T cells were related to changes in plasma cortisol concentration. Smith and Myburgh [156] reported no change in resting plasma cortisol concentration but found that $CD4^+$ and $CD8^+$ T cell counts and $CD16^+/CD56^+$ NK cells decreased following 4 weeks of intense training. Makras et al. [157] observed an increase in urinary cortisol concentration, an increase in $CD4^+$ T cell count and a decrease in neutrophil count at rest after 4 weeks of military training. Ortega et al. [158] found that neutrophil phagocytic activity was higher in female athletes compared with non-athletes. In the athletes, neutrophil phagocytic activity correlated positively with plasma cortisol concentration, whereas it correlated negatively with plasma ACTH concentration. Findings from the study by Cunniffe et al. [159] suggest that elevated salivary cortisol concentration with training may reduce salivary immunoglobulin A concentration, resulting in increased susceptibility to upper respiratory illness. Some of the variability among these studies may result from differences in the physical fitness of study participants, training loads and blood sampling times.

The effects of chronic training on cortisol and immune responses to acute exercise are also variable. Verde et al. [160] reported that changes in serum cortisol concentration, $CD3^+$ T cell counts and lymphocyte proliferation after acute exercise were all attenuated following 3 weeks of intense training. Lancaster et al. [161] discovered that 2 weeks of intense training reduced plasma cortisol concentration but did not alter lymphocyte production of the type 1 cytokine IFN-γ or the type 2 cytokine IL-4. In contrast with these findings, other research indicates no effect of chronic training on exercise-induced changes in plasma and salivary cortisol concentration, immune cell counts or salivary immunoglobulin A concentration [150, 152, 162].

A small number of studies have examined changes in plasma or urinary catecholamine concentrations and immune cell counts following chronic training. Imrich et al. [150] found no changes in plasma catecholamine concentration or immune cell counts at rest or in response to acute exercise following 6 weeks of training.

Hooper et al. [163] reported that both the number of circulating neutrophils and plasma noradrenaline concentration were elevated in swimmers showing symptoms of overtraining compared with swimmers who were not overtrained after 6 months of training. However, it is unclear whether these responses were linked in any way. Mackinnon et al. [151] observed that urinary norepinephrine concentration decreased, whereas plasma noradrenaline and leukocyte counts at rest did not change following 4 weeks of intense training. Makras et al. [157] found that the ratio of adrenaline to noradrenaline in urine increased after 4 weeks of military training. This response correlated positively with $CD4^+$ T cell counts and correlated negatively with neutrophil counts.

Biological Significance of Interactions Between the Endocrine and Immune Systems

Dhabhar [1] proposes the following analogy to explain the possible significance of acute stress on the immune system. Within minutes of the onset of stress, catecholamines stimulate the body's 'soldiers' (i.e., leukocytes) to leave their 'barracks' (i.e., spleen, lung, bone marrow, lymph nodes) and enter the 'boulevards' (i.e., blood vessels and lymphatics). As stress proceeds, glucocorticoids are released which stimulate leukocytes to exit the bloodstream and enter potential 'battle stations' (i.e., skin, lung, gastrointestinal and urinary-genital tracts, mucosal surfaces and lymph nodes) in preparation for immune challenges that may occur in response to stressful stimuli [1].

In the context of exercise, the factors that stimulate the release of stress hormones are most often non-harmful. These factors may include demands for (1) increased blood flow to contracting muscle (to deliver oxygen and nutrients) and skin (for thermoregulation) and (2) release of energy substrates from the liver and adipose tissue (e.g., glucose, fatty acids, amino acids) to support muscle metabolism. Interaction between the endocrine and immune systems during exercise can therefore be considered as rather non-specific. However, stress hormones may (incidentally) prime immune cells to respond to infectious pathogens and/or airborne pollutants that invade mucosal surfaces lining the respiratory tract.

Dhabhar [1] proposed that the effects of stress on the immune system and general health depend on the duration of exposure to stress. Acute and intense stress may enhance immune function, and mild stress of moderate duration may promote immunosurveillance, while chronic stress may cause immune dysregulation [1]. Immunoprotection resulting from acute stress may lead to more effective wound healing, responses to vaccination and resistance to infection and cancer. Immunopathology resulting from severe acute stress or persistent stress may promote pro-inflammatory and autoimmune diseases. Immunosuppression resulting from chronic stress may reduce the effectiveness of wound healing and vaccination and increase the risk of infection and cancer. By contrast, chronic stress may reduce the risk of pro-inflammatory and autoimmune diseases by suppressing aspects of immune function that contribute to such conditions (e.g., T lymphocyte activity, cytokine production) [1].

Both acute exercise [164] and chronic training [165, 166] increase antibody production in response to vaccination. Mild repeated stress resulting from chronic training also improves the rate of wound healing [167], decreases the risk of upper respiratory illness [168] and reduces the prevalence and severity of various chronic diseases [169]. Although more work is needed to define their precise role, it is likely that stress hormones mediate some of these benefits of exercise.

Summary

A variety of non-harmful stimuli during exercise induce the release of stress hormones. These stress hormones influence many physiological systems, including the immune system. Stress hormones act to mobilise immune cells into the circulation and can increase or decrease the activity of these cells. The precise nature of the inter-

action between stress hormones and immune cells likely depends on multiple factors, including the intensity and duration of exercise, the physical fitness of exercising individuals and environmental conditions. Some stress hormones (e.g., catecholamines) influence immune cell activity mainly during exercise, whereas others (e.g., cortisol) may have a more delayed effect on immune function during the later stages of exercise and/or after exercise. Nutritional interventions such as carbohydrate and caffeine supplementation can alter the secretion of stress hormones during exercise, but these alterations do not always result in changes in immune function. Immunoendocrine interactions during exercise may serve to promote some aspects of health. However, further research is needed to understand the biological significance of such interactions in more detail.

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Effects of Female Reproductive Hormones on Sports Performance

16

Constance M. Lebrun, Sarah M. Joyce, and Naama W. Constantini

Introduction

Over the past 30 years, the involvement of women and girls in physical activities and competitive sports has increased exponentially. This is largely a consequence of Title IX of the United States Educational Assistance Act (enacted in 1972), which mandated institutions receiving federal monies to provide equal access for women to funding for extracurricular activities, including opportunities for participation, financial resources for scholarships, and qualified coaching. Equally as important, there have been progressive changes in societal and cultural views worldwide toward the acceptance of female athletes into the sporting arena—historically and traditionally a male bastion. Women now compete at the highest levels in most sports, some of which were previously played by men only, such as ice hockey, wrestling, and rugby. Along these lines, the 2012 Summer Olympic Games in London marked the introduction of women's boxing as a full-participation sports.

Yet scientific knowledge regarding a woman's unique physiology—from childhood through puberty and adolescence, across the reproductive lifespan, and into the postmenopausal years—has not kept pace with this explosion in sports participation by girls and women. In particular, there still remains a multitude of unanswered questions about the effects of the female reproductive hormones—estrogen and progesterone—on various aspects of athletic performance. *Athletic performance* itself is a multifaceted entity—a complex and intricate kaleidoscope of cardiovascular, respiratory, metabolic, endocrinological, and psychological factors that all must interact to enhance and facilitate sporting success. The relative importance of each varies with the specific demands of the particular discipline.

Athletes and coaches have long postulated that altered athletic performance might result from the hormonal swings of the female menstrual cycle (MC). Early studies were retrospective and nonspecific, with substantial recall bias in terms of MC phase and status [1–3]. Researchers have investigated the influence of the MC on substrate metabolism, cardiorespiratory function, thermoregulation, psychological factors, and musculoskeletal injury rates. The individual sex steroids estrogen and progesterone can have antagonistic, synergistic, or additive

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effects, and furthermore, their relative concentrations change during the course of an ovulatory menstrual cycle [4, 5]. In addition, hormonal levels have been shown to increase with exercise [6–8]. Both oral contraceptives (OCs) and hormone replacement therapy (HRT) can provide a stable and controllable hormonal milieu for training and competition. However, the combinations of exogenous hormones in these formulations introduce yet other potential modifying factors. This chapter integrates what is already known in this controversial field of inquiry [9–16], with new emerging evidence and suggestions for interesting future directions. The reader is referred to additional reading and summative reviews [17–22] for further details on select individual investigations.

Physiology of the Menstrual Cycle

A well-defined, predictable pattern of hormonal fluctuations takes place over the course of an ovulatory menstrual cycle [23, 24]. In eumenorrheic females, an average menstrual cycle (MC) lasts 28 days, but may range from 20 to 45 days. The three main phases—*follicular*, *ovulatory*, and *luteal*—are based on ovarian function and controlled by pituitary hormonal signals (Fig. 16.1) [4]. Intricate feedback mechanisms involve the gonadotropins—luteinizing hormone (LH) and follicle-stimulating hormone (FSH)—and the female sex steroids. Some studies further divide the cycle into five discrete phases, including an early and late follicular phase and an early, middle, and late luteal phase (see Chap. 8 by

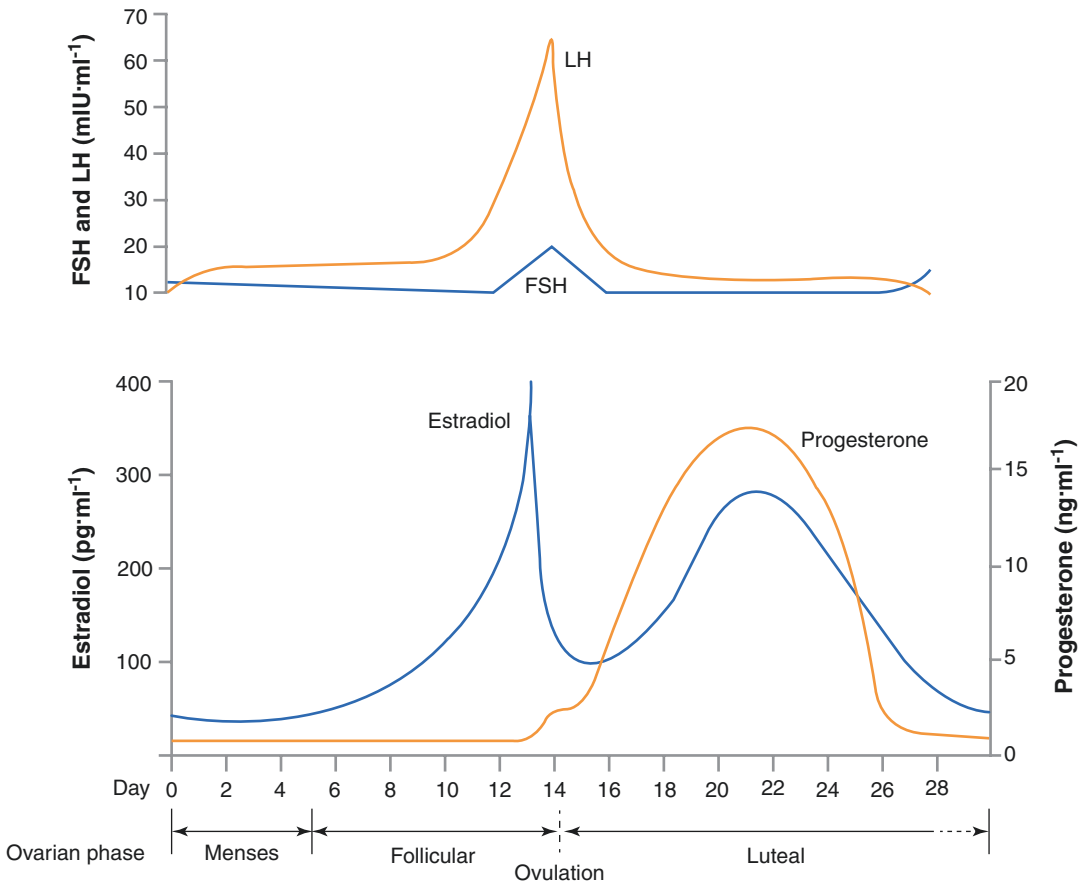

Fig. 16.1 Hormonal changes and phases of the menstrual cycle. *FSH* follicle-stimulating hormone; *LH* luteinizing hormone

Liu et al. [25]. Varying concentrations of estradiol and progesterone differentiate the phases of an ovulatory MC: both are low during the follicular phase (FP), estrogen is high and progesterone low during the ovulatory phase (OP), and both are high in the luteal phase (LP).

Oral Contraceptives

Oral contraceptive pills provide steady levels of exogenous estrogen and progestin, which act primarily by disrupting the normal hypothalamic-pituitary-ovarian (HPO) axis to suppress ovulation (Fig. 16.2). Current recommendations state that age alone does not constitute a medical reason for denying any contraceptive method including OCs and progestogen-only injectables, as this concern must be balanced against the advantages of avoiding pregnancy in adolescents below 18 years [26]. OCs can be safely prescribed right up until the perimenopausal years in nonsmoking women. In addition to the prevention of pregnancy, OCs are sometimes utilized by female athletes to manipulate timing of menses around important competitions. However, it is estimated that the prevalence of OC usage in the athletic population matches that within the general community [27]. Newer formulations such as Seasonale even make it possible to even completely withhold menstruation. This OC is administered continuously for 3 months, allowing only four *periods* or withdrawal bleeds per year, thereby decreasing premenstrual syndrome (PMS) and concomitant menstrual discomfort and pain, as well as blood loss and iron deficiency [28–30].

Contemporary low-dose combination pills have a three- to fourfold decrease in estrogen content and a tenfold decrease in progestin compared with earlier generation OCs, usually containing between 15 and 35 μg ethinyl estradiol (EE) and less than 1 mg progestin [31]. In *monophasic* preparations, estrogen and progesterone doses are fixed over the entire pill cycle, while in *biphasic* or *triphasic* formulations, amounts vary to mimic normal physiologic cyclical patterns. Most OCs incorporate EE as the synthetic estrogen, although some older formulations contained mestranol (i.e., a semisynthetic estrogen).

Synthetic progestins, derived either from 19-nortestosterone or from 17-OH progesterone derivatives and 19-norprogesterone derivatives, have been chemically refined to decrease unwanted side effects. *First-generation* progestins include norethindrone and norethindrone acetate, norethynodrel, and ethynodiol diacetate. Levonorgestrel and norgestrel are *second-generation* drugs from the *gonane*

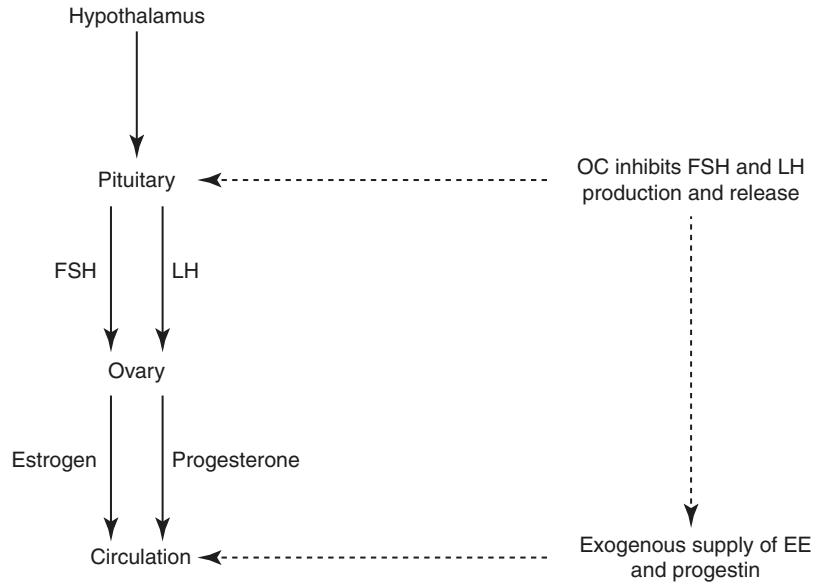

Fig. 16.2 Suppression of endogenous female sex steroid hormones by combined oral contraceptives (OCs). *FSH* follicle-stimulating hormone; *LH* luteinizing hormone; *EE* ethinyl estradiol

group, with norgestrel the more potent and the more androgenic. The *estrane* group comprises norethisterone and its metabolites, as well as norethisterone prodrugs: norethynodrel, lynestrenol, and ethynodiol acetate. *Third-generation* progestins are deliberately more selective for progestin receptors, with consequently a less negative impact on lipoproteins and atherogenesis of vessel walls. These include desogestrel (and its active metabolite 3-keto-desogestrel or etonogestrel), norgestimate, and gestodene. *Fourth-generation* progestins are dienogest (a hybrid progestin, with antiandrogenic actions) and drospirenone (derived from spironolactone, giving it anti-mineralocorticoid and progestogenic properties not found in most synthetic progestins). There are other new progestins, with varying pharmacological profiles and activities [32].

For women unable to tolerate these combined regimens or for those with medical contraindications to estrogen use, there are progestin-only preparations, such as progestin-only *minipills*, or injectable depot medroxyprogesterone acetate (DMPA) or Depo-Provera [33]. Subdermal slow-release capsules containing levonorgestrel (Norplant) or etonogestrel (Implanon) provide rapidly reversible contraception for up to 5 years with only a 1% failure rate, but require special physician training on implantation and removal of the capsules. Increased and irregular intermenstrual bleeding with progestin-only preparations may, in itself, be disruptive for athletic training and competition. There is little existing information on the use of these contraceptive methods in adolescent athletes and/or any associated effect on performance. Additional methods of long-acting reversible contraception include intrauterine devices (IUDs) containing either copper or levonorgestrel (Mirena®) and the contraceptive vaginal ring or transdermal patch [34].

Physiological Effects of the Female Sex Steroid Hormones

The female sex steroid hormones are all derivatives of cholesterol. Within the estrogen group of 18-carbon steroids, *17β estradiol* is the major form, and *estrone* and *estriol* are less potent. In women, they are secreted primarily by the ovaries and to a lesser extent by the adrenals [35]. As discussed, synthetic forms include ethinyl estradiol and mestranol. The other major female hormones are the *progestins*: endogenous *progesterone* and *first-*, *second-*, *third-*, and now *fourth-generation* synthetic progestins in OCs. The various female sex steroids (endogenous and exogenous) exert a myriad of diverse and complex effects on multiple physiologic parameters, with the potential to influence athletic performance. Therefore, considerations for exercise performance in women such hence differ significantly from men [36].

Cardiovascular Function

All estrogens are known to have important actions on the cardiovascular system [37, 38]. They alter plasma fibrinolytic activity and platelet aggregation, resulting in a detrimental increase in thrombosis. Conversely, they also confer protection against atherosclerosis by decreasing total cholesterol and low-density lipoprotein (LDL) levels and increasing high-density lipoproteins (HDL). Estrogen enhances vasodilation of the vascular smooth muscle of coronary arteries and peripheral vascular beds [39, 40], in turn increasing blood supply to the heart and muscles. At least theoretically, there is potential here for enhancement of cardiac function and aerobic performance. Estrogen controls production and release of nitric oxide (NO), now known to be the endothelium-derived relaxation factor [41], and can act as a calcium channel blocker [42, 43]. In addition, estrogen is thought to be protective against inflammation [44, 45]. Many of these actions are antagonized by progesterone, making this an extremely complex area of physiology [46].

Overall, estrogen is viewed as protective against cardiovascular disease and hypertension [47]. Therefore, there is mounting concern that women with functional hypothalamic amenorrhea (and consequently low estradiol levels) have an increased risk of cardiovascular disease, through changes in the above mechanisms [48–54]. Amenorrhea in female athletes is associated

with endothelial dysfunction and an unfavorable lipid profile [55–57]. One study showed that folic acid supplementation improved vascular function in amenorrheic athletes [58]. By replacing estradiol with an exogenous form of estrogen, OCs may ameliorate this endothelial (and vascular) dysfunction and, by extension, potentially modify this increased risk [59].

Respiratory Function

Sex hormones are involved in central neural control of breathing [60] and can also affect the lungs and the airways. Investigations have consistently shown increased minute ventilation (VE) and augmented ventilatory response to hypoxia and hypercapnia during the LP, when hormone levels are highest [61, 62]. Other researchers have documented changes in carbon dioxide (CO_2) sensitivity [63] and pulmonary carbon monoxide diffusing capacity [64]. Similar changes in ventilation and respiration are seen in pregnancy [65, 66], due to elevated progesterone levels. Different exogenous synthetic progestins in OCs may also have some influences on the respiratory system [67].

Substrate Metabolism and Energy Sources

Estrogen (and progesterone to a lesser extent) promotes glycogen uptake and storage in the liver and muscle (mostly type I fibers) and during exercise spares glycogen stores by shifting metabolism toward free fatty acids (FFA) [68–74]. This occurs by increased lipid synthesis, enhanced lipolysis in muscle through induction of lipoprotein lipase (LPL), and greater utilization of FFA (Fig. 16.3). Such metabolic adaptations may be advantageous for women during ultraendurance exercise in comparison with their male counterparts [75, 76], by reducing reliance on muscle glycogen and increasing oxidative capacity [68]. Women respond similarly to men to a protocol of carbohydrate (CHO) loading to supercompensate muscle glycogen stores, when fed the same relative amount of CHO [77].

Glycogenolysis (hepatic release of glucose) and gluconeogenesis help to control blood glucose levels along with peripheral glucose uptake. Progesterone may promote translocation of the GLUT-4 glucose transporter to the plasma membrane [73]. It has been hypothesized that the drop in blood glucose represents an inability to maintain blood glucose homeostasis, rather than increased utilization [78]. Paradoxically, however, both estrogen and progesterone suppress gluconeogenic output during exercise, which may potentially compromise performance in the latter stages of ultralong events, unless energy replacement supplements are adequate [22]. Some have suggested that it may be more relevant to supplement energy intake during exercise with protein when progesterone is elevated, compared with estrogen, because progesterone promotes protein catabolism, while estrogen suppresses it [79]. Similarly, estradiol and progesterone seem to have opposing effects in terms of lipid metabolism, with greater lipid oxidation when estradiol is used alone (Fig. 16.3). A detailed review of these complex metabolic processes, and the relative roles of hormones in regulating CHO and fat utilization at rest and during exercise, is beyond the scope of this chapter but is available elsewhere [73, 80].

Estrogen also acts in concert with growth hormone (GH), catecholamines, and insulin to regulate glucose metabolism [81] by decreasing insulin-binding capacity, resulting in deterioration in glucose tolerance and insulin resistance [82]. Transdermal estradiol has been shown to change glucose metabolism, through decreased gluconeogenesis and changes in epinephrine secretion and glucose transport [83]. Metabolic effects of progesterone include relative glucose intolerance and insulin resistance during the LP and pregnancy (when progesterone is high), with greater dependence on fat as a substrate, as shown by higher circulating FFAs [84], lower respiratory exchange ratios (RERs), and lower blood lactate levels during submaximal exercise [71, 85]. Progesterone also appears to induce peripheral insulin resistance through actions on insulin receptors [81, 82], facilitated by the presence of estrogen. Female athletes with diabetes may need

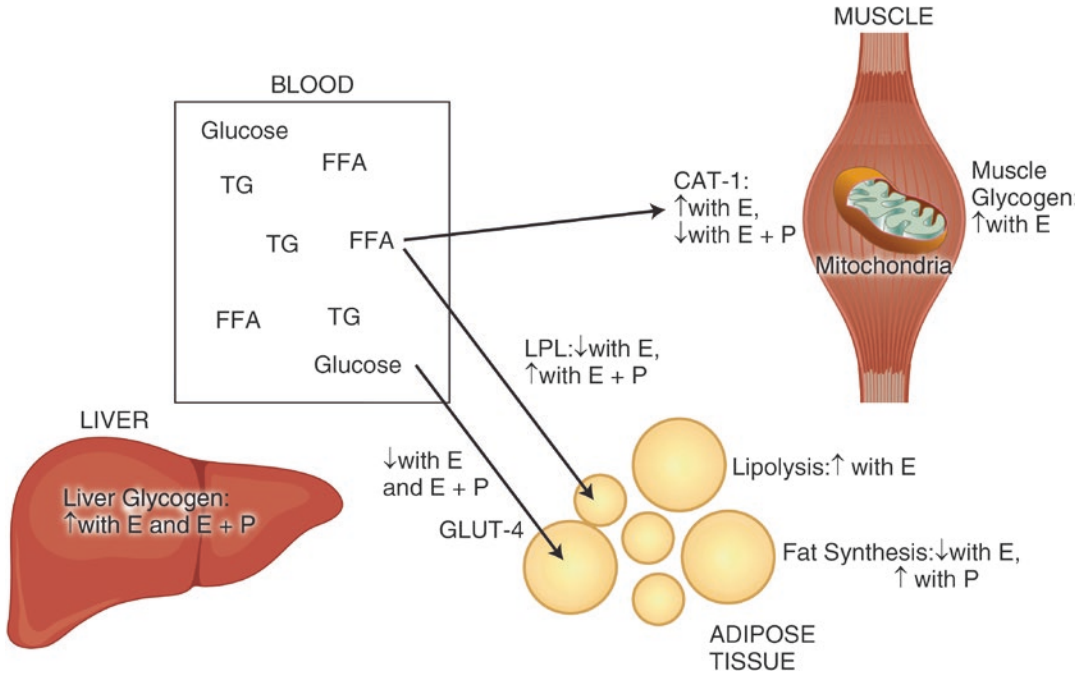

Fig. 16.3 Overview of the effects of estrogen (E) and progesterone (P) on pathways of carbohydrate and fat metabolism; ↑, increased flux through pathway; ↓, decreased flux through pathway; *TG* triglycerides; *FFA* free fatty acids; *LPL* lipoprotein lipase; *GLUT-4* glucose transporter type 4; *CAT 1* mitochondria carnitine acyltransferase 1

to be cognizant of such menstrual cycle influences, in order to optimize glucose control during training and competition.

There are varying concentrations and types of synthetic progestins in OCs, which also have the potential to impact hormonal responses and substrate utilization during exercise. The more androgenic progestins (norgestrel and levonorgestrel), in combination with ethynodiol diacetate, cause a decrease in glucose tolerance and an increase in insulin levels, but ethynodiol diacetate and norethindrone do not seem to have this effect. The newer *third-generation progestins* (i.e., desogestrel, gestodene, and norgestimate) have been designed to have less impact on glucose tolerance and insulin levels, as have *fourth-generation* progestins [86–88].

Deterioration of glucose tolerance and hyperinsulinemia are seen with DMPA, but norethindrone alone does not seem to have any significant effects. Approximately 25–50% of insulin-dependent diabetics will have an increase in insulin requirements while taking OCs, although there is great intraindividual variability [89]. Low-dose monophasic OCs, limiting the amount of androgenic activity of the progestin, may be the best choice for women with insulin-dependent diabetes mellitus (IDDM). More information is needed on the impact of third- and fourth-generation progestins on glucose tolerance, insulin responses, and any clinical implications for exercising females.

Body Composition, Weight, and Bone Mineral Density

Other actions of estrogen include deposition of fat into the breasts, buttocks, and thighs, typically female characteristics. In terms of fluid balance and plasma volume, estrogen causes sodium and chloride retention, resulting in edema, weight gain, and an increase in blood pressure. Levels of both estrogen and progesterone are high in the

end-luteal phase (i.e., immediately premenstruation). Through a complex feedback mechanism involving the renin-angiotensin system and aldosterone, progesterone in particular can cause fluid retention and increased body weight, possibly hindering performance [90]. In one study, however, sodium retention during the LP did not correlate significantly with the typical premenstrual symptoms of subjective breast tenderness and bloating [91]. In another 1-year prospective cohort study, monitoring of fluid retention during the MC in 62 healthy women revealed a peak on the first day of flow (when estradiol and progesterone levels are low), rather than premenstrually, possibly suggesting a lag of fluid dynamics in response to previous higher hormone levels [92]. Administration of OCs has variable effects on weight, body composition [93], and fluid dynamics, depending on the relative androgenicity of the progestin used [94].

Importantly for bone mineral density (BMD), estrogen facilitates both gastrointestinal calcium absorption and calcium uptake into the bone. Chronic estrogen-deficient states (such as menopause or amenorrhea and other forms of menstrual dysfunction) increase susceptibility to alterations in BMD. This can lead to either osteopenia or frank osteoporosis, with an increased risk for fractures, including stress fractures in younger individuals (a definite hindrance to training and competition!). The problem here is really twofold: lack of estrogen causes increased bone resorption, but equally and even more importantly, low energy availability (intentional or inadvertent) leads to menstrual dysfunction [95, 96]. Low energy availability interferes with bone formation through the insulin growth factor-growth hormone (IGF-GH) axis (see Chap. 11 by Loucks). This is most worrisome in the young adolescent athlete, who should be building up peak BMD during the critical years of rapid growth; even endurance training or weight-bearing exercises are not completely protective [97]. There is an ongoing need to educate athletes, coaches, and physicians about this important issue [98].

Traditional postmenopausal HRT has been designed to counteract this process and replace the beneficial effects of estrogen in maintaining BMD. Because of the other energy-dependent mechanisms involved, it remains controversial whether or not administration of OCs is protective in younger women with functional hypothalamic amenorrhea [99–101] or if the length of previous exposure is a factor [102, 103]. Influences of synthetic progestins on bone density are dependent on biochemical configuration and relative potency. Progesterone injections (DMPA) induce amenorrhea, with detrimental effects on BMD, which have been shown to be only partially reversible on discontinuation [104]. It therefore behooves health-care providers to be aware of such potential effects, especially in younger female athletes, for whom stress fractures (especially in high-risk areas, such as the femoral neck) can be catastrophic. Little to no research has been done on BMD and contraceptive hormone-releasing patches, IUDs, or vaginal rings.

Thermoregulation

Progesterone has long been recognized to have thermogenic action. It causes an increase in basal body temperature (BBT) of 0.3–0.5 °C during the LP [105–108] and during pregnancy [108] through a central action on the preoptic neurones [109]. This central effect on body temperature also increases minute ventilation [110]. There are numerous associated physiological mechanisms: altered skin blood flow [111, 112], a higher threshold for cutaneous vasodilatation, delayed onset of sweating [113, 114], and decreased thermal conductance [115]. The increased BBT is postulated to result from an alteration or *shift* in set-point temperature related to the MC [116], but there may be individual differences in these temperature changes [117]. Estrogen modifies the temperature effects of progesterone [118]. Exogenous progestins in OCs can also affect centrally thermoregulatory mechanisms and shift baseline core BBT and the threshold for the active vasodilator system to a higher internal temperature, with associated implications for exercise performance in the heat [119].

Psychological Factors: Estrogen and the Brain

There is increasing evidence that estrogen mediates different aspects of cognition, alertness, and mood, possibly through changes in the availability of neurotransmitters in the brain, such as serotonin. This is especially important during competition, where peak mental functions are required. Alterations in 5-hydroxytryptamine (5-HT) and serotonin pathways may also play a role in dysphoric PMS [120], and there may be associated changes in functional ability [121], which might increase risk for musculoskeletal injury. There have been reports of MC alterations in color vision performance, sleep-related memory consolidation, etc., but only with small numbers of subjects and mostly without hormonal verification. Brain size and morphology have been found to change over the MC, with significant grey matter volume peak and CSF loss at the time of ovulation, but with no correlation to estradiol or progesterone hormone levels [122].

It has also been proposed that estrogen benefits cognitive function and verbal memory in postmenopausal women, again through an effect on neurotransmitters, such as serotonin [123]. Recent research has shown an inverse relationship between blood glutamate levels (which have neurotoxic properties) and levels of plasma estrogen and progesterone [124]. Several current reviews explore these concepts in more detail [125, 126]. The potential effects, if any, of a prolonged hypoestrogenic state on cognitive function in amenorrheic athletes are purely speculative and hypothetical at this time.

Researchers have linked gender differences in ischemic brain injury to estrogens [127] and have even examined clinical measures of concussion during the MC [128]. Progesterone is also thought to possibly have neuroprotective benefits [129]. There is potential for OCs to positively affect cognition, attention, mood, and various other psychological parameters, such as mental rotation and verbal fluency, depending on the specific chemical formulation [130–132]. This is a fertile area for future research, especially since brain function under a varying human estrogenic environment is another source of modulation of exercise responses [133].

Female Reproductive Hormones and Athletic Performance

Physical fitness is frequently defined in terms of aerobic capacity, anaerobic capability, muscle endurance and strength, flexibility, and body fat percentage, but actual *athletic performance* is much more complex, with neuromuscular, sensorimotor, psychomotor, cognitive, and psychological functions all coming into play (Fig. 16.4). There are significant genetic inheritance effects on various components such as peak oxygen uptake and anaerobic power [134]. Therefore, both *nature* and *nurture* (genetic factors and training) are involved in determining athletic prowess [135]. Finally, the human spirit is of such tenacity that the ultimate determinate of success and athletic performance may actually reside within the brain—according to the central governor model (CGM) of Noakes [136, 137].

As detailed in previous sections, both estrogen and progesterone have significant effects on the metabolic, thermoregulatory, cardiovascular, and respiratory systems. Therefore, it is not unreasonable to conjecture that cyclical endogenous hormonal variations of the MC might influence performance. When comparing research findings, it is critical to consider the population being studied and the training status of the women (untrained, recreationally active, or elite athletes). Small subject numbers lead to inadequate power to detect significant differences, and individual variability could conceivably skew the results. Contradictory findings may be explained by the variety of testing protocols, differences in exercise intensity levels used, wide circadian variation in hormonal secretion, and discrepancies in the timing of testing, as well as the state of hydration, nutrition, and fitness of subjects. Much of the early research is fraught with methodological inaccuracies, the most sig-

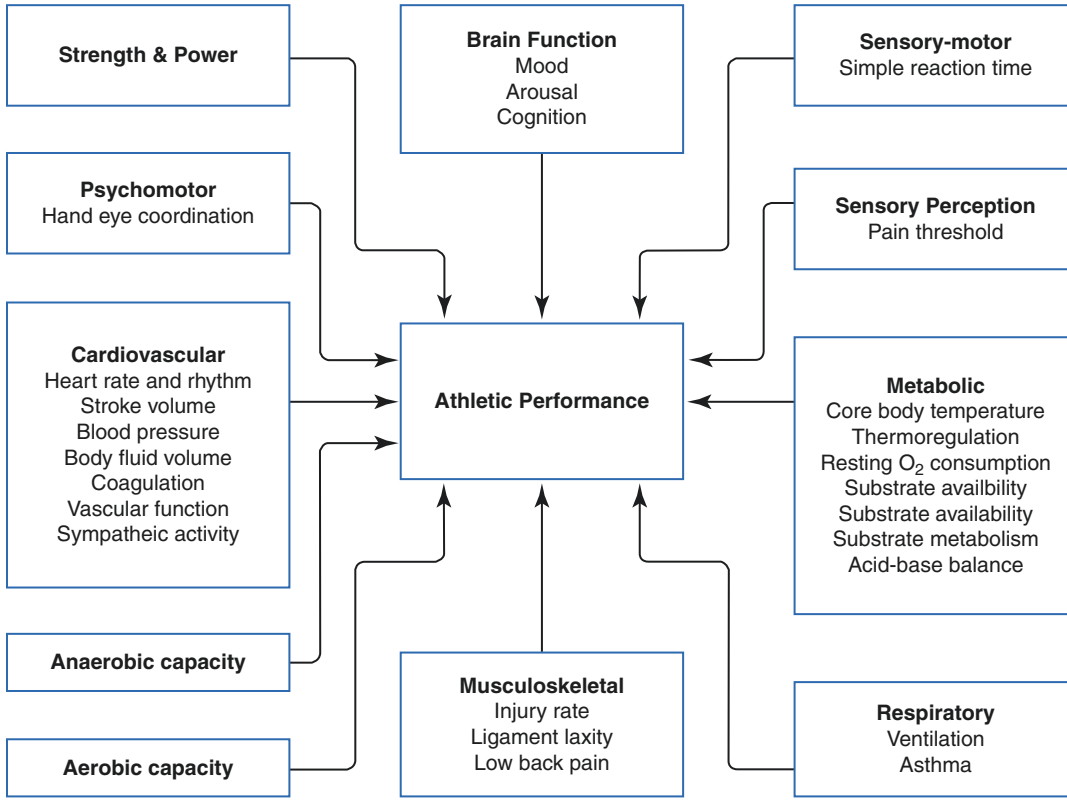

Fig. 16.4 Components of physical performance that may be affected by menstrual cycle fluctuations in endogenous hormones

nificant being inconsistent definitions and documentation of MC phases (see Chaps. 1 and 8 by Hackney et al. and Liu et al.).

Cardiovascular Function and Athletic Performance

Cardiovascular Function and the Menstrual Cycle

Some physiological parameters can potentially be affected during the MC by the different cardiovascular effects of estrogen and progesterone. There are transient changes in plasma volume (PV), peaking within 2 days of the estimated day of ovulation and progressively increasing during the LP [138]. Hemoglobin concentration may affect oxygen delivery to the tissues, but studies have shown no change [9], increased resting hemoglobin [139], or a decrease in hemoglobin in the LP [140]. Also, in terms of actual oxygen utilization, increased body temperature during the LP causes a rightward shift of the oxyhemoglobin dissociation curve, while the concomitant LP increase in ventilation shifts the curve leftward due to an increase in pH, quite probably leading to no net effect.

Peripheral hemodynamics and renal function are affected through various mechanisms by both MC phase [141] and OC use, with additional effects during exercise [142]. Measurements of tissue Doppler imaging-derived myocardial performance index during the menstrual and the LP in 27 women aged 19–42 documented that endogenous estrogen improved combined systolic and diastolic function in both the left and right ventricles [143]. Another study tested 19 eumenorrheic women with two incremental tests to voluntary exhaustion on a cycle ergometer and did not show any differences in cardiorespiratory

measures, substrate metabolism (lactate), or performance (defined as power output and oxygen consumption or VO_2), but did find a greater ventilatory drive in LP [144].

Decreased estrogen levels increase peripheral resistance and decrease exercising muscle blood flow [37, 38, 43]. Blood pressure is reduced and systemic vascular reactivity increased during the late FP, just prior to ovulation [145, 146]. Both MC [147, 148] and OCs [148] alter sympathetic nerve activity during orthostatic stress in young healthy women.

Conversely, higher estradiol levels have been shown to lower cardiovascular responses to stress, most likely through effects on arterial wall tone and a decrease in beta-receptor sensitivity to catecholamines [149]. Progesterone may act to increase cardiac excitability during the LP, as documented by a greater number and duration of episodes of paroxysmal supraventricular tachycardia (SVT) [150]. A higher heart rate (HR) and rating of perceived exertion (RPE), at the same intensity of exercise, have been seen in many studies, suggesting greater cardiovascular strain during the LP. However, given the overriding adaptations of the cardiovascular system to exercise, it is not surprising to find few observed alterations in actual athletic performance, despite this increase in HR and RPE. Within the last decade, MC phase has been demonstrated to have an influence on timing of acute coronary events, with increases during the early FP [151, 152], possibly due to alterations in adrenergic control. Additionally, estrogen may have a beneficial effect on myocardial ischemia in women with coronary artery disease (CAD) [153]. Given the increasing numbers of masters-level female athletes training and competing, some of these findings become more pertinent.

Oral Contraceptives and Cardiovascular Function

Synthetic hormones also have significant cardiovascular effects. This was suggested by very early studies in subjects taking higher-dosage OCs [154, 155]. Theoretically, such estrogen-mediated cardiovascular changes (e.g., increases in preload and stroke volume caused by increased PV) might augment effective cardiac output (CO) and enhance performance. Higher CO has been found at rest and during exercise, with or without concomitant alterations in blood pressure and vascular volume, in women on several different higher-dosage combination pills [90, 156]. It is likely that the newer formulations of synthetic progestins in OCs have less impact, as they have fewer side effects.

An echocardiographic study of 31 young women did not find any differences in resting left ventricular structure and function attributable to either hormonal variation over the MC or with a combined OC pill [157], although estradiol levels were not measured directly. Studies have also found no significant alterations in cardiac index or pulmonary artery distensibility in inactive women taking OCs [154, 158]. Heart rates at rest and during exercise generally do not change with administration of OCs; however, increases in *office* blood pressure (from 2 to 7 mmHg systolic and from 1 to 3 mmHg diastolic) have been documented during the first few months of use, diminishing over time and reversible on discontinuation of OCs. This finding has primarily been associated with low-dose monophasic OCs containing levonorgestrel, and not with triphasic preparations or OCs with the newer progestins. Detrimental changes in serum lipids are also minimized with the newer progestins and are attenuated by regular exercise [159].

Exercise has an anticoagulation effect and has been observed to act synergistically with some OCs to increase fibrinolytic activity. However, estrogen also enhances platelet aggregation and increases various coagulation factors, facilitating thrombogenesis; this effect is decreased with formulations containing 35 μg of EE or less [160]. The newest progestins may be associated with a greater incidence of venous thromboembolism [161, 162], compounded in women with factor V Leiden thrombophilia. Because of the overall increased risk of cardiovascular disease in women using OCs, current prescribing guidelines should be carefully followed [163].

Respiratory Function and Athletic Performance

Respiratory Function and the Menstrual Cycle

Minute ventilation (VE) and respiratory drive are generally increased under the influence of higher progesterone levels during LP [63, 140]. Curiously, more recent research has suggested that estradiol mediates cyclic changes in angiogenesis and causes neovascularization in the pulmonary vascular bed, leading to decreased diffusing capacity [164]. In one early study, a higher ventilatory rate during the LP was associated with greater oxygen demand, subjective dyspnea, and perceived exertion and a decrease in maximal exercise response during the LP, but only in the nonathletes [62], suggesting that these responses may be overcome with increased training.

Conversely, another early study found no significant differences in VO_{2max}, HR, time to exhaustion, or maximal ventilation between MC phases in five moderately trained women [165]. Relative ventilatory threshold (VT), however, occurred at a significantly higher percentage of VO_{2max} in the early FP compared with the mid-luteal phase. Many others since then have documented either no MC variation in ventilation or mid-luteal elevation in resting minute ventilation (VE), but generally no changes in VE during submaximal exercise. These are well reviewed in a recent research publication [166]. This latter group also did not find any MC effect on sensitivity to chemical stimuli, either isocapnic hypoxic ventilatory response (iHVR) or hypercapnic ventilatory response. They concluded that any hormone-mediated influences are of insufficient magnitude to exceed the inherent variation in these chemosensitivity measures, and that feed-forward and feedback mechanisms during exercise override the effects of naturally occurring changes in sex hormones. It appears that neither exercise VE nor physical performance in women at altitude is affected by MC phase [167], possibly for similar reasons, although some have found ventilatory alterations with higher work output, but no change in VO_{2max} [168]. Menstrual cycle-induced modulation of the ventilatory responses to exercise may be altered under acute hypobaric-hypoxic conditions [169].

There is also a documented link between female sex steroids and airway function: variously termed *perimenstrual asthma* [170], *premenstrual asthma* (*PMA*) [171], or *menstrual-linked asthma* (*MLA*). Overall, an estimated 33–52% of asthmatic women report premenstrual worsening of asthma symptoms, and an additional 22% report their asthma to be more severe during menses [172]. This can have significant clinical impact for female athletes with asthma [173–176].

The role of endogenous and exogenous hormones in asthma is postulated to involve various mechanisms [177]. Airway hyperresponsiveness is exacerbated during premenstrual phase, due to the withdrawal of high levels of progesterone (a smooth muscle relaxant) and estrogen or both. In terms of airway tone, estrogens increase the production of nitric oxide (NO), an endogenous bronchodilator, in the human bronchial epithelium [178]. Estrogens also have anti-inflammatory effects, so they can modulate the increased airway wall inflammation found in MLA [179]. Recently, a large cross-sectional population health questionnaire of women registered in an asthma database (540/1260 – a 43% response rate) found an 11% incidence of self-reported MLA (worse with menstruation) as well as an increased association with other autoimmune-type diseases, such as rheumatoid arthritis, eczema, and heart disease [180]. In this study, women with MLA compared to women without MLA also reported more urgent/emergent asthma-related health-care visits per year, more emergency room visits, and higher asthma-related absenteeism and used almost twice the number of β_2 agonist rescue doses per day.

The multifactorial etiology of MLA has implications for pharmacotherapy [181, 182]. Short- and long-acting β_2 agonists are helpful [183], as are leukotriene antagonists [184]. OC use has been reported to reduce the prevalence of current wheeze in women with a history of asthma [185], with a significant trend linked to duration of use [177]; conversely, however, OC administration has also been found to exacerbate MLA [186].

Oral Contraceptives and Respiratory Function

Ventilatory changes similar to those of the LP of the MC, along with increased oxygen consumption [187], have also been documented in women taking the older higher-dose OCs, with some evidence for accommodation over time [188]. However, studies using low-dose OCs did not show any associated ventilatory changes, but documented a slight decrement in aerobic capacity [189, 190]. A recent pilot study of cardiorespiratory fitness in 12 endurance-trained runners suggested that OC use might attenuate MC-induced ventilatory changes as compared with non-OC users [191], thus allowing for more consistent cardiorespiratory fitness throughout the (artificial) cycle.

Substrate Metabolism

Estrogen and progesterone promote glycogen uptake and storage. Muscle biopsies have documented increased muscle glycogen storage during the LP [192], with greater fat use and oxidation at ovulation postulated to be due to higher estrogen levels. Glycogen repletion after exhaustive exercise has been noted to increase during the FP [68]. Some investigators have found a lower RER during the LP, suggesting increased reliance on FFA for fuel [68, 139]; however, most have not found this to be the case [113, 114, 193–195]. This may be dependent on exercise intensity, which shifts the metabolic demands from aerobic to anaerobic fuel sources. For example, during the LP in nine eumenorrheic women, lower CHO use and oxidation rates, in association with greater lipid use and oxidation, were documented with 10-min submaximal treadmill exercise intensities at 35% and 60% VO_{2max}, but not at 75% VO_{2max} [70].

Blood lactate generally increases in proportion to exercise intensity and the anaerobic contribution to glycolysis. A number of early studies with hormonal documentation suggested decreased lactate production during the LP [6, 78, 140], associated with an increase in endurance time [6, 139], but many others have not demonstrated any significant MC differences in lactate [144, 165, 193–197], or any enhanced LP performance. In a study of nine athletes, no differences were found in resting blood lactate or in running time to exhaustion, but recovery lactate levels were significantly lower during the LP [197]. This suggests preferential metabolism of lipids and a reduction in CHO metabolism, but confounding factors are energy demand and nutritional status. A CHO-loading diet can supercompensate muscle glycogen stores in the early FP to values attained in the LP [198–200]. There may also be mechanisms related to other hormones; for example, in a study of eight women performing three maximal exercise tests with simultaneous determination of lactate threshold during early and mid-FP, and mid-LP, there was no effect on lactate threshold, but there was a significant correlation between LT and the epinephrine breakpoint [201].

Theoretically, endurance performance could also be enhanced during the LP, through increased muscle glycogen stores [68, 192] and the effects of MC on CHO and lipid metabolism [84]. Consistent with these findings, high estrogen levels during the LP are associated with sparing of muscle glycogen, in comparison with the FP [80]. Hypothetically, such advantage might be lost in amenorrheic athletes [202]. Dissociation of blood glucose homeostasis during exercise has also been shown to occur [78]. An additional factor to consider is fluctuation with the time of day [203, 204]. Contradictory research (especially for endurance performance) can be explained by differences in definitions and exercise protocols for endurance, variability in subject fitness, preexercise hydration, nutritional status, and initial muscle glycogen stores [205].

A different study found substrate oxidation and GH responses to exercise to be independent of MC phase and status [195]. However, exercise-associated GH release is attenuated in amenorrheic athletes, due to decreased growth hormone-releasing hormone (GHRH) response to exercise, compared to eumenorrheic athletes This is particularly relevant to adolescent female athletes, since the prevalence of athletic (hypothalamic) amenorrhea among these athletes is

4–20 times higher than the general population, most frequently also associated with energy deficiency. Reduced exercise-induced GH response in these athletes may be important, since it potentially indicates reduced effectiveness to training stimuli.

Glucose tolerance deteriorates during the LP [81], but it has been suggested that the cyclic changes in metabolic control are attributable to mechanisms other than the variations in insulin sensitivity, such as energy intake and caloric expenditure. Other investigators have not shown a substantial change in blood glucose over the MC [68, 195, 206]. It may be more appropriate to look at E/P ratio (pmol/nmol) during the LP as well as the absolute magnitude of increase in ovarian hormones.

Substrate turnover studies using radioactive tracer isotopes have very precisely examined the kinetics of glucose and lipid metabolism, using glucose rate of appearance and glycerol appearance, respectively, and have found lower CHO utilization during the LP than the FP [207–209]. Another group did not find any MC phase effect on glycerol or palmitate kinetics during 90 min of moderate exercise [210]. This complex subject is well reviewed elsewhere [22].

Synthetic steroids in OCs appear to increase glucose flux, but not overall CHO and lipid oxidation rates, during moderate-intensity exercise, with greater metabolic effects than endogenous ovarian hormones [211, 212]. Increased TG mobilization is not matched by an increase in oxidation but rather by reesterification. When CHO use predominates (such as in a postprandial state or during moderate-intensity exercise), whole-body plasma FFA turnover does not appear to be affected by either MC or OC, suggesting that the hierarchy of regulators of fatty acid oxidation is exercise intensity > recent CHO nutrition > synthetic hormones > endogenous ovarian hormones.

The interaction of OCs with energy metabolism systems is complex, and potential ergogenic or deleterious effects are not well defined. Although glucose production is determined primarily by insulin, other hormones, such as thyroxine, catecholamines (epinephrine and norepinephrine), cortisol, and growth hormone (GH), can have a counterregulatory effect. GH levels are increased by high doses of estrogen, hypoglycemia, and endogenous opioids and decreased by progestins. At rest, fasting blood glucose, serum triglycerides, basal serum prolactin, and GH may be higher in women taking combination OCs. Other studies have shown lower blood glucose levels at rest and during exercise in OC users and an increase in FFA levels during mild exercise [206]. During prolonged exercise, women on OCs have been shown to have an elevated GH response to exercise and lower glucose and CHO use, with a shift more toward metabolism of FFA [165]. Overall, there appears to be a possible glycogen-sparing effect of OCs, with the magnitude dependent on the specific chemical formulation. These alterations in substrate utilization, although minor, might still have an effect on aerobic performance, particularly in the elite female athlete.

Thermoregulation

Although some early studies did not show any cycle phase differences in response to short-term exercise or heat exposure [113, 114], others demonstrated that the normal hormonal fluctuations of an ovulatory MC may increase the propensity for heat stress during the LP [213–216]. This can happen even at rest, i.e., with passive heat exposure [217], but can be somewhat ameliorated with acclimation and physical training [218]. Short-term endurance training in previously untrained subjects improves heat loss responses by decreasing the threshold temperatures, with this effect occurring within a month of training and disappearing within a month of cessation of training [219]. The degree of increase in sweating with training differs among body sites (chest, thigh, back, and forearm) and might be affected by MC phase.

The length of endurance performance in humans is related to a critical internal temperature [220], which is why precooling strategies before exercise, and in between bouts of exertion in the heat, are found to be physiologically advantageous

[221, 222] and to decrease perceived exertion [223]. A higher initial core body temperature during the LP may therefore theoretically increase the risk for heat accumulation when exercising in hot weather, thus decreasing time to fatigue [224, 225]. An early study of seven eumenorrheic women [226] did not find any variation in sweat loss, sweat rate, and steady-state exercise metabolism rate during a 2-h cycle ergometer exercise at 30% of maximal VO_2 uptake in the heat, during mid-FP, menstrual flow, midcycle (including ovulation), and mid-LP. However, this heat stress test was conducted *after* heat acclimation. Elevated skin temperature also has an adverse effect [227], as does a high starting ambient temperature [228].

Thresholds for shivering and sweating vary over the course of an ovulatory MC, with LP enhancement of the heat loss response, but with no associated impact on performance [113]. Actual metabolic heat production does not seem to change from the FP to the LP, possibly due to decreased skin thermal conductance [115]. A greater temperature threshold and larger gains for sweating have been reported during the LP compared with the FP [229], as well as a greater sweat rate [230]. In the latter study, women exercising in a warm and humid environment (32 °C and 80% humidity) with adequate water intake seemed able to adapt to the luteal phase increase of BBT, through reduced urinary volume and increased sweating rate.

Plasma volume (PV) dynamics during passive heat stress and exercise are also affected by MC phase [231]. In women exercising at 80% $VO_{2\,peak}$ on a cycle ergometer and passive heat stress of 50 °C (dry environment), more fluid shifted out of the vasculature during the LP, and there was a greater fluid loss during exercise in the FP, yet the final PV was not different between phases. It has been proposed that the thermoregulatory vasodilator response is attenuated by increasing exercise-induced vasoconstrictor tone in proportion to exercise intensity [111]. Therefore, under extreme conditions, and at high ambient temperature and humidity, there may be significant implications for women participating in prolonged endurance activities (e.g., marathons, ultramarathons, and triathlons) during the LP [216].

For unacclimatized game players, although the performance of intermittent, high-intensity shuttle running in the heat was unaffected by MC phase, it was influenced by OC use. Athletes on OC ran further (improved performance) on days 15–28 compared to days 1–14, despite higher rectal temperatures, HR, and GH but lower plasma glucose. However, the numbers were small—seven women studied during the MC and eight on OC [232]. Interestingly, OCs may also impact thermoregulatory responses to exercise [229, 233–235] by increasing BBT by 0.2 °C during the days on active OC pills, as compared with the days off OCs. There also appears to be an elevated HR during exercise [233]. A potential advantage of OC use, then, is to make thermoregulatory responses more uniform across the cycle (at least for 21 days), thereby decreasing any cyclic performance alterations due to changes in core BBT. However, it appears that the administration of synthetic progestins in OCs still causes higher core BBT at rest and during exercise, similar to the influence of endogenous progesterone during the LP.

Physical Capacity, Strength, and Reproductive Hormones

Physical Capacity, Strength, and the Menstrual Cycle

Physical work capacity during the MC has been investigated [196, 236, 237], but without hormonal documentation, it is difficult to give much credence to these studies. However, there is a suggestion from these early studies that HR response to repetitive lifting and an isometric endurance lift were both greater by 7–10 beats per minute in the postovulatory phase, possibly as a result of the elevation in temperature [237, 238]. Superior achievement in hip strength (flexion and extension) and standing broad jump during the premenstrual phase [1], isometric handgrip endurance of forearm contraction during the ovulatory phase [239], maximal voluntary contraction of handgrip during the FP [240], and handgrip strength during menses [241] have all been reported in the past, but most investigators

have not noted any significant MC effects [3, 242–244]. A prospective study with hormonal measurements did not find any variations in isokinetic strength of knee flexion and extension between FP and LP [245].

Muscle strength decreases with the onset of menopause, and it is thought that estrogen may have an inotropic effect on muscle strength [246, 247]. Estradiol promotes secretion of GH, a known anabolic hormone. Some early research suggests that estrogen increases the ability of muscles to contract by about 10%, with a peak in strength just before ovulation [239, 248]. Contrary to this finding, women being treated with gonadotropin injections for in vitro fertilization did not have any change in maximal strength and fatigability of the first dorsal interosseous muscle, despite the iatrogenic acute and massive fluctuations in estrogen [249]. One group did not find any variation in either muscle strength or endurance with changing hormone levels across three MC phases [250]. Others have documented maximal muscle contraction during the ovulatory phase, but without any significant changes in muscle strength, fatigability, or electrically stimulated contractile properties [251]. Since then, a corroborating study [252] also found improved muscle strength at ovulation, with isokinetic peak torque knee flexors improved by ~9 N m and maximum voluntary isometric contraction knee extensors by ~5 N m.

There are no clear mechanisms for these noted effects, but a recent publication reviews this area in much more detail [253]. In an elegant experiment sequentially assessing limb blood flow and skin and deep tissue temperature change during the MC, isometric endurance was shown to be lower during the end of the FP, due to (1) the cyclic variation in muscle temperature, (2) direct effects of the MC on circulation, and (3) direct effects of the MC on muscle [254]. More recent work has attempted to link changes in electromyography variables over the MC to increased muscle fatigue [255, 256].

Progesterone does not appear to have substantial effects on either muscle strength or function, but testosterone definitely has anabolic actions in females [257]. Although not systematically studied, testosterone varies over the MC under the control of luteinizing hormone (LH), and also from peripheral conversion of androstenedione, with very little actually produced by the ovaries. Concentrations are lowest in the early FP, highest just prior to or at the time of ovulation, and then fall during LP [258]. Dehydroepiandrosterone (DHEA) and its sulfoconjugate DHEA-S, which are secreted by the adrenal glands, as well as androstenedione, secreted by adrenals and ovaries, are all of physiological importance in women [259] and peak prior to or at the time of ovulation [35]. Exhaustive physical exercise (an ergocycle test at 75% of their VO_{2max} until exhaustion) induces an increase in circulating DHEA-S and testosterone in young women [260, 261].

Studies looking at effects of resistance exercise on circulation of androgens in women are still contradictory: both estradiol and progesterone increase after a single bout of resistance exercise in mid-LP, but not in early FP [259]. These different responses have prompted some investigators [262] to suggest periodization of training cycles during different phases of the MC to maximize anabolic effects. An early study modeled *menstrual cycle-triggered training* (MCTT) in seven healthy women—every second day in the FP and once per week in the LP—vs. *regular* training periodization (every third day over the entire MC without regard for cycle phase) for a total of 4 weeks [263]. Trainability of isokinetic strength of one-leg knee extensor muscles was slightly greater during FP, compared to when the respective leg was trained for 4 weeks without regard for the cycle phase (33% increase vs. 13% increase). It is known that estrogen may govern the regulation of a number of downstream genes and molecular targets [264], improving the intrinsic quality of skeletal muscles by enabling fibers to generate force through myosin binding to actin during contraction [265]. Estrogen may reduce protein catabolism; in contrast, progesterone influences amino acid oxidation and protein degradation with increased protein catabolism, greater in LP than in FP, both at rest and during exercise.

Oral Contraceptives and Strength

At one time it was hypothesized that the androgenic component of the OCs might be ergogenic and help to increase muscle strength. In 1987 the International Olympic Committee (IOC) actually contemplated banning compounds containing norethindrone, a more androgenic progestin. Fortunately, this situation was successfully challenged and overruled. An early study suggested that OCs might have an effect on static muscle function [240], but to date there is no consensus on this matter. Other studies did not find any significant MC phase differences or changes with OCs in various strength parameters [239, 245, 248, 266–268], even with OCs containing progestins of higher androgenicity [269]. Similarly, a prospective study of strength and torque production in collegiate women softball and water polo athletes participating in a 12-week strength development program did not demonstrate any positive impact of taking combination OCs [270].

The thermogenic effect of progesterone [229], which may have a detrimental impact on forearm isometric endurance and muscle force, is minimized in athletes taking OCs [240]. Some researchers believe that increased GH response to exercise in women on OCs would potentiate the effects of a training program [271]. However, recent work has questioned the effect of OC administration on serum androstenedione and the bioavailability of testosterone, and suggested a potential negative impact on the synthesis and breakdown of myofibrillar proteins [272]. This may also be related to the effect of MC and OCs to attenuate delayed onset muscle soreness (DOMS) 48 h postexercise [273], through a potential protective role of estrogen in decreasing production of prostaglandins [274].

Aerobic Capacity and Reproductive Hormones

Aerobic Capacity and the Menstrual Cycle

For the most part, aerobic performance, as measured by maximal oxygen capacity (VO_{2max}) and submaximal exercise responses, does not change significantly during an ovulatory MC [140, 144, 165, 168, 194, 201, 245, 275]. Despite theoretical metabolic advantages, mostly to do with substrate metabolism, or enhanced glycogen stores during the LP [68], only a few studies have suggested enhanced endurance performance [68, 139].

Earlier work, with hormonal documentation of MC phase, is well summarized elsewhere [253], with predominately comparable findings. For example, a comparison of eight eumenorrheic and eight amenorrheic runners doing one maximal and one submaximal treadmill run (40 min at 80% VO_{2max} treadmill run), during early FP and mid-LP (confirmed by urinary and serum hormone levels), did not find any phase or group differences in oxygen uptake, minute ventilation, HR, RER, RPE, time to fatigue (maximal), or plasma lactate [194]. A few small studies have measured a slight decrement in aerobic capacity [245] and exercise efficiency [114] during high-intensity exercise in the LP. The latter paralleled a 5.2% increase in oxygen consumption, a 5.6% increase in metabolic rate, and a decrease in net efficiency of 5.3% [114]. Testing protocols differ, generally without any standardization of other variables, such as circadian rhythms, nutritional and hydration status, glycogen stores, caffeine ingestion, and exercise during the 24 h prior to testing. In all likelihood, these confounding factors contribute more to changes or enhancement of performance than hormonal influences of either MC or OC. In addition, running economy (RE), defined as the rate of oxygen consumption (VO_2) during a given submaximal steady-state running speed, has been suggested as a better measure of performance than VO_{2max} [25].

Investigations of work performance and MC have looked at physiological and psychological determinants, temperature, pain perception, and so on [237, 276, 277]. There is an entire body of literature in this area [278]. Most reports are based on surveys or cross-sectional data, without hormonal verification of cycle phase, but are thought-provoking, nonetheless.

In a study of MC effects on exercise in sedentary young women, 14 subjects with a peak VO_{2max} < 45 mL/kg/min were subjected to an incremental test to exhaustion and steady-state

submaximal cycle ergometer during two phases [275]. Time to exhaustion, maximum power output and total work done, absolute VO_{2peak}, VE, respiratory frequency and HR, and lactate and lactate threshold did not differ between phases. However, as workload increased, plasma lactate, carbon dioxide output, and RER were all lower during LP, while oxygen uptake was higher. During the steady-state tests at submaximal intensities (at workloads of 25% and 75% of menstrual cycle phase-specific VO_{2peak}), findings were similar. Exercise performance did not change between MC phases, but metabolic responses suggested greater dependence on fat as the energy source during LP.

Oral Contraceptives and Aerobic Capacity

Performance enhancement has been reported anecdotally in 8% of women on OCs [2], but conversely a detrimental impact on VO_{2max} was found after 2 months on a higher-dose OC, reversible on discontinuation [279]. Women taking a low-dose monophasic OC (0.4 mg norethindrone) for 6 months had a 7% decrease in maximal VO_2 uptake and 8% deterioration in exercise performance (as measured by oxygen pulse or volume of oxygen consumed per heart beat) [189]. In seven women taking a lower-dose triphasic OC for 2 months, compared with a similar group on placebo, there was a smaller decrement in VO_{2max} (4.7%) not evident in the placebo group, who experienced a 1.5% increase over the same time period [190]. Neither anaerobic capacity nor aerobic endurance was altered in this particular study. In another investigation of ten moderately trained women, prospectively randomized to placebo ($n = 3$) or an OC containing 1 mg norethindrone and 35 μg ethinyl estradiol ($n = 7$) for 21 days, neither the cycle phase nor low-dose OC had any significant adverse effects on ventilatory measures or performance during a maximal treadmill test or endurance run [280]. A separate research group has been studying *within OC* differences: 13 female cyclists (mean peak VO_2 53.0 ± 5.6 mL/kg/min) had alterations in several physiological variables between the tests done during pill consumption and during early and late withdrawal phases, but had no performance differences in a 1-h cycle endurance test [281].

Others have looked at aerobic capacity during various MC phases (10 women) compared with low-dose OC (5 women) [282]. Again, there were no differences between mid-FP and late LP or OC test results at two time points. However, there was altered metabolism *between groups* (plasma ammonium was higher in the non-OC group) and *within the OC group* (blood lactate and ammonium were higher within the first week compared with the second week on OCs), believed to represent differences in substrate metabolism. Another study of ten women on a monophasic OC found a somewhat lower (3–5.8%) decrease in VO_2 when participants were on early and late OC use, compared to off OC, during a 12-min treadmill run (4 min each at 7, 8, and 9 km/h) [283]. This was actually associated with an improved running economy.

More contemporary work investigated peak exercise capacity in six moderately active women during the FP and LP and after 4 months on the same triphasic OC [284]. There were no significant MC changes, but all subjects experienced decrements in VO_{2peak} (average: 11% L/min; 13% mL/kg/min), as well as decreased time to peak exercise (14%) and peak power output (8%). These were not different between tests during OC and the week off OC (low and high ethinyl estradiol levels), suggesting a persistence of OC effects. Finally, contrary to the above findings, one study comparing two monophasic OCs with differing dosages of synthetic progestins (single-blind, randomized, counterbalanced, crossover study) suggested a higher VO_{2peak} with usage of OCs for more than 6 months, with the effect more pronounced in the higher progestin OC [285]. Obviously, much more accurate research is needed.

Anaerobic Capacity and Reproductive Hormones

Anaerobic Capacity and the Menstrual Cycle

Anaerobic capacity refers to the maximal amount of adenosine triphosphate (ATP) resynthesized via anaerobic metabolism during a specific bout

of short-duration exercise. There is a paucity of research in this area, but for the most part there is either no difference in anaerobic power output during the different phases of the MC or greater anaerobic capacity and peak power during the LP. Older studies suggested no MC effects on an anaerobic endurance test on a cycle ergometer (no hormonal analyses of cycle phase) [286] or a 600-yd run test, with postexercise blood tests [287]. Another investigation without hormonal verification measured mean power output and peak power output during a modified Wingate test as greater in MF than in either ML or menstrual phases [288]. Others have reported poorer performances during the menstrual phase in a 50-m swim exercise [289] and standing broad jump [1].

It appears that the activity and fitness level of the women may be a factor. Previous studies (using cycle history only) have shown some decrement in performance during menstruation, but *more active women* (collegiate athletes) [290] were less influenced by MC phase than women defined as *fairly active* (11% less anaerobic capacity and 6% less anaerobic power in FP than in LP) [291] or healthy *active* women [242]. Fifteen sedentary females (ages 19–23 years) performed a Wingate test on a Monark 818E ergometer with 75 g/kg load on the seventh, 14th, and 21st cycle days randomly. There were no differences in peak power, mean power, and fatigue index, nor any correlations with estradiol or progesterone levels [292]. Studies of fit athletes (VO_{2max} > 50 mL/kg/min) with more precise hormonal documentation showed no changes in the anaerobic treadmill speed test or AST (subjects run at 3.52 m/s at a 20% grade until unable to maintain the set pace—average times approx. 28–29 s) between FP and LP [245].

A small study of six women doing ten 6-s sprints on a cycle ergometer during the FP and LP revealed no differences in peak power, oxygen intake, or capillary blood lactate. However, average work was greater in LP, as was the recovery VO_2 between sprints [293]. A different protocol tested eight females doing repeated 30-s sprints with 2-min rest periods at three phases—FP, just prior to ovulation (midcycle), and LP (high endogenous hormones). There were no measured changes in peak power output, recovery, lactate, blood pH and ammonia, or estimated plasma volume [294]. Maximal accumulated oxygen deficit (MAOD) was assessed in 12 women with an average VO_{2max} of 34.9 mL/kg/min, during repeated submaximal cycling exercise at 50%, 60%, 70%, and 80% of VO_{2max} for 10 min, followed by repeated sprint cycling for three times at 120% of VO_{2max}, with 20-min rest between sprints. Again, no significant differences were found in any physiological parameters [295].

Oral Contraceptives and Anaerobic Capacity

In a model of performance using OCs to simulate the MC, five female rowers were tested for anaerobic power (10-s all-out effort) and anaerobic capacity (1000-m row) at two time points in each of three OC cycles: on OC days 16–18 (high exogenous estrogen and progesterone levels) and OC days 26–28 (i.e., during the week off OCs) [296]. Peak power output was higher, and rowing performance better, at the low (exogenous) hormone levels. Pre- and postexercise glucose concentrations, plasma resting, and postexercise TG were also lower at this time. Endogenous hormones were not significantly different between phases.

A study using a multijump test, squatting jump test, and force velocity test on a cycle ergometer saw no differences between MC phases, consistent throughout three different time phases in a normal cycle—menstruation (days 1–4), mid-FP (days 7–9), and mid-LP (days 19–21) (confirmed by serum progesterone)—or three testing times in a subsequent OC cycle; however, these particular tests might not have adequately stressed the lactic acid system [297]. One of the largest studies examining the effects of reproductive hormones on anaerobic performance tested seven women with normal menstrual cycles during the menses and LP (documented by urinary LH levels), and 17 women on OCs (OCs) [268]. No significant cycle phase differences were found in the women with regular cycles, or between active-pill (high hor-

mone) and withdrawal phases in the OC users on the Wingate cycle test and associated parameters (such as peak power, anaerobic capacity, and power decline), or in power for the Margaria-Kalamen staircase test. Another group compared nine OC users and eight normally menstruating subjects (NM) in an all-out 30-s sprint on a treadmill and did not find any difference in peak and mean power output [298]. However, the integrated GH was greater in the OC group, suggesting that the high-androgenicity OCs in this study caused a higher GH response to sprinting. Again, this begs the question of some type of anabolic effect of OCs through the effects on GH secretion during exercise.

Of note in many of these studies, however, is that the women are on different OCs, both multiphasic and monophasic. One group of investigators [282] studied five recreationally active women doing an intermittent running protocol (20-s sprints of increasing speeds with 100-s passive recovery until fatigue). Performance was the same after 2 weeks of OC, but higher lactate levels were found during the first week of testing, suggesting a possible alteration in metabolism with OC. Yet in a subsequent study of nine untrained women doing intermittent treadmill running followed by a run to exhaustion, there were no differences in performance or in energy metabolism between tests conducted during menstruation and after OC for 19–21 days [299]. In conjunction with anaerobic performance, the anaerobic threshold [300] appears to be affected by usage of low-dose OCs.

Female Reproductive Hormones and Overall Sports Performance

Menstrual Cycle and Overall Sports Performance

Optimal sports-specific performance is the primary goal of every athlete. Success in events such as long-distance running and cycling is more dependent on aerobic capacity, whereas sprinting activities require anaerobic capacity. Weightlifting obviously necessitates great strength, while fluid retention might be critical for weight-dependent sports as well as for those where the body has to be lifted off the ground (high jumping, gymnastics, etc.). Early studies were based on subjective feelings, and relied on subject recall of menstrual status (a substantial source of error and bias), without measurement of hormone levels [301, 302]. Many athletes described a decrement in performance during the premenstrual and menstrual phases, while others reported improved performance [303, 304]. Perimenstrual symptoms, such as abdominal or low back pain, fatigue, or nervousness during menstruation, may also come into play. Survey questionnaires of NCAA athletes [305] and 241 Turkish athletes [306] did not yield any clarifications. Nevertheless, gold medals have been won and world records set at all different phases of the menstrual cycle.

Some previous studies have addressed MC and performance in specific sports [9]. For example, swimming speed was found to be highest during menstruation and lowest during the premenstrual period [289, 307]; cross-country skiers performed better in the early LP and in the late FP [308]; and in runners, no effect of MC phase on aerobic parameters or perceived exertion was identified [194]. A more recent study of performance in a cycling time trial found it to be enhanced during the late FP, with the preovulatory surge in estradiol and suppressed progesterone [309].

Several contemporary studies on rowers should be reassuring for elite female athletes in this sport: 11 rowers were tested on a 1-h ergometer exercise at 70% VO_{2max} in the FP and LP, finding no effects on energy expenditure, heart rate, blood lactate, or substrate oxidation (RER) [310]. Other work by this same group compared 24 rowers—8 competitive cyclic athletes, 7 recreationally trained cyclic athletes, and 9 recreationally trained rowers taking OC during 2 incremental tests to voluntary exhaustion. Higher values of VE/VCO_2 were documented during the LP at both intensities in the cyclic athletes, compared with *FP* in the group on OCs (not really phases then), but no other significant differences were found, particularly not in any performance variable [311].

Oral Contraceptives and Overall Sports Performance

The question of whether OCs help or hinder overall athletic performance still remains largely unanswered [21]. Studies have documented fewer musculoskeletal injuries in women taking OCs, likely secondary to amelioration of PMS symptoms and dysmenorrhea [312–314]. Presently, extended- and continuous-use oral contraceptive regimens may give even better cycle control and reduce performance-disrupting dysmenorrhea and PMS symptoms, as well as the more severe premenstrual dysphoric disorder (PMDD). Current formulations include Seasonale, with 91 consecutive days of active OCs [315], and Lybrel (levonorgestrel/ethinyl estradiol), with continuous active OCs for 1 year. Both regimens are safe and efficacious [316, 317], but current usage by athletes and any effects on performance are unknown.

As mentioned above, endurance capacity (1-h rowing ergometer test at an intensity of 70% VO_{2max}) was not significantly different in eight rowers on a monophasic OC, during the active-pill and non-active-pill phases [318]. Another study did not demonstrate any effects on an endurance test in women taking a triphasic OC, during similar testing phases [296]. Several different performance tests were examined in ten female team sports players using a monophasic OC: during pill consumption and during early and late withdrawal phases [319]. Only reactive strength during a drop jump landing from the 45-cm height varied; it was higher during the OC consumption phase. The authors postulated a possible implication of hormones on neuromuscular timing and the stretch-shortening cycle, which are both important for sprinting and jumping performance.

The most recent research in this area raises some other interesting questions. Swimmers performed a 200-m time trial in three *phases* of a monophasic OC: during consumption and both early and late withdrawal phases. Swim times were not different, but there was decreased blood lactate and increased pH during the withdrawal phase, postulated to occur because of increase in fluid retention, plasma volume, and cellular alkalosis [320]. There are potential implications for coaches and elite-level athletes when basing training programs on lactate levels.

Female Reproductive Hormones and Sports Injuries

ACL Injuries

The incidence of sports injuries in females is nearly tenfold that of males. This has been found in ball games, running, biking, military training, and other physical activities. Noncontact injuries to the anterior cruciate ligament (ACL) occur at a rate 4–6 times that of males participating in the same jumping and cutting sports, such as soccer and basketball. Postulated mechanisms that may be impacted by the female hormones (estrogen, progesterone, and relaxin) include anatomical and biomechanical factors, neuromuscular control, ligament laxity, knee instability, and others; these have been reviewed in detail by ACL research groups [321–323] and even by the IOC Medical Commission [324]. Most studies focus on knee injuries and the changes in ACL laxity throughout the MC. Estrogen and progesterone are believed to affect tensile properties of ligaments [325] as well as neuromuscular function. Estrogen has potential mechanical effects on the collagen-rich structures that contribute to joint stability. For example, increases in knee joint laxity at the time of ovulation have a detrimental effect on mediolateral knee joint loading during cutting maneuvers, which is subject specific [326]. This is thought to lead to increased joint loads [327].

Both estrogen and progesterone receptors have been found in synoviocytes in the lining of the knee, fibroblasts in the stroma of the ACL [328], and cells in the blood vessel walls of the ligaments in both men and women [328–330]. There are also relaxin receptors in the ACL [331, 332]. The importance of these findings and any causal relationship to an increased incidence of ACL injury is still under debate, although a recent prospective study monitored 143 NCAA Division I athletes over their 4-year career. Those elite

female athletes sustaining ACL injury had elevated serum relaxin levels and therefore may have been at increased risk [333].

Much of the early information came from animal studies [334], later replicated in humans [335]. The results of in vivo assessment of knee joint laxity in humans are somewhat mixed [336–338], documenting either no changes with MC or, similar to animal model studies, increased compliance at times of high estrogen (i.e., ≈ovulation) [338] and/or during the LP [339, 340]. No changes in ACL laxity were found among three phases of the MC in high school-aged girls [341], including at the time of ovulation (determined with the OvuQuick™ One-Step Ovulation Predictor (Quidel Corp., San Diego, CA)). However, because ovulation lasts 24–36 h in a normally cycling female, this phase in the menstrual cycle is more correctly referred to as *near ovulation* to reflect this.

Various investigators have attempted to characterize high- and low-risk MC phases (Table 16.1). An early study, using only interviewer-administered questionnaires to 28 women, reported a significant statistical association between the MC and likelihood of ACL injuries (greater during the ovulatory phase and less during the FP in 28 women) [342]. Interestingly, a group of researchers reanalyzed the data and found a different chi-square value, which was not significant [343]. The authors responded appropriately with a retraction but maintained that there were fewer injuries than expected in the FP and more in the ovulatory phase. These original investigators published a later study using urinary hormone levels for cycle phase verifications and validated their hypothesis of greater than expected incidence of ACL injuries during midcycle (ovulatory phase), but less than expected during the LP [344]. Other researchers have also shown the risk to be greater in the premenstrual period or late LP [345, 346], but there are little objective data using large numbers of subjects. There appears to be some periodicity to these serious injuries, regardless of OC status, but this is dependent on the method of analysis [347, 348]. Teenaged female athletes have been shown to be more prone to ACL injuries during the FP [341, 349]. More than half of the respondents in the first study also reported feelings of diminished athletic performance during menstruation, which is in agreement with the findings of greater variability during a cyclical athletic movement. Several studies in skiers found an increased incidence during the preovulatory phase: a matched case-control study of alpine skiers (with verification of cycle phase) [350] and a self-reported questionnaire [351].

Tissue qualities are also affected by estradiol concentrations [352]. Using hormonal measurements, investigators found lower musculotendinous stiffness (MTS) during cyclical hopping tests at the ovulatory phase, in contrast to the menstrual phase and FP [353]. They postulated that the resultant increase in compliance leads to greater reliance on reflexive responses from the contractile component of the muscle, due to decreased contribution from passive elastic structures. This would increase electromechanical delay and therefore risk of injury. A reduction in

Table 16.1 Hormonal risk factors for ACL injury during the menstrual cycle

Study design	Injuries/subject	High-risk phase	Hormones	References
Questionnaire	28	Preovulatory[a]	No	[342]
Case series	65	Preovulatory	Urine	[344]
Prospective cohort	46/69	Menstrual	No	[345]
Prospective cohort	17/23	Late LP	No	[346]
Unmatched case-control	37/38	FP	Salivary	[335]
Case series	83	Preovulatory	No	[348]
Matched case-control	46/91	Preovulatory	Serum	[350]
Descriptive	18/37	Ovulatory	No	[349]
Matched case-control	93/186	Preovulatory	No	[351]

FP follicular phase, *LP* luteal phase

[a]Later found not to be significantly different (see Ref. (343))

knee stiffness of approximately 17% at ovulation was associated with increased knee laxity in another study [354]. Such MC changes have not been found in other tissues, such as the gastrocnemius [355], patellar tendon [356], or Achilles tendon [357]. Some researchers have found no changes in muscle and tendon properties in knee extensors and plantar flexors [358]. More recent work has examined variations in varus/valgus and internal/external rotational knee laxity [359]. Muscle stiffness of, and neuromechanics of, the hamstrings have also been studied at different MC phases and with OCs [360–362].

Various neuromuscular performance characteristics, such as gender differences in muscle strength, recruitment order, and peak torque production, also likely contribute to the higher ACL injury rate in women [363]. Some have found no variation across MC [337, 364], while others have documented alterations in neuromuscular control patterns during landing or postural control [365]. Knee joint kinesthesia, as measured by performance in a square-hop task, improved at ovulation when compared to the pre- and menstruation phases of the cycle [366]. Sympathetic neural responses to upright tilt have also been noted [367]. Given that muscle forces influence knee joint dynamic restraint, neuromuscular control of the lower limb musculature will dictate the propensity for ACL injury [368]. A previous study reported modified co-contraction patterns of the gluteus maximus and semitendinosus at different stages of the MC, with increased synchronicity of the contraction between the two muscle groups around ovulation [369]. Knee joint position sense accuracy decreased during menses [370], but others have found no differences in knee or hip loading, using a variety of measures, during across MC phases or with OCs [371]. Currently there is much ongoing sophisticated research on landing biomechanics [372].

Additionally, PMS, with its effects on balance and motion perception, may be important. Motor skill performance may decrease during the premenstrual phase [121]. A connection between the MC cycle and soccer injuries [313], with fewer injuries in women on OCs [314], has been reported, but these authors used a faulty calendar system for calculating MC phase, which may have underestimated the incidence of injuries.

Other mechanisms of increased susceptibility to injury during specific MC phases (and/or while taking OCs) include variations in muscle strength [266], neuroendocrine activation [373], motoneuron excitability and anterior tibial displacement [374], and, most recently, tibial acceleration variability [359, 375]. Risks can be ameliorated with various neuromuscular training programs, including plyometrics, proprioceptive exercises, and correction of dynamic valgus (drop jumps). Improved knowledge of predisposing factors will also facilitate screening of athletes at increased jeopardy of sustaining ACL injuries [376].

Keeping in mind the above mechanisms, OCs use may stabilize the hormonal milieu and thus may function to either passively or actively stabilize the knee joint [377, 378]. To this end, OC use was found to influence injury risk in some [348], but not all, studies [379], in addition to neuromuscular properties [378, 380] and sporting performance [319]. During steady-state running, female athletes who do not take OCs exhibit greater variability in gross mediolateral acceleration at the proximal tibia during menstruation compared to during ovulation [378]. Given that mediolateral tibial acceleration can be used to represent varus/valgus movement of the knee, and that valgus collapse is a commonly cited gender-specific risk factor for ACL injury, further research is needed to examine the ideal level of movement variability in this population around the time of menstruation. While OC ingestion may diminish the somewhat significant association between ACL injury and the ovulatory phase, there is no consensus on any proven protective effects [351].

Much work remains to be done in the investigation of sex hormones and anterior knee laxity. Variations in the MC, with different hormonal profiles, will also have an impact [381]. There is great difficulty with validated outcome measures using serum urine or salivary progesterone in combination with MC data [382]. In addition, there may be a phase delay of hormonal effects, or they may be more cumulative and chronic,

rather than acute. There are many excellent reviews on this important topic [323, 338, 383–385].

Summary

Although understanding of the unique physiology of the female athlete has increased, there are still many unanswered questions. Both endogenous and exogenous female sex steroids can influence numerous cardiovascular, respiratory, and metabolic variables but most likely have minimal impact on the athletic ability of most recreational athletes. In elite athletes, however, even a statistically nonsignificant change can mean the critical difference between first and second place. There appears to be individual variability in the response of different performance parameters to MC phase and/or OC administration. In particular, there may be subtle alterations in substrate metabolism and increased susceptibility to heat stress under conditions of high heat and humidity. The latter is most important for female athletes competing in endurance events. Similarly, MC phase and OC use may have some implications in terms of management of MLA, prevention of ACL tears, and the response to periodized strength and endurance training. These areas warrant further scientific investigation.

Although the majority of research to date suggests that regularly menstruating female athletes do not need to adjust their MC to maximize performance, it is difficult to extrapolate controlled laboratory findings from a study population to an individual competitor on the playing field. It is critical for each woman to monitor her own physiological responses and to *listen to her body*. For women with menstrual dysfunction and/or the need for contraception, OCs may provide a stable hormonal milieu for training and competition, and predictable onset of menstrual bleeding. Potential side effects (and any concomitant impact on performance) can be minimized with the lower-dose triphasic pills and the newer progestins. Continuous pills are another promising new option. Further large-scale, prospective, randomized clinical trials are needed on trained athletes, using accurate hormonal measurements for verification of MC phase, to further elucidate the short- and long-term effects of cycle phase and OCs in exercising women.

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Endocrine Implications of Relative Energy Deficiency in Sport

17

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Introduction

Relative energy deficiency in sport (RED-S), a concept introduced by Dr. Constantini in 2002 and coined by the International Olympic Committee (IOC) in 2014, refers to the potential health and performance consequences of inadequate energy availability in the athlete [1, 2]. RED-S is an expansion of important work on the female athlete triad (triad), which is the interrelationship of energy availability (EA), menstrual function, and bone health [3]. As research on female and male athletes has shown that low EA can lead to other negative health and performance consequences [2, 4], RED-S was proposed to broaden the triad and to include male athletes in addition to female athletes. The RED-S models describe 10 potential health and 10 possible performance-related effects of low EA [2]. See Fig. 17.1a, b.

In this chapter, we briefly describe the RED-S health and performance models and then specifically focus on the endocrine implications of RED-S or what the RED-S model lists as "menstrual function," "bone health," "metabolic," and "endocrine" effects [2]. The chapter by Loucks (see Chap. 11 this book) is dedicated to low EA's effects on reproductive hormones in women, so in this chapter we will concentrate on some of the less well-known endocrine consequences of low EA, based on current literature. We will describe sex differences, gaps in knowledge, and areas for future research and treatment of RED-S.

Relative Energy Deficiency

Based on pioneering work of Loucks and others, EA has been defined as energy intake (EI) (kcal) minus exercise energy expenditure (EEE) (kcal), normalized to fat-free mass (FFM, measured in kg), or the dietary energy remaining for normal physiological functioning after what is utilized for exercise [5]. During times of low EA or relative energy deficiency, energy is diverted away from non-vital processes, such as fat storage, growth, development, and reproduction [6]. See Chap. 11.

The RED-S concept is illustrated in two models: a "health consequences" of RED-S diagram and a "potential performance consequences" of RED-S diagram, both of which place energy in the center, illustrating that inadequate EA affects various physiological systems [2, 7].The original 2014 IOC consensus statement on RED-S suggested that there were not only "menstrual

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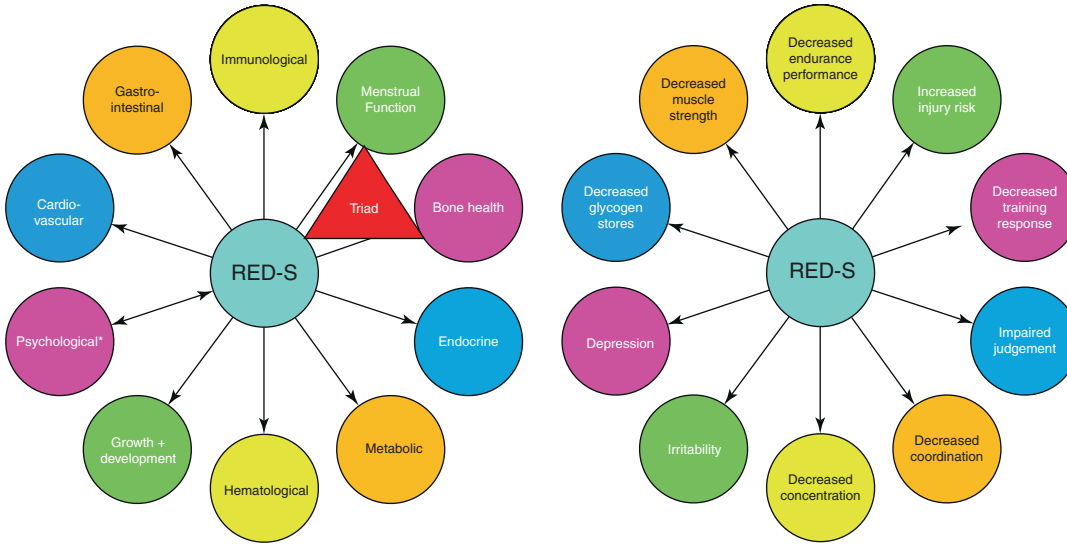

Fig. 17.1 Potential health and performance consequences of RED-S [2]. (Adapted from Constantini [1])

function" and "bone health" effects of relative energy deficiency but also other "endocrine" consequences, "metabolic" changes, "hematologic" effects, "growth and development" detriments, "psychological sequelae" (and psychological contributions to relative energy deficiency), "cardiovascular" changes, "gastrointestinal" issues, and "immunological" suppression (see Fig. 17.1a) [2]. Such adaptations were then proposed to affect various aspects of performance, which were described in a "potential performance consequences" of RED-S diagram. These include "increased injury risk," "decreased training response," "impaired judgment," "decreased coordination," "decreased concentration," "irritability," "depression," decreased glycogen stores," "decreased muscle strength," and "decreased endurance performance." See Fig. 17.1b [2].

While altering hormonal secretion to suppress reproductive function in times of food scarcity is an important survival adaptation, many of the hormonal and physiologic changes that occur during relative energy deficiency are interrelated and clearly not ideal for an athlete's health and performance. Female athletes in leanness sports (weight-class sports and activities in which thinness is thought to confer a competitive advantage) have been shown to have higher rates of low EA than athletes in non-leanness sports [7]. However, in one study of 1000 female athletes (ages 15–30 years) using surrogate markers for low EA, it was recently shown that up to 47% of female athletes were at risk for low EA, regardless of body mass index or sport [8]. Data concerning low EA in male athletes are less robust, but emerging research suggests male athletes in leanness sports (e.g., wrestling, lightweight rowing, jockeys) and athletes enduring high training loads (open-weight rowing) are also at greater risk of low EA [9, 10].

Endocrine Effects of Relative Energy Deficiency

Because of the difficulty in measuring low EA and the likely individual variability of adequate EA cutoffs, some of the described endocrine effects of RED-S have been based on individuals thought to be in low EA states. Women with anorexia nervosa (AN) and athletes with functional hypothalamic amenorrhea (FHA) are typically characterized by insufficient EA, and these conditions have frequently been used as low EA surrogates to improve understanding of hormonal effects of relative energy deficiency [6, 11].

Athletes in leanness sports have been assumed to have lower EA than their counterparts in non-leanness sports, and comparisons of leanness vs. non-leanness sport athletes have aided in elucidating hormonal changes in RED-S [5, 12, 13]. Investigations have involved states of chronic low EA, while other research has been performed prospectively with short-term EA manipulation. Finally, while there is an abundance of literature available on low EA/RED-S in females relative to males, work in male athletes is growing. Throughout the remaining chapter, we highlight the endocrine effects of RED-S in both sexes, as determined by direct EA measurement, but also include preliminary studies that have utilized EA surrogates to help inform future research.

Hypothalamic-Pituitary-Gonadal Axis (GnRH, LH, FSH, Estrogens, Progesterone, Androgens-Testosterone, Dehydroepiandrosterone)

Females

It is well-established that low EA disrupts the hypothalamic-pituitary-gonadal (HPG) axis through a variety of mechanisms. Most importantly, in low EA states, aberrant gonadotropin-releasing hormone (GnRH) pulsatility at the hypothalamus leads to decreased luteinizing hormone (LH) pulse frequency at the pituitary, resulting in menstrual cycle disruption in women, and eventually FHA [11]. FHA is a form of amenorrhea classified as persistent anovulation that is not attributable to an organic cause, meaning it can be reversed after behaviors that impede menstruation are corrected (e.g., extreme dieting, excessive exercise, psychological, and/or physical stress) [11, 14].

In seminal work by Loucks and Thuma, manipulation of EA via diet and exercise led to dose-response changes in LH pulsatility [15]. LH pulse frequency decreased, and amplitude increased during low EA (10 and 20 kcal • kg^{-1} FFM • day^{-1}), though mean 24-hour LH and FSH concentrations were unchanged [15]. Reduced estradiol was noted at severely low EA (10 kcal • kg^{-1} FFM • day^{-1}). In further work, LH pulse frequency has been found to be highest in eumenorrheic nonathlete controls, lower in eumenorrheic athletes, and lowest in amenorrheic athletes. Findings are inconsistent in LH pulse amplitude and total LH secretion, possibly due to the lack of differentiation of the causes of amenorrhea in some studies (e.g., FHA vs. polycystic ovary syndrome), as well as differences in timing and methods of sampling [16–18]. In younger eumenorrheic nonathletes, eumenorrheic athletes, and amenorrheic athletes (ages 14–21 years), no difference was found between groups in overnight LH pulse frequency nor any difference in LH parameters between the two athlete groups. However, lower LH pulse amplitude and total pulsatile secretion were found in the amenorrheic athletes versus nonathlete controls [19].

In a study in which athlete participants were believed to have relative energy deficiency compared to the nonathletes based on exercise testing and food and training records, urinary estradiol and progesterone were predictably low in the amenorrheic athletes, but luteal phase progesterone was also lower in the eumenorrheic athletes vs. eumenorrheic nonathletes [20].

In studies of testosterone levels in female athletes with relative energy deficiency or surrogates for low EA, testosterone has been found to be similar, elevated, or decreased compared to those thought to have adequate EA [18, 21–24]. Similar to concerns about LH and FSH determination in oligo/amenorrheic athletes assumed to have relative energy deficiency, future understanding of androgen changes (e.g., testosterone and dehydroepiandrosterone) in female athletes in states of low EA requires ruling out other causes of hyperandrogenism.

Males

As with female athletes with FHA, male athletes in leanness sports are at increased risk for lower total, free, and basal testosterone levels, a state termed the "exercise-hypogonadal male condition

(EHMC)" [25–28]. However, studies of LH and testosterone secretion in male athletes at high risk for EHMC are inconsistent. In a study by Hooper et al., long-distance male runners with a mean EA of 27.2 kcal • kg^{-1} FFM • day^{-1} were compared to nonathletes with a mean EA of 45.4 kcal • kg^{-1} FFM • day^{-1} [29]. Both groups underwent frequent hormonal sampling over 4 hours. No differences in mean LH pulse frequency, amplitude, or total LH concentrations were noted between groups, but mean total testosterone was significantly lower in the runners vs. non athletes [29]. Another study noted lower LH pulse amplitude and AUC (from frequent sampling over 6 hours) and lower basal total serum testosterone concentrations in distance male runners vs. controls [30]. Yet another found lower LH pulse amplitude and frequency (frequent sampling over 8 hours) in marathoners vs. healthy controls but comparable total testosterone concentrations between groups [31]. Interestingly, when given increasing doses of exogenous GnRH, the marathoners also had a suppressed LH response [31]. Hackney et al. reported a trend toward higher LH concentrations, no difference in LH pulse frequency or amplitude (frequent sampling over 4 hours), and lower total and free testosterone in male endurance athletes vs. nonathletes [32]. Later, Hackney et al. studied male endurance runners (averaging 95–140 km/week for over 10 years) versus sedentary controls and compared LH and testosterone levels pre- and post-GnRH infusion (serial sampling for 3 hours after infusion) [33]. LH was slightly less responsive to the GnRH infusion, and testosterone levels were significantly lower in the endurance athletes vs. controls. These results suggest both a central adaptation (pituitary response) and peripheral adaptation (testicular response) to GnRH stimulation in the endurance-trained men [33].

In a study of men in a team, mixed ultra-endurance race over 800 km (median duration 6.3 days), total and free testosterone, and FSH were significantly decreased immediately post-race compared to pre-race, but not LH concentrations [34]. EI and EEE were estimated in a subset of athletes, with a resultant energy deficit of ~40,000 kcal over the race or ~6349 kcal · day^{-1} if they completed the race in the median 6.3 days [34]. After a 1230 km road race, male cyclists had significantly decreased serum testosterone (−67%), which remained suppressed after 12 hours of recovery [35]. It is important to recognize that individual athletes in a variety of sports may be at risk for EHMC, as male athletes participating in non-leanness sports (e.g., American football) have also been reported to have lower testosterone concentrations [36–38]. In a study of hormone levels in 454 male elite athletes within 2 hours of completion of a major national or international competition, low testosterone concentrations (<10 nmol/L) were seen in 25.4% of the athletes in 12 of the 15 sports, including powerlifting, football (American soccer), ice hockey, and handball [38]. Military studies have also supported the findings of low EA effects on hormonal profiles of male athletes. For example, after 8 weeks of US Army Ranger School, characterized by high-energy expenditure, energy restriction, and sleep deprivation, male soldiers demonstrated an average 70% decrease in serum total testosterone.

Estradiol has been less examined in male athletes. In a study of male endurance athletes versus untrained controls, Hackney found no difference in resting estradiol concentrations over 4 hours between the two groups [32]. In the above elite athlete study, one quarter of the estradiol measurements in the men were below the detectable level of quantification (34 pmol/L) [38]. In a study of Division 1 male collegiate golfers, runners, and off-season wrestlers, only the runners had low estradiol levels [13]. Progestins are low at baseline in men and therefore have had limited study in male athletes. Urinary progesterone excretion post-Ironman race was not significantly different than pre-race levels in male triathletes [39]. Further research, with careful attention to EA measurements and hormonal pulsatility, is needed to improve our understanding of the effects of acute and chronic relative energy deficiency on the entire HPG axis in male athletes of various sporting disciplines. Please see Chap. 7.

Bone Health

When exploring endocrine effects of RED-S, it is important to include the bone, which is both an endocrine organ (bone secretes osteocalcin and fibroblast growth factor 23) and an endocrine target (acted upon by a variety of hormones, including most of those included in this chapter) [40, 41]. Women with AN and female athletes with oligomenorrhea/amenorrhea have lower bone mineral density (BMD), impaired bone microarchitecture, and altered bone turnover markers compared to those without eating disorders and eumenorrheic counterparts [42–44]. Such athletes have been reported to have decreased estimates of bone strength and higher fracture rates compared to eumenorrheic athletes and controls [45, 46]. Additionally, EA and estrogen status were found to have independent and combined effects on BMD, bone geometry, and bone strength estimates in exercising women [47]. Women prospectively subjected to short- and long-term periods of low EA via dietary and exercise manipulation were shown to have negative effects on bone turnover markers [48, 49].

Less work has been published on the effects of relative energy deficiency on the bone in male athletes. Males participating in leanness sports (including cyclists, jockeys, and runners) have consistently been found to have lower BMD than those in non-leanness sports [10, 44, 50]. A study in jockeys specifically reported decreased BMD, cortical area, and tibia strength/strain indices [51]. Similar to female athletes, markers of low EA (BMI $\leq$17.5 kg • m^{-2} and expected body weight < 85%) have been associated with decreased BMD in young male runners [52, 53]. Another study found that male athletes with testosterone concentrations within the bottom quartile of subjects (yet still within normal range) had higher lifetime histories of bone stress injuries compared to those with testosterone levels above this level. Our group found that estradiol concentrations, BMI, and resistance training were more important predictors of BMD in male athletes than testosterone concentrations were [13].

In examining bone metabolism, a significant decrease in N-terminal pro-peptide of type 1 collagen (a bone formation marker) was found in male distance runners after 3 days of 60 min/day of treadmill running in a low EA state (50% of estimated energy needs), but not when they experienced adequate EA [54]. Papageorgiou et al. manipulated EA (45 vs. 15 kcal • kg^{-1} FFM • day^{-1}) in men and eumenorrheic women via EI and had them perform daily treadmill running (EEE of 15 kcal • kg^{-1} FFM • day^{-1}) [48]. The women had lower bone formation and higher bone resorption marker concentrations in the low vs. adequate EA conditions. Interestingly, the bone turnover marker concentrations were not significantly different between the two conditions in men [48].

Interestingly, Ackerman et al. [13] reported that estradiol levels, BMI, and resistance training participation were more important determinants of BMD in male athletes than were testosterone levels [13]. More work is needed in various male athlete populations with different EA conditions to better understand the relative effects of EA,

Growth Hormone/Insulin-Like Growth Factor 1

Growth hormone (GH), produced by the anterior pituitary, is necessary for bone and muscle anabolism and exerts many of its effects via the peptide insulin-like growth factor 1 (IGF-1), whose production it stimulates at the liver. Females with AN have elevated levels of GH, concomitant with reduced levels IGF-1, suggesting GH resistance at the liver in low EA states [55–57]. GH is also important for carbohydrate, protein, and fat metabolism, independent of IGF-1. Thus, increased levels of GH are critical during low EA, as they can help maintain euglycemia, largely through fat mobilization. It seems a useful survival adaptation that growth is suspended (decreased serum IGF-1 and increased GH resistance at the liver) and fuel accessibility is enhanced (increased serum GH) in times of relative energy deficiency [58]. In fact, after women with AN were administered with recombinant human GH daily for 12 weeks, they had less fat mass and unchanged IGF-1 levels [59].

When energy was restricted to 10 or 20 kcal • kg^{-1} FFM • day^{-1} in eumenorrheic, untrained women, GH concentrations increased, and IGF-1 levels decreased compared to their levels during an adequate energy state (45 kcal • kg^{-1} FFM • day^{-1}) [15, 17]. Laughlin and Yen reported that both amenorrheic and eumenorrheic athletes had higher 24-hour mean GH concentrations than eumenorrheic nonathletes, but the three groups had comparable IGF-1 concentrations [16]. When differences in IGF-binding protein 1 levels were considered, the amenorrheic athletes had the lowest bioactive IGF-1 levels [16].

Case studies of men with AN have demonstrated increased GH levels [60, 61]. Similarly, male wrestlers had higher GH secretion during their competitive season, a time of relative energy deficiency and weight loss [62]. At the season's end, the wrestlers' GH concentrations were elevated, and their IGF-1 concentrations were reduced, consistent with patterns in AN [62]. When bodybuilders experienced relative energy deficiency by restricting their EI for 11 weeks, they had significant decreases in IGF-1 levels [63]. Male cyclists competing in the aforementioned ultra-endurance race had low IGF-1 levels that were strongly associated with their relative energy deficiency [35]. Additionally, male Olympic endurance athletes had higher IGF-binding protein 1 compared to non-endurance athletes, consistent with less bioavailable IGF-1 [64].

Thyroid Hormones

Both thyroid excess and deficiency can inhibit growth and reproduction [65]. During times of relative energy deficiency, the hypothalamic-pituitary-thyroid axis adapts in order to reduce energy expenditure, and a "sick euthyroid" profile is common [42]. Women with FHA and AN and athletes with amenorrhea have consistently been reported to have decreased triiodothyronine (T3) levels but variable levels of thyroxine (T4) and thyroid stimulating hormone (TSH), compared to eumenorrheic controls [6, 55, 57, 66–74].

When 27 eumenorrheic women performed supervised aerobic exercise over 4 days but consumed food resulting in four different levels of EA (10.8, 19.0, 25.0, and 40.4 kcal • kg^{-1} FFM • day^{-1}), total and free T3 concentrations decreased between 19 and 25 kcal • kg^{-1} FFM • day^{-1}, and free T4 and reverse T3 increased between 10.8 and 19 kcal • kg^{-1} FFM • day^{-1} [68]. When 46 women were randomized to "low" or "normal" EA (8 vs. 30 kcal • kg^{-1} FFM • day^{-1}) incorporating no exercise, low-intensity exercise, or high-intensity exercise (i.e., six different testing conditions), total and free T3 decreased in the low EA conditions, total T4 and reverse T3 increased, but free T4 was unchanged, all regardless of exercise quantity or intensity [75].

In elite endurance track and field athletes, amenorrheic women had significantly lower free T3 concentrations than their eumenorrheic counterparts, and men with the lowest quartile testosterone levels had lower T3 concentrations than the male athletes with testosterone values above this cutoff [27]. When elite male runners were compared to healthy, nonathlete, lean men, TSH and TSH/free T3 ratios were lower in the runners [76]. Leptin was implicated in the adaptive response of the hypothalamic-pituitary-thyroid axis, as the free leptin index was independently associated with the TSH/free T3 ratio [76]. Interestingly, there were no differences in free T3 and T4 concentrations between the athletes and nonathletes [76]. T3 has been suggested as a clinical marker for relative energy deficiency in athletes, but more work is needed in both sexes to better understand thyroid function changes in acute and chronic low EA conditions of various severities. Chapter 6 in this book provides additional discussion on elements of exercise and thyroid function.

Cortisol

The hypothalamic-pituitary-adrenal (HPA) axis highly influences energy balance, affecting energy intake, storage, and mobilization. States of emotional and physiologic stress, including low EA, can lead to HPA activation, resulting in

increased cortisol release from the adrenal cortex. Extremely low fat/low BMI and high adiposity/high BMI states activate the HPA axis [77]. Cortisol has been implicated in fat accumulation during energy excess, but paradoxically, cortisol also has catabolic properties to the muscle and bone, and levels rise with stress, prolonged exercise, and energy deficiency [65, 78, 79]. In animal models, decreased EI activates vagal signaling from the GI tract to the hypothalamus, resulting in increased cortisol-releasing hormone (CRH) production. Increased CRH not only increases adrenocorticotropic hormone (ACTH) production at the pituitary but also directly modulates hypothalamic GnRH release, which inhibits pituitary LH pulsatility [65]. In amenorrheic athletes, it has been difficult to elucidate if GnRH modulation is predominantly the direct result of increased CRH or increased cortisol, in other words, if hypercortisolemia a contributor or simply a biomarker of reproductive dysfunction in amenorrheic athletes [66, 80, 81].

In a study of amenorrheic athletes, eumenorrheic athletes, and eumenorrheic nonathlete controls, no differences in ACTH secretion or cortisol pulse frequency were noted among the groups, but 24-hour urine cortisol concentrations were greater in the amenorrheic athletes vs. the two eumenorrheic athletes and nonathletes [20]. Other groups have found greater baseline cortisol, as well as overnight cortisol pulse amplitude, mass, half-life, and total concentration in amenorrheic athletes compared to eumenorrheic athletes and nonathletes [18, 80, 82]. De Souza et al. reported higher baseline cortisol levels in amenorrheic athletes vs. eumenorrheic athletes and nonathletes but a blunted cortisol response to ACTH stimulation in those with amenorrhea [83]. The three groups had comparable peak cortisol levels after stimulation, with the authors suggesting a normal limitation to maximal adrenal secretory capacity [83]. Stress from exercise alone can also increase cortisol, as demonstrated by Laughlin and Yen's findings of higher 24-hour serum cortisol concentrations in both amenorrheic and eumenorrheic athletes compared to eumenorrheic nonathletes [16]. Another group found no significant differences between amenorrheic and eumenorrheic athletes in cortisol levels at baseline or after various exercise intensities but did report blunted catecholamine (norepinephrine and epinephrine) secretion after high-intensity exercise in the amenorrheic athletes [78].

Studies have demonstrated that women with FHA frequently have both relative energy deficiency and increased psychological stress [11]. Interestingly, Michopoulos et al. reported treatment of FHA with cognitive behavior therapy (CBT) led to improved cortisol levels and menstrual restoration in some women, suggesting that CBT decreased cortisol levels and improved exercise and eating habits [84].

Hooper et al. performed a cross-sectional study of distance male runners with low EA (mean 27.2 ± 12.7 kcal • kg^{-1} FFM • day^{-1}) compared to nonathletes with adequate EA (45.4 ± 18.2 kcal • kg^{-1} FFM • day^{-1}) and found that cortisol concentrations were not different between the groups [29]. Because of the interplay between EA, physical and psychological stress, and the complexities of CRH, ACTH, and cortisol signaling, much more work is needed to understand cortisol's responses and effects in relative energy deficiency in female and male athletes.

Dietary Intake Regulating Hormones

Various hormones are involved in caloric intake via appetite regulation and/or behavioral (reward related) food ingestion. The effect of relative energy deficiency on some of the most prominent of these hormones is described below.

Leptin

Leptin is an anorexigenic adipokine that is lower in states of relative energy deficiency, including in women with AN, amenorrheic athletes, and female athletes experiencing increased EEE without concomitant increases in EI [19, 24, 85–89]. Leptin correlates with absolute fat mass in

women regardless of EA, menstrual status, and exercise level [19, 86]. Leptin is also important for reproduction, with levels positively predicting estradiol and testosterone and overnight leptin secretory parameters positively predicting LH secretory parameters in adolescent female athletes and nonathletes [19, 24]. Additionally, although it has negative effects on appetite, when administered in adjusted doses, leptin has been shown to resume ovulatory cycles in some women with FHA [90, 91]. Cross-sectional human studies have not found a correlation between leptin and BMD in normal weight children, adolescents, or postmenopausal women, but interventional studies have reported positively effects on BMD in people with leptin deficiency [92, 93].

Acute decreases in leptin have been found in male athletes after intense exercise in running, rowing, and swimming endurance events of various durations [94–97]. In a small study by Koehler et al., six male habitual exercisers were subjected to four separate, 4-day EA conditions: low EA of 15 kcal • kg^{-1} FFM • day^{-1} (achieved via diet alone or diet and exercise) and higher EA of 40 kcal • kg^{-1} FFM • day^{-1} (achieved via die alone or diet and exercise) [98]. Fasting leptin decreased by 53–56% after both low EA conditions, but not after the higher EA conditions [98]. In one study of male Olympic athletes, men in leanness sports (e.g., triathlon, gymnastics, and skating) had lower leptin levels compared to men in non-leanness sports (e.g., handball, ice hockey, and snowboarding) [64]. Male marathoners had lower leptin levels and fat mass vs. sedentary males; as in women, a positive correlation between leptin and total fat mass occurred in both groups [96]. After 5 days of military training that led to relative energy deficiency, men experienced drops in serum leptin to about 1/3 of their baseline [99]. In another study, healthy men were subjected to 72 hours of fasting, which led to serum leptin and total testosterone levels dropping to about 10% and 60% of baseline, respectively [100]. When the men were given replacement doses of recombinant leptin during fasting, the total testosterone concentrations did not significantly change, suggesting analogous effects of leptin on the HPG axis in men as in women [100].

Adiponectin

Adiponectin is another adipocyte-derived anorexigenic hormone. In most studies, females with AN and amenorrheic athletes have higher adiponectin concentrations than normal weight controls [23, 101–103]. Higher levels of adiponectin have been reported in female athletes vs. nonathletes and correlate positively with lean mass and negatively with BMI, fat, and BMD in various studies, but results are inconsistent [23, 87, 101, 104].

We are unaware of any research examining adiponectin concentrations in males in chronically low EA states. Cross-sectional studies controlling for BMI and fat demonstrated a positive association with physical activity and adiponectin in men, but studies of adiponectin levels immediately after exercise sessions have not controlled for BMI or body composition and have found either decreases or no changes in adiponectin levels [104]. For example, in a 180 km ultramarathon, it was estimated that the athletes experienced a mean energy deficit of 5000 kcal; immediately post-race, adiponectin levels were not significantly different from baseline, but did drop slightly 17–22 hours later [97]. Adiponectin and other adipokines likely impact both the HPG axis and bone metabolism, acting as important links between EA, reproductive status, and the bone, but much more work is needed to understand this relationship in both women and men [105].

Ghrelin

Ghrelin, an orexigenic hormone predominantly produced in the stomach fundus, acts on the hypothalamus and pituitary and modifies the secretion of various hormones including GnRH, LH, FSH, ACTH, and GH. For example, when the active form of ghrelin, acylated ghrelin,

attaches to the GH secretagogue receptor 1a (GHSR1a), GH is released [58]. Increased level of ghrelin has been reported in women and adolescents with AN and in amenorrheic athletes, and various ghrelin pulse parameters have correlated negatively with fat mass in these populations [19, 24, 70, 106–108]. Scheid et al. reported an increase in ghrelin that correlated with decreases in body weight, BMI, fat-free mass, and EA in women subjected to a 3-month decrease in EA via diet and exercise [109]. When synchronized swimmers intensified their training, but had a drop in EI (therefore EA), they too demonstrated increased ghrelin concentrations [89]. Overnight ghrelin pulsatile secretion was higher, and leptin and LH pulsatile secretions were lower in amenorrheic athletes than eumenorrheic nonathletes [19]. There were independent associations of ghrelin and leptin secretion with LH secretion, suggesting that both hormones likely contribute to decreased LH pulsatility and amenorrhea in athletes with relative energy deficiency [19].

Exogenous ghrelin infusion increased caloric intake, as well as hunger and appetite scores in subjects who were normal weight and those who were obese [110]. A higher ghrelin dose was needed to increase caloric intake in the normal weight subjects than the obese subjects [110]. A separate study found that hunger scores increased less in patients with AN vs. controls after ghrelin infusion, suggesting different responses to ghrelin in individuals with different BMIs, or even ghrelin resistance in lower weight populations [111]. Ghrelin is also known to stimulate gastric motility, yet women with low EA, such as those with AN, often experience constipation [58]. After women with AN were treated for 4 weeks with a GHSR1a agonist, they had significantly decreased gastric emptying time and a trend toward weight gain compared to those who received placebo, suggesting that GHSR1a agonist treatment could potentially overcome ghrelin resistance [112]. Women with AN and FHA have high dietary restraint and a high drive for thinness; thus, it will be important to determine if female athletes with decreased EA have a physical and/or psychological suppression of ghrelin's ability to stimulate appetite and caloric intake [113].

In a study of males with AN and healthy controls, ghrelin was not significantly different between groups [114]. Nor were differences in ghrelin noted in male athletes subjected to low EA conditions (15 kcal • kg^{-1} FFM • day^{-1}) vs. adequate EA conditions (40 kcal • kg^{-1} FFM • day^{-1}) [98]. More studies are needed to compare ghrelin's role in women vs. men, particularly athletes with relative energy deficiency.

Peptide YY

Peptide YY (PYY) is an anorexigenic hormone secreted by the intestine in response to caloric intake, acting at the hypothalamus to decrease appetite and caloric intake. PYY levels are higher in adolescent and adult women with AN and in amenorrheic athletes compared to controls, with levels positively correlated with percent body fat and negatively with BMI and resting energy expenditure (REE) [23, 115–117]. It is unclear if these elevated levels of PYY in states of relative energy deficiency are inappropriate and contribute to the decreased hunger sensation reported by those with low EA, such as women with AN [58, 118]. In vitro and in vivo animal studies have found that PYY inhibits ghrelin-activated neurons and LH secretion from the pituitary [119, 120]. In adolescent female athletes (regardless of menstrual status) and healthy controls, PYY levels negative correlated with testosterone concentrations [23].

Men with AN were reported to have higher PYY levels than healthy controls [114]. We are unaware of any study of PYY in male athletes with relative energy deficiency. It has been proposed that PYY contributes to disordered eating behaviors, ghrelin resistance, and the disruption of GnRH in AN and other states of relative energy deficiency and may also have a negative effect on the bone [58, 113]. More work is needed in humans to clarify such relationships.

Oxytocin

Oxytocin is a peptide produced by the hypothalamus and secreted by the posterior pituitary and has various functions. In addition to stimulating lactation and uterine contraction in women, it is also anorexigenic, inhibiting reward-related eating behaviors, suppressing the hypothalamic-pituitary-adrenal (HPA) axis, altering the glucoregulatory response to caloric intake, and potentially having anxiolytic and antidepressant effects [121–123]. Oxytocin is also thought to contribute to sodium and fluid regulation [124]. Nocturnal oxytocin secretion is decreased in women with AN and amenorrheic and eumenorrheic athletes compared to eumenorrheic nonathletes [125, 126]. In adolescent amenorrheic athletes, oxytocin is positively associated with markers of EA, including weight, BMI, and REE [127].

Oxytocin levels did not significantly change after a group of male professional cyclists and a group of sedentary controls both exercised to exhaustion, but oxytocin levels were lower in the athletes at baseline, during, and postexercise [128]. Increases in pre- to post-run oxytocin levels were reported in male athletes who participated in a 56 km ultramarathon [129]. With the various theorized roles of oxytocin in food intake, fluid balance, stress, and mood, future work is needed in both female and male athletes to better understand its dominant effects in RED-S.

Insulin

Insulin, a polypeptide produced in the pancreas, stimulates the uptake of glucose into the liver, muscle, and adipose tissue and has an anabolic effect on various tissues, including the bone [130]. Insulin also affects GnRH signaling, and frequent hormonal sampling studies have shown that total insulin pulsatility concentrations correlate with LH pulsatility [16, 18]. In states of low EA, downregulation of insulin typically occurs presumably to enhance substrate availability [65]. Amenorrheic athletes were found to have lower insulin levels and enhanced insulin sensitivity compared to eumenorrheic athletes and nonathletes [16, 18]. Similarly, female runners with luteal phase defects had lower insulin levels vs. ovulatory runners and nonathlete controls [131].

In male bodybuilders experiencing decreases in EA over 11 weeks to reduce body fat in preparation for competition, insulin significantly decreased, with insulin levels strongly correlating with lean mass [63]. In the Koehler et al. study discussed previously, with the four different 4-day EA conditions, significant decreases in insulin (by about 1/3) were noted in both low EA states in the male exercisers [98]. Similar results were noted in the aforementioned 72-hour fasting study, where insulin levels were reportedly lower on the final day compared to baseline [100]. Unlike its effects on testosterone, concomitant leptin administration during the fast did not prevent the insulin drop [100].

Amylin

Amylin is a peptide hormone produced in the pancreas and brain. Research suggests amylin inhibits gastric emptying and glucagon secretion, enhances satiety, and has a positive effect on bone [132]. Women with AN were found to have reduced fasting levels of amylin compared to healthy controls, and amylin was noted to correlate positively with the spine and hip BMD [133]. We are not aware of any studies of amylin in female or male athletes.

Incretins

Glucagon-like peptide one (GLP-1) and gastric inhibitory peptide (GIP) are incretins, gut hormones that stimulate insulin and decrease glucagon release. Both hormones are reportedly lower in females with AN compared to controls [133–135]. However, GLP-1 levels were not significantly different among amenorrheic athletes and ovulating athletes and controls [115]. Study of incretins in relative energy deficiency is needed in female and male athlete populations.

Metabolic Effects of Relative Energy Deficiency

Resting metabolic rate (RMR), the number of calories the body uses to maintain physiologic function at rest, has been reported to be lower in oligo/amenorrheic athletes vs. eumenorrheic athletes and controls [70, 136, 137]. In a study of elite endurance athletes, those with EA < 45 kcal • kg^{-1} FFM • day^{-1} had lower RMR than those with EA ≥ 45 kcal • kg^{-1} FFM • day^{-1} [137]. Similar findings have been reported in male athletes. In one study, despite similar EEE, BMI, and body composition, male endurance athletes with low EI consumed an average of 1490 fewer kcal • day^{-1} than an adequate EI group (1116 to 1395 kcal · day^{-1} below calculated energy needs) [138]. The low EI athletes had RMRs that were 8% lower than the adequate EI group, suggesting an energy-conserving adaptation to maintain BMI and physiologic function [138]. In a study of elite open-weight male rowers who underwent 4 weeks of intensified training (increased EEE) without concomitant adjustments in EI (therefore decreased EA), significant decreases in absolute and relative RMR were observed, along with significant reductions in total body mass and fat mass [139]. In a study of male cyclists, triathletes, and distance runners who were divided by resting metabolic rate (RMR) (RMR_{ratio} < 0.90 and RMR_{ratio} > 0.90), there were no significant differences in 24-hour EA between the two RMR groups [140]. But those in the lower RMR_{ratio} group spent more time during the study period in an energy deficit greater than 400 kcal and had greater single-hour energy deficits vs. subjects in the normal RMR_{ratio} group; greater single-hour energy deficits were associated with higher cortisol concentrations and lower testosterone/cortisol ratios [140].

Multiple studies have found that amenorrheic athletes have lower BMI compared to eumenorrheic athletes and nonathlete controls [24, 46, 85]. One study reported that ovarian-suppressed collegiate swimmers had slightly higher BMI and fat mass vs. eumenorrheic swimmers [141], but more robust literature has demonstrated that amenorrheic athletes have lower absolute and relative body fat compared to their eumenorrheic counterparts [24, 80, 85]. Male collegiate distance runners were reported to have lower BMI than off-season wrestlers and lower BMI and fat mass compared to golfers, suggesting that the degree of inadequate EA likely affects body composition [13]. However, changes in metabolism, with the severity and duration of relative energy deficiency, need to be considered when comparing body composition in athletes with different EA.

Summary and Conclusions

It is well-established that low EA or relative energy deficiency has negative effects on the menstrual function and bone health of female athletes, as has been researched and described for decades by Triad. However, RED-S expands upon that concept, illustrating that relative energy deficiency can happen to any athlete, male or female, participating in a leanness or non-leanness sport, with effects on various interrelated hormones, resulting in numerous health and performance consequences. The focus on endocrine changes in this chapter illustrates the complexity of the hormonal signaling at play.

As awareness of the prevalence and consequences of RED-S expands, a better understanding of hormonal pathways leading to health and performance detriments will be paramount. Much of the work done in this field has not involved direct EA measurements, but has instead used low EA surrogates, making assumptions based on menstrual status, BMI, type of sport, and training or competition duration. Food and training logs to estimate EA rely on self-report, with known shortcomings. Many of the hormones discussed previously have few studies in athletes, particularly male athletes. Future studies of RED-S should be prospective, with direct EA component measurements, proper hormonal sampling techniques, more pulsatile hormonal testing, diverse populations, and both short- and long-term study designs. A better understanding of the order and pattern of disruption of many of the hormones appreciated in states of relative energy deficiency will improve our screening and treatment of RED-S in our athletes (Table 17.1).

Table 17.1 Summary of endocrine changes using direct or surrogate measures of low-energy availability. Adapted from Elliott-Sale KJ, et al. [6]

	Females	Males
Hypothalamic-pituitary-gonadal axis		
LH	↔, ↓	↑, ↔, ↓
FSH	↔	↓
Estradiol	↓	↓, ↔,
Testosterone	↑, ↔, ↓	↔, ↓
Progesterone	↓	
Growth hormone/IGF-1 axis		
GH	↑	↑
IGF-1	↔, ↓	↑, ↓
IGF binding protein-1	↑	↑
Hypothalamic-pituitary-thyroid axis		
TSH	↔	↔
T3	↓	↓
Free T3	↓	↓
T4	↑, ↔, ↓	↓
Free T4	↔, ↓	↓
Hypothalamic-pituitary-adrenal axis		
Cortisol	↑, ↔	↔
Energy homeostasis/appetite regulation		
Leptin	↓	↓
Adiponectin	↑, ↔	
Ghrelin	↑	↔
Peptide YY	↑	↑
Oxytocin	↓	↓
Insulin	↓	↓
Amylin	↓	
Metabolism		
Resting metabolic rate	↓	↓

↑ denotes a higher level/increase with low EA, ↓ denotes a lower level/decrease with low EA, ↔ denotes no difference/change with low EA. Examples of surrogates for low EA include eating disorders, menstrual dysfunction, low BMI, participation in leanness sports, and prolonged exercise

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Vitamin D and Exercise Performance

18

Joi J. Thomas and D. Enette Larson-Meyer

Introduction

In 1645, Daniel Whistler first described the physical manifestation that came to be known as rickets in the equivalent of a PhD dissertation. Francis Glisson, an English physician, reported similar observations 5 years later in one of the first pediatric texts published in London [1]. In this text, *A Treatise of the Rickets*: *Being a Disease Common to Children*, he gave a complete description of rickets and also differentiated between rickets and infantile scurvy. He did not, however, note the importance of diet or the origin of the disease in his clinical description. As early as 1822, however, Sniadecki published observations that children in Warsaw, Poland, had a higher incidence of rickets while children living in rural areas outside of the city had a much lower prevalence of the disease [2]. He hypothesized that the development of rickets was due to a lack of adequate sun exposure. Rickets was also observed with a high prevalence in the UK and India in children of the very rich who were kept from the sunlight. It was also seen in children living in highly industrialized areas like London and New York City.

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Close to the beginning of the twentieth century, there were three major discoveries regarding vitamin D. The first, from both UK and US researchers, was that vitamin D was a dietary compound [2]. McCollum and Davis conducted extensive investigations with development and growth in rats fed a variety of diets [3]. They noted that diets consisting simply of purified proteins, carbohydrates, and fats were insufficient to promote growth in young rats. These studies led the way for the work of Sir Edward Mellanby. Mellanby induced rickets in dogs through their diet to show that McCollum and Davis were working with a compound other than vitamin A, as previously thought [4]. Mellanby noted that some of the fastest-growing dogs in his experiments were fed diets low in vitamin A. He was also one of the first investigators to note the antirachitic effects of cod liver oil which is rich in vitamin A. McCollum was later able to eliminate vitamin A and correctly identify a previously unidentified compound, referred to as "calcium-depositing vitamin" in earlier journal articles, which he named vitamin D [5]. McCollum focused on the antirachitic properties of this vitamin and was able to show that cod liver oil, even after it had been oxidized, was still effective in preventing and curing rickets in rats. His work focused on comparing a variety of dietary fats, including cod liver oil, butterfat, and several vegetable oils including peanut, rapeseed, olive, and coconut oil.

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The second major discovery was the discovery of the reported benefits of ultraviolet light by Steenbock and Black [6]. Steenbock and Black irradiated rations of hog millet with a mercury arc lamp, which produces a broad spectrum of light including UVB, and fed it to rats. Rats fed with the irradiated rations had increased growth and significantly higher ash content in the femur compared to the controls. This was further supported by the work of Huldschinsky who showed that children with rickets improved after exposure to a mercury arc lamp [7]. Infants with rickets in a hospital in New York City also showed complete recovery from rickets following exposure to sunlight on the roof of the hospital [7]. This included complete eradication (or disappearance) of signs including beading of the ribs, bone deformity detected by X-ray, and increased deposition of inorganic salts at the epiphyses of the long bones. The infants were exposed to sunlight for 15 min to 1 h 4–5 days a week depending upon the weather [8]. This important breakthrough led to the realization that irradiating certain foods and even the skin of animals and humans can be beneficial. As early as 1923, Steenbock and Black noted that the properties of the irradiated substances were related to calcium, which we now recognize as a classic function of vitamin D [6]. This discovery of irradiating foods led to the irradiation of milk, the first food to be fortified with vitamin D.

The final early twentieth-century discovery concerning vitamin D resulted in a Nobel Prize for Adolf Windaus in 1938. Windaus was a steroid chemist from Germany, and his contribution to the vitamin D puzzle was identification and chemical synthesis of vitamin D [9]. Through a series of irradiation experiments, Windaus was able to determine that ergosterol is an intermediary compound to the active form of vitamin D. With this knowledge and the ability to fortify foods, rickets was essentially eradicated from the US population. Vitamin D became a miracle vitamin and was added to a myriad of foods including dairy products, cereal and breads, hotdogs, soda, and peanut butter [2]. Schlitz Brewery even introduced beer containing vitamin D (Fig. 18.1) and marketed it as "the beer containing sunny energy in both summer and winter" and advertised with the slogan "keep sunny summer health, drink Schlitz all winter."

Europe followed suit with vitamin D fortification, but after World War II, fortification procedures weren't closely regulated, and accidental over-fortification of vitamin D in milk led to vitamin D intoxication in some children and infants [2]. This led to legislation that prohibited the fortification of vitamin D in foodstuffs in many European countries. Even today some European countries still ban the fortification of dairy products but allow vitamin D fortification in margarine and cereals.

Physiology of Vitamin D

Although labeled a vitamin by McCollum, vitamin D actually acts like a hormone [1]. There are four major factors that classify vitamin D's actions as a hormone. The first is that vitamin D is metabolized into more than 41 metabolites, most importantly 25-hydroxyvitamin D (25(OH) D) and 1,25-dihydroxyvitamin D (1,25(OH)2D). The formation of 1,25(OH)2D is also regulated in the kidney. Furthermore, the main metabolites are transported outside the cell through circulation by lipoproteins, albumin, and vitamin D-binding protein (DBP) and inside the cell through the vitamin D receptor (VDR). The final factor involves the identification of the VDR as a nuclear transcription factor which regulates transcription of a large number of genes.

Vitamin D, Calcium Regulation, and Bone Health

Vitamin D is classically recognized as playing an important role in calcium homeostasis with the target organs being the bone, intestine, and kidneys. Vitamin D stimulates calcium transport from these organs to the blood. Production of the active form of vitamin D (1,25(OH)2D) is stimulated by parathyroid hormone (PTH) [10].

Fig. 18.1 1936 Schlitz advertisement for vitamin D beer

Vitamin D serves to increase serum calcium concentrations in three ways [11]. First, it induces gene expression of the proteins involved in active intestinal calcium absorption which includes the protein calbindin. Calbindin is a calcium-binding protein in the intestine and has been localized primarily in absorptive cells of the mucosa. This supports the role of calbindin as a facilitator of calcium diffusion [12]. This occurs through the cell interior towards the basolateral membrane. Vitamin D also allows mobilization of calcium from bone when it is absent or deficient in the diet. This action is through stimulation of osteoblasts to produce receptor activator nuclear factor-κB ligand (RANKL). RANKL then subsequently stimulates osteoclastogenesis and activates resting osteoclasts. Activated osteoclasts increase bone resorption. It has been reported, in vivo, that both 1,25(OH)D and PTH are required for this to occur [11]. The final event is enhanced calcium absorption in the distal renal tubules which also requires both vitamin D and PTH. The distal tubule is responsible for reabsorption of the last 1% of the filtered load of calcium and can represent a significant amount of calcium retention (e.g., as high as 7 g).

Additional Roles of Vitamin D

In addition to the classic targets for vitamin D, more recent research has discovered other roles for this hormone (Table 18.1). Vitamin D is now known to play a role in immune modulation and reproductive function and may protect against multiple sclerosis, certain cancers, diabetes, high blood pressure, and cardiovascular disease. Grandi et al. reported an inverse association between circulating 25(OH)D concentration and cardiovascular disease incidence and mortality [27]. Covic et al. reported an association between low VDR activation and increased risk of hypertension [28]. While the exact mechanism for the protective effect of vitamin D in diabetes mellitus has yet to be elucidated, proposed mechanisms include pancreatic β-cell dysfunction, chronic inflammation, and peripheral insulin resistance due to compromised status [29]. VDR has been identified in pancreatic islets, indicating a possible role for vitamin D in insulin secretion [30]. VDR has also been identified in human sperm and human testis, while vitamin D has been shown to be necessary for estrogen biosynthesis in both male and female gonads [25, 31, 32]. Vitamin D and VDR are both thought to play a role in skeletal muscle growth differentiation and function [33]. The binding of 1,25(OH)2D to the VDR also results in enhanced transcription of several proteins. A summary of the effects of vitamin D can be seen in Table 18.1.

VDR has been identified in pancreatic islets, indicating a possible role for vitamin D in insulin secretion [30]. VDR has also been identified in human sperm and human testis, while vitamin D has been shown to be necessary for estrogen biosynthesis in both male and female gonads [25, 31, 32]. Vitamin D and VDR are both thought to play a role in skeletal muscle growth differentiation and function [33]. The binding of

Table 18.1 Effects of vitamin D on various systems

Effects of vitamin D	
Bone [13–16]	Decreases the risk of osteoporosis and osteoporotic fractures
	Hypothesized to be the best predictor of fracture risk
	Increases bone mineralization in adolescents
Intestine [12, 17, 18]	Increases calcium and phosphorus absorption in the small intestine
	Increases synthesis of calbindin
	Promotes intracellular calcium transport
Kidney [11, 19]	Increases calcium reabsorption
Skeletal muscle [20–24]	Decreases risk of falls
	Inhibits type II muscle fiber atrophy
	Inhibits fatty degeneration and infiltration of fat, fibrosis, and glycogen granules
	Deficiency promotes nonspecific muscle pain
Reproductive system [25, 26]	Increases the likelihood of in vitro fertilization success
	Indirectly reduces the risk of preeclampsia by regulating calcium homeostasis
	Positively influences sperm function

1,25(OH)2D to the VDR also results in enhanced transcription of several proteins. A summary of the effects of vitamin D can be seen in Table 18.1.

Vitamin D Synthesis and Metabolism

Both exogenous and endogenous cholesterol can be used as a precursor for vitamin D synthesis. In the initial step of endogenous synthesis, acetyl CoA and acetoacetyl CoA react to form hydroxymethylglutaryl CoA (HMG CoA). HMG CoA is acted upon by HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis, to create mevalonate. Mevalonate is phosphorylated to farnesyl phosphate and subsequently converted to squalene and squalene 2,3-epoxide. Squalene 2,3-epoxide is then converted to lanosterol and finally to cholesterol which is the precursor to 7-dehydrocholesterol. Cholesterol is stored in the membrane of skin cells and converted to 7-dehydrocholesterol upon exposure to UVB light (wavelength 290–315 nm) [34].

Vitamin D is synthesized cutaneously from UVB rays when 7-dehydrocholesterol is converted to cholecalciferol (previtamin D_3) [1, 2, 10]. This complex conversion involves photochemical and thermal reactions, and no enzymes are involved. Cholecalciferol is transported to the liver by vitamin DBP where it is hydroxylated in the liver to 25(OH)D by cytochrome 25-hydroxylases. This conversion involves the addition of a hydroxyl group on carbon 25. 25(OH)D is hydroxylated to 1,25(OH)2D by 1α-hydroxylase in the cytochrome of the kidney under the direction of PTH when serum calcium and phosphorus concentrations drop. 1,25 (OH)2D is the active form of vitamin D. 1α-hydroxylase tightly regulates the production of 1,25(OH)2D.

At least four different isoforms of the 25-hydroxylases have been identified [1]. These four enzymes are all microsomal cytochrome P_{450} (CYP) isoforms (CYP2DIII, CYP2D25, CYP3A4, and CYP2R1). Although four enzymes have been identified, CYP2R1 is considered to be the key enzyme because a homozygous mutation was reported in a patient with low circulating concentrations of 25(OH)D and exhibiting classical symptoms of vitamin D deficiency, including rickets [35]. In contrast to the 25-hydroxylases, there is only one 1α-hydroxylase (CYP27B1). CYP27B1 is most abundant in the kidneys. Production is positively regulated by low serum calcium concentration, elevated PTH, and growth hormone and IGF-I and negatively regulated by phosphate, fibroblast growth factor 23 (FGF23), calcitonin, and 1,25(OH)2 [1]. Although the kidney seems to be the only site for production of serum 1,25(OH)2D, CYP27B1 is expressed in the skin, monocytes, placenta, and bone cells and thought to produce paracrine and autocrine actions [36]. This has been identified by immunohistochemical and Western blot analyses in both normal and diseased tissues. Although the function of CYP27B1 in several extrarenal tissues, including the adrenal medulla, brain, pancreas, and colon, remains to be elucidated, the staining pattern may indicate intracrine actions in peripheral tissue related to vitamin D [36]. While 4 25-hydroxylases have thus been discovered, only one gene has been identified in the catabolism of both 25(OH)D and 1,25(OH)2D. This multifunctional gene, CYP24A1, converts both metabolites to either calcitroic acid after initial 24-hydroxylation or a side chain lactone after 23-hydroxylation [35, 37]. Calcitroic acid is secreted in the bile. Figure 18.2 gives an overview of vitamin D synthesis and metabolism.

Cutaneous Synthesis

Several factors affect the synthesis of vitamin D (see Table 18.2). Time of day can alter synthesis of vitamin D with decreased synthesis in the early morning and late afternoon. Optimal synthesis is seen from 10:00 to 15:00 in the spring, summer, and fall [2]. Air pollution can block UVB rays and decrease synthesis, as can time of year. Although the sun is closest to the earth from November to February, the zenith angle is more oblique, so the UVB photons pass through a greater distance of the ozone layer, which allows the ozone to more efficiently absorb the photons [2]. This decreased

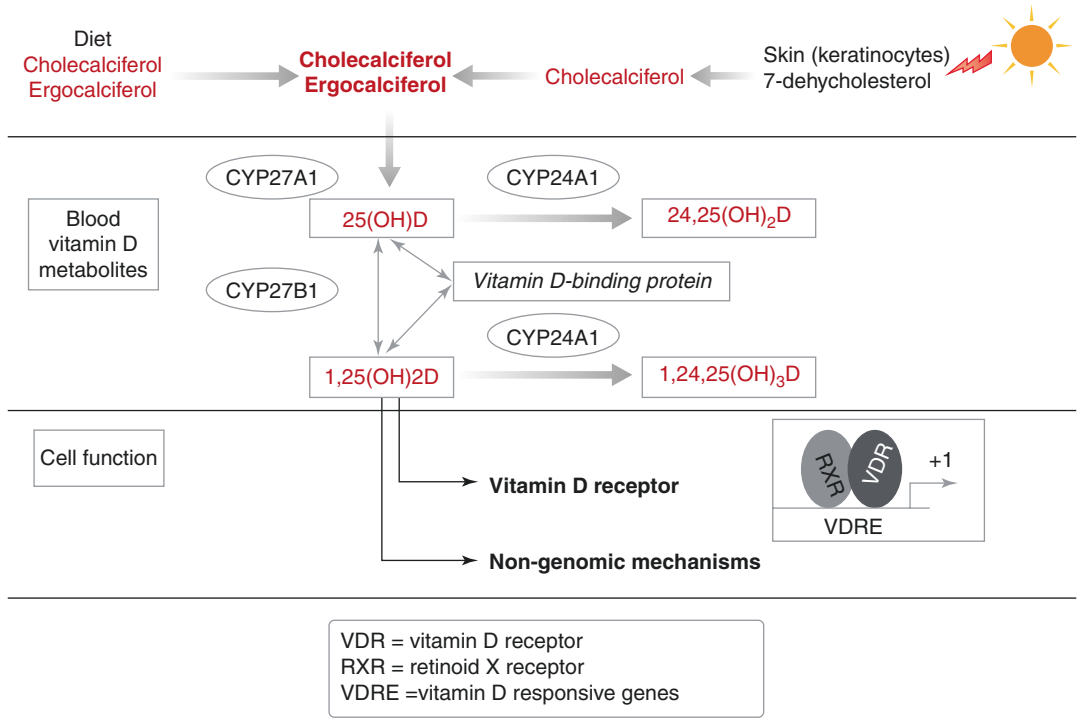

Fig. 18.2 An overview of vitamin D synthesis and metabolism. (Modified from Elsevier Limited [38])

Table 18.2 Factors associated with decreased vitamin D synthesis. (Adapted from Holick [2])

Factors that decrease cutaneous synthesis of vitamin D
Latitudes >37° north or south
Increased melanin content in the skin
Air pollution and cloud cover
Zenith angle of the sun (November to February)
Proper sunscreen application
Time of day (early morning and late afternoon)
Increased age

synthesis is especially seen in latitudes above 37° where the UVB photons may be decreased from 80% to 100% during the winter months [39]. Increased melanin content serves as a natural skin protectant but also decreases vitamin D production. Wearing 1 ounce of SPF 8, the recommended amount to cover the entire body surface, can decrease cutaneous vitamin D synthesis more than 95%, whereas wearing SPF 15 can reduce the capacity more than 98% [2]. As the skin ages, less 7-dehydrocholesterol is available, and this also serves to decrease synthesis [40]. Adipose tissue is thought to serve as an irreversible "sink" for vitamin D which does not appear to affect the synthesis of vitamin D, but it does affect the availability, once it has been produced.

Dietary Intake of Vitamin D

Very few foods are natural sources of vitamin D. It is estimated that only 100–200 IU of vitamin D comes from food sources daily (Table 18.3) even in athletic populations [20, 41]. A cohort study in Finland found that fortifying milk with vitamin D was still not sufficient in resolving hypovitaminosis D in young healthy men [42]. Of the few food sources, oily fish are among the best sources. These include salmon, herring, and sardines. Cod liver oil, which has been considered critically important for bone health for hundreds of years, is also a very good source. Beef liver and irradiated mushrooms are also sources of vitamin D. Egg yolks may also contain vitamin D, but the total amount is highly variable, and they are not generally considered a good

Table 18.3 Vitamin D values of selected foods

Vitamin D source	Serving size	IU per serving size (IU)
Fortified orange juice	8 oz. (1 cup)	137
2% milk	8 oz.	100
Skim milk	8 oz.	100
Fortified soy milk	8 oz.	100
Fortified rice milk	8 oz.	100
Sardines packed in oil	1 can (3.75 oz.)	178
Salmon—pink	½ fillet (124 g)	522
Cod liver oil	1 tbsp	1360
Atlantic herring	1 fillet (143 g)	546
Portabella mushroom		
Irradiated	1 mushroom cap (84 g)	375
Nonirradiated	1 mushroom cap (84 g)	8

USDA National Nutrient Database for Standard Reference, Release 24

source because of their high cholesterol content. Fortified foods include milk (dairy and nondairy), margarine, orange juice, and some breads and cereals. Table 18.3 lists several food sources of vitamin D and the IU in a serving.

Vitamin D-Binding Protein

Vitamin D and all of its metabolites are transported in the circulation bound to vitamin DBP. DBP is highly polymorphic and belongs to the albumin superfamily that includes albumin, α-albumin, and α-fetoprotein. This family is characterized by unique cysteine residue arrangements which have the ability to form disulfide bonds with other distally located cysteine residues [43]. The functional domains differentiated by these bonds are distinct from albumin and help to define the physiologic roles of DBP. DBP is synthesized predominantly by hepatic parenchymal cells, but other cells can also produce DBP. DBP has been detected in cerebrospinal fluid, seminal fluid, saliva, and breast milk in addition to plasma [44, 45]. Researchers originally thought that DBP detected in extrarenal tissue was artifact from plasma contamination during preparation, but this has been disproven. The presence of DBP in tissue is now thought to be indicative of the numerous functions of vitamin D in renal tissue [46].

Several functions for DBP have been identified. DBP depolymerizes and binds actin and tightly binds 25(OH)D [46, 47]. It has been shown that 25(OH)D binds to DBP with a tenfold higher affinity than 1,25(OH)2D [44]. Additionally, DBP also binds fatty acids, controls bone development through osteoclast activation, and modulates immune and inflammatory responses, including leukocyte C5a-mediated chemotaxis and macrophage activation [43, 44, 48]. The introduction of another name for DBP, macrophage-activating factor (GcMAF/DBP-MAF), highlights the importance of its macrophage-stimulating activities [49]. There is also evidence to show that DBP is associated with the surface of cells which include neutrophils, fibroblasts, monocytes, B and T cells, B lymphoblastoids, placental cytotrophoblasts, human sperm, and smooth muscle cells [44].

DBP has potential therapeutic properties, separate from its actions on vitamin D. In addition to binding actin intracellularly, DBP also acts as an extracellular actin scavenger. The serum protein gelsolin (GSN), which is an actin-binding protein, acts with DBP to scavenge actin following cell lysis and is usually seen with physiologic stress. Following such tissue injury, intracellular actin is released into the circulation which can result in damage to the microvasculature through microemboli [46]. Both actin-DBP and actin-GSN complexes have been detected in the circulation following tissue injury [50]. DBP binds to G-actin and prevents further nucleation and polymerization [48]. This binding sequesters G-actin and prevents it from polymerizing into F-actin. DBP is unable to bind with F-actin but (GSN) can both bind and sever the filaments. By removing actin from circulation, DBP is thought to attenuate clot formation and prevent the consequences of actin toxicity [45].

Vitamin D Receptor

The VDR is a nuclear protein that binds 1,25(OH)2D. VDR expression has been identified in nearly every human tissue, which gives further evidence to the importance and myriad functions of vitamin D. The VDR is a ligand-

activated transcription factor and is a member of the superfamily of nuclear receptors for steroid hormones [51]. The VDR has been identified in mammals, birds, amphibians, and fish with a calcified skeleton. Genome mapping has identified 2776 positions occupied by the VDR and 229 genes that have significant changes in expression in response to vitamin D [52]. Although almost all nucleated cells express the VDR, its expression is variable. A few tissues and cells have either low or absent VDR, and these include red blood cells and some highly differentiated brain cells including Purkinje cells of the cerebellum [1]. It is seen with a high degree of homology in ligand binding, functionality, and structure. In nonchordate species such as crabs, mollusks, and octopus, the VDR is undetectable [1].

The VDR functions through heterodimerization with any of the three retinoid X receptor (RXR) isoforms (RXR alpha, beta, and gamma), much like the other members of group I nuclear receptors (NR). 1,25(OH)2D binds to the VDR, and the complex is then able to modulate the expression of target genes. Once bound with 1,25(OH)2D, VDR is phosphorylated, and the surface conformation is reconfigured. This is thought to result in the release of corepressors. Corepressors are substances that inhibit the expression of genes indirectly through interaction with repressor proteins. The VDR then recruits RXR and binds to vitamin D-responsive elements (VDREs). Many of the genes that are regulated by vitamin D will have multiple VDREs [53]. Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1-alpha) has also been shown as a coactivator for the VDR and can augment ligand-dependent VDR transcription [54].

Methods of Measurement and Optimal Concentrations

Vitamin D concentration is assessed by measuring 25(OH)D, the inactive form of vitamin D. Serum assays can be done on 1,25(OH)2D, but this is not an accurate portrayal of an individual's vitamin D status. 1,25(OH)2D has a shorter half-life than 25(OH)D. 25(OH)D has a half-life of ~2–3 weeks in circulation, while the half-life of 1,25(OH)2D is ~4–6 h in circulation [55]. The circulating concentration of 25(OH)D is a thousandfold higher than the circulating concentration of 1,25(OH)2D. Most importantly, in vitamin D insufficiency, elevated PTH stimulates the hydroxylation of 1,25(OH)2D, which increases circulating 1,25(OH)2D concentration. In fact, it is not uncommon to find normal or even elevated serum 1,25(OH)2D in vitamin D-insufficient or vitamin D-deficient individuals [2, 55].

Common methods of assessing vitamin D status in serum include radioimmunoassay and chromatography techniques. Radioimmunoassay methods detect antibodies that are directed against $25(OH)D_3$ and of $25(OH)D_2$. Chromatographic methods, which include liquid chromatography–tandem mass spectrometry (LC–MS), separate and quantify $25(OH)D_2$ and $25(OH)D_3$ from its epimers. There are advantages and disadvantages to both. Immunoassay methods are less expensive and have a shorter turnaround time. They also can be automated, require fewer skilled personnel, and employ more user-friendly equipment. Chromatographic methods are more expensive, require more skilled personnel, and have a much longer turnaround time. On the other hand, chromatographic methods are able to differentiate between $25(OH)D_2$ and $25(OH)D_3$, while immunoassay methods can only assess total 25(OH)D [56]. Because of the many advantages, notably cost, and shorter turnaround time, immunoassays are often the method of choice for assessing vitamin D status. In the past, concerns were raised because of the reported variability between labs, often due to a difference in methods of assessment, continuing to confound the diagnosis of hypovitaminosis D [57]. Recent work has shown that this variability has decreased markedly as techniques and equipment improve, but there is still a move towards international standardization [56, 57].

Although methods of assessment have improved, there continues to be much debate on the appropriate serum 25(OH)D concentration for ideal health (Table 18.4). Many researchers

Table 18.4 Concentrations of 25(OH)D

25(OH)D concentrations for health [2, 13, 55, 58, 59]	
Status	Concentration
Optimal	>40 ng/mL (>100 nmol/L)
Sufficient	≥30–32 ng/mL (>75 to 80 nmol/L)
Insufficient	21–29 ng/mL (51–74 nmol/L)
Deficient	≤20 ng/mL (50 nmol/L)
Toxicity	>150 ng/mL (374 nmol/L) and hypercalcemia

agree that a 25(OH)D concentration less than 20 ng/mL (50 nmol/L) is indicative of deficiency, 21–29 ng/mL (51–74 nmol/L) is suggestive of insufficiency, and greater than 30 ng/mL (75 nmol/L) identifies sufficiency [2, 13, 55]. Researchers further agree that maintaining serum 25(OH)D concentration of 30–32 ng/mL (75–80 nmol/L) or greater is sufficient in the general population [58]. This is because intestinal calcium absorption is maximized at concentrations at or above 32 ng/mL (80 nmol/L) [59]. Bischoff-Ferrari et al. have reported evidence from clinical trials that fracture prevention efficacy is optimized when 25(OH)D was ≈ 40 ng/mL (100 nmol/L) [60]. They further reported that serum 25(OH)D concentration of 36–40 ng/mL (90–100 nmol/L) indicated the best results for lower-extremity strength in older adults. These and other studies in breast cancer have led some researchers to speculate that optimal concentrations are greater than 40–50 ng/mL (100–125 nmol/L) which is believed to be the serum concentration in which the human genome developed, i.e., when humans spent time outdoors without sunscreen [61].

Maintaining adequate to optimal stores of vitamin D has been found to be critical for health. Supplemental vitamin D (500–600 IU/day) has been associated with a 40% reduction in the risk of developing multiple sclerosis, and vitamin D deficiency is common in patients with autoimmune diseases [62]. It has been proposed that supplementing individuals with higher doses of vitamin D (800 IU/day) can decrease the incidence of autoimmunity [63]. Additionally, vitamin D intake (>500 IU/day) was seen to have an inverse relationship with the development of breast cancer in premenopausal women [64].

Vitamin D and Immunity

Vitamin D is suspected to play a strong role in autoimmune rheumatic diseases (Table 18.5). This includes rheumatoid arthritis, undifferentiated connective tissue disease, and systemic lupus erythematosus [66]. Low serum 25(OH)D concentrations are often correlated to the severity of the disease. 1,25(OH)2D can exert its effect on several immune cell types including dendritic cells, macrophages, and T and B cells (Table 18.6). This includes both systemic and locally produced 1,25(OH)2D [37]. Additionally, the identification of VDR in cells of the immune system and the presence of 1α-hydroxylase in dendritic cells and macrophages suggest that vitamin D has regulatory autocrine and paracrine functions, particularly at sites of inflammation [83]. There is also evidence that 1,25(OH)2D is an immunosuppressive agent that enhances the pathogenesis of T helper 1-mediated autoimmune diseases including inflammatory bowel disease and experimental autoimmune encephalomyelitis [84, 85].

In the T cells of VDR knockout mice, an inflammatory phenotype is expressed in comparison to cells of control mice. Knockout T cells proliferate twice as much in a mixed lymphocyte reaction and transfer a more severe form of inflammatory bowel disease compared to wild-type controls [86]. Additionally, increased expression of IL-1β and TNF-α in the colon of both young (5-week-old) and old (9-month-old) VDR knockout mice was seen compared to wild-type controls [87].

1,25(OH)2D appears to significantly inhibit adaptive immune cells. This is seen in the inhibition of T-cell proliferation and the decrease in the production of IL-2, interferon-γ (IFN-γ) mRNA, and protein in T cells [37]. There are also increases in IL-4 and IL-10 in T cells [37].

Hypovitaminosis D

Vitamin D insufficiency and deficiency have been documented worldwide and have been deemed a pandemic by some researchers [2, 13,

Table 18.5 The relationship between vitamin D and disease states

Vitamin D and various diseases and disorders	
Periodontal disease [60, 65]	25(OH)D concentration associated with attachment loss (reduction in connective tissue which attaches the tooth to the alveolar bone) in individuals >50 years
Autoimmune disorders [62, 66–69]	Association between high 25(OH)D concentration and lower incidence of multiple sclerosis (MS) and MS-related disability
	Vitamin D intake may be protective in MS development
	25(OH)D concentration associated with the severity of rheumatic arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis
	25(OH)D concentration lower in patients diagnosed with mixed connective tissue disease (MCTD)
	Fibrosis of the connective tissue was inversely related to 25(OH)D concentration in individuals with systemic sclerosis
Cardiovascular diseases [28, 70–74]	25(OH)D concentration associated with decreased incidence of myocardial infarction
	25(OH)D concentration associated with cardiovascular events even after controlling for other factors associated with coronary artery disease
	25(OH)D concentration determined to be an independent inverse predictor of end-stage renal disease (ESRD)
Diabetes [29, 75, 76]	Vitamin D supplementation (2000 IU/day) improved β-cell function in individuals at risk for type II diabetes
	Low 25(OH)D and calcium concentrations negatively influenced glycemia
Cancer [77–81]	Vitamin D intake and 25(OH)D concentration inversely associated with colon or rectal cancer development
	Increased 25(OH)D concentration may be protective against breast cancer incidence, especially in women >60 years
Respiratory infections [82]	25(OH)D concentration inversely associated with risk of developing tuberculosis
	Low 25(OH)D concentration associated with increased incidence of upper respiratory infections (URIs)
	25(OH)D concentration may reduce the risk of developing asthma

Table 18.6 Effects of vitamin D on immunity

Effects of vitamin D on immunity [37, 82]	
Monocytes and macrophages	↑ IL-1
	↑ Proliferation
	↑ Cathelicidin
	↑ VDR, CYP27B1
Dendritic cells	↓ Maturation
	↓ MHC class II
	↓ CD40, CD80, CD86
	↓ IL-12
	↑ IL-10
Effector or memory T cells	↓ IL-2, IFN-γ, IL-17
	↓ Cytotoxicity
	↓ Proliferation
	↓ $CD4^+$:$CD8^+$ T-cell ratio
	↑ IL-4, IL-10
	↑ T_R1-cell and T_{REG}-cell generation
B cells or antibody-secreting cells (ASCs)	↓ Proliferation
	↓ IgG, IgM production
	↓ Plasma-cell differentiation
	↑ VDR
	↑ CYP24A1

88]. In North India, 96% of neonates [89], 91% of apparently healthy school girls [90], and 84% of pregnant women [89] were found to have serum 25(OH)D concentration less than 20 ng/mL (50 nmol/L). Up to 70% of adolescent girls in Iran [91] and 80% of adolescent girls in Saudi Arabia [92] had 25(OH)D concentration less than 10 ng/mL (25 nmol/L). In the National Health and Nutrition Examination Survey (NHANES), which evaluated over 20,000 US men and women from 2002 to 2004, 29% of men and 35% of women between the ages of 20 and 49 were found to have serum 25(OH)D less than 20 ng/mL (50 nmol/L) [93]. In a study of young Finnish girls, 9–15 years of age, 67.7% had serum 25(OH)D concentrations less than 15 ng/mL (37.5 nmol/L) during the winter months, and 3 months of supplementation with 400 IU/day of vitamin D was unable to prevent hypovitaminosis D [94]. In Australia, a study that investigated serum 25(OH)D concentration in recently arrived immigrants from Africa reported that 53% of the

participants had concentrations less than 10 ng/mL (25 nmol/L) and 92% had concentrations less than 20 ng/mL (50 nmol/L) [95].

Vitamin D deficiency is also a growing concern for athletes. Hamilton et al. [96] investigated Middle Eastern sportsmen and found that 91% were vitamin D deficient (<20 ng/mL (<8 nmol/L)) and the entire cohort was insufficient (<30 ng/mL (<12 nmol/L)). Constantini et al. investigated the prevalence of vitamin D insufficiency and deficiency among young Israeli athletes and dancers [97]. They reported that only 27% of the cohort (n = 98) was vitamin D sufficient (≥30 mg/mL). Additionally, vitamin D insufficiency was reported in 48% of athletes participating in outdoor sports (tennis, soccer, running, triathlon, and sailing) and 80% of athletes participating in indoor sports (dancing, basketball, swimming, Tae Kwon Do, judo, gymnastics, and table tennis). Our lab looked at vitamin D insufficiency and deficiency over the course of the year in collegiate athletes and found that 12.2%, 63.6%, and 20.0% of athletes were either vitamin D insufficient or deficient in the fall, winter, and spring, respectively [98].

Lovell [99] investigated elite Australian gymnasts and reported that 15 of the 18 gymnasts studied had vitamin D concentrations below optimal (<30 ng/mL (75 nmol/L)). The group mean was 22.4 ng/mL (56 nmol/L). Also of particular importance was that 13 of the 18 gymnasts had experienced a bony stress injury within the previous year. Garciá and Guisado investigated serum 25(OH)D concentrations in male professional basketball players [100]. They reported a mean serum 25(OH)D concentration of 47.8 ± 21.8 nmol/L immediately following the winter months. Serum 25(OH)D concentrations were associated with vitamin D intake, independent of sun exposure. They concluded that professional basketball players were at a higher risk of hypovitaminosis D after winter. Lehtonen-Veromaa et al. investigated the incidence of hypovitaminosis D and effects of supplementation in young (9–15-year-old) Finnish female athletes [94]. In the cohort, 13.4% of the athletes had severe hypovitaminosis D (<20 nmol/L), and 67.7% of the athletes had moderate hypovitaminosis D (20–37.5 nmol/L) at baseline. Additionally, 2.2% of the participants had serum 25(OH)D concentrations less than 10 nmol/L. One year later, after a minimum of 3 months of supplementation of 400 IU/day, 9.1% still had severe hypovitaminosis D, and 63.4% of the athletes had moderate hypovitaminosis D.

Research has shown that there is seasonality associated with vitamin D status [101, 102]. Hall et al. observed a significant difference between winter and summer serum 25(OH)D concentrations in a cohort of 72 individuals of both African and European ancestry [103]. They further reported a difference in serum 25(OH)D concentrations based upon skin pigmentation (reflectance). Participants of European ancestry had significantly greater serum 25(OH)D concentrations than those of Hispanic, African, and North and South Asian descent. These findings were consistent throughout the year. Snellman et al. investigated the seasonality of vitamin D in a twin study and reported a significant difference between summer and winter serum 25(OH)D concentration [101]. Collectively these studies suggest that latitude and elevation [98], skin pigmentation [103], time of day during training, percent body fat [104], and time of year [98, 101] may all influence the serum 25(OH)D status of athletes. Other important factors may include indoor training and amount of skin exposed during training.

Vitamin D Receptor and Skeletal Muscle

Identification of the VDR in skeletal muscle was an important discovery. Simpson et al. [105] first identified the receptor in cultured myoblast cells from rats which was confirmed by Boland [106] shortly thereafter who identified the receptor in myoblast cells in chicks. VDR has a high specificity for 1,25(OH)2D, and this property was instrumental in the initial identification. The binding shown by Boland was both specific and nonspecific, but the specific binding had a high affinity and a low capacity (Fig. 18.3) [106]. The binding affinity refers to the strength of the interaction, and capacity refers to the actual amount

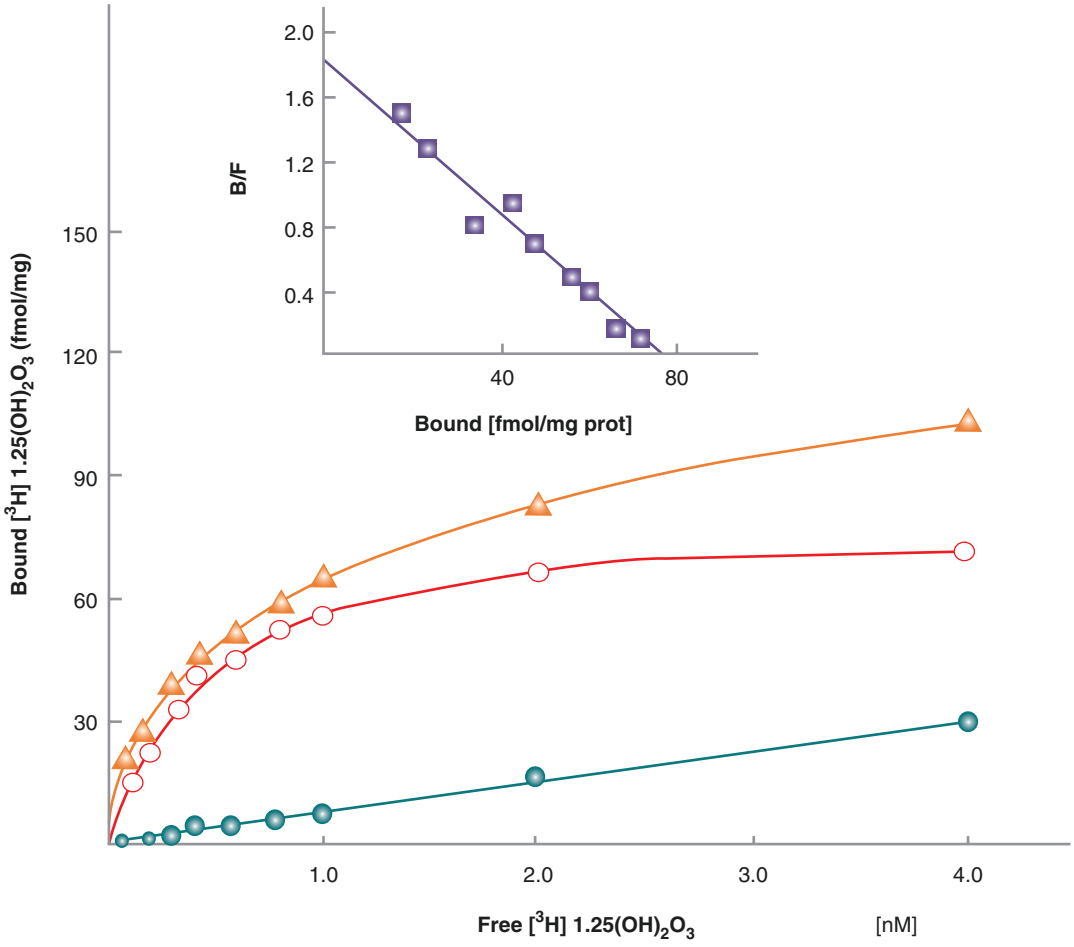

Fig. 18.3 Embryonic chick skeletal muscle myoblasts: saturation analysis of (3H) 1,25-dihydroxyvitamin D3-binding cytosol from embryonic chick skeletal muscle myoblasts. Cytosol (1.0 mg protein) was incubated with increasing concentrations (0.01–4 nM) of (3H) 1,25-dihydroxyvitamin D3 in the presence or absence of 100-fold molar excess of radioinert 1,25-dihydroxyvitamin D3 at 4 °C for 16 h. Bound and free 1,25-dihydroxyvitamin D3 was separated with hydroxyapatite. The saturation plot is of total (*triangle*), specific (*dot*), and nonspecific binding (*filled circle*). (Modified from Elsevier Limited [106])

of the sample that binds to the medium. This evidence was in support of the theory that skeletal muscle is a target organ with direct physiological actions for 1,25(OH)2D. Simpson et al. [105] also identified the VDR but were able to show additionally that VDR concentration decreased after myoblast cells fused and differentiated into myotubes and that DNA synthesis and cell proliferation of the myoblast line were inhibited by 1,25(OH)2D. These findings are indicative of direct physiological action of vitamin D in skeletal muscle, at least in the myoblast stage.

More recently, Endo et al. [107] worked with VDR null mice to investigate skeletal muscle development. They found that VDR null mice had significantly smaller muscle cells (approximately 20% in diameter) at 3 weeks than their wild-type littermates and more prominent changes in the muscle cells at 8 weeks. This suggests that the effects may be either additive based on systemic metabolic changes or progressive based upon the length of time with no VDR. Additionally, VDR null mice had increased expression of embryonic- and neonatal-type

myosin heavy chain (MHC) at 3 weeks which was still expressed at 8 weeks only in VDR null mice. Type II MHC was expressed equally between the VDR null mice and wild-type littermates.

These findings were further supported by the increased expression in VDR null mice of Myf5, E2A, and myogenin [107]. Myf5 is responsible for regulating muscle differentiation, E2A further differentiates into genes E12 and E47, and myogenin is responsible for the coordination of skeletal muscle development and repair. This increased expression of MyoD transcription factors suggests that the downregulation of myogenic differentiation factors require VDR and 1,25(OH)2D.

It is also likely that these morphological changes are due to a primary physiological rather than a secondary effect. In the aforementioned series of studies, the authors put forth three lines of evidence to support the direct physiologic role of VDR actions in skeletal muscle. Firstly the VDR null mice developed apparent morphological abnormalities in skeletal muscle and a deregulated pattern of muscle gene expression before weaning. Secondly, the same changes were still observed in older rescued VDR null mice fed with a high-calcium diet, and thirdly, direct negative regulatory effects of 1,25(OH)2D on muscle gene expression were at least in part reproduced in cultured myoblasts in vitro [107]. The authors postulate that this may have implications in adult models as well, particularly in cases of remodeling following injury, denervation, or immobilization. Further research, however, is necessary to confirm these earlier findings and fully elucidate the impact of VDR on differentiating myotubes.

Vitamin D and Sarcopenia

Sarcopenia is the term used to describe the loss of muscle mass and strength that is often associated with aging. This is considered to be an important connection with impairment and may be a factor in disability of the aged and elderly [108]. Sarcopenia is thought to exhibit a disproportionate atrophy of type IIa fibers and a decrease in the synthesis rate of myosin heavy chain proteins [108, 109]. There may also be a loss of growth factors, an increase in catabolic factors, or a combination of the two. Growth factors include neural growth factors, growth hormone, and estrogens and androgens, while catabolic factors may include inflammatory cytokines.

Vitamin D has been indicated to have a role in reducing the age-related decline in muscle function. Several well-designed clinical studies in older men and women have reported correlations between reduced 25(OH)D concentration and reduced muscle strength, gait speed, grip strength, and muscle mass [21, 110–113]. Lower 25(OH)D concentration has also been shown to correlate with increased PTH concentration and increased risk of falls [21, 33].

More recent studies have focused on the role of vitamin D in older populations, particularly regarding sarcopenia. In a prospective study of community-dwelling, older men and women (62 ± 7 years), Scott et al. [114] reported that individuals with 25(OH)D less than 20 ng/mL (50 nmol/L) had lower average leg strength, leg muscle quality, and appendicular lean mass. A higher 25(OH)D concentration was also modestly but significantly associated with greater muscle mass and was also predictive of greater muscle strength and muscular quality. In a 3-year longitudinal study, Visser et al. reported that individuals with baseline 25(OH)D concentration of less than 10 ng/mL (25 nmol/L) were more likely to develop sarcopenia compared to those with 25(OH)D concentration of at least 20 ng/mL (50 nmol/L) [110].

Rejnmark reviewed 16 randomized controlled trials that assessed muscle function after vitamin D intervention [113]. Overall, supplementation between 800 and 1600 IU/day of vitamin D was not found to improve grip strength, but several trials reported improvement of gait speed and body sway. It is important to note that of the 16 trials, only 1 involved patients less than 50 years of age (10–17 years old). Randomized controlled trials that have investigated various levels of vitamin D supplementation have received mixed results. Moreira-Pfrimer et al. reported a 16.4% increase in strength of hip flexors (SHF) and a 24.6%

increase in strength of knee extensors (SKE) in a group of institutionalized elderly (≥60 years of age) receiving calcium/vitamin D treatment for 6 months [111]. The participants in the treatment group were supplemented with 1000 mg/day of calcium throughout the trial and received 150,000 IU of vitamin D once a month for the first 2 months and then 90,000 IU once a month for the next 4 months. This treatment increased serum 25(OH)D concentrations from an average of 18 ng/mL (46 nmol/L) to 34.9 ng/mL (87 nmol/L). This was compared to no improvement in a calcium/placebo group in the absence of physical training. Bischoff et al. reported a 49% decrease in falls in elderly women residing in a long-term geriatric care facility, after receiving a calcium/vitamin D treatment of 1200 mg/day and 800 IU/day, respectively, for 12 weeks [21]. Although the individual strength scores did not report a significant improvement in musculoskeletal function in the calcium/vitamin D treatment group, there was a significant improvement in overall muscle function when comparing groups over time.

Although several studies have reported a significant increase in muscular strength and function with vitamin D supplementation, there are other studies that have reported no significant improvements with supplementation. Cordless et al. reported no change in muscle strength or activities of daily living following 6 months of supplementation with 9000 IU/day of vitamin D_2 which increased status from 7.2 ng/mL (18 nmol/L) to 48 ng/mL (120 nmol/L) [115]. The subjects, who were elderly patients at an in-care facility on a geriatric ward, also had no reported improvement in mental assessment scores as compared to the placebo treatment group. Latham et al. investigated whether a single oral dose of 300,000 IU of vitamin D_2 would reduce falls and improve physical health in frail older people after hospitalization [116]. After 6 months of treatment which increased average serum 25(OH)D concentrations from 15.2 ng/mL (38 nmol/L) to 24 ng/mL (60 nmol/L), there were no significant changes reported in strength, balance, and a timed walking test. There were also no reported differences in number of falls during the 6-month trial period, compared to the placebo group.

The variability of the results of these studies may be attributed to several factors. Firstly, there was a wide range in the age of the participants (50–99 years). Research has shown a decrease in skeletal muscle VDR associated with aging. Secondly, while there was a significant improvement in mean serum 25(OH)D concentrations in all of the studies, many of the individual participants were still considered vitamin D deficient or insufficient. Finally, several of the trials did not report PTH concentrations which, if elevated, would have drastically impacted the results. Additional research investigating the role of vitamin D in sarcopenia is essential, particularly randomized clinical trials that explore the role of vitamin D supplementation on muscle strength and function.

Vitamin D and Exercise Performance

The role of vitamin D on athletic performance was first investigated by German researchers in the 1920s [117]. In the late 1920s, German sports teams were using UV radiation as an artificial ergogenic aid. By the 1930s, Russian researchers followed suit. One study reported a 7.4% improvement in 100-m dash time for male students undergoing physical training and irradiation treatment [117]. This was compared to a 1.7% improvement in men undergoing the same physical training but without irradiation treatment. This was followed by abundant research in Germany, albeit not of the quality of research of today's standards, on UV irradiation and physical performance which included studies on gymnasts, swimmers, untrained adults, and even schoolchildren [117].

In the USA, the first published study involving irradiation and physical performance was conducted in 1945 when Allen and Cureton irradiated 11 male college students for 10 weeks and compared them with a control group undergoing identical physical training [118]. The treatment group had a 19.2% improvement in cardiovascular fitness compared with a 1.5% improvement in the control group. Rosentswieg [119, 120] inves-

tigated the effects of ultraviolet radiation on both endurance and strength in 23 college-age women. While trends towards an improvement in both strength and endurance were noted, these improvements were not significant. In both studies, however, participants were tested within 4 h of the radiation treatment, which may not have been enough time for the ultraviolet exposure to increase vitamin D availability.

Recently, Ward et al. [121] investigated the relationship of 25(OH)D and PTH with muscle power and force in 12–14-year-old postmenarcheal females from an inner city middle school ($n = 99$). The low average 25(OH)D concentration (11.6 ng/mL) and elevated PTH concentration (4.87 pmol/L) indicated that the majority of girls were vitamin D deficient. Vitamin D status was strongly predictive of jump velocity, jump height, power, and body mass-adjusted force (expressed as N/kg) but not absolute force. In this study, weight was used as a quadratic term in the model because a linear regression didn't hold across the whole weight range. Although none of the girls had any symptoms of vitamin D deficiency, vertical jumping may detect subclinical effects of 25(OH)D status. Jumping mechanography was used as a marker of muscle function because previous reports had suggested that proximal muscles are most often affected in vitamin D deficiency and proximal muscles are the most important muscle groups in the jumping mechanism [113].

These results suggest that young vitamin D-deficient individuals may not be able to maximally generate force which, in turn, may not be able to maximally load and develop bones. Typically, when an individual jumps, the maximum force generated is 3–3.5 times an individual's body weight, which wasn't supported in this study. These results may be of particular importance for various populations. In younger populations this may result in abnormal bone mineralization, whereas it may decrease performance in athletic populations and impair bone health maintenance in older populations.

In a follow-up study, Ward et al. supplemented a subpopulation of the original cohort ($n = 72$) over the course of 1 year with four doses of 150,000 IU of vitamin D (approximately 1650 IU/day) and retested them for muscle jump velocity [122]. After 1 year of supplementation, efficiency of movement was increased by 5% in the treated group, but no other improvements were found. Improvement in efficiency of movement, however, suggests a higher flexibility and increased muscular coordination due to treatment. It is worth noting that there was also a significant baseline 25(OH)D by group interaction for jumping velocity which was driven by greater change in jump velocity in those with the lowest baseline 25(OH)D concentrations [122]. Because the vitamin concentrations in the treated group were still suboptimal (56.0 nmol/L), there may have been a significant change if the vitamin D concentration was within the optimal range.

El-Hajj Fuleihan et al. investigated the effects of 1 year of supplementation with two different doses of oral vitamin D (1,400 or 14,000 IU/week) in school-aged children (10–17 years of age) [112]. They found a significant increase in lean mass in premenarcheal girls. Consistent trends for increased bone mineral density (BMD) and/or bone mineral content (BMC) were reported. There were significant increases for trochanter BMC in both treatment groups and at the lumbar spine BMD in the lower treatment group. No significant improvements, however, were found in lean mass, BMD, or BMC in boys or postmenarcheal girls.

Summary

Vitamin D has a long and interesting history that began with the first description of rickets in 1645 and continues today with research investigating its role in immunity, chronic disease prevention, and even muscle function and athletic performance. As a vitamin that "acts like a hormone," vitamin D plays a long-recognized role in calcium homeostasis targeting the bone, intestine, and kidneys and a more recently recognized role in immune modulation and reproductive function. It may protect against multiple sclerosis, certain cancers, autoimmune rheumatic disorders, diabetes, high blood pressure, and cardiovascular disease.

Vitamin D can be obtained in the diet or can be synthesized in the skin from UVB light. Very few foods contain vitamin D including oily fish like salmon, mackerel, and sardines; cod liver oil; and fortified foods like milk, margarine, and juice. Several factors influence cutaneous synthesis including latitude, elevation, skin pigmentation (reflectance), age, time of day, season, and sunscreen use. Because of these factors, vitamin D insufficiency and deficiency have been observed worldwide in various populations. Future research is needed to elucidate the role of vitamin D in both muscle function and athletic performance.

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19 The Effects of Altitude on the Hormonal Response to Physical Exercise

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Introduction

One of the most important roles of the endocrine system is to allow adaptation to new environmental conditions. The neuroendocrine system "feels" and "informs" the body on such environmental conditions and then triggers biological responses to induce adaptive processes. Most of the research studies performed in acute or chronic hypoxic conditions focused on hormones involved in water and electrolyte balance or on the adrenergic system, whereas scanty data on the other endocrine axes are available. Hormones act through membrane or intracellular receptors (nuclear or cytoplasmic). Hypoxia has been reported to modulate the expression of membrane and nuclear receptors in terms of both down- and upregulation events. The study of altitude effects on endocrine variations induced by physical exercise is an extremely interesting but complicated field. Most studies show limitations due to (1) the low reproducibility of experimental protocols, (2) the number of subjects enrolled, and (3) the fact that the identification of changes of a single hormone is impaired by the concomitant adaptation of the whole endocrine system to hypoxic conditions. Moreover, several environmental factors affect both the endocrine and metabolic response at the same time. Based on the foregoing points, in this chapter we summarize literature data and our personal experience with respect to altitude exposure. To that end, the main variables involved in affecting the hormonal response to physical exercise at high altitude (HA) are reported in Fig. 19.1.

Growth Hormone/Insulin-Like Growth Factor-I Axis

Physical activity (PA) is an important environmental regulator of the growth hormone (GH)/insulin-like growth factor (IGF)-I axis activity [1, 2]. The GH response to exercise is dependent on the duration and intensity of the exercise bout, the fitness level of the exercising subject, the refractoriness of pituitary somatotroph cells to the exercise stimuli, and other environmental factors [3–5]. The neuroendocrine pathways that regulate GH secretion during exercise include the cholinergic, serotoninergic, α-adrenergic, dopaminergic, and opioidergic systems [6–8]. The exercise-induced GH release is influenced by fluid intake, environmental and nutritional factors, as well as some pathological states [9–12]. Gender regulates the relationship between exercise intensity and GH release, since GH secretion

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Time of exposure	Acute/chronic (hours/days/weeks/altitude natives) Morning/evening/night
Altitude and environmental factors	Actual altitude, partial oxygen pressure, temperature, humidity, solar radiation exposure
Training status	Neuromuscular and resistance adaptation
Duration and intensity of workload	Mild/sub-maximal/strenous exercise
Energy and hydroelectrolitic balance	Nutritional and metabolic status Food intake (appetite suppression / caloric restriction / reduced caloric consumption) Dehydration / hypohydration / overhydration
Psychological attitude	Deal with danger, stress, anxiety and self-confidence
Others	Age Gender Quality of sleep

Fig. 19.1 Factors involved/influencing the hormonal response to physical activity at high altitude

is greater in women than in men [13]. The acute GH response to aerobic or resistance exercise is reduced with age [8].

Exercise leads to increases in IGF-I levels, which are likely to occur also via GH-independent mechanisms [14]. Hemodynamic or metabolic effects of exercise per se might play a role, although long periods of exercise training are able to stimulate IGF-I gene expression [15].

The influence of altitude-induced hypoxia on the somatotroph response to PA has been clearly described in different experimental conditions. Similarly to other hormonal axes, many variables have been shown to be involved in altitude-induced hypoxia, such as the actual altitude, the duration of exposure, the training degree of the subjects investigated, and the type of exercise performed.

There is scanty knowledge about the response of the GH/IGF-I axis to extremely HA [16, 17], while more is known about the metabolic adaptation. The metabolic adaptation occurring during HA exposure is mainly characterized by an increased dependence on blood glucose as a fuel with a concomitant increase in insulin sensitivity and lipolysis coupled with a decreased reliance on lipid substrates [18–20]. Our research group recently suggested that this metabolic profile could be determined by remarkable changes in GH/IGF-I axis function [17]. That is, we found that well-trained acclimatized climbers show clear-cut increases in mean GH concentration right after strenuous PA, and this agrees with evidence that PA represents a neuroendocrine-mediated stimulus of somatotropic secretion [2, 6] as well as with the enhancement of the GH response to GH-releasing hormone recorded in subjects chronically living at HA [21]. Accordingly, it had been also reported that low-altitude natives adapted to HA show a more marked GH increase than non-acclimatized subjects [22].

Interestingly, our study just noted the change in GH status was coupled with a concomitant increase in mean total IGF-I and IGFBP-3 levels [17]. Indeed, IGF-I is the best marker of GH status although IGFBP-3, a GH-dependent IGF-I-binding protein, also reflects chronic variations in the status of somatotropic function [23]. The clear increase in IGF-I and IGFBP-3 together with the enhancement of mean GH levels therefore clearly points toward increased activity of an

anabolic axis like the GH/IGF-I at HA. In fact, increased activity of the GH/IGF-I axis is likely to trigger protein anabolism and might also play a role in the adaptations occurring in glucose and lipid metabolism at HA [19, 24].

Though performed in a simulated setting of hypoxia, a very interesting study by Engfred and colleagues underlined the importance of training in the modulation of hormonal responses to exercise in hypoxic conditions [25]. Quite surprisingly, a 5-week training-induced changes in GH response to exercise in a group of previously untrained subjects, studied in a hypobaric chamber at a simulated altitude of 2500 m, were not influenced by hypoxia per se [26].

Although elevated GH levels [26–28], with maintained circadian rhythm [26], have been widely described, and no changes in resting GH concentrations of sea level residents were observed during acute exposure to HA [27, 28], the somatotroph response to exercise has been shown to be influenced also by acute exposure to hypoxia, again suggesting a relationship with metabolic aspects. In fact, GH concentrations during hypoxic exercise (20 min at 750 kpm/min on a cycle ergometer) performed in a hypobaric chamber (decompressed to a simulated altitude of 4550 m) have been shown to be higher than under normoxic conditions [29]. Moreover Yan et al. found that the more severe is the hypoxia, the greater the GH response and the isometric strength gain [30]. This hormonal response to acute resistance exercise tends to a substantial reduction following training, probably due to the neuromuscular adaptation, which decreased the metabolic stress thus reflected in the mitigated GH responses. However, even when reduced, induced GH response was significantly higher during severe hypoxia than both in normoxia and moderate hypoxia [30].

Together with other hormonal and metabolic responses, involving glucose, free fatty acids, cortisol, and insulin, these changes in GH/IGF-I axis seem to be aimed to increase fat mobilization and gluconeogenesis, in order to optimize energy substrate availability [29]. Interestingly, more than 30 years ago, Raynaud and colleagues hypothesized a modification of GH secretory pattern during submaximal exercise in hypoxic conditions, in the presence of normal absolute hormone concentrations [27]. In fact, compared with lowlanders, in highlander natives (3800 m), the rate of GH increase at the beginning of a submaximal exercise session was faster and earlier, but the mean maximal value reached at the end of the exercise bout was similar. Moreover, the GH response pattern in lowlanders during the early stages of exposure to hypoxia resembles that of highlanders [27]. The potential underlying explanations of these observations include an alteration of the hormonal clearance through a more pronounced reduction of hepatic blood flow or a difference in the state of the pituitary gland prior to the exercise bout. However, the subsequent studies did not allow the researchers to reach definitive conclusions.

In another study by Van Helder and colleagues, the balance between oxygen demand and availability was suggested to be an important regulator of GH secretion during exercise [31]. In five normal men, performing seven sets of seven squats at a load equal to 80% of their seven repetition maximum, these authors found a plasma GH increase during and after the completion of the exercise, coupled with a significant linear correlation between GH changes and the corresponding oxygen demand/availability ratio [31]. Interestingly, the existence of a significant correlation between changes in plasma GH levels and the demand/availability ratios over a wide variety of exercise (aerobic and anaerobic, continuous and intermittent, weight lifting and cycling), in both fit and unfit subjects under normoxic and hypoxic conditions, has been demonstrated.

Accordingly, almost the entirety of the studies investigating somatotroph function at HA, where the oxygen demand/availability ratio is increased, particularly when individuals perform PA, observed an increase in GH secretion. Therefore, this increase could play a role in modifying the endocrine–metabolic response to exercise to satisfy the increased needs at HA.

The additive stimulatory effect of hypoxia per se on the GH response to exercise has been also described in simulated conditions [32], though not in terms of hepatic IGF-I production.

Differently from chronic conditions, the acute exposure to hypoxia has been shown to blunt the GH response to submaximal PA in untrained individuals, but not in trained subjects [33]. In line with this observation, a previous study indicated that the hormonal response, including GH, to exercise is influenced by hypoxia and physical training, mainly via changes in the relative workload [34].

These data once again emphasized the central and critical role of physical fitness in the modulation of hormonal and metabolic adaptive responses to exercise alone as well as in altitude-induced hypoxic conditions.

Prolactin

Prolactin is most of all considered fundamental for lactation by endocrinologist, although it has been recognized to have important metabolic activities too. In fact, it is well-known that prolactin stimulates insulin synthesis and release and that pathological hyperprolactinemia is characterized by hyperinsulinism and insulin resistance both reverted by normalization of prolactin levels [35, 36]. Moreover, prolactin also acts as an important connection between the endocrine and the immune system, being also produced by extrapituitary sites including immune system and being involved in lymphocyte survival, activation, and proliferation [37]. The endocrine/paracrine prolactin has been shown to stimulate the immune cells by binding to prolactin receptors, which are expressed on many cells of the immune system, including hematopoietic stem cells, T cells, B cells, monocytes, macrophages, NK cells, neutrophils, and thymic epithelial cells [38]. Prolactin is implicated in lymphoproliferation, cytokine production, and antibody secretion, but it not seems to be essential, since both prolactin-deficient and prolactin receptor-deficient mice have normal hematopoiesis [39–41].

Data on prolactin and hypoxia as well as PA are scanty and not concordant. Hypoxia per se, independently of concomitant PA, has been shown to apparently influence prolactin secretion [42, 43], but prolactin resting levels at HA are reported to be both elevated [42] and decreased [43, 44].

Some studies reported that prolactin levels transiently increase with exercise, and this response is proportional to the exercise intensity [6, 45, 46], and others indicate that prolactin increments occur only when the anaerobic threshold is reached and appear to be correlated with pro-opiomelanocortin derivatives, ACTH, and beta-endorphins [47, 48]. Moreover, the prolactin increase may be related to changes in body temperature and dehydration, become exaggerated by stress, or be reduced with habituation and hypoxia exposure and is unresponsive to some metabolic events [6, 17].

Other studies suggested that dopamine, the main factor involved in prolactin regulation, and possibly noradrenaline (i.e., norepinephrine), inhibit prolactin secretion at HA [49]. However, while in hypoxia conditions noradrenaline consistently increases, dopamine changes are inconsistent, being either reported unchanged or increased [50–52]. Another potential prolactin modulator at HA is erythropoietin that could promote dopamine release and, therefore, inhibition of prolactin secretion [53].

Currently there are very few studies that specifically investigate the role of altitude on the prolactin response to exercise. Nonetheless, in agreement with the observation that prolactin levels are influenced by oxygen availability, an inhibition of exercise-induced blood prolactin response has been described after acute exposure to hypoxia [54].

The results of the different studies are however not completely concordant: for example, recently a study by Verratti et al. in a group of young women participating in 14 days of trekking at HA documented that prolactin levels differed significantly during the expedition, with lower values at sea level after the expedition than at altitude [55]. This finding makes the oxygen availability theory not completely applicable, as alterations in prolactin levels seemed to persist and magnify even after exposure to hypoxia. However, it is noteworthy that these data are driven from a study group composed only of

women, whereas the majority of other studies focused on men. Future investigations should examine if elements of sex dimorphism exist for this hormone.

Interestingly, prolactin changes were similar to those observed for TSH with the lowest levels after the return to sea level: these results could be explained considering the common modulation of TRH not only on the TSH but also on prolactin production [55].

As another element influencing prolactin secretion is cold, exposure to low temperature has been shown to diminish the exercise-induced prolactin response [56], though it does not seem to influence baseline levels [42].

In our model of maximal exposure to altitude over a period of 2 months, in association with a vigorous PA, prolactin levels increased but persisted within the normal range [17]. An adaptive metabolic purpose could explain this significant increase in lactotropic secretion that followed the exposure to HA, in agreement with a previous study [42]. In fact, prolactin has been shown to markedly affect glucose metabolism [35], but, on the other hand, chronic stressful conditions are known to increase prolactin secretion most likely via central neuroendocrine mechanisms [57].

In a study of chronic exposure *(3–12 months)* to HA, prolactin levels were not different from sea level values in acclimatized men, similarly to HA native residents [58]. On the other hand, another study on women reported a decrease in basal prolactin levels, related to the degree of hypoxemia [43], in agreement with the low serum prolactin concentration, described in native HA women [44].

Eventually, a recent study performed on young male adults, exposed to hypoxia at HA (5380 m) for 12 months when undergoing a military service, showed that prolactin levels significantly decreased after 6 months of exposure to hypoxia, re-increased after adaptation at 12th month, and then returned to normal range after 6 months at the sea level. Hence, HA hypoxia appears to cause a temporary and reversible reduction of prolactin levels [59].

However, considering the scanty data available, definitive conclusions about the actual prolactin response to exercise after chronic exposure to HA cannot be firmly drawn. Nevertheless, prolactin secretion in response to exercise during acute or short-term exposure to HA seems to be preserved, though attenuated, accordingly to the reduced baseline prolactin levels shown at altitude. The underlying mechanism is likely represented by an enhanced dopaminergic or noradrenergic tone. In fact, an alteration at the hypothalamic level appears to be less likely since prolactin response to thyrotropin-releasing hormone stimulation was not altered by exposure to HA [60].

Thyroid Function

The thyroid function changes secondary to PA represent a complex physiological response, which is influenced by several individual and environmental factors. Levels of thyroid-stimulating hormone (TSH), thyroxine (T4), free T4 (fT4), triiodothyronine (T3), and free T3 (fT3) have been reported to be unaffected, increased, or decreased varying with the type and duration of exercise, ambient temperature, and energy intake [61–63]. Although these divergent findings are difficult to interpret due to the highly variable exercise sessions and to procedural limitations, one of the more consistent findings is reverse T3 increase, particularly when a caloric energy deficiency is associated with exercise [64].

It is plausible to hypothesize a role of thyroid function in the adaptive process to altitude hypoxia, considering the well-known ability of thyroid hormones to increase oxygen availability by increasing ventilation and cardiac output as well as red blood cell mass. Moreover, thyroid hormones are known to increase levels of 2,3-diphosphoglycerate in erythrocytes, facilitating the unloading of oxygen to tissues through a rightward shift in the oxyhemoglobin dissociation curve [65].

Accordingly, an increase in thyroid hormone release at HA has been described by many authors [17, 21, 66–74]. In particular, most authors agree that HA induces an elevation in plasma concentration of both free and total T4

levels [17, 21, 66–74]. T3 has also been shown to be increased [69, 71–73], although to a lesser extent than T4. On the other hand, some authors reported an increase in reverse T3 (rT3) only [70, 75], suggesting the possibility of a hypoxia-induced inhibition of T4 to T3 conversion, with a concomitant rise in rT3 concentration and in T4/T3 ratio [70, 75]. An increase in corticosteroid secretion could explain these changes, similarly to other stressful conditions. In fact, marked physical exhaustion due to HA-related demanding physical work could negatively influence thyroid hormone levels [75]. Moreover, cold per se may also contribute to inhibit thyroid hormone secretion. In fact, T3 which plays a pivotal role in cold habituation decreases with cold exposure, whereas T4 and TSH remain unchanged [76].

The interrelationship between HA, hypoxia, PA, and thyroid axis has been extensively studied [17, 70, 72–74, 77]. Although the effects of PA per se are not unambiguous [61–63, 77], environmental conditions have been reported to play a relevant role [78]. A study in which subjects had a short-term stay at extreme HA during Mt. Everest climbing found an increase in total T4 and T3 concentrations in association with an increase in TSH levels [70]. On the other hand, a significant elevation of free T4 levels after 3 weeks at 4300 m without any change in TSH levels has been reported too [74]. In a study by our research group, after a 2-month stay at HA, we confirmed the lack of change in TSH levels as well as an increase in fT4 and a significant reduction of fT3 levels that were below the lowest limit of the typical normal range [17]. These findings suggest a HA-induced low T3 syndrome that would reflect an impairment of peripheral fT4 to fT3 conversion under chronic exposure to HA hypoxia. Indeed, it is reasonable to hypothesize that prolonged exposure to hypobaric hypoxia at extreme HA induces a low T3 syndrome that would also be explained by the status of negative energy balance caused by strenuous PA [77] and characteristic of HA exposure [17, 79, 80]. Moreover, however, other authors found no difference in fT3 levels after exercise at moderate HA, while fT4 did differ significantly with higher levels at sea level, after 14 days of HA trekking [55]. Furthermore, a dissociation at HA between TSH (unchanged) and thyroid hormone (increased) levels has also been reported by several authors [17, 21, 60, 68, 69, 71, 72, 74, 81].

Several explanations for the TSH-independent T4 rise have been proposed. Pituitary dysfunction has been likely excluded, since the TSH response to TRH administration has been shown to be preserved at altitude [21, 68, 70, 72], although at extreme altitude an increased TSH response to TRH was found, suggesting that the severe hypoxic stress or the association with other stressors (such as cold) could influence the pituitary response [70]. A change in hormone levels can be caused by either a modified secretion rate, a disturbed metabolic clearance, or hemoconcentration and vascular fluid shift. The T4 rise at HA cannot be simply explained by dehydration and hemoconcentration evaluated by the concentration of total plasma proteins. Actually, in contrast with a potential decreased T4 clearance, the T4 degradation rate has been shown to be increased during acute exposure to altitude. A potential role of increased thyroxine-binding globulin [71, 82, 83] or enhanced ß-adrenergic stimulation [67] has also been hypothesized but not definitely demonstrated.

Conversely, a divergent finding was documented by Verratti et al., who found a slight increase in TSH at altitude which they explained as the adaptation to the need to increase oxygen availability. The subsequent decrement observed at sea level after the trek has been interpreted as a feedback effect: indeed, the fT4 at that determination showed the highest value [55].

The impact of caloric restriction on the endocrine response to PA at HA has been elegantly investigated by Barnholt and colleagues [74]. During 3 weeks at 4300 m, they found no difference in fT4 (increased) and TSH (unchanged) secretory patterns between a group of active subjects adequately fed to maintain body weight and another under caloric restriction. The authors hypothesized that the hypoxic stimulus at altitude is capable of overriding the fall in T4 induced by caloric restriction [74].

On the whole, thyroid response to PA at HA seems to be increased with respect to sea level in

different experimental models and during different types of exercise, likely contributing to the adaptive process. However, some environmental factors associated with altitude exposure, such as cold, could negatively modulate thyroid function. The apparent dissociation between TSH and thyroid hormones is not fully understood, but does not likely reflect an alteration at the pituitary level (i.e., more so driven by peripheral events).

Gonadal Function

The effects of PA on the reproductive axis in males vary with the intensity and duration of the activity, the fitness level of the individual, and his nutritional metabolic status. In short, intense exercise usually increases, while prolonged exercise usually decreases serum testosterone (TEST) levels [84–86]. The exercise-associated increment in circulating TEST does not seem to be mediated by luteinizing hormone (LH). Possible mechanisms such as hemoconcentration, reduced clearance, and/or increased TEST synthesis may be involved [87, 88]. Both central and peripheral mechanisms may explain the TEST decrease during and subsequent to more prolonged exercise, including decreased gonadotropins, decreased or increased prolactin levels, and alterations in TEST production and/or secretion [84, 89–91].

Few studies have investigated the impact of altitude on the relationship between gonadal hormones secretion and PA. In fact, most of the studies describing gonadal function at altitude do not analyze the concomitant specific effect of PA. Moreover, most of the studies specifically focus on this topic only in males.

TEST resting levels have been reported to be increased after acute (few days) exposure to moderate altitudes [92, 93] by some authors, but not by others [42, 94]. An activation of adrenal function could contribute to the increase in TEST levels, whereas increased prolactin and estradiol concentrations could account for a decrease in TEST secretion [17, 42, 94].

On the other hand, chronic exposure to hypoxia apparently does not influence TEST levels in adult HA natives [95], although an earlier increase in TEST and subsequent onset of puberty have been described in young HA males [96].

Some authors have reported HA-induced increase in progesterone levels but no change in pituitary, gonadal, and adrenal hormones in subjects who had a prolonged stay at HA but were not performing any PA [58]. Accordingly, progesterone has been suggested to positively modulate the hypoxic ventilatory response in polycythemic HA residents [97]. Other data conversely reported that a prolonged exposure to HA was coupled with an increase in prolactin but decrease in LH and TEST levels [42].

We have reported a significant TEST decrease, associated with a concomitant increase in progesterone, in climbers exerting strenuous PA at HA [17]. Accordingly, a reversible spermatogenic and Leydig cell dysfunction has been found in members of an Himalayan HA expedition [98]. In line with these results, Jiang et al. conducted a study on a group of young male soldiers exposed to hypoxia during intense military training over 12 months and documented a transient and reversible decrease in LH and TEST levels [59]. High altitude hypoxia-induced declines would probably be due to the decreased mitochondrial steroid dehydrogenase activity in acute and chronic hypoxia. At the control 6 months after exposure, LH levels returned comparable to those before exposure; the serum TEST level was still significantly higher than that before exposure, probably because of a rebound effect [59]. Therefore chronic hypoxia at HA causes adverse effects on reproductive hormones, but these effects are reversible [59].

TEST decrease could most simply reflect a stress-induced depression in the function of the gonadal axis, mainly due to the combined negative influence of hypoxia and strenuous PA. In fact, reduced TEST levels have been recorded in men performing PA in hypoxic conditions [17, 42, 74, 99] as well as in subjects undergoing endurance training [84]. Moreover, this HA gonadal profile would be negatively affected by prolactin increase [17, 100], and the fact that the GH/IGF-I axis is concomitantly activated while TEST is decreased [17] may explain the lack of anabolism and the increased dependence on glucose utilization.

The increase in progesterone levels in hypoxic conditions at HA could be viewed as a stimulus for the respiratory drive. In fact, progesterone has been suggested to be a potent respiratory stimulant in the physiological regulation of breathing by increasing the sensitivity of the respiratory center to carbon dioxide (CO_2) [101, 102]. Moreover, this positive progesterone effect would be favored by the concomitant decline in TEST levels that are known to exert a downregulation of progesterone receptors [101].

It has been clearly suggested that the chronic TEST response to PA at HA may also be modulated primarily by caloric consumption [74]. Acutely, TEST levels increased regardless of energy balance, but when food intake was controlled in order to maintain body weight at 4300 m, a gradual rise in serum TEST concentration persisted [74].

However, in line with the hypothesis that effects of altitude and caloric restriction would oppose each other, the influence of chronic altitude exposure on TEST levels seems to be mitigated when the energy balance is negative. In fact, some studies taken after periods of intense trekking or diminished caloric intake [58, 98] often show a decrease in pituitary–testicular hormone release that may be the result of the confounding influences of a negative energy state rather than altitude. Therefore, the steady decline of TEST levels over time in negative conditions of energy balance may represent an adaptive response of the reproductive system to a low-energy, catabolic state [103].

Interestingly, during intense anaerobic exercise, the relationship between LH and TEST is modified, since TEST increases without any significant elevation of LH [104]. On the other hand, resting HA levels of LH and follicle-stimulating hormone (FSH) tend to decrease [42, 92, 94, 105], but without concomitant variations in TEST secretion, likely reflecting modulations of other mediators [42], as occurred during intense PA. In contrast to these findings, Verratti et al. documented a significant increase in LH levels after high moderate altitude trekking, while plasma concentrations of FSH, total TEST, prolactin, and estradiol were unmodified at sea level after the hypoxic experience, with respect to baseline values at sea level [106].

When lowlanders acclimatized to 3542 m then trekked to an extreme altitude of 5080 m, plasma TEST decreased but then progressively normalized after 6 months of staying at 6300 m [58]. Concomitantly, LH levels after trekking to 5080 m was higher than at an altitude of 3542 m but decreased thereafter during prolonged residence at extreme altitude. Also in these subjects, plasma progesterone was increased after a 6-month stay at extreme altitude [58]. The potential impairment of reproductive function (i.e., spermatogenesis) has been recently investigated in male mountaineers involved in an expedition at HA (5900 m) [107]. The authors concluded that exercise at high altitude might be associated with a direct transitory testicular dysfunction, resulting in a reduced number of ejaculated sperm, mostly due to a defective spermiation [107]. This result was confirmed in the study by Verratti et al., where a short exposure to hypoxia (5 days) combined with exercise at altitude induced a significant reduction in sperm forward motility at sea level after the expedition. The underlying mechanism supposed was, because of the short-term exposure, that the reduced forward motility may result from the effects of the acute altitude hypoxia on spermatozoa during the epididymal transit where they mature acquiring their motility [106]. A transient alteration in semen quality was also documented in a group of young soldiers exposed to chronic hypoxia (12 months) during intense military training at high altitude: all the adverse effects on semen caused by hypoxia were reversible, when controlled 6 months after exposure to high altitude [59].

On the whole, considering the TEST decrease in the short term and the subsequent normalization of gonadal hormone profiles observed at high altitude, as well as the unlikely presence of an insufficient pituitary function [60], a transitory Leydig cell dysfunction could be hypothesized, mainly due to HA hypoxia per se (although a potential hypocaloric state influence must be also acknowledged).

As far as females are concerned, to our knowledge there is just one study focusing on the effect

of PA at altitude on gonadal hormone secretion [55]. Verratti et al. analyzed seven regularly menstruating, lowlander native women living at sea level participating in 14 days of trekking HA. They found that TEST level was lowered by HA and was restored after the end of the expedition; hence, this reduction can be attributed to a direct effect of hypoxia. Progesterone decreased significantly in all participants at the end of the expedition, although most of the participants were in the luteal phase. The important inhibitory effect on progesterone secretion became evident as no ovulation occurred in any woman during the expedition. There were no significant differences instead in estradiol, FSH, and LH levels collected at sea levels and during or after the expedition [55].

A recent review article by Shaw et al. [108] reported that HA natives (women) in general have a later menarcheal age with an early termination of the cycle (i.e., menopause). However, there was no evidence suggesting if hypoxia has a causative role or rather it could be due to different nutritional intake, physical workload exposure, improper hygiene, and/or cultural factors [108]. Some research groups have focused their investigations on migrant populations: when HA native females migrate to lower altitude. This was not found to change the menarcheal age as their physiology was already adapted to the earlier condition, but when lowlanders move to HA, onset of menstruation is delayed, suggesting perhaps in part a role of hypoxia [108]. Interestingly, high- and lowlanders also differ in menstrual pattern, with there being a greater length of their cycles at HA, due to a longer follicular phase perhaps due to a delayed follicular recruitment [109].

Reproductive hormonal profiles also vary between lowlander women and HA natives, the latter showing higher FSH levels and apparently a lower production of estrogen and progesterone [108, 109]; however, data are scanty on this topic and more research is needed.

As far as fertility is concerned, altitude seems to affect reproductive capacity for conception too as HA natives had lower fertility rates. Furthermore, HA migratory population showed higher fertility when relocated to a lower altitude. This may at least partially be due to oxidative stress caused by overabundance of reactive oxygen species (ROS) following hypoxia exposure, with detrimental effects on ovarian function and consequently negative reproductive outcome.

In both genders, therefore, a rapid change in gonadal hormone profiles and reproductive functional has been documented in response to the HA hypoxic environment exposure. These alterations could result in reproductive and fertility impairments. To what extent (magnitude) hypoxia plays as a direct or indirect factor in these developments remains to be determined. Figure 19.2 summarizes the reproductive hormonal changes with PA and HA exposure.

Sympathoadrenal System

The sympathoadrenal system plays a critical role in regulating a number of physiological functions necessary to control the stress imposed by PA and by altitude exposure, such as heart rate, vascular resistance, stroke volume, and blood pressure [110].

An increase in the plasma concentration of catecholamines during dynamic as well as during static exercise has been reported in humans [61]. Work intensity, relative workload, and the duration of exercise are the major determinants of the sympathoadrenergic response to exercise [34, 111]. Similarly the acute exposure to reduced partial pressure of oxygen at HA stimulates the sympathoadrenal system, likely stimulating arterial chemoreceptor, although some studies did not show an acute hypoxia-induced modification of resting plasma or urinary noradrenaline (i.e., norepinephrine) [112].

A differential adaptive response between sympathetic neural activity and adrenal medulla activity has been shown during exposure to HA, as described by Mazzeo and co-workers [113]. On arrival to 4300 m, adrenaline (i.e., epinephrine) arterial concentration was significantly increased both at rest and during prolonged low-intensity

	High altitude acute exposure	High altitude chronic exposure
GH/IGF-I Axis	GH response to submaximal physical exercise is blunted in untrained individuals, but not in trained subjects	Increase in GH response to exercise
Prolactin	Inhibition of exercise-induced prolactin response after acute exposure to hypoxia, though it does not seem to influence baseline levels	Prolactin increase is reduced with habituation and levels persist within the normal range or decreased
Thyroid function	Increase in total T4 and T3 concentrations associated with an increase in TSH levels	Significant elevation of free T4 levels and reduction of free T3 levels below the lowest normal limit without any change in TSH levels
Gonadal function	Divergent results on testosterone levels modification alter acute exposure to high altitude in men Reduced number of ejaculated, mostly due to a defective spermiation	Testosterone initially decrease but then progressively normalize. Concomitantly, LH augments but then decreases during prolonged residence at extreme altitude
	In woman, testosterone and progesterone levels decrease at high altitude, no differences in oestradiol and FSH/LH values	No studies focusing on changes of female gonadal axis alter chronic exposure to hypoxia in literature

Fig. 19.2 Summary of main metabolic and reproductive hormonal alterations during physical exercise and high altitude exposure. *Legend*: GH growth hormone, IGF-I insulin growth factor-I, T3 triiodothyronine, T4 thyroxine, TSH thyroid-stimulating hormone, FSH follicle-stimulating hormone, LH luteinizing hormone

exercise. On the contrary, arterial norepinephrine concentrations were lower than those observed at sea level in resting conditions, while were increased to similar values after only 45 minutes of submaximal exercise [113]. Plasma epinephrine and norepinephrine levels during mild exercise are not affected by the inhalation of gas mixtures containing a concentration of oxygen (19–13%) equivalent to an altitude of 700–3700 m above sea level. However, the higher the intensity of exercise (>50% of VO_2max [maximal oxygen uptake]), the greater the increment of plasma epinephrine and norepinephrine levels during hypoxia [114].

A strong correlation between catecholamines and glucose/lactate turnovers has been reported [18, 113, 115]. Thus, circulating norepinephrine concentration correlates with the glucose rate of appearance [18], allowing an increased use of blood glucose during hypoxic exercise. It is important to underline that exposure to cold environments could also stimulate the release of catecholamines, which, in turn, stimulate thermogenesis [83].

The most important modification of the adrenergic system in response to prolonged hypoxia is the desensitization of β-adrenergic receptors. This leads to a lower heart rate response to adrenergic activation which is similar to that observed in response to physical training [82]. Therefore, a chronic exposure to HA could cause an abolition of the adrenergic system acute modifications. In fact, residents at HA show plasma catecholamine concentrations similar to those in lowlanders [116]. In contrast, most studies on subjects staying more than 1 week at HA reported an increased sympathoadrenergic activity, suggesting that the duration of hypoxic exposure is one of the major determinants of the sympathoadrenergic response to exercise [117].

Along these lines, Woods et al., in a recent study on 14 healthy British military servicemen and women aged 22–35 years, described alteration in markers of physiological stress (as plasma normetanephrine, metanephrine, cortisol, high-sensitive cardiac troponin T, and N-terminal brain natriuretic peptide) during exercise under conditions of normoxia, normobaric hypoxia, hypobaric hypoxia, and genuine HA [118]. Plasma metanephrines are stable markers of catecholamine secretion and have previously been reported to reflect physiological stress and the sympathoadrenal response to cycling exercise [119]. In this study by Woods, plasma normetanephrine increased under all experimental conditions with exercise. When comparing hypoxic environments, the only significantly different response was exactly in plasma normetanephrine, suggesting that there may be subtle differences between environments in the sympathoadrenal response [118].

A study from Barnholt and colleagues pointed toward the importance of energy balance in modulating the hormonal response to exercise even at HA [74]. In fact, they showed that the expected acute altitude-induced epinephrine increase in subjects under caloric restriction was significantly lower than that experienced within subjects in adequate energy balance [74]. On the other hand, these authors found that norepinephrine levels rose gradually over the first days of exposure in both groups, suggesting that the sympathetic nervous activity increased independent of energy intake status. The adrenergic pattern secretion described by Barnholt is in line with the proposed "dissociation" theory between the adrenal medulla and sympathetic response to HA exposure [120]. Therefore, it could be hypothesized that hypoxia per se can act directly on the adrenal system to secrete epinephrine based on the severity of hypoxemia, even before sympathetic activity is elevated [121]. The dampened epinephrine rise in physically active subjects under negative energy balance [74] supports the concept of a negative interactive effect between hypoxia and caloric restriction on the adrenal medulla.

Importantly, this adrenergic pattern in response to exercise at HA may diminish the body compensatory response to reduced arterial oxygen, placing calorie-restricted sojourners at a greater risk for altitude sickness and decrements in physical performance. Furthermore, the negative influence of caloric restriction on epinephrine availability could also reduce muscle glycogenolysis directly and hepatic glucose production indirectly, thus decreasing carbohydrate availability and use upon acute altitude exposure.

Renin–Angiotensin–Aldosterone System

Considering the central role of renin–angiotensin–aldosterone system (RAAS) in the physiological regulation of fluid balance, volemia, and blood pressure, it is not surprising that this was one of the first endocrine systems to be investigated in HA adaptive processes. In fact, the first indirect information suggesting a decrease in aldosterone at altitude is derived from four subjects during an expedition to the Himalayas in 1956 [122]. A subsequent study in seven subjects exposed for 24 days at 4350 m confirmed this previous observation [123].

Nevertheless, also due to the several variables involved in the fine-tuned regulation of RAAS, the subsequent studies were unable to draw definitive and concordant conclusions about the actual functional modifications of RAAS at altitude, in particular during concomitant PA. In fact, although almost all studies describe a low plasma aldosterone concentration (PAC), they report variable responses in terms of plasma renin activity (PRA) in hypoxic conditions: resting PRA was found increased [124, 125], unchanged [16, 126–128], or decreased [92, 129–131]. On the other hand, PAC or aldosterone urinary excretion was more consistently decreased [123, 126, 127, 129–132], although some authors report an unchanged [128] or even increased [16, 92, 124] aldosterone response, possibly resulting from reduced sodium intake.

Exercise has been shown to represent an activator of RAAS [133–135], in particular in terms of absolute rather than relative work load [136]. Hypoxia blunts the exercise-induced increase in PRA and aldosterone secretion [130, 132], although when adequate hydration is maintained,

RAAS seems to be inhibited by mild and prolonged exercise and is not influenced by hypoxia [137]. It has been shown that, after a prolonged exposure to hypoxia, RAAS response to exercise was exacerbated under re-exposure to normoxia [138, 139]. Concomitantly, a decrease in resting PRA and an abolished response to exercise have been suggested after 3 weeks at HA, likely due to a blunted response of renal β-receptors. This phenomenon has not been observed in HA natives, in whom PRA concentration at rest was increased, though the aldosterone response to renin during exercise was attenuated [116].

Very recent data reported by Cooke et al. [140] showed no change in angiotensin-converting enzyme (ACE) activity with acute (during a trek to HA) or more chronic hypoxic exposure (simulated altitude of 4800 m) and suggest that the changes seen in aldosterone occur through a non-ACE-dependent mechanism. This mechanism does not appear to be through a reduction in aldosterone synthase activity, and alternative explanations remain to be explored, such as a downregulation of adrenal angiotensin II receptors or an upregulation of angiotensin I converting enzyme 2 (ACE2) expression [140].

Moreover, the RAAS response to altitude hypoxia can be also modulated by other associated stressors, such as the stress of a rapid ascent to altitude [124], exposure to cold, danger exposure, and/or strenuous exercise [16].

Altogether, it could be concluded that normal acclimatization to HA is associated with a clear suppression of RAAS, likely via multiple mechanisms, such as modified renal perfusion pressure and blunted adrenergic responsiveness. Moreover, once again, the importance of diet and hydration status can deeply modulate the exercise-induced modifications in RAAS function during hypoxic conditions.

Hypothalamus–Pituitary–Adrenal Axis

The hypothalamus–pituitary–adrenal (HPA) response to hypoxia has long been investigated due to its possible involvement in HA acclimatization. Whereas acute exposure to moderate altitude does not appear to increase plasma glucocorticoid levels, more severe hypoxia does result in an increase in adrenocorticotrophin hormone (ACTH) and corticosteroids in various species [117].

Accordingly, a more marked rise in plasma and urinary cortisol has been shown in many studies. This may precede the onset of symptoms in subjects developing acute mountain sickness (AMS) [126]. The diurnal rhythm of cortisol is maintained at HA and is accompanied by a parallel variation in AMS score [141]. After 1 week at 6542 m, plasma cortisol has been found elevated at first and then decreased with subsequent acclimatization, further supporting an association with AMS [141].

Exercise represents a potent physiological stimulus on the HPA axis [142]. Glucocorticoids exert many beneficial effects in exercising humans, increasing the availability of metabolic substrates for the need of energy of muscles, maintaining normal vascular integrity and responsiveness and protecting the organism from an overreaction of the immune system in the face of exercise-induced muscle damage [143].

Exercise intensity and duration are the major factors affecting the activation of the HPA axis [104, 144]. The response of the HPA axis to PA is independent of age and gender and is affected by hypohydration, meals, and time of day [143, 145–147].

When the HPA axis is repeatedly challenged by exercise (i.e., training), adaptation processes are activated in order to protect the body from the severe metabolic and immune consequences of increased cortisol levels [148].

Under moderate hypoxia the cortisol response to PA was found to be variable, i.e., unchanged [149], augmented [126], or decreased [150]. Moreover, the cortisol response to exercise is correlated with ACTH in normoxic, but not in hypoxic conditions [150], suggesting a decreased adrenal sensitivity to ACTH when exercising in hypoxia.

However, ACTH is not the only controller of cortisol secretion during hypoxic stress. In fact, other factors can also interfere, such as adrenal blood flow (higher in spontaneous ventilation), stimulation of pulmonary stretch receptors

(which can inhibit ACTH response), and low adrenal tissue partial pressure of oxygen (pO_2) [151]. In addition to the effects of intrinsic characteristics of the exercise stimulus, many other stress factors could modulate the hormonal response to exercise (i.e., intensity and/or oxygen availability, hydration, nutrition, low temperature). For example, as frequently observed at HA, corticosteroids also become elevated during hypothermia. An inverse relationship between 11-hydroxy-corticosteroid plasma concentrations and the degree of hypothermia exists. In fact, in one study, the highest corticosteroid concentrations were measured in hypothermic individuals who died, with respect to those who survived [78]. Interestingly, another study hypothesized that the cortisol response to exercise, also in altitude conditions, could be influenced by the degree of stress in athletes, secondary to a potential state of anxiety and/or low self-confidence [152].

All these data suggest that acute HA exposure does not significantly modify the physiological HPA response to PA. There is some evidence for an increase in pituitary ACTH content and number of corticotropic cells in the anterior pituitary in rats chronically exposed to hypoxia, suggesting a chronic activation of the HPA axis [117]. Indeed, pituitary and adrenal hypertrophy have been demonstrated in rats exposed to a simulated altitude of 5500 m [153]. In humans, plasma and urinary cortisol both increase with the time and level of exposure to altitude [117]. Also salivary cortisol and dehydroepiandrosterone sulfate (DHEA-S) levels significantly increase at HA [154], but they maintain their physiological diurnal fluctuations, which are characterized by peak levels in the morning and a declining pattern thereafter. Measurement of DHEA-S concentration may provide important information about mechanism of the body's adaption to the corticoadrenal activation, which is characteristic of the psychophysical stress response. In fact, the ratio of cortisol to DHEA-S has been associated with very general measures of well-being [155] and could reflect the degree to which an individual is buffered against the negative effects of stress.

Little information is available about the effect of lifelong exposure to hypoxia on cortisol concentration in humans. Along this line, Maresh and co-workers [156] reported a greater cortisol response to a simulated altitude of 4760 m in lowlanders as compared to moderate-altitude natives, suggesting some adaptation of the system to chronic hypoxia. However, the cortisol response to maximal exercise was similar in both groups whether tested at their respective residence altitude or in hypobaric chamber, suggesting that the stimulus provided by exercise alone, and not the hypoxic environment, is responsible for increased cortisol levels after exercise [157].

Altitude per se has been shown to exert a strong influence over the HPA axis [16, 92]. Barnholt and colleagues confirmed an acute and persistent increase in cortisol levels in active subjects over a short-term stay at HA [74]. This pattern of cortisol secretion has been shown to be accentuated when altitude exposure is associated with another stress, such as caloric restriction [74]. On the other hand, other studies showed no significant changes in cortisol secretion with respect to sea level [17, 58]. This lack of significant changes in cortisol and ACTH levels can be explained by the low reliability of a single basal evaluation of cortisol, as performed in many studies, to adequately investigate the HPA axis function and therefore to exclude some stress-induced derangement. Such discrepancies in different studies [16, 17, 58, 92] and the difference in acute cortisol response depending on energy balance [74] may reflect a varying response based on substrate availability. In fact, cortisol has a catabolic effect on fat and proteins and is known to increase circulating free fatty acids, glycerol, and amino acids [158]. Therefore, since energy deficiency at altitude has been shown to increase dependence on lipid metabolism [159], elevated cortisol concentrations may help to compensate for the early changed glucose response in calorie-restricted subjects by providing free fatty acids as an alternate fuel and/or stimulating gluconeogenesis via elevated precursor availability (e.g., glycerol and amino acids).

HA exposure is an environmental stress condition that induces also several physiological adaptive immune responses. Evidence over the

past few decades documents the existence of an association between endocrine system and immunity. The sympathoadrenal and the HPA axes, known to play a central role in the body's homeostasis at HA, are the two pathways by which the brain communicates with the periphery in order to regulate the magnitude of an innate or adaptive immune response [160]. In vivo and in vitro data show that immune changes are strongly influenced by the stress response, particularly through catecholamines (the hormone epinephrine and the neurotransmitter/hormone norepinephrine) and cortisol, although this evidence is still to be fully demonstrated during HA exposure [161]. In their work Ermolao et al. [162] evaluated the relationships between stress hormone response and immunological alterations during both acute and prolonged HA exposure (5050 m). Their results suggest a possible influence of catecholamines and cortisol on the cellular and functional alterations observed in the immune system during acute and prolonged stay at HA. During HA exposure, the neurotransmitter, epinephrine, and cortisol secreted by the sympathoadrenal and the HPA axes show characteristic time course and functions, although the presence of a large variability in the experimental design (e.g., field vs laboratory studies, altitude, modalities, time of exposure, etc.) often leads to several differences in the observed results.

The presence of a reciprocal influence between the neuroendocrine and the immune system has been fully demonstrated. In particular epinephrine, as well as cortisol, can modulate lymphoid functions, and the presence of β2-adrenergic receptors within lymphoid organs suggests a direct influence on the immune cells. From these findings, it can be hypothesized that the stress response experienced may be involved with the immune regulation also during HA exposure.

Antidiuresis System

The relationship between antidiuretic hormone (also called vasopressin [AVP]) system and altitude exposure, during which relevant changes in body water distribution and electrolytes occur, is a well-accepted fact. Changes in fluid balance are an important part of the process of acclimatizing to HA. Plasma osmolality generally increases with a period of time at HA [163], and hypoxia seems to alter AVP regulation by raising the osmotic threshold and increasing AVP responsiveness above that threshold [156]. AVP secretion is also modulated by the stress response even without an osmotic stimulus. In fact it has been shown that it increases during exercise and its response is modulated by the intensity and duration of exercise [164] and different degrees of hypoxia, as previously stated. For example, short-term exercise fails to increase AVP, which instead increases after 2 hours of a similar workload [164]. Likewise a very short-term exposure (20 minutes) to mild hypoxia (about 3400 m) was found to result in a significant reduction in AVP which returned to sea level values with more severe hypoxia (5000 m). On the contrary, as soon as hypoxia began to be intolerable (i.e., presence of nausea, headache respiratory distress), AVP increases, edema worsens, and antidiuresis was present, most likely because the adaptive role of AVP is being present as long as altitude is tolerated [117].

It is important to note that technologically, the available AVP bioassays are quite unreliable. However, in the last years, thanks to the availability of copeptin measurement, the problem has been solved in part. Copeptin is released along with AVP as part of the large peptide precursor, PRoAVP, and hence is a useful surrogate measure. Copeptin, in fact, is more stable both in vivo and ex vivo and can be stored in a variety of media. Recently some studies focused on modulation and alteration of copeptin at HA and during exercise. Mellor et al. [165] found that copeptin and AVP are correlated at HA; however, their response to exercise at HA is less pronounced compared with that of sea level until extreme altitude (> 5000 m) is reached. The significant rise in AVP and copeptin at 5000 meters following exercise was the largest seen at any altitude, yet it seems due to non-osmotic stimulation, supporting the importance of exer-

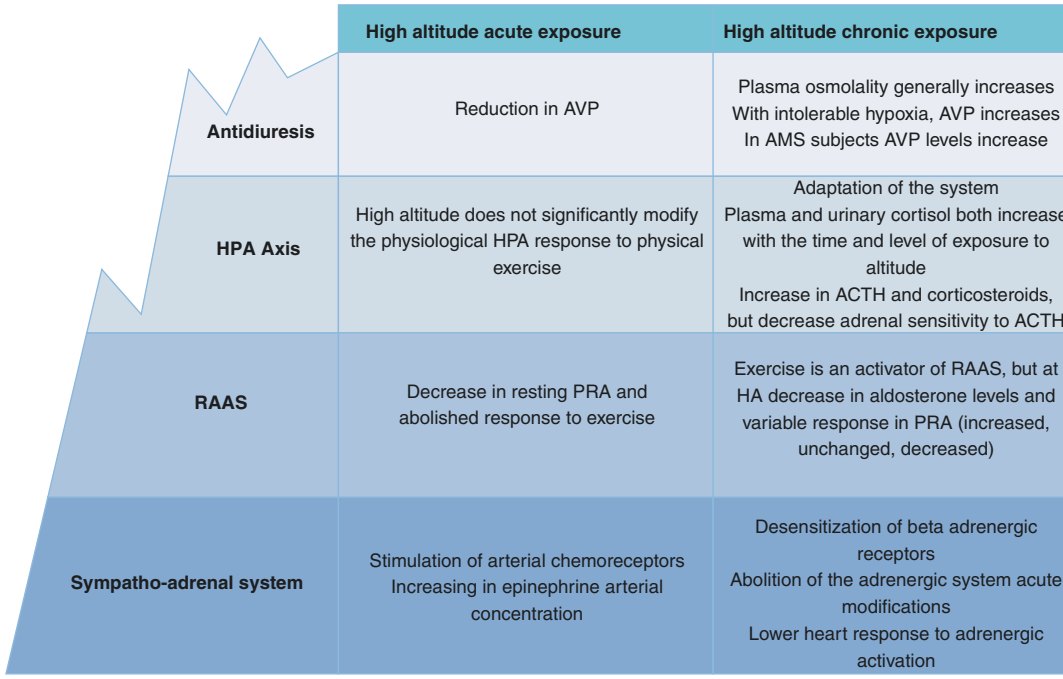

Fig. 19.3 Summary of main hormonal alterations in water–electrolyte (hydration) and blood pressure balance during physical activity and high altitude exposure. *Legend*: AVP/ADH vasopressin, AMS acute mountain sickness, HPA hypothalamus–pituitary adrenal axis, ACTH adrenocorticotropic hormone, RAAS renin–angiotensin–aldosterone system, PRA plasma renin activity

tion in its release. Possible non-osmotic causes behind the copeptin and AVP rise may include exertional headache or nausea, anxiety, or the stress of an abrupt climb to a new altitude. But, these researchers did not find a correlation among copeptin, AVP, and AMS [165]. Different results were found by Bärtsch et al. [166] who examined AVP levels in subjects before and during a sojourn of 3–4 days at 4559 m both at rest and during 30 minutes of exercise on a cycle ergometer. No significant changes occurred at rest, both in subjects susceptible or not to AMS, while, during the exercise test, a significantly greater increase in AVP levels was observed in subjects developing AMS.

The most important action of AVP is to facilitate the reabsorption of water from the glomerular filtrate. It is obvious that water deprivation stimulates and a salt-free diet decreases AVP secretion. These could therefore be confounding factors in the regulation of AVP response in HA exposure, and researchers need to address these factors carefully. Figure 19.3 summarizes main hormonal alterations in fluid volume–hydration and blood pressure balance during PA and HA exposure.

Hormonal Modulation of Appetite–Energy Balance

Subjects exposed to HA lose significantly overall body weight from fat mass as well as lean mass, particularly if involved in increased PA, such as climbing or trekking to HA [79, 80]. As a consequence, an energy imbalance occurs, likely reflecting increased energy expenditure and decreased, or at least inadequate, food intake probably due to hypoxia-related reduced satiety [79, 80]. Appetite suppression ("high altitude anorexia") has been observed during acute exposure to both simulated [167] and terrestrial

altitude [168]. This effect appears to be maintained during chronic altitude exposures [169] which is associated with significant decreases in energy intake, body mass, and physical performance at altitude [170]. Several circulating hormones have been implicated in the development of altitude-induced anorexia, including glucagon-like peptide-1, peptide YY, and pancreatic polypeptide [171].

In this context, significant variations in the secretion of leptin and ghrelin, as two of the major hormones involved in the regulation of energy balance, appetite, and food intake as well as in peripheral metabolism [172], are also expected with altitude exposure. In fact, a decrease in body weight is generally associated with leptin reduction and ghrelin increase, while the opposite picture is associated with body weight excess [172]. However, the data available so far are discrepant and confusing, since leptin levels have been reported as either increased [173], decreased [17, 174], or unchanged [74], while a trend toward decreased [173] or unchanged [17] ghrelin levels at HA has been described. In particular, an increase in leptin coupled with ghrelin decrease has been detected after acute exposure to HA [173], but other authors reported that prolonged HA exposure is associated with a reduction of leptin concentrations, likely due to the loss of body mass (i.e., fat mass) and the strong hypoxia-related sympathetic activation [174]. Findings of Bailey et al. [175] suggest that appetite perceptions and plasma acylated ghrelin may be suppressed in response to just 50 minutes normobaric hypoxic exposure during exercise, and the same acute suppression has been detected in another study [167] after 7 hours exposure to simulated altitude (4000 m). One major limitation of the current research about ghrelin at HA is the measurement of total ghrelin concentration, rather than the constituent components of acylated and desacylated ghrelin which have opposing effects on appetite regulation.

Cold/extreme temperature is another important variable that must be considered in energy balance at HA. In fact maintenance of the core temperature requires additional energy for the thermoregulation during and after exercise if environmental temperatures are outside the thermoneutral zone, either below (cold) or above (warmth/heat) [176]. Hormonal evaluations in this scenario are sparse. The analysis of plasma hormone concentrations in the few available studies showed that acylated ghrelin levels increased during cold exposure, whereas leptin levels decreased during immersion in cold water [177]. In future research it would be interesting to evaluate changes in leptin concentration after exercise in cool and neutral environments, in order to better assess the role that ghrelin and leptin may play in the regulation of short-term energy intake after exposure to the cold.

Increasing exercise intensity may increase energy expenditure, and evidence suggests high-intensity exercise produces greater short-term reductions in appetite compared to moderate-intensity exercise [178]. Extreme HA exposure in association with strenuous PA does not allow the normal physiological response of leptin and ghrelin to significantly decrease body weight and cause negative energy balance. Also glucagon-like peptide-1 (GLP-1) is involved in food intake regulation and energy balance; in particular GLP-1 concentration seems to be unaffected by short exposure to hypoxia combined with exercise. In one study the response of GLP-1 to hypoxia has been investigated, and fasting concentration of GLP-1 did not seem to differ compared to normoxia following overnight exposure to a simulated altitude of 4100 m, while there was a tendency for GLP-1 to be higher 40 minute post-meal. This might suggest that hypoxia does not influence GLP-1 in the absence of feeding [179].

Finally, it is important to note that the wide variation in study results can once again derive from different research methodologies employed and the role of potential confounders alone or in combination (e.g., cold exposure, weight loss, diet) as well as the intrinsic characteristics of the exercise bout could explain, at least in part, the differences observed in leptin and ghrelin secretion in these conditions relative to HA exposure.

Conclusions

The adaptation to a new environment needs an information system which firstly informs the body on the environment characteristics and secondly triggers biological responses that may be more or less "adaptive" to new external conditions. The neuroendocrine system represents one of the most important body networks for such adaptation.

HA means both hypoxia and low-temperature exposure are a sufficient trigger per se for endocrine secretions. A further stimulus is represented by PA. Obviously the activation of the neuroendocrine system has a central role in such scenarios. Indeed, the strong endocrine response to HA can improve oxygen delivery via cardiorespiratory and hemopoietic adaptations and induce an adaptive response in favor of enhanced energy preservation and activation of the immune system. New papers have been published in the literature in recent years, but overall the data available on the effects of HA on endocrine responses to PA persist at being scanty and, most of all, nonhomogeneous. Hence not entirely clear conclusions cannot be drawn at this time on PA with HA exposure. For this reason to many researchers this topic remains of high interest in an attempt to more clearly understand the role of HA exposure and hypoxia on humans.

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20 An Introduction to Circadian Endocrine Physiology: Implications for Exercise and Sports Performance

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Introduction: Biological Rhythms

Biological rhythms are physiological and behavioral phenomena which recur regularly in living organisms. Though intrinsically determined (endogenous) and persisting independent of external influences, ultimately, the timing and amplitude of biological rhythms are influenced by their interaction with environmental cues. Biological rhythms are involved in the maintenance of homeostasis, which entails anticipating, not just reacting, to environmental demands. The various components of mammalian biologic signaling and communication systems such as the endocrine system, central nervous system, autonomic nervous system, immune system, as well as intestinal tract all function in a rhythmic and integrated manner [1].

The time interval for completion of one cycle, i.e., the period, is the basis of classification of biological rhythms into one of three classes—circadian, ultradian, and infradian. Biological rhythms that occur approximately every 24 h are circadian, those with periods considerably shorter than 24 h are ultradian, and those with periods considerably longer than 24 h are infradian [2].

The process of entrainment is described as aligning one's biological circadian rhythms to the environment's 24-hour rhythm imposed by the earth's rotation around its own axis. This can be accomplished by certain cues or zeitgebers as they are referred to, light being the most important one [3].

The Body Clock

Mammalian circadian rhythms are orchestrated by nerve cells in the SCN, small paired nuclei located in the hypothalamus. The neurons of the SCN (also referred to as the circadian pacemaker or "biologic clock") generate circadian rhythms via a negative feedback loop of clock gene expression [5–7].

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Light entrainment and shifting of the SCN rhythms are mediated primarily through the retinohypothalamic tract (RHT) which projects from the retinal ganglion cells to the SCN. Interruption of the RHT eliminates light (photic) entrainment [8], as well as phase shifting induced by exposure to bright light. The classical photopigments, namely, rhodopsin and cone opsins, are not primarily required for entrainment to the light/dark cycle [9]. Melanopsin is the major photopigment implicated in time-keeping mechanisms (entrainment, phase shifting) and pupillary responses [10–12]. Output tracts from the SCN are distributed primarily to several nuclei in the hypothalamus, most importantly to the paraventricular nucleus, and to a lesser extent to the midline thalamus and basal forebrain [13]. The projection to the paraventricular nucleus is part of the multisynaptic pathway (involved in the modulation of melatonin secretion by the SCN) which passes from the SCN through the paraventricular nucleus, the intermediolateral cell column of the spinal cord (containing sympathetic outflow fibers), the superior cervical ganglion, to the pineal gland [14].

Circadian clocks are comprised of autoregulatory feedback loops which are based on the transcription and translation of clock genes. Mammalian circadian clock gene consists of the heterodimers, CLOCK (paralogue NPAS2) and BMAL1 (also known as ARNTL). CLOCK-BMAL1 are basic helix-loop-helix (bHLH)-PER-ARNT-SIM (PAS) transcription factors [15, 16]. Rhythmic genes contain E-boxes to which the CLOCK-BMAL1 complex can bind and regulate the transcription of repressor proteins called period (PER1, PER2, and PER3) and cryptochrome (CRY1 and CRY2) [16–18]. The transcription of PER and CRY starts in the morning and is finished by late afternoon/evening, and by evening, PER and CRY proteins are formed [19]. Thereafter, at night, these proteins will interact with serine/threonine kinases, casein kinase 1δ (CK1δ) and CK1ε [19–21], and with each other and then translocate to the nucleus where they repress their own transcription by binding to CLOCK-BMAL1. Eventually, the transcription of PER and CRY will stop as the repression continues. Their levels eventually start to fall off since the half-lives for PER and CRY are short and also because they undergo degradation by proteasomes via ubiquitylation by E3 ligase complexes [20, 22]. Eventually the repressor complex is turned over, and the negative feedback loop stops. At this point, CLOCK-BMAL1 can initiate a new transcription cycle the next morning. In studying human sleep-timing disorders, the PER2 and CK1δ have been implicated which further signify the role of clock genes [23, 24]. NR1D1 and NR1D2 encode REV-ERBα and REV-ERBβ, respectively, and these are nuclear receptors that can be activated by the CLOCK-BMAL1 complex [25–27]. REV-ERBα and REV-ERBβ will compete with RORα, RORβ, and RORγ at the RevDR2 and ROREs (retinoic acid-related orphan receptor binding elements) [25–27]. This leads to the repression of CLOCK-BMAL1 which leads to a rhythmic gene expression with PER genes [25] leading to a production of positive feedback and negative feedback loops of activators and repressors [28]. The third feedback loop under the control of CLOCK-BMAL1 involves D-boxes. PAR-bZip (proline and acidic amino acid-rich basic leucine zipper) contains three factors, D-box binding protein (DBP), hepatic leukemia factor (HLF), and thyrotroph embryonic factor (TEF). D-boxes with nuclear factor interleukin-3 regulated (NFIL3), also known as E4BP4, will interact with DBP, HLF, and TEF and is driven by REV-ERB-ROR loop [29, 30]. Based on the phases of expression of E-boxes, ROREs, and D-boxes in the promoter and enhancer regions, the three feedback loops are able to generate a cycle of transcription of the clock genes [31].

Peripheral Clocks

In addition to the master circadian pacemaker in the SCN (the body clock), peripheral oscillators employing very similar molecular machinery exist in every tissue. The SCN and the peripheral oscillators communicate via hormonal signals and autonomous nerve signals (Fig. 20.1). Approximately 10% of the human genome is

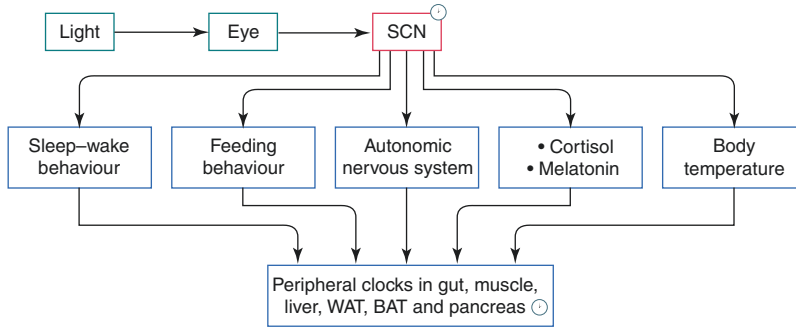

Fig. 20.1 The circadian timing system is composed of a central clock in the suprachiasmatic nucleus (SCN) located in the hypothalamus of the brain and peripheral clocks in other brain areas and peripheral tissues. The circadian rhythms in these clocks are generated by a molecular transcriptional–translational feedback loop. The light signal, reaching the SCN via the retina and the retinohypothalamic tract, is the most important zeitgeber for the SCN. The SCN synchronizes peripheral clocks through neural, endocrine, temperature, and behavioral signals. BAT, brown adipose tissue; WAT, white adipose tissue. (Modified from Springer Nature: Nature Reviews Endocrinology. Circadian clocks and Insulin Resistance. Stenvers and Scheer [4], Copyright 2019)

controlled by a circadian rhythm, notably in the SCN, the liver, and the heart [32, 33] (Fig. 20.2).

Hormonal

Levels of many hormones along with tissue responsiveness to these hormones oscillate over an approximately 24-hour period in constant dim light conditions and exactly 24-hour period in natural "entrained" conditions [34]. These oscillations are controlled by three processes and include (a) intrinsic circadian clock and timekeeping mechanisms, (b) sleep/wake cycle, and (c) feeding/fasting state [34].

An illustrative example of circadian fluctuations of endocrine factors is the hormonal regulation of the hypothalamic–pituitary axis (HPA). These hormones include cortisol, growth hormone, prolactin, thyroid hormone, and gonadal steroids [35–39]. Other hormones, such as insulin and adipokines, primarily affect the feeding/fasting cycle. These circadian fluctuations of endocrine factors usually oscillate in time-of-day-dependent (diurnal) patterns [40, 41]. Although the daily oscillations of hormones have been recognized for some time, the understanding of the daily oscillations of the sensitivity of the receptors to which they bind is limited. However, receptor responsiveness to ACTH and insulin has been investigated. Circulating levels of ACTH do not correlate with the circulating levels of cortisol. This is due to a diurnal rhythm of the adrenal ACTH receptors sensitivity which determines when ACTH is able to stimulate cortisol secretion [42]. Circulating blood glucose also exhibits a time-of-day-dependent pattern that is referred to as a "dawn phenomenon," which is characterized by an uptick of blood glucose between 0500 h and 0800 h without any food intake. This phenomenon is exaggerated in type 1 and type 2 diabetics and must be considered when dosing medication [43–47].

Melatonin

Melatonin is primarily secreted by the pineal gland. However, cells in other organs and systems (e.g., gastrointestinal tract, bone marrow, skin, leukocytes, and membranous cochlea) have also been reported to release melatonin in a less robust manner [48]. Melatonin as an SCN-dependent output signal is a chemical marker of biological night [51]. Melatonin functions as a feedback signal in binding to melatonin receptors (MT1 and MT2) on the SCN and can be used to phase shift circadian sleep–wake rhythms and to entrain circadian sleep–wake rhythms in blind individuals [52, 53].

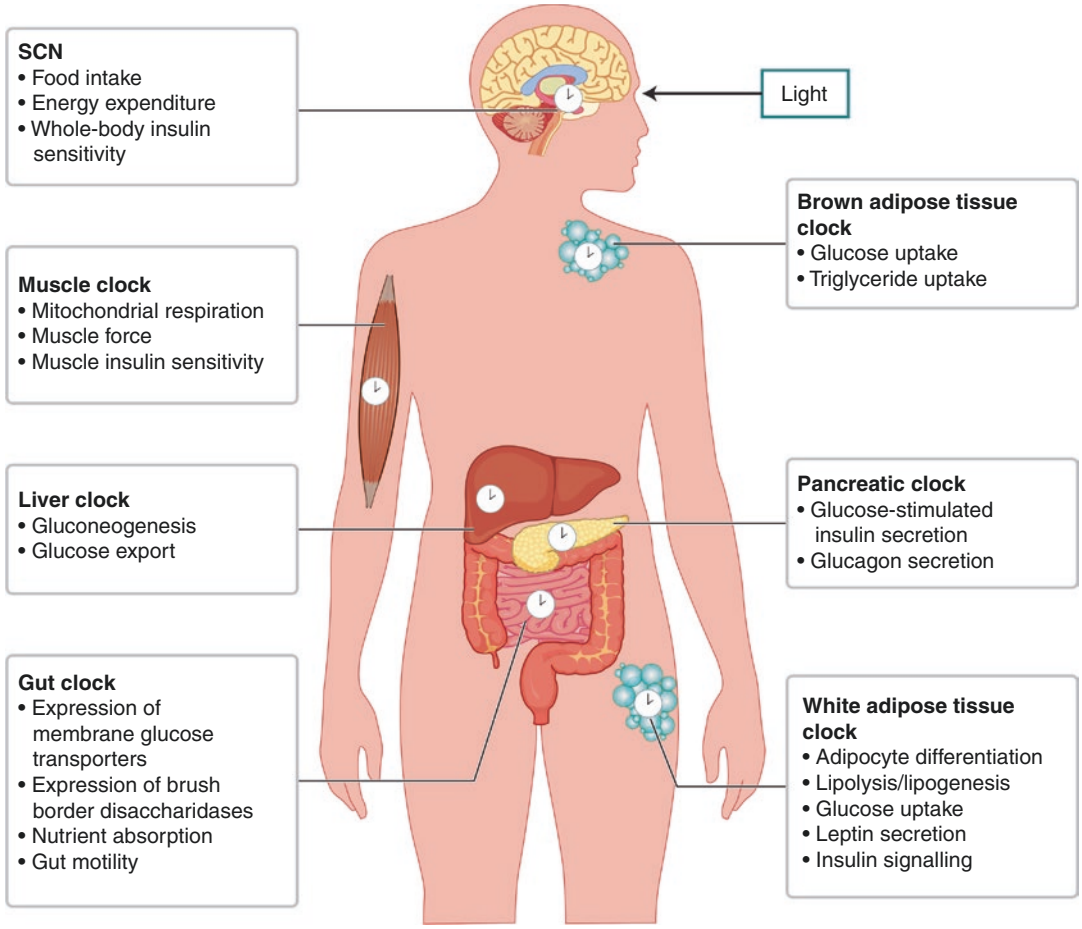

Fig. 20.2 The molecular clock consists of a transcriptional translational feedback loop involving the clock proteins CLOCK, ARNTL, PER, and CRY and the nuclear receptors NR1D1, NR1D2, and ROR. The central and peripheral clocks are responsible for a variety of functions. SCN, suprachiasmatic nucleus. (Modified from Springer Nature: Nature Reviews Endocrinology. Circadian clocks and Insulin Resistance Stenvers and Scheer [4], Copyright 2019)

Growth Hormone (GH)

Signals affecting GH release also begin in the hypothalamus. GH is controlled via the balance between growth hormone-releasing hormone (GHRH) and somatostatin (SMS), both of which are released by the hypothalamus [49, 50]. GHRH is stimulatory and SMS is inhibitory in nature. GH is released by the anterior pituitary and its role is mainly metabolic in nature. GH essentially opposes insulin and therefore increases blood glucose, decreases glucose utilization, and increases lipolysis [51]. Not only does GH have a circadian rhythm; it also has an ultradian rhythm along with sexual dimorphism. In adult females, GH is secreted uniformly throughout the day without significant peaks with increased circulating levels at night. On the other hand, adult males have very distinct peaks throughout the day and increased circulating levels at night [35, 52]. Children have similar differences between males and females, but the peaks are significantly larger than in adults suggesting that GH variation is not determined by gonadal steroids [53, 54].

Cortisol

Circulating cortisol exhibits a circadian rhythm which is thought to be a result of the modulatory action of the biological clock on the hypothalamic–pituitary–adrenal (HPA) axis. The signal for cortisol release begins in the hypothalamus where corticotropin-releasing hormone (CRH) is secreted and travels through the hypophyseal portal vasculature to the anterior pituitary gland where it signals the release of ACTH into the circulation, which subsequently reaches the adrenal glands and stimulates the release of cortisol [42]. In "diurnal" species (those regularly awake during the day and asleep during the night), cortisol level begins to rise during the night, and the highest levels are usually attained in the early morning after which the level begins to decline during the day, with a trough during the early part of the night [55]. Interrupted sleep during the night and nocturnal sleep deprivation are both associated with elevated cortisol in the circulation. Cortisol has a time-of-day-dependent rhythmic release which begins with a steady rise during the sleep phase and peaks between 0700 and 0800 h [56]. The steady rise is in "anticipation" of increased activity during wakefulness, with a peak that presumably helps the body handle stress associated with waking [42, 56].

Prolactin

The plasma concentration of prolactin is higher during the internal night and reflective of three different types of oscillations, namely, two ultradian rhythms and a circadian oscillation [1]. One of the ultradian rhythms has a high amplitude pulsatile quality clustered during the night and early morning hours. In both men and nonpregnant women, a significant percentage of daily secretion of prolactin occurs during rapid eye movement (REM) sleep, and REM sleep activity on the EEG is in turn promoted by prolactin [57]. One study [58] also indicated that exercise (90-min cycling at 70% of maximal oxygen consumption) performed between 4:30 and 6:00 PM resulted in increased nighttime blood levels of prolactin when compared to a day without exercise.

Testosterone

In healthy men, levels of circulating testosterone vary with the time of day such that peak concentrations are observed between 6 and 8 AM, while trough levels are measured between 6 and 8 PM, but this variation is blunted in older men in whom overall testosterone in circulation is also reduced [59]. Testosterone levels begin to rise at night (from 21:00 h), and levels fall during the day; this is responsible for the early morning peaks and evening troughs. The findings from the study by McMurray et al. [60] suggest that the early morning peak of testosterone might be augmented by engaging in heavy-resistance exercise (three sets of six exercises to exhaustion) between 7 and 8 PM the previous day. In addition, fragmented sleep can disrupt the rhythm of testosterone secretion, and the nocturnal rise in testosterone may be attenuated [61] (Fig. 20.3).

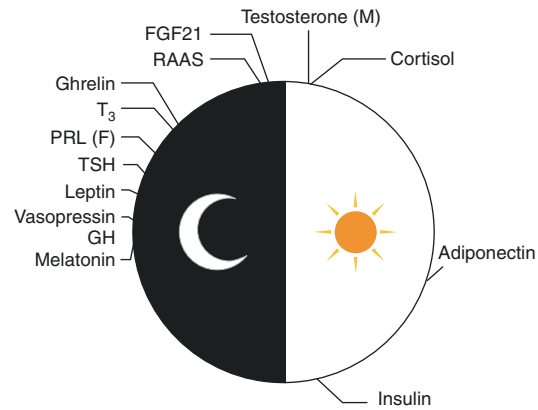

Fig. 20.3 Peak level timing of major hormones. GH, growth hormone; TSH, thyroid stimulating hormone; PRL, prolactin; T_3, triiodothyronine; RAAS, renin-angiotensin-aldosterone system; FGF21, fibroblast growth factor 21; (F) females only, (M) males only. *(*Modified from Springer Nature: Nature Reviews Endocrinology. Circadian clock control of endocrine factors. Gamble et al [34], Copyright 2014*)*

Metabolic Regulation

Food intake, energy expenditure, and insulin sensitivity are under the control of the master clock in the SCN and reciprocally feed into the SCN, but they are further modulated locally by peripheral clocks which run in sync or out of sync with the master clock [4]. Specifically, insulin secretion is regulated by the pancreatic peripheral clock, and insulin sensitivity is regulated by muscle, adipose, and liver peripheral clocks [4]. Pancreatic beta islet cells and intact islets have a circadian rhythm [116], as demonstrated in humans [117, 118] and rodents [119–121]. Insulin secretion can be modulated by various hormones displaying circadian variation, such as cortisol [62] and melatonin [63, 64]. GH is also regulated by SCN via the sleep/wake cycle, and its rhythm is opposite to that of insulin sensitivity, particularly in the liver and muscle [65, 66]. Eight weeks after an SCN lesion, rodents become insulin resistant [67, 68].

As blood glucose levels increase, a signal is sent to the pancreatic beta islet cells to secrete insulin for utilization of the circulating glucose and protein synthesis in the skeletal muscle, liver, and fat [69, 70]. Insulin, being an anabolic hormone, promotes triglyceride synthesis by inhibiting fatty acid beta oxidation and using these fatty acids to synthesize triglycerides [70].

Human studies using misalignment protocols reveal a circadian rhythm for glucose tolerance [88, 89]. This is consistent with the diurnal peaks in the morning and dips in the evening in insulin sensitivity [71] that is the opposite of mitochondrial oxidative capacity in skeletal muscle which dips in the morning and peaks in the evening [72].

Insulin resistance can develop as a result of misalignment between the circadian rhythm and sleep/wake cycle or nutrition intake and is influenced by certain environmental, behavioral, or genetic factors [4]. Some of the known factors that have been implicated in the past include mutations in the clock genes, altered light exposure disrupting the light/dark rhythm, sleep debt, shift work, and transmeridian travel [4].

Metabolism and Peripheral Clocks

Peripheral clocks have been demonstrated in all domains of metabolic activity—processing and absorption of nutrients, synthesis of complex compounds, regulation, consumption (e.g., skeletal muscle), and storage (e.g., white adipose tissue). Intestinal cells contain a molecular clock which is synchronized with food intake, thereby regulating intestinal motility and nutrient absorption [73–75]. An intrinsic liver clock is central to the control of the metabolism of lipids and glucose, as suggested by microarray [32, 76], proteomic [77, 78], and metabolomic [79–81] studies. One of the functions of the liver clock is to maintain euglycemia, and it does so by synchronizing gluconeogenesis and glucose output with the daily feeding periods [82, 83]. Signals from the autonomic and endocrine [84] systems help the intrinsic liver clock synchronize with the SCN [76]. The liver clock is highly reactive to the timing of food intake [85].

A peripheral molecular clock in skeletal muscle is synchronized with other clocks by food intake and exercise as well as the SCN-generated signals [86–92]. White adipose tissue (WAT) also contains a circadian clock that is synchronized by light, SCN [93], and the diet [84, 94]. Glucose uptake in adipose tissue of rodents shows a circadian rhythm [95], and WAT in the skin of obese individuals manifests a diurnal rhythm for insulin sensitivity which peaks at noon [96].

Exposure to Light Alters Metabolism

Rodent studies have illustrated that exposure to dim light during night has a drastic effect on the metabolic state and leads to obesity and glucose intolerance because of a disruption in the rhythmic fasting/feeding cycle and locomotor behavior [97–99]. This effect of nighttime light exposure is not limited to rodents as studies of humans revealed similar findings including increased rates of obesity [100, 101] and the development of type 2 diabetes [102]. Bright light can also be used to decrease insulin resistance in a time-dependent manner in healthy

human beings in an environment with timely caloric intake and physical activity [88, 89].

Chronotype

Individuals with an evening chronotype (including a tendency to prefer activity in late afternoon to evening, having a significant problem waking up or falling asleep early, self-reported performance deficits in early morning) have an increased risk of developing type 2 diabetes [129, 130]. It has been suggested that this may be associated with a desynchrony between the environmental/social demands and our intrinsic circadian rhythm controlled by the SCN [129, 130].

Central and peripheral clocks play a vital role in glycemic regulation, i.e., insulin resistance and glucose tolerance. This is evidenced by the fact that exposure to dim light during the night and sleep disruptions lead to glucose intolerance [4]. Insulin resistance has also been associated with jet lag, shift work, "social jet lag," sleep disruptions including amount and quality of sleep, and extreme evening chronotypes [4].

Glucose and fatty acid release and uptake from the circulation are also dependent on the circadian rhythm. The system of glucose production by the liver, carbohydrate consumption, and the glucose uptake by skeletal muscle is very fragile, and any mismatch can lead to higher glucose levels in the peripheral circulation [4]. This, in turn, may perturb lipid metabolism (i.e., lipolysis and lipogenesis responsiveness to internal and external synchronizing signals).

Desynchrony in Metabolic Disorders

By using either 20-h days, during which a person is awake for 13 h and asleep for 7 h, or 28-h days, during which a person is awake for 20 h and asleep for 8 h, it is possible to misalign the central and peripheral clocks with the sleep/wake cycle in a lab setting [66]. An approximate 180-degree shift occurs after three cycles of a 28-hour sleep/wake schedule. Therefore, a full return to the cycle and back to alignment of inner circadian rhythm with sleep/wake rhythm can occur after an additional three cycles of 28-h days. This allows an experimenter to compare subjects in misalignment and full alignment to examine which endocrine factors follow inner circadian rhythm and which follow the behavioral sleep/wake rhythm. Scheer et al. were able to do exactly this and determined that during misalignment, leptin, insulin, and norepinephrine do not follow the 24-h inner circadian rhythm, whereas cortisol, glucose, and epinephrine were not affected by the misalignment and continued to follow a 24-h circadian rhythm [103]. As a side note, it must be mentioned that the experimental protocol may influence all these hormones. It was observed that the levels of leptin decreased during misalignment to physiologically significant levels after 25 days [104]. Even the transcripts in the blood decreased after three cycles in healthy volunteers during misalignment [105]. The 28-h protocol to misalign the rhythms can also affect glucose utilization and insulin secretion due to the inability of the pancreatic beta islet cells to compensate for the misalignment, thus leading to insulin resistance and glucose intolerance [103]. Leproult and colleagues went a step further to report a worsening in insulin resistance when circadian misalignment occurs in subjects that have been sleep deprived [106].

The effects seen in the endocrine system of subjects who have been misaligned experimentally closely resemble the effects seen in shift workers. Shift work leads to circadian misalignment which affects the rhythms in circulating melatonin and cortisol levels [107–109]. There are many negative side effects of shift work including the development of cardiometabolic syndrome [110] which is most likely because of these individuals' higher levels of glucose and insulin postprandially, and they also have been found to have higher levels of triacylglycerol during the night shift [111]. The incidence of cardiovascular disease, gastrointestinal disease, and cancer has all been found to be increased in these patients [103, 110, 112–115], thus highlighting the importance of grasping the concept of synchronization of the inner circadian rhythm with the external environmental/behavior factors and the endocrine system.

Any pathology that can affect the endocrine system can cause misalignment among various internal oscillators. For example, the intrinsic rhythm in the white blood cells of individuals with diabetes is altered [116]. However, the rhythm of insulin sensitivity is not affected in type 2 diabetes, but there is no rhythmicity observed in the secretion of insulin from the beta cells [117]. Furthermore, shorter insulin secretion rhythms have been consistently documented in first-degree relatives of type 2 diabetics [118]. Animal models of metabolic dysfunction have confirmed these clinical findings and proven that metabolic and circadian rhythm abnormalities have a reciprocal detrimental effect [119–123]. Circadian rhythms have also been shown to be altered by the implementation of short-term high-fat diets [124, 125].

Food Intake and Food Restriction

Damiola and colleagues report that the peripheral clocks can be phase shifted by restricting food intake to a specific period during the sleep phase in rodents [126]. In addition, this type of feeding can induce circadian clock gene oscillations in peripheral tissues of CLOCK mutant mice [127]. Recent studies have demonstrated that increased caloric intake at the end of the day or during the sleep phase of the cycle can lead to misalignment of the metabolic rhythm and cause cardiometabolic disease [99, 128–132]. Nighttime eating is associated with obesity [133]. Additionally, Qin et al. reported that metabolic dysfunction ensues following increased nighttime caloric intake due to the uncoupling of insulin secretion and blood glucose levels [134], which is consistent with the findings of Kudo et al. that liver clock function and consequentially cardiometabolic parameters can be improved by restricting energy intake to active phase [122].

For a period of 16 weeks, obese subjects in that study were restricted to eating within a 10-hour window. This resulted in a weight loss of 3 kg which lasted for a year [135]. In a recent study, patients with diabetes who were restricted to eating during a window of 6 h that ended at 3 PM had a significant drop in insulin resistance as compared with individuals who had a feeding period of 12 h [136].

Immune System

Accumulating research implicates the immune system in the regulation of physiological effects of exercise, performance, mood and motivation, negative effects of trauma, and recovery after training and competition. Mediators of the immune system display circadian oscillations, possibly "informing" the host to anticipate greater microbial threats and physiological vulnerabilities, so the host can better protect against infection and clear harmful cellular elements and tissue fragments [137]. Hematopoietic cells in the circulation exhibit a circadian pattern, along with hormones, based on the rest–activity phase of the species [138]. The rest–activity phase is determined by whether a species is diurnal or nocturnal. Specifically, during the resting phase, hematopoietic stem cells along with progenitor cells and mature leukocytes increase in the peripheral circulation [138, 139], with the exception of CD8+ T cells, which do not [140]. On the other hand, during the active phase, proinflammatory cytokines tumor necrosis factor (TNF) and interleukin-1β (IL-1β) peak in the circulation along with adrenaline, noradrenaline, and glucocorticoids [138, 139].

Even though the hematopoietic cell count increases during the resting phase, the migration of these cells from blood to tissue does not occur until the active phase at which point they migrate to the tissues. Peripheral clocks generate circadian rhythms of cells in peripheral tissues which could oscillate autonomously but interlock with other oscillators and the master circadian clock. Immune cells such as macrophages and lymphocytes have their own internal clocks [141–143]. Synchronization of oscillators in the body occurs via the autonomic nervous system and hormonal signals [42, 144]. Glucocorticoids, adrenaline, and noradrenaline play a major role in this process. These hormones are under the control of ACTH via the HPA axis and released by the adre-

nal glands. Noradrenaline can also be released by local sympathetic nerves. The SCN sends a signal via the HPA axis to the paraventricular nucleus in the hypothalamus inducing the release of ACTH from the pituitary gland which controls the adrenal glands [42, 144]. Glucocorticoids promote anti-inflammatory mechanisms and inhibit proinflammatory mechanisms by binding to glucocorticoid receptors which are present in all cells except the SCN [145]. Catecholamines, via α- and β-adrenergic receptors, increase the number of specific immune cells, including neutrophils and natural killer cells, in addition to upregulating humoral response of the immune system [145].

The increase in leukocytes at the onset of the active phase [146] overlaps with peaks in cardiovascular ischemic events, including myocardial infarctions, known to occur during early morning hours [147]. Antimicrobial efficacy also shows a diurnal variation and is more robust under a rhythmic daily lighting pattern. Animals with cecal ligation and puncture-induced rat model of sepsis had better outcomes under alternating 12-hour day and night lighting pattern compared to constant light conditions (such as in a modern intensive care unit), which had significant reduction in survival rates [148].

Measurements in Circadian Physiology

Some of the common methods used in circadian rhythm research will now be described briefly.

Constant Routine

It is difficult to separate the contributions of (a) external (environmental) factors, (b) sleep vs. wake, and (c) internal circadian rhythms to any observed diurnal rhythm. The circadian pacemaker (or "biological clock") in the suprachiasmatic nucleus of the brain "drives" rhythms in physiology and behavior, but light, temperature, food, posture (external factors), as well as stress level and motivation (internal factors) all affect the pacemaker. Specifically, if a physiological variable (e.g., body temperature or melatonin) is measured in the presence of an intact sleep–wake cycle, then any data derived from such measurements may not be attributable to the circadian pacemaker alone because the underlying endogenous rhythm (i.e., the one attributable to the circadian pacemaker) may have been "masked" by the periodic behaviors involved in the sleep–wake cycle [149]. Additionally, the overlap between sleep–wakefulness alternations with circadian rhythms makes it difficult to disentangle their relative contributions to observed oscillating phenomena. Thus, special procedures had to be developed in an animal or human subject, and *constant routine* protocols were designed to address this problem [150].

With the use of a *constant routine* protocol, the aim is to control for external factors through design and for sleep–wake-related factors statistically [149]. In a *constant routine*, ambient light and temperature are kept constant, and the subject is required to maintain constant wakefulness while food and water are administered at fixed, short, and regular intervals. *Constant routines* lasting more than 24 h are usually needed to evaluate an entire circadian cycle, for example, a study of the circadian variation in temperature might require a *constant routine* of about 28 h [151]. Maintaining wakefulness for that duration is challenging. Apart from the *constant routine* described above, many other less demanding protocols have also been used in studies of circadian physiology. Examples include shorter protocols, multiple nap protocols [152], and protocols that allow periodic changes in posture (e.g., bathroom breaks [153]).

Forced Desynchrony

As described above, the interpretation of circadian contribution to diurnal variation in physiology can be confounded by sleep and other "masking factors," and the *constant routine* protocol is used to circumvent this problem. However, the sleep deprivation resulting from the *constant routine* procedure (even if results are

adjusted statistically for it) can impact on the interpretation of results. For this reason, the *forced desynchrony* protocol may be more suitable because it avoids sleep deprivation effects and at the same time controls for the effects of the relevant masking factors [154].

In a *forced desynchrony* protocol, the sleep–wake cycle is "forced" into a state of desynchrony with the circadian pacemaker by subjecting individuals to a period of sleep–wake schedule that is drastically different from the "normal" 24 h in entrained conditions. Common durations of "artificial days" in forced desynchrony protocols are either short (20 h) or long (28 h). The circadian pacemaker cannot adapt to these long or short periods of alternation between rest and activity and begins to oscillate at its own intrinsic period (free running [155]). An important feature of a *forced desynchrony* protocol is that the proportion between sleep and wake (1:2) remains constant, activities are allowed, and usually sleep deprivation is minimized. Usually the participants in such a protocol stay in a sleep-monitoring laboratory for the duration of the protocol; lighting levels are dimmed during periods of wakefulness and almost extinguished during periods of rest and typically do not have access to clock-bearing devices or cues that might convey information regarding the time of day. Since the circadian pacemaker is "free running" and desynchronized from the sleep–wake cycle, data (e.g., core body temperature (CBT), plasma melatonin, or cortisol) can be collected over successive circadian cycles to compute separately the circadian (process C) and sleep–wake (process S) contribution to the variable under investigation (see Fig. 20.4).

Dim Light Melatonin Onset

Measurement of circulating melatonin is a preferred marker of circadian phase, and this is because melatonin is relatively less influenced by biochemical and physiological factors. For instance, the core body temperature minimum (CBTmin) can be significantly influenced by caloric intake and physical activity, but these factors exert a negligible effect on melatonin [157]. However one factor that has been known to significantly affect the level of melatonin is bright light, which effectively suppresses its secretion [158]. Thus, measuring melatonin onset requires exposure to dim light. In an entrained healthy human subject in stable environments (e.g., no transmeridian travel, no shift work) in dim light, levels of melatonin in the blood begin to rise abruptly a couple of hours before the onset of sleepiness that precedes nocturnal sleep and reaches the highest level during the first part of the night; the beginning of the rise is called dim light melatonin onset (DLMO). Because plasma and saliva melatonin are highly correlated [159], salivary onset of melatonin is now commonly used to measure DLMO. Normal DLMO usually occurs between 19:30 and 22:00 h for adults and 19:00 and 21:00 h for children aged 6–12 years [160].

Core Body Temperature

CBT has been used as a marker of circadian rhythms in conditions when sleep–wake, activity, and diet can be maintained constant. The circadian rhythm of CBT is such that the maximum level is reached toward the end of the biological day and the minimum level toward the end of the biological night, at approximately 4–5 AM for the majority of individuals [161]. Although CBT is easily measured, it is also influenced by "masking factors," which means that *constant routine* or *forced desynchrony* protocols are necessary when CBT is used as a marker of central circadian rhythm.

Morningness–Eveningness Questionnaire

The first self-administered questionnaire to evaluate whether an individual was a "morning" or "evening" person (this is known as chronotype) was created by Horne and Ostberg [162]. Morningness could be conceptualized as a natural tendency to go to sleep and wake up early (so-called larks) and be most alert in the early

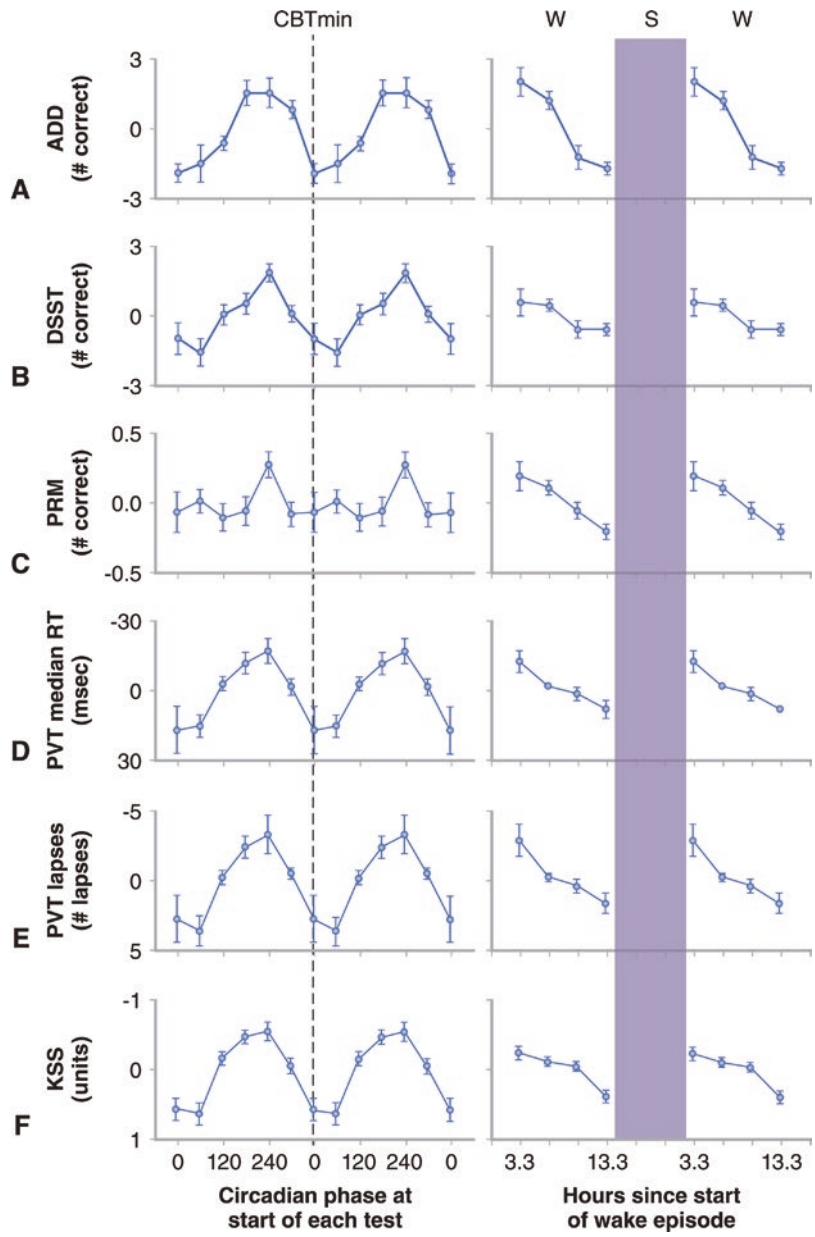

Fig. 20.4 Circadian (plotted on the *left*) and homeostatic (plotted on the *right*) variation in cognitive function. For each graph, lower-lying data points indicate poorer performance on that neurocognitive measure. Addition/calculation test (ADD), digit symbol substitution test (DSST), probed recall memory test (PRM), psychomotor vigilance task ((PVT) which consists of the median reaction time and total number of lapses), and Karolinska sleepiness scale (KSS) scores all exhibit a pattern of maximal scores near the core body temperature maximum and minimal scores near the core body temperature minimum (CBTmin). Minimal scores are also recorded with increasing duration of wakefulness (W in the figure) as homeostatic pressure builds up from the point of awakening from sleep (S in the figure). Note that even though the trend of circadian variation was observed in the probed recall memory test, statistical significance was not reached for this measure. (Modified from The American Physiological Society: Am J Physiol-Regulatory, Integrative and Comparative Physiology. Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. Wyatt et al. [156]. Copyright 1999)

morning, while eveningness is a natural tendency to stay up late (so-called owls), wake up later, and feel most alert later in the evening. Since the introduction of Horne and Ostberg's morningness–eveningness questionnaire, several other questionnaires have now been introduced including an Athlete's Morningness–Eveningness Questionnaire [163]. Morningness–eveningness status of an individual has been associated with certain variants in circadian genes, such as CLOCK [164] and PER genes [165].

Sleep

Although discussing sleep and sleep impairment in relationship to hormones and athletic activity is beyond the scope of this chapter, we cannot completely avoid mentioning it considering interactions between circadian rhythms and sleep on physiology and performance. Poor sleep quality is prevalent among athletes [166].

Interaction Between Circadian Rhythms and the Sleep Homeostat

Sleep is regulated via an interaction between the circadian process and the sleep homeostat, and this is the so-called two-process model [167]. The circadian process is sometimes referred to as "process C" and the homeostatic process as "process S." The homeostatic and circadian processes act antagonistically to consolidate wakefulness during daytime and sleep during nighttime. The homeostatic process can be conceptualized, similar to hunger and thirst, as a buildup of pressure for sleep when a person is awake and the dissipation of the pressure when asleep. Toward the morning after a period of sleep at night, there is very little homeostatic pressure for sleep, but the body clock reduces its firing to elevate the threshold for waking and thus consolidates the sleep state. The homeostatic pressure for sleep (similar to appetite for food or thirst) gradually builds up as the day progresses. The circadian process elevates the threshold for falling asleep [168] and thus consolidates wakefulness.

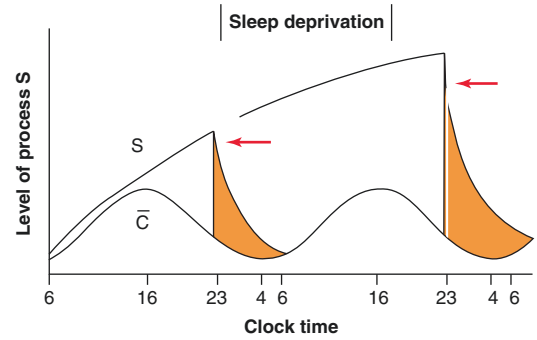

Fig. 20.5 Two-process model of sleep showing the saturation of appetite for sleep with time and the exponential decrease in process S during sleep. This explains why sleep debt accumulated over several hours can be paid with a short nap. (Modified from Elsevier: Sports Medicine Clinics. Sports chronobiology consultation: from the lab to the arena. Postolache et al [163], Copyright 2005)

Because homeostatic pressure for sleep decreases exponentially during sleep, a significant amount of accumulated sleep debt (from sleep deprivation) can be paid by a short nap (see red arrow in Fig. 20.5).

Circadian Rhythms and General Human Performance

Circadian rhythm effects have been observed in relation to both cognitive and physical performance in human subjects. For example, in a study that employed a 20-h *forced desynchrony* protocol, participants demonstrated a circadian pattern of performance in tests of psychomotor vigilance, short-term memory, addition/calculation, digit symbol substitution, and alertness [169]. After controlling for homeostatic effects, peak performance was recorded near the maximum of the CBT shortly before the onset of melatonin secretion, while significant dip in performance occurred around the time of CBT minimum shortly after melatonin maximum secretion (Fig. 20.4) (process C). After controlling for circadian effects, cognitive performance scores also decreased with increasing hours of wakefulness (process S).

The study by Freivalds et al. [170] evaluated the circadian variation in performance-related

capabilities over a 25-h period. Specifically, they measured variation in elbow flexion strength (practically relevant to manual handling of materials), simple reaction time, maximum information processing rate, physiological tremor, and critical eye–hand tracking capacity. Though the amplitudes were small, circadian variation was recorded for all the above measures of job performance-related variables. Performance scores were generally better during the day and evening times when compared to night or morning times. In the study by Teo et al. [171], the circadian rhythm of cortisol and testosterone was evaluated in relation to strength and power performances at four different time points during the day (8:00 h, 12:00 h, 16:00 h, and 20:00 h). Power performance (maximal force production and peak power output in countermovement jump and isometric mid-thigh pulls) exhibited peak performance at 16:00 h when compared to the other time points. Unexpectedly, no relationship was found between rhythmicity of cortisol or testosterone blood levels and rhythmicity in power performance.

Mental Performance

Prolonged wakefulness affects cortical excitability which results in changes in brain functional activity [172], and circadian rhythmicity also induces predictable changes in cortical excitability (Fig. 20.4). Subjects with the most variability in cortical excitability have the highest peaks in cortisol secretion, most likely due to a robust wakefulness maintaining circadian rhythm [177] (Fig. 20.6).

Circadian Rhythms and Athletic Performance

Evidence from several studies indicates that athletic performance in many types of sports exhibit circadian rhythms with peak performance usually noticed during late afternoon and worse performance in the morning [173]. For example, cyclists, runners, shot putters, swimmers, and badminton players have been shown to perform better in the late afternoon compared to the morning [174–176]. In addition, during major sports competitions, it appears that more world records are broken by athletes competing in the early evening compared to those competing in the morning [177]. However, it is important to note that many of the studies that have reported a diurnal rhythm in athletic performance have an important limitation—they did not control for confounding by "masking factors." Therefore, the observed rhythm in athletic performance could have arisen due to environmental and behavioral characteristics that are unrelated to circadian regulation. For example, worse performance in the morning could be because of ambient temperature, sleepiness and lethargy, as well as relative stiffness of joints after an overnight bed rest.

Three studies have been conducted [173] in which "chronobiological protocols" were employed to mitigate against the impact of "masking factors" in the evaluation of circadian rhythms of athletic performance. In the first study [178], subjects participated in a 4-day 5-a-side soccer match in which 5-min breaks were allowed at the end of every hour. Activity of the subjects (measured by a modified motion analysis method [179] and heart rate) exhibited a circadian rhythm with peaks in the afternoons (average 17:00 h) and troughs in the early morning (average 5:00 h).

In another study, Callard et al. [180] measured torque developed by voluntary isometric contractions of the knee extensors during cycling and at rest. The measurements at rest and during cycling were done on 2 different days a month apart. The variation in torque was circadian with peaks at around 19:00 h when measured every 4 h both at rest and during cycling.

A constant environment, elimination of sleep, and constant amount of activity were part of the protocol employed in these two studies and can therefore be viewed as close approximations to the *constant routine* protocol described earlier in this chapter.

The third study (Kline et al. [176]) subjecting experienced swimmers to an "ultrashort" sleep–wake cycle in which they were allowed to sleep in the dark for an hour and mandated to stay

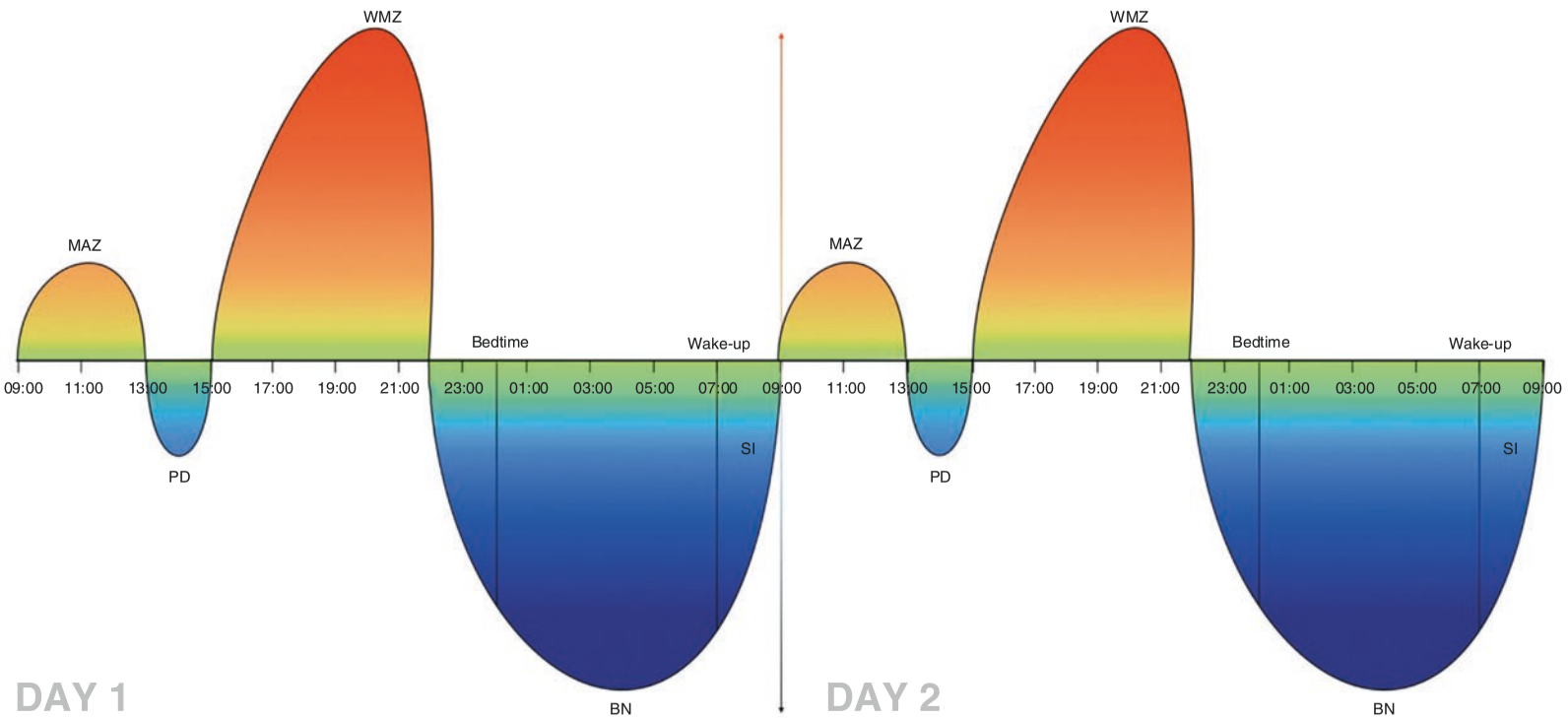

Fig. 20.6 Graphic representation of the overall performance in a typical individual (neutral chronotype) over a 48-hour period. The x-axis illustrates the time of the day. Peak performance is seen during wake maintenance zone (WMZ) during late afternoon/evening (a time where napping is very difficult even with accumulated sleep debt). Performance is lowest during the biological night (BN). Morning alertness zone (MAZ) is the interval between waking up to approximately lunch time, representing a time with improved performance relative to BN. However immediately after waking up, there is an interval of performance at its worst, called sleep inertia (SI), lasting 20–30 min for most individuals and up to 1–2 h in few individuals. The postprandial dip (PD) is a drop in performance that occurs midday in a sizable proportion of individuals, most often in anticipation of feeding, in some individuals made worse by lunch. Orange represents peak performance. Yellow is good performance. Green is baseline. Light blue is lower performance. Dark blue is the lowest performance

awake for 2 h in dim light. Measurements of the time required to complete a 200-m swim was done over a 50–55-h period such that a total of 6 measurements were obtained for each swimmer. The swimmers' performance had a circadian peak in the internal late evening/early night, around 23:00 h (Figure 20.7a). Of interest, the period of peak performance in all three studies tends to overlap with the timing of maximal CBT (see core body temperature above), and the worst performance, with CBTmin.

In summary, performance generally peaks (except in those with an extreme morning chronotype) in the early evening at a time when the circadian pacemaker is firing frequently to counteract the effect of accumulated wakefulness. This period is called "wake maintenance zone," as it is the period when it is difficult, if not impossible, to fall asleep for majority of non-sleep-deprived individuals, despite an accumulated duration of wakefulness and thus increased "appetite" for sleep. The characterization of the individual circadian rhythms of athletic performance could be important when making recommendations regarding the optimal time, internal and external, for competing, training, practicing, and recovering for elite athletes, and "working out" for most of us, for maintaining health and productivity.

Jet Lag

More than 30 million Americans have been estimated to embark on air travel across five or more time zones each year [181]. Altered mood, fatigue, and poor cognitive performance are some of the symptoms associated with transmeridian travel, with mismatched capabilities and environmental demands, and secondary sleep loss through reduced sleep propensity [182]. Misalignments between "internal" biological rhythms, environmental light/dark cycles, and rest–activity societal demands are the main

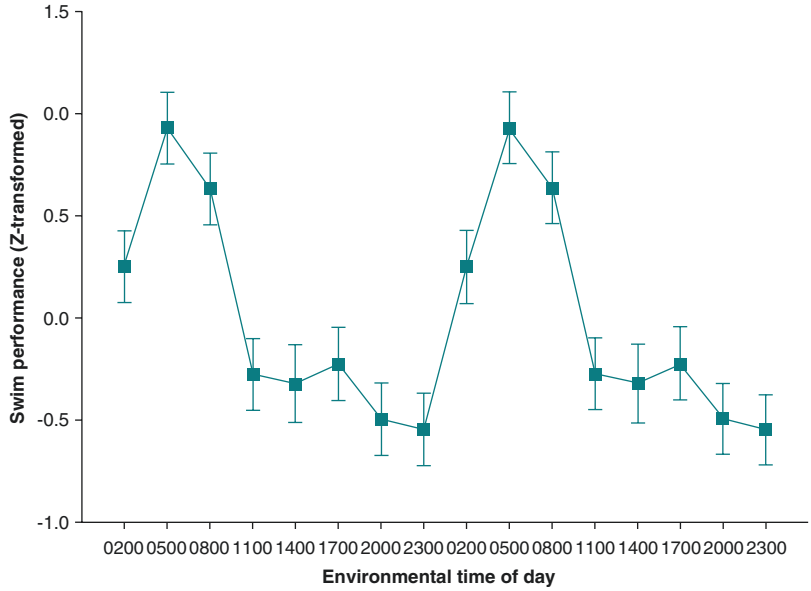

Fig. 20.7 Swimmer performance as a function of environmental time of day. Means and standard error for z-transformed performance values are shown, with lower scores representing better performance (as time: to complete given distance). The cyclic nature of performance across time is evident, with repeatedly and significantly faster swimming occurring during the 1100, 1400, 1700, 2000, and 2300 trial times compared with the earlier monitored times of 0200, 0500, and 0800. (Modified from The American Physiological Society: Journal of Applied Physiology. Circadian variation in swim performance. Kline et al. [176]. Copyright 2007)

mechanisms involved in jet lag symptoms and implicated in the development of various health problems as well as having adverse effects on alertness, attention, learning, and memory. It is also likely that desynchronization between peripheral circadian oscillators and the pacing of the SCN contribute to the physiological changes in jet lag. Appropriately timed exposure to bright light, light avoidance, melatonin, or melatonin agonist administration are some chronobiology-based interventions for preventing and treating symptoms of jet lag.

Jet lag has been documented to have a negative impact on the performance of athletes. For example, in Major League Baseball, home teams playing against teams that had traveled eastward hit 1.24 more home runs [183]. The decreased athletic performance associated with jet lag may be due to a number of processes including (1) peak performance time for an athlete and time of performance demands (training and competition) may become misaligned due to the effects of transmeridian travel on the circadian system; (2) sleep disturbances associated with transmeridian travel and the subsequent sleep debt; and (3) the general malaise associated with jet lag [163, 184].

Jet lag has been linked with certain illnesses strongly associated with lower life expectancy [185]. For instance, certain cancers have been associated with chronic jet lag [186, 187]. Repeated jet lag is also known to contribute to metabolic abnormalities, such as glucose intolerance. It appears that the gut microbiome may be contributing to metabolic abnormalities in jet lag, as experimentally, performing fecal transplant from humans chronically exposed to transmeridian travel, to mice, resulted in the mice to become intolerant to glucose [188].

Light exposure and avoidance, melatonin administration for shifting (excellent efficacy) and improving quality of sleep (weak efficacy), management of sleep debt, and intermittently administering sleep-promoting and alerting pharmacological agents are common elements of practice of jet lag consultation [163, 189]. It is important, however, to consult the latest antidoping regulations before prescribing any pharmacological agents to athletes. Timed exercise and meals may also have a role in the management of phase shifting, but their role is less important as light exposure and avoidance. However, timed rest–activity and exercise, as well as timing of meals, may have a high relevance to synchronization between peripheral clocks among themselves and the SCN clock.

Interventions

Bright Light

Light is the main entrainment agent in most species, and exposure to single pulses of light at certain times of the day can result in phase shifting of circadian rhythms [190, 191]. Light pulses administered 2–6 h before the CBT minimum usually result in phase delay of the circadian rhythm, while administration of light 2–6 h after the temperature minimum phase advances the circadian rhythm. The wavelength of the light is also important as blue light has a more potent effect on shifting circadian rhythms than red light [219]. The circadian phase-shifting potential of light can be useful in the treatment of circadian rhythm sleep disorders including shift work, delayed or advanced sleep phase, and jet lag disorders (this is pertinent for athletes engaged in transmeridian travel to competition sites) (see section "Jet Lag").

Light exposure regulates not only the timing and quality of our hormonal rhythms, sleep, and wakefulness (a largely SCN-dependent process) but also our mood, an SCN-independent process.

The discovery of ipRGCs, melanopsin-containing retinal ganglion cells, and the knowledge of the spectral sensitivity of the circadian system led to the understanding of a photoreceptor system other than that of rods and cones with an increased response to low-wavelength visual light (blue-green domain). A finding consistent with the observation that many blind individuals have the capability to detect light, to achieve entrainment, to phase shift, and to regulate melatonin secretion in the absence of vision [192]. A

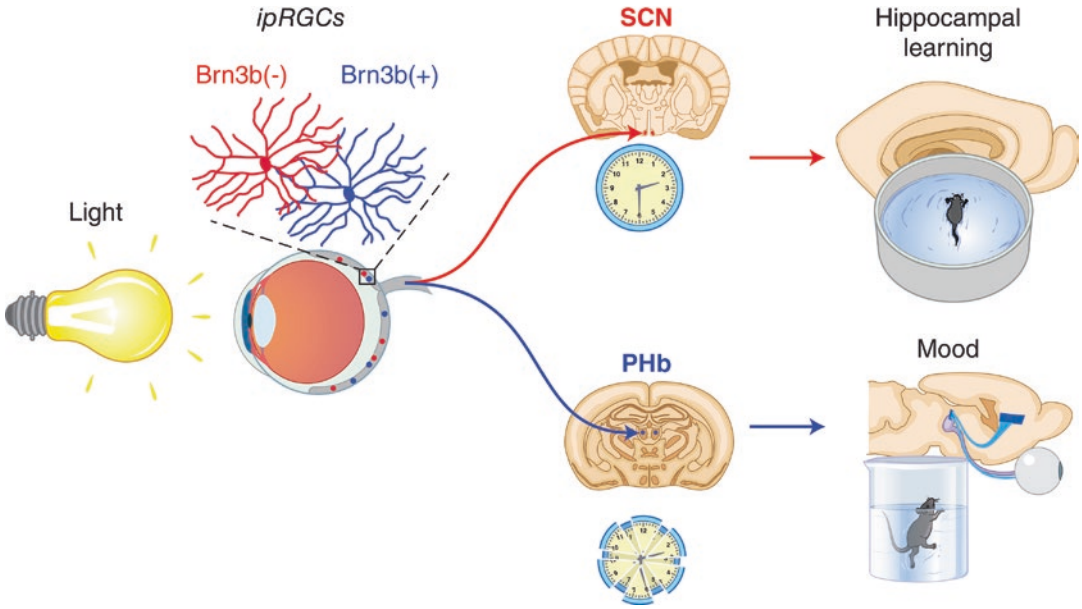

Fig. 20.8 Effect of light on learning and mood. Two distinct pathways that project via ipRGCs to suprachiasmatic nucleus (SCN) in the hypothalamus to the hippocampus for learning and perihabenular nucleus (PHb) in the dorsal thalamus to the ventromedial prefrontal cortex for mood via Brn3b(−) (red) and Brn3b(+) (blue), respectively. (Modified from Elsevier: Cell. Light Affects Mood and Learning through Distinct Retina-Brain Pathways. Fernandez et al. [195], Copyright 2018)

study with mice lacking rods and cones demonstrated they were able to process light for photoentrainment as well as other non-image forming (NIF) functions and was consistent with the presence of unique photoreceptor system [9]. Although the ipRGCs are unique photoreceptors, as melanopsin-containing retinal ganglion cells, they can also receive and process input from rods and cones [11, 12, 193, 194]. In the rodent brain, ipRGCs project to SCN, subparaventricular zone, and intergeniculate leaflet for circadian rhythm control. They also project to the ventrolateralpreoptic area (VLPO) and lateral hypothalamus to modulate sleep. Finally, as recently reported, they also project to the medial amygdala and lateral habenula for direct mood regulation [195, 196] (Fig. 20.8). Evidence for a direct mood effect of bright light independent of circadian shifting mechanisms has been suggested by several studies on the early antidepressant effect of light. For instance, Reeves et al. reported that only 1 h of bright blue light had an antidepressant effect as compared to 1 h of placebo light [197].

Light exposure may also have undesirable effects. First, exposure to the wrong timing may have detrimental effects from a circadian standpoint. For instance, exposure to late afternoon, evening, and early nighttime light in individuals who tend to have problems falling asleep on time and waking up on time may exacerbate a sleep–wake phase delay. In general, it is likely beneficial to limit blue bright light exposure from computers, tablets, and phones in the evening and, if evening exposure cannot be avoided, to turn on a nocturnal blue light filter app. Also, of practical interest, individuals who read an actual book as compared to those reading from a screen have a shorter sleep latency and report being more alert the following day [198] (Fig. 20.9).

For a more detailed review of light exposure and avoidance in relationship to jet lag, please see the reviews by Postolache and Oren [189] and Postolache et al. [163].

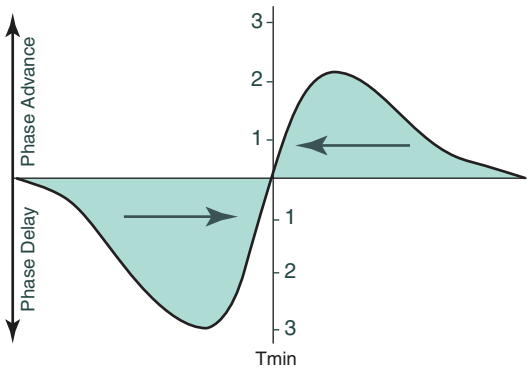

Fig. 20.9 Phase response curve (PRC) to the bright light phase advances (positive values) and delays (negative values) is plotted against the timing of the center of the light exposure relative to the core body temperature. Phase delays occur when light exposure occurs before body temperature minimum (Tmin: in most people 4–5 AM under entrained conditions). Phase advances occur when exposure to light takes place after Tmin. All phase shifts have a greater amplitude in temporal proximity to the Tmin. Units on the y-axis are hours. The maximal phase delay (2.7 h) exceeds the maximal phase advance (2.3 h)

Exercise

In addition to improved amount and quality of sleep [199], exercise induces phase shifts in circadian rhythms of melatonin [200] and in the rhythms of the peripheral clocks in muscle tissue [89, 90]. Hackney and Viru [201] carried out a study of the cortisol profile in physically active individuals randomly exposed to daytime high- or moderate-intensity exercise and no exercise conditions, respectively, using a crossover design with 1-week washout and measuring daytime and nighttime cortisol levels. The nocturnal levels of cortisol were lower in the daytime exercise conditions and lower after anaerobic (i.e., high intensity) daytime exercise conditions as compared to aerobic (i.e., moderate intensity) daytime exercise conditions. Thus, daytime exercise appears to suppress nocturnal cortisol secretion. The main limitation of the study was the confounding by sleep and wakefulness, factors that were not controlled by design and not adjusted for statistically.

Furthermore, scheduled voluntary wheel running and forced treadmill running induce entrainment and phase shifting in rodents [202–204]. Also, in humans, exercise has been shown to have phase-shifting effects. For instance, Van Reeth et al. [205] reported a phase delay in the circadian rhythms of thyrotropin and melatonin with a single bout of exercise (alternating arm and leg ergometry at 40 and 60% of maximal O_2 consumption) centered from 5 h before to 4 h after the body temperature minimum, in 300 lux constant light conditions. The findings by Van Reeth et al. [205] were later replicated by Behr et al. [206] in both younger and older adults (without a significant age-group effect) using DLMO as a circadian phase marker.

In another study, Buxton OM et al. found higher-intensity (stair climbing at 75% VO_2 max) but shorter duration (1 h) exercise had a similar phase-delaying effect on the thyrotropin rhythm and of interest moderate-intensity exercise delayed the circadian rhythm of melatonin more than the higher-intensity exercise [195].

As light could have been a confounding factor in these studies, a developed protocol replicated the phase-delaying capacity of nocturnal exercise in very dim light conditions (three 45-min bouts of cycle ergometry with 0.65 lux in the line of sight) on melatonin circadian rhythms [207] each night. When different timings of scheduled exercise were added to the previous protocol (morning, afternoon, and night exercise sessions), nocturnal exercise significantly phase delayed, while early evening exercise phase advanced melatonin onset rhythms [200]. However, it appeared that only the first bout of evening exercise significantly advanced the melatonin circadian rhythm, as the effects were diminished below significance on subsequent nights. In another study conducted in a temporal isolation facility, in which participants were subjected to an imposed external cycle of 23-h and 40-min duration (participants were subjected to 12 of these cycles), 2 h of exercise twice per day (cycling and rowing to a heart rate of 140 beats/min), a phase advance of the melatonin rhythm of 1.6 h, was observed, and the phase difference (i.e., between exercise and "no exercise" states) was significant 6 days after the start of the exercise [208].

Because exercise has potential phase-shifting effects, it brings up the issue of whether exercise can enhance or inhibit circadian phase shifting that occurs with light exposure. The best available data suggests that it does not [206, 209] and is consistent with the more potent effects of light as a zeitgeber for phase shifting as compared to exercise.

In regard to re-entrainment (of considerable importance for prevention and treatment of jet lag), it appears that timed exercise has a significant entrainment accelerating effect on sleep–wake cycles, but, in contrast to light, not on circadian rhythms of melatonin [210].

Conclusions

1. Predictable hormonal (e.g., melatonin and cortisol) and electrophysiological changes occur with a period of approximately 24 h.
2. These changes are associated with changes in performance, cognitive and physical, simple and complex.
3. Best performance occurs during the end of the internal (biological) day (i.e., evening), except in individuals who score very high on morningness.
4. Measuring circadian rhythms is challenging, because of masking effects of sleep–wake and environmental effects.
5. The most important and potent signal for shifting circadian rhythms is exposure to bright light. Other factors with therapeutic potential include rest–activity, exercise, melatonin administration, and feeding restriction.
6. Knowledge of circadian factors is likely to (a) improve our understanding and ability to predict fluctuations in performance, (b) minimize effects of jet lag and sleep loss on athletic performance in competitive athletes, (c) use simple nonpharmacological techniques to shift circadian rhythms to avoid competing during individual circadian "dips" in performance, and (d) choose wisely the timing of sleeping, eating, and exercising for everyone, for maximizing health, wellness, and longevity.

Glossary

Circadian phase This is the phase of the circadian cycle, representing the timing of the internal biological clock and is usually estimated by measuring onset–offsets, troughs or peaks, or changes in slopes (i.e., increasing vs. decreasing) in circadian markers such as hormones (melatonin, cortisol, prolactin) or CBT. Circadian phase measurement should be performed in controlled dim light conditions. In animal research, rest–activity in dim light conditions is used to estimate circadian phase.

Circadian rhythms A full 24-hour period rhythm that exists in all biological cells under a constant environment. It is derived from the Latin words circa and *dies*, meaning around and 1 day, respectively.

Chronobiology The science concerned with the study of biological rhythms.

Chronotype The natural tendency for an individual to either go to sleep and wake up early and perform best in the morning (a morning person AKA larks) or to go to sleep and wake up late and perform best in the evening (evening person AKA owls). The chronotype has been related to clock gene polymorphisms, to circadian sleep disorders, to mental and physical health.

Daily (diurnal) rhythms Diurnal rhythms refer to variations in a physiological parameter with time of day. In contrast with circadian variations which are usually endogenous (i.e., intrinsically generated by the body clock; see below) but influenced by external light–dark cycle, diurnal variations can be either driven by the biological clock, external light–dark cycle, the sleep–wake cycle, or their interaction. These can be physiologic, hormonal, or behavioral in nature. They are measured with the light/dark cycle and sleep/wake cycle.

Entrainment The process by which biological rhythms are synchronized (by timing signals) to a 24-h environmental cycle (usually the day/night cycle). Under entrainment or entrained conditions, circadian rhythms usually oscillate with a period of 24 h, induced by exposure to time cues (i.e., zeitgebers).

Free-running rhythm A non-24-h rhythm seen in the absence of timing signals, most importantly in dark (or very dim light) conditions, which expresses the intrinsic circadian period of the circadian pacemaker (the suprachiasmatic nuclei, see below). Almost all animals have the internal circadian period slightly different than 24 h, either slightly shorter (as in most rodents) or slightly longer (as in humans, among other animals). Animals with longer than 24-h circadian rhythms tend to phase delay from 1 day to another in the absence of exposure to light during morning hours.

Jet lag A circadian rhythm sleep disorder consisting of sleep difficulty at night, daytime sleepiness, impairment of daytime function, gastrointestinal disturbance, and general malaise associated with transmeridian air travel, resulting from a desynchrony between external time cues, sleep–wake, and timing of endogenous rhythms.

Masking Obscuring of circadian rhythms (driven by the circadian pacemaker) by external or internal factors. For example, physiological processes and behaviors (such as opening and closure of eyelids, activity, food intake) associated with the sleep–wake cycle, exposure to bright light, eating, drinking, and standing could all be related to the rhythm of a physiological variable, though the rhythm is primarily generated endogenously by the circadian pacemaker. Therefore, measuring the rhythm of the variable (e.g., CBT) in the presence of an intact sleep–wake cycle and all the other external factors could "mask" the true contribution of the circadian pacemaker to the rhythm and lead to inaccurate measurements.

Misalignment protocol (forced desynchrony) When an extreme non-24-hour cycle, like a 20-hour or a 28-hour cycle, is implemented to disentangle endogenous circadian rhythms and sleep–wake cycles.

Morningness–eveningness (ME) The natural tendency for an individual to either go to sleep and wake up early and perform best in the morning (a morning person) or to go to sleep and wake up late and perform best in the evening (evening person). The ME has been related to clock gene polymorphisms and to circadian sleep disorders.

Nadir This is the lowest point of a biological rhythm, e.g., the nadir of CBT is the lowest point on the CBT rhythm and is usually around 2 h before habitual wakening time in most individuals with a stable circadian rhythm.

Peripheral clocks Autonomous clocks that exist in all cells of the body complementing the SCN which has a central clock. When aligned, these clocks work harmoniously with the master clock as well as with the environmental timing, and each other.

Phase advance Positioning of a circadian rhythm earlier relative to clock time or other circadian markers.

Phase delay Positioning of a circadian rhythm later relative to clock time or other circadian markers.

Phase response curve (PRC) A graphical illustration of the relationship between the timing of exposure to a zeitgeber or other intervention (on the *x*-axis) and the shifting induced by the exposure to the zeitgeber or other intervention. Conventionally, on the *y*-axis, positive values represent phase advances, and negative values represent phase delays. For example, the circadian phase-shifting effects (advance or delay, depending on the time of exposure) of bright light or melatonin administration on any marker of circadian rhythms (such as CBT or the nocturnal melatonin secretion measured in blood or the onset of nocturnal melatonin secretion in saliva) have been presented as phase response curves. On the *x*-axis, timing is usually measured in relationship to a circadian marker—such as core temperature trough or the onset of melatonin secretion (i.e., internal timing) rather than external timing. Determination of circadian phase response curves is very demanding in terms of funds and time—requiring highly controlled conditions and minimizing exposure to zeitgebers.

Postprandial dip (or early afternoon dip) The dip in performance observed during the midafternoon hours (incorrectly called postprandial), because it occurs in anticipation and not as a physiological reaction to the main meal of the day. For most individuals, performance measures (physical and cognitive) exhibit an increase from a low at the morning wake time

to peak levels in the early evening time, but in some individuals a dip in performance is observed during the midafternoon hours.

Seasonal affective disorder A form of depression that occurs in the fall and winter with spontaneous remission in spring and summer.

Suprachiasmatic nuclei (SCN) A group of brain cells located bilaterally above the optic chiasm in the anterior basal hypothalamus and demonstrated to be the site of the master circadian oscillator ("body clock") that synchronizes, as a conductor does with the orchestra, circadian rhythms of peripheral tissues, organs, and cells.

Wake maintenance zone This is the time of the day (usually in the late evening) when the propensity for sleep is lowest and wakefulness or arousal is increased. The wake maintenance zone (also referred to as the forbidden zone for sleep) is mediated by the circadian pacemaker.

Zeitgeber The name given to any external time-signaling stimuli that help maintain periodic regularity in circadian rhythms. It is a German word which literarily means "time giver." Light is considered the most potent zeitgeber. Other zeitgebers are exercise, food, temperature, and social interactions.

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The Role of Hormones in Exercise-Induced Muscle Hypertrophy

21

Julius E. Fink

Introduction

Skeletal muscle has several important functions in the human body. It is not only necessary for daily physical functions such as standing, walking, or holding things, it also acts as major glucose uptake and amino acid storage organ regulating several internal functions such as metabolism. In view of the multiple health benefits of skeletal muscle as a tissue, it is imperative to maintain a healthy amount of muscle, especially in the elderly. Resistance training is widely recognized as the most effective way to increase muscle mass. However, the exact mechanism of resistance training-induced muscle hypertrophy is not completely understood to this day. Past research showed that besides mechanical stress, metabolic stress is an important trigger for muscle mass growth [1]. Appropriate metabolic stress triggers enhanced protein synthesis [2], muscle fiber recruitment [3, 4], hormonal responses, and muscle cell swelling [1].

However, it is not clear how resistance training-induced hormonal elevations lead to muscle hypertrophy. Past literature suggests that improved protein synthesis, decreased protein breakdown [5], satellite cell activation [6], wingless/integrated (Wnt) signaling pathway [7], and sex hormone-binding globulin (SHBG) receptor binding [8] all might be the downstream reactions of resistance training-induced hormonal elevations ultimately leading to muscle hypertrophy. Besides, resistance training-induced hormonal elevations could also stimulate non-genomic pathways leading to increased intracellular calcium [9] and force production [10], improving training intensity and muscular adaptations [11].

Hormonal Elevations After Resistance Training

During the last decade, the effects of resistance training-induced acute hormonal increases including growth hormone)GH), testosterone, free testosterone, and insulin-like growth factor-1 (IGF-1) on muscle hypertrophy have been widely investigated [12–17].

Endogenous testosterone release seems to respond well to training protocols that include large muscle groups with moderate intensity, high volume, and relatively short rest periods [18]. Previous research showed a positive correlation (r = 0.76) between acute resistance training-induced testosterone increases and muscle cross-sectional area [11, 19]. Nevertheless, resistance training (RT)-induced acute testosterone elevations may last only for about 60 min,

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and peak values generally do not go beyond 22.5 nmol/L [20].

On the other hand, GH release seems to respond to smaller muscle groups with low to medium intensity and short rest periods [21], with peaks between the period immediately after and 15 min into recovery from resistance training, following a steady decrease to baseline values around 60 min post-resistance training [15, 21, 22]. Stimulations via resistance training can increase blood GH up to 24 ug/L for large muscle groups [22] and up to 12 ug/L for small muscle groups [21]. Depending on the study, strong positive correlations between GH and muscle fiber typesI ($r = 0.74$) and II ($r = 0.71$) [13] or weak positive correlations (fiber typesI ($r = 0.36$) and II ($r = 0.28$)) [13, 17] have been observed. Recent studies demonstrated that SHBG increases total androgen levels via hypothalamic-pituitary feedback and prolonged half-life [23], while resistance training enhances SHBG [24], indicating potentially a possible role of SHBG in resistance training-induced anabolic pathways.

Besides the hormones above, myokines seem to be affected by resistance training too. Myokines such as irisin, interleukin-15, brain-derived neurotrophic factor, leukemia inhibitory factor, fibroblast growth factor 21, and secreted protein acidic and rich in cysteine seem to be secreted in response to exercise, leading to changes in metabolic disorders via enhanced AMP-activated protein kinase signaling, glucose uptake, and lipolysis [25].

However, several recent studies negating any correlation between acute resistance training-induced hormonal elevations and muscle hypertrophy exist too [26, 27]. One study suggests that rather than circulating (testosterone, free testosterone, dehydroepiandrosterone, dihydrotestosterone, insulin-like growth factor, free IGF-1, luteinizing hormone, and GH) or intramuscular hormones, intramuscular androgen receptor content linearly correlates with lean body mass both pre and post 12 weeks of resistance training ($P < 0.01$), type 1 cross-sectional area ($P < 0.05$), and type 2 cross-sectional area ($P < 0.01$) [27] (Table 21.1).

Table 21.1 Correlation between resistance training-induced hormonal elevations and muscle hypertrophy

Authors	Target hormones	Findings
Ahtiainen et al. [19]	C, FT, GH, T	Positive relation between the difference in acute T responses before and after a 21-week training period with changes in muscle CSA
Fink et al. [28]	GH, IGF-1, T	No interdependence between hormonal changes and CSA
Fink et al. [29]	GH	No interdependence between hormonal changes and CSA
McCall et al. [13]	C, GH, IGF-1, T	No relation between GH and CSA but a relation between GH with types I and II muscle fiber hypertrophy
Mitchell et al. [30]	FT, GH, IGF-1,	No interdependence between hormonal changes and muscle fiber hypertrophy
Mangine et al. [11]	C, GH, I, IGF-1, T	Positive relation between T and muscle growth
Morton et al. [26]	C, DHEA, DHT, IGF-1, LH, T	No interdependence between hormonal changes and muscle fiber hypertrophy
Morton et al. [27]	AR, DHEA, DHT, FT, GH, IGF-1, FIGF-1, LH, T	AR but not circulating nor intramuscular hormone affect RT-induced muscle hypertrophy
Rønnestad et al. [14]	C, GH, T	Improved CSA increases in a state of high-circulating hormonal concentration as compared to low hormonal concentration
West et al. [16]	C, GH, I, IGF-1, T	No changes in anabolic signaling or acute postexercise MPS response in a state of high-circulating hormonal concentration
West et al. [15]	C, GH, I, IGF-1, T	No enhanced total CSA or muscle fiber size in a state of high-circulating hormonal concentration
West et al. [2]	C, GH, IGF-1, T	Weak relation between C and muscle fiber area and between GH and muscle fiber area

Abbreviation key: *AR* androgen receptor, *C* cortisol, *CSA* cross-sectional area, *DHEA* dehydroepiandrosterone, *DHT* dihydrotestosterone, *FIGF-1* free insulin-like growth factor-1, *FT* free testosterone, *GH* growth hormone, *I* insulin, *IGF-1* insulin-like growth factor-1, *LH* luteinizing hormone, *MPS* muscle protein synthesis, *T* testosterone

The debate about the correlation between systemic hormones and muscle hypertrophy is still ongoing, with recent studies supporting such a correlation existing [11, 31]. The reason for those discrepancies among studies is probably related to different measurement and result analysis methods, for instance, regression models vs. partial least squares-structural equation modeling (PLS-SEM), acute increases post-training vs. resting levels pre−/post-training period, absolute values vs. percent increases, absolute increases vs. area under the curve, magnetic resonance imaging vs. muscle biopsy for CSA assessment, dual-energy X-ray absorptiometry (DXA) vs. mathematical formula, etc. All those factors increase the odds of discrepancies among studies, since scan results should only be compared (i.e., more accurately) when measured by the same method.

Mechanism of Hypertrophy Induced via Hormonal Elevations

It is widely recognized that mechanical tension plays a major role in muscle mass regulation [32] via conversion of mechanical stimulus into biochemical reactions, with skeletal muscle being mainly regulated via the multi-protein phosphorylation cascade of mammalian target of rapamycin complex 1 (mTORC1) [33]. The mTORC1 composed of upstream (insulin receptor substrate 1 (IRS1), protein kinase B (Akt), tumor sclerosis complex 2 (TSC2)) and downstream (mTOR, ribosomal S6 kinase 1 (p70S6k), ribosomal protein S6 (RPS6)) signaling triggers the anabolic mechanism. In addition to resistance training, growth factors such as insulin and insulin-like growth factor-1 (IGF-1) seem also to trigger the mTORC1 mechanism. The association between circulating hormones, mTORC1 signaling, and muscle hypertrophy is not completely understood yet. Steroidal hormones such as testosterone pass through the sarcolemma and enter the cell where they bind to androgen receptors triggering gene transcription within the nucleus leading to muscle protein synthesis and local production of growth factors such as IGF-1 [34]. Indeed, testosterone administration has shown to improve intracellular reutilization of amino acids, therefore increasing muscle protein synthesis [35] while inhibiting muscle protein breakdown [36], which might be due to inhibited ubiquitin-proteasome activity or antagonism of glucocorticoids via either ligand-receptor or DNA binding blockage [34, 37]. Besides those anabolic mechanisms, testosterone might increase quiescent satellite cells and therefore improve DNA necessary for muscle growth [6]. A recent study demonstrated correlations between muscle mass and androgen receptor with Wnt5a protein levels, a member of the wingless/integrated (Wnt) signaling markers, believed to regulate myogenesis [38], which showed further increases after treatment with testosterone and a non-aromatizable synthetic testosterone derivative (trenbolone), suggesting hormone-induced muscle hypertrophy regulated via the Wnt pathway [39]. On the other hand, it has been suggested that GH leads to hyperplasia that is an increase in the number of myofibers as opposed to conventional hypertrophy which means an increase in the size of myofibers. However, this theory has never been proven in human studies and is limited to animal studies [40]. As a nonsteroidal hormone, GH acts via two pathways: the direct receptor pathway and the indirect pathway via IGF-1 conversion in the liver. In the direct pathway, GH binds to the GH receptor triggering intracellular signaling via Janus kinase and signal transducers and activators of transcription (Stat) pathway affecting most tissues. However, the main anabolic effects of GH are indirect via IGF-1 conversion. IGF-1 acts mainly via the PI3K/Akt pathway to induce muscle hypertrophy (Fig. 21.1). SHBG has been thought as a regulator of concentration of certain free circulating steroids. However, recent research suggests that SHBG has a signaling role for steroids at the cell membrane [41]. Unbound SHBG binds to SHBG receptors (SHBG-R) on cell membranes where it can bind to steroids. This tripartite complex made of steroid-SHBG-SHBG-r triggers adenylyl cyclase and induces cAMP and the following intracellular signal transduction.

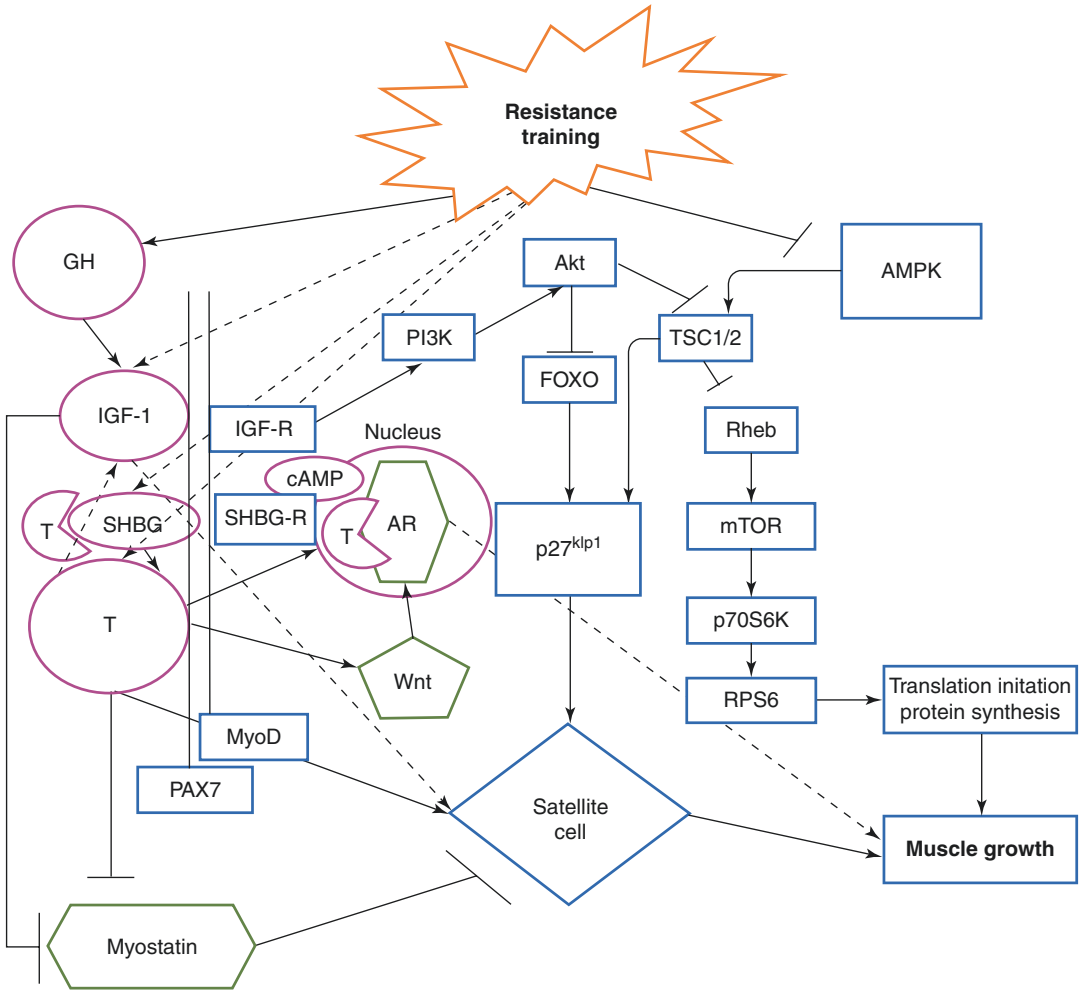

Fig. 21.1 Hypothesized mechanisms by which resistance training induces muscle growth. Akt, protein kinase B; AMPK, 5′ adenosine monophosphate-activated protein kinase; AR, androgen receptor; FOXO, Forkhead box O; GH, GH; IGF-1, insulin-like growth factor-1; IGF-R, insulin-like growth factor receptor; mTOR, mammalian target of rapamycin; p70S6K, ribosomal protein S6 kinase; $p27^{kip1}$, cyclin-dependent kinase inhibitor 1B; Rheb, Ras homolog enriched in brain; RPS6, ribosomal protein S6; SHBG, sex hormone-binding globulin; SHBG-R, sex hormone-binding globulin receptor; TSC1/2, tuberous sclerosis 1/2

Mechanical Stress vs. Hormone-Induced Hypertrophy

As we mentioned in the paragraph above, resistance training triggers anabolic mechanisms mainly via mechanical stress. Anabolic pathways regulated via hormones seem to be closely related to the pathways involved in the mechanical stress-induced pathways. Several genes are activated and necessary in both pathways, making it difficult to investigate each pathway in a disconnected environment. However, resistance training-induced acute physiological increases of hormones may not trigger anabolic signaling but rather permit those mechanisms to take place [42]. On the other hand, chronic supraphysiological levels of anabolic hormones seen in amateur and professional athletes using androgenic anabolic steroids (AAS) do enhance anabolic responses and lead to supraphysiological muscle mass [43] (see Chap. 28, "Hormones as Performance-Enhancing Agents" by E.J. Richmond and A.D. Rogol).

Initially developed for the treatment of muscle wasting diseases, AAS have soon been discovered by strength athletes and bodybuilders seeking supernatural bodies. According to a survey of 500 AAS users, more than half of the users confirmed using more than 1000 mg of T or similar AAS weekly. Besides AAS, 25% of the users seem to use GH and insulin, while more than 99% confirmed the onset of side effects related to AAS [44].

It has been shown that the supplementation of supraphysiological doses of testosterone (600 mg/week) enhances muscle mass and strength regardless resistance training in healthy men as compared to the control group [45], demonstrating the anabolic effects of supraphysiological levels of testosterone on body mass and functions. Several other studies testified the anabolic effects of different AAS such as trenbolone, stanozolol, or nandrolone [46–48]. Even though several AAS exist, only a few are approved for humans. For instance, trenbolone is used as growth promoter in cattle [49]. The strong effects of this drug have been verified in several animal studies [46, 50, 51], making it notorious among athletes. Furthermore, the combination of AAS and protein hormones might lead to even larger enhancements in body composition as compared to single use [52], indicating synergistic effects of AAS and other performance-enhancing drugs. Notably, the concomitant use of GH, insulin, and AAS is believed to trigger body enhancements surpassing the use of AAS only by far. As a matter of fact, the combination of testosterone and GH improves muscle gene IGF-1 expression [52], while insulin inhibits proteolysis [53].

Similar to naturally occurring testosterone, AAS have several anabolic and performance-enhancing properties:

- Increase in satellite cell and myonuclear number
- Increased protein synthesis [54]
- Decreased protein breakdown [54]
- Increased nitrogen retention [54]
- Increase in red blood cells [55, 56]

Since the 1930s, researchers aimed to discover new chemical structures minimizing side effects and maximizing anabolic effects of AAS, in other words, increasing anabolic while decreasing androgenic effects. The muscle building potency of AAS is often measured by the anabolic to androgenic ratio of a given drug. This ratio has been calculated based on the growth rate of the *levator ani* muscle versus the prostate in rodents after AAS administration [57].

The most widely used AAS nowadays are divided into three groups [54]:

1. Testosterone derivatives (testosterone, methyltestosterone, methandrostenolone, chlorodehydromethyltestosterone, fluoxymesterone, boldenone): AAS in this category have typically a high rate of aromatization of androgens into estrogens.
2. Dihydrotestosterone derivatives (stanozolol, oxandrolone, oxymetholone, mesterolone, methenolone, drostanolone): Because of their 5DHT molecule, these drugs do not aromatize and convert into estrogen.
3. Nandrolone derivatives (nandrolone, trenbolone): Compounds in this group show the highest anabolic to androgenic ratio. Side effects caused by progesterone-like activity might occur.

From the information above, we can extrapolate that steroid and protein hormones can induce anabolic pathways leading to muscle hypertrophy if the serum levels are high enough, that is, supraphysiological via exogenous administration. However, resistance training-induced acute physiological hormonal increases might not trigger major anabolic responses, but rather allow other anabolic mechanisms to function properly and might be considered as markers for the intensity of mechanical or metabolic stress of a given resistance training protocol.

Conclusion

The above discussion points to the hypothesis that resistance training-induced hormonal elevations might not directly correlate with muscle hypertrophy, be it total measured as cross-sectional

area, as fiber-type hypertrophy, or as muscle protein synthesis. However we know that supraphysiological levels of testosterone or other AAS lead to increased muscle mass and strength even without additional mechanical stress. Therefore it seems that the anabolic pathway triggered by hormonal elevations requires a certain threshold in serum concentrations and bioavailable duration. Indeed, resistance training-induced elevations in hormones are of short duration and do not raise circulating concentrations above physiological ranges. On the other hand, administration of supraphysiological doses of AAS and other protein hormones leads to chronic increased levels of circulating anabolic hormones going beyond physiological ranges. The anabolic pathway induced via resistance training can be looked at a machinery triggered by mechanical stress requiring a certain level of circulating hormones to function properly and which can be enhanced by supraphysiological hormonal elevations.

Even though testosterone and other anabolic hormones can trigger anabolic pathways via non-genomic pathways, the role of androgen receptors in hormone-induced muscle hypertrophy might be critical. The number of androgen receptors increases in response to resistance training as well in response to higher androgen levels. More androgen receptors allow more genomic activity of circulating steroids and the following transduction into anabolic signals. Therefore not only serum hormone levels but also androgen receptor content might dictate to which extent a certain resistance training protocol can induce muscle growth. In conclusion, it is suggested that resistance training induces muscle hypertrophy mainly via mechanical stress and the following mTOR pathway alongside the androgen receptor-regulated pathway via increased levels of circulating hormones and androgen receptor content. However, resistance training protocols causing severe mechanical stress are often those leading to the strongest hormonal elevations; therefore mechanical and hormonal anabolic signaling might go hand in hand with regard to resistance training-induced muscle hypertrophy.

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22

Endocrine Responses to Acute and Chronic Exercise in the Developing Child

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Introduction

Physical activity plays a vital role in the developing child. Physical activity stimulates somatic growth, influences muscle development, strengthens bones, and contributes to the development of the cardiovascular, respiratory, and thermoregulatory systems. Many of these processes are regulated by the endocrine system: the release of hormones related to growth and development, as well as metabolism. For example, participating in normal physical activity during the day stimulates a pulsatile release of growth hormone (GH) from the anterior pituitary gland. GH, in turn, stimulates muscle development and bone growth. GH also stimulates the use of fat as an energy source during the exercise, which in turn, influences body composition. This is just one example illustrating the importance of the study of pediatric exercise endocrinology as it relates to the understanding of the biological process of growth and development.

The study of pediatric exercise endocrinology has advanced in the past decades expanding the scope of studies to understand responses to exercise sessions of different modalities. The research available suggests that some hormones respond to acute aerobic exercise similarly in children and adults with some differences in magnitude of the change; however, the responses of other hormones are associated with changes related to sexual maturation specifically as it relates to resistance exercise. In adults, excess adiposity modifies some of the metabolic hormones' responses to exercise, and the limited available evidence suggests that the same occurs in youth. Training studies have focused on understanding either changes in hormones that are associated with exercise performance (cortisol, testosterone [TEST], GH, and insulin-like growth factor-1 [IGF-1]) or changes in metabolic hormones related to childhood obesity, namely, insulin and leptin. Therefore, the purpose of this chapter is threefold: first to present hormonal responses to aerobic and resistance exercise in children and if these hormonal responses change as the child goes through sexual maturation, second to describe the present state of knowledge as to whether the degree of excess adiposity influences exercise-induced endocrine responses, and third to describe any known endocrine adaptations to exercise training in children and adolescents.

Acute Hormonal Responses to Aerobic Exercise

Catecholamines

The catecholamines include epinephrine, norepinephrine, and dopamine. In many places outside of the United States, norepinephrine and epinephrine

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are commonly referred to as noradrenaline and adrenaline, respectively. There are actually two sources of these amines: the sympathetic nervous system (SNS), which releases predominantly norepinephrine and the adrenal glands, which secrete mostly epinephrine. The catecholamines have far-reaching effects during exercise influencing many systems within the body. Their response to exercise is intensity and duration dependent. During exercise, larger changes are normally seen in norepinephrine vs. epinephrine in adults compared to children, and this is related to the muscle mass involved [1].

During short-term high-intensity exercise, like graded exercise testing or a maximal aerobic power test (VO_2max) usually lasting 10–15 minutes, studies have shown that catecholamines respond similarly or slightly less in children compared to adults [1, 2]. In particular, Lehmann and coworkers showed that norepinephrine and epinephrine increases with maximal exercise in boys were 25% lower than in adults but fairly comparable during submaximal exercise if accounting for differences in absolute workload [2]. The authors suggested that in children vs. adults, there is a lower maximal capacity of the sympathetic nervous system and the anaerobic system. The lower glycolytic capacity in children has been linked to lower epinephrine release [3].

During submaximal exercise Eliakim et al. noted that 20 minutes of interval exercise (2:1 rest ratio) resulted in a 250% increase in norepinephrine and a doubling of epinephrine [4]. Delamarche showed sex differences in catecholamine release between girls and boys during 60 minutes of endurance exercise [5]. Boys' concentrations were higher than girls and reached peak (specifically norepinephrine) sooner (15 minutes vs. 60 minutes into the exercise). But in general, both sexes responded to acute submaximal exercise with an increase in catecholamine within 10 minutes [5]. In summary, catecholamine release during maximal and submaximal exercise in children presents similar characteristics to adults, but the overall absolute concentrations may be lower in children than adults.

Insulin

In healthy children, glucoregulation is not a major issue during short-term aerobic exercise as most studies find that blood glucose is unchanged or even slightly increased [2, 5]. However, aerobic exercise does affect circulating insulin levels. The key functions of insulin include increasing cellular uptake of glucose, increasing glucose use for metabolism, and reducing beta-oxidation of fats, particularly in striated muscle [6]. During exercise, increases in sympathetic nervous system activation and the adrenal release of the catecholamine inhibit the release of insulin from the pancreas. This decrease in circulating insulin allows for better utilization of fats for energy and prevents most nutrients from being stored when they are needed to produce ATP [6]. Although insulin declines, muscles can uptake glucose from the blood via the contraction-stimulated glucose transporters [7].

In general, several studies have demonstrated that the concentration of insulin decreases in response to acute aerobic submaximal exercise in children [4, 5, 8, 9] as it does in adults. Delamarche and colleagues (1994) studied insulin concentrations in response to 60 minutes of cycling exercise at 60% VO_2max in prepubertal girls and boys [5]. This study showed a 66% decrease in insulin concentrations within 15 minutes of the exercise session. Similarly, Viru et al. showed a decrease in basal insulin concentrations in girls who completed a 20-minutes cycling test also at 60% VO_2 max [9]. Viru and coworkers followed prepubertal girls for 3 years and found that that fasting insulin concentrations increased from prepuberty to mid-puberty and then decreased at the end of puberty if the children were of normal weight [9]. However, the results of Viru and coworkers demonstrated that the decrease in insulin concentrations in response to acute exercise was independent of both, pubertal stage and age. Interestingly, an earlier study by Wirth et al. showed different exercise-induced insulin responses: a decrease in response to acute exercise in prepuberty, no change during mid-puberty, and an increase at the end of puberty [10]. Last, an early study evaluating boys' responses to a maximal graded exercise

test showed that only those boys at pubertal stage 5 showed a significant decrease immediately after exercise [11]. However, this study had a very low number of participants in each stage [11]. However, Pomerants showed that boys displayed an acute decrease in insulin in response to 30-minutes cycling at 95% ventilatory threshold which was more pronounced in those boys in Tanner stages II–V [12]. Hence, there appears that possibly puberty could affect observed decreases in insulin in response to acute exercise, but at this moment, studies present conflicting results.

In recent years the high public interest and appeal in high-intensity interval training (HIIT) exercise led to two studies evaluating acute responses by a research group out of the United Kingdom. Cockcroft and colleagues compared insulin responses during a HIIT session in which postpubescent adolescents completed eight bouts 1-minute long at 90% of their peak power output (PPO) separated by 75 seconds of rest and a moderate-intensity bout long enough to match the work output (~ 206 kcal expended) and examined time-course changes in insulin sensitivity [13]. These authors showed no differences in fasting insulin 24 or 48 hours postexercise in any condition but a lower area under the curve (AUC) for insulin during an oral glucose tolerance test (OGGT) for both exercise sessions compared to the control condition [13]. In young boys' mean ages of 7–10, similar exercise bouts elicited no effect in insulin concentrations immediately after exercise or during an OGTT administered 10 minutes after exercise [14]. In this study the authors indicated the boys had normal insulin resistance and hence perhaps limiting the effect of exercise [14]. This lack of immediate decrease in insulin has also been observed in children of normal weight in response to a resistance exercise stepping protocol in which insulin became lower than rest after 60 minutes into recovery [15]. In summary, insulin concentrations decrease within 15 minutes during aerobic exercise in children, with responses possibly affected by degree of maturity as well obesity and/or insulin resistance.

Glucagon

Glucagon is a pancreatic hormone and has the primary role of stimulating glycogenolysis in the liver and lipolysis at the adipocyte to help sustain metabolic homeostasis. The primary stimulus for the release of glucagon is low blood glucose levels, but increased sympathetic nervous system stimulation of the alpha cells also causes the release of glucagon. Unlike insulin, few studies have evaluated the changes in glucagon during acute exercise in children. In 1994, Delamarche showed a slight increase (10%) in glucagon in response to moderate-intensity cycling in 8–11-year-old children [5]. Glucagon concentrations rose within 15 minutes into the exercise, remained elevated at 30 minutes, and declined by 60 minutes into the exercise session. No differences between the sexes were observed in these responses. The 10% increase could have been related to hemoconcentration (known to occur with exercise) and not a result of greater production. Galassetti and colleagues studied the response of glucagon in adolescent girls and boys (11–15 years) in response to intermittent cycling at ~80% VO_2 max [16]. Glucagon concentrations increased at the end of the 30-minute bout; a change similar to those observed in adults. In contrast, two studies did not show an increase in glucagon in response to exercise. Garlaschi et al. showed no change in glucagon during 30 minutes of aerobic exercise in children [17]. Rubin et al. showed a lower glucagon concentration immediately postexercise compared to baseline in children ages 8–11 years who did a 30-minutes intermittent cycling exercise at 80% of their peak heart rate [8]. The results of two of these studies suggest that in children glucagon increases during exercise, but the other two studies do not support this response; therefore, these contradictory outcomes suggest more research is needed on this hormone to delineate expected responses to exercise for this hormone.

Cortisol

In response to physiological or psychological stress, the pituitary gland releases the adrenocorticotrophic hormone that stimulates the release of cortisol from the adrenal cortex. Its major roles

during exercise are to aid in lipid mobilization, protein breakdown, and glucose formation in the liver. In adults, cortisol increases in response to exercise bouts if exercise intensity is greater than 50% VO_2 max (moderate intensity) and may decrease during exercise if the intensity is lower than 50% VO_2 max [18].

Several studies evaluated changes in cortisol during aerobic exercise in children and presented either an increase, a decrease, or no change in response to the exercise bout. Del Corral showed in 10-year-old male children exercising at 70% VO_2 max, compared to resting conditions, cortisol concentrations increased by 43% and 57% at 15 and 30 minutes, respectively [19]. After 15 minutes of recovery from exercise, cortisol concentrations remained elevated compared to rest. Viru et al. also showed an increase in cortisol in response to 20 minutes of cycling exercise at 60% VO_2max in young girls [9].

Several studies showed no change in circulating cortisol concentrations in response to high exercise intensity. Two studies used an intermittent cycling bout with ten, 2-minute exercise intervals separated by 1 minute of rest at an intensity of approximately 80% peak oxygen uptake VO_2peak [4, 16] which is the peak oxygen used at the highest workload completed in a graded exercise test. Both studies included children with a wide age range and showed that cortisol concentrations were lower 1 hour into the recovery from exercise compared to baseline and immediately postexercise. However, the resting concentrations of cortisol (~ 14 μg • dL^{-1}) were relatively high compared to other studies that evaluated this response. Rubin et al. using the same intermittent protocol in children ages 8–11 years old also showed no immediate cortisol response to exercise, high cortisol concentrations at rest, and a steady decrease during the 1-hour-long recovery [8]. Sills and Cerny who examined 30 minutes of continuous (50% VO_2max) or interval (100% VO_2max at 1:1-minute work-rest ratio) exercise also found no significant effect of either exercise protocol on cortisol [20]. As all of these studies were done in the morning, it is possible that cortisol concentrations were in their morning peak and then decreased following the natural circadian rhythm. If this was the case, the exercise-induced rise in cortisol was perhaps masked by the morning's peak levels.

More recent studies have examined changes in cortisol in response to HIIT, in 2014 Engel compared the responses between boys (most on Tanner stage II) and young men in salivary cortisol reporting comparable changes (~170% increase compared to baseline) [21]. The protocol consisted in completing four Wingate bouts 30-second long separated by a 2-minute recovery using a two W/kg resistance and was conducted in the evening [21]. Another study in trained adolescent cyclists compared a HIIT protocol to a prolonged duration (90-minute), moderate-intensity (60% PPO) protocol [22]. The HIIT required completing four bouts 4-minute long at 90–95% PPO separated by 3-minute rest. HIIT increased salivary cortisol 30 and 60 minutes postexercise, with a decrease after 180 minutes of recovery [22]. Lastly, another study evaluated salivary cortisol responses to protocols of different modalities in boys ages 11–13y matched by total work [23]. For the HIIT protocol, boys completed twice three bouts of boxing (30-second long) and three bouts of cycling (30-second long), all bouts separated by 30-second rest. Salivary cortisol increased from pre-exercise to postexercise approximately 1–1.5-fold [23]. Thus, these three studies show the immediate increase in cortisol in response to HIIT, supporting the notion of an intensity threshold for increases in cortisol.

With regard to the role of puberty or maturation, a cross-sectional study in 235 children ages 2.2–18.5 years showed that there were no associations between baseline cortisol concentrations and either age, sex, or growth [24]. In contrast, in a longitudinal exercise study, Viru et al. showed that the cortisol response to acute exercise was the highest (about a 55.5–66.2% increase) during mid-puberty and developmental stages II and III in comparison to stages IV and V [9]. Hackney and colleagues have recently shown no difference in the magnitude of cortisol increases between adolescents in Tanner stages IV and V and adults completing a graded cycling test [25].

As shown in the previous section, the response of cortisol to exercise is highly variable. Different mechanisms are involved in its secretion depending on the duration of exercise and the intensity [26]. In general, the studies reviewed that showed no exercise-induced cortisol response included intermittent protocols at a moderate/high intensity and were conducted mostly in the morning when the circadian rhythm of cortisol may cause participants to have high pre-exercise concentrations. However, protocols using intermittent exercise at HIIT clearly show increases in cortisol when studies were conducted late morning or in the evening. In summary, studies in youth show increases or no change in cortisol in response to acute aerobic exercise with changes dependent on the intensity and duration of exercise and the time of the testing session. While the only longitudinal study that evaluated the role of pubertal maturation in modulating exercise-induced responses showed a larger magnitude of response as girls matured [9], cross-sectional studies have not supported this finding [25].

Growth Hormone and Insulin-Like Growth Factor Axis

The GH-IGF-1 axis is a hormonal axis that involves GH release from the pituitary and IGF-1 from the liver. This axis is involved in a number of physiological functions including muscle hypertrophy, body composition changes, bone mineral density, and cognitive functioning. The release of GH is controlled by the hypothalamus; it presents a circadian pattern, being greatest the release early after onset of sleep [19]. Once in circulation, GH stimulates hepatic release of IGF-1, which enhances bone growth and development and protein synthesis.

The GH-IGF-1 axis responds acutely to the stress of exercise. Several studies have demonstrated increases in GH in response to aerobic exercise: maximal, submaximal intermittent, and submaximal continuous [4, 8–10, 12, 17, 20, 27, 28]. The increase in GH in response to acute exercise is dependent on pubertal status [9, 10, 29, 30]. Children in more advanced pubertal stages respond with larger peak GH concentrations ranging from 6.9 ± 4.2 to 28.5 ± 14.3 μg • L^{-1} than those children in early puberty [30]. However, it is important to note that not all children in early pubertal stages respond to the stress of exercise with increased GH; and this should be considered when analyzing results [28, 30]. It could be expected that 5–10% of children in early puberty will not respond with increased GH in response to acute exercise.

The reason for the increase in circulating GH during exercise is not well understood. The increase is not related to blood glucose levels, as most studies show increased GH without hypoglycemia [31]. Two studies have shown that GH release is not related to GH-releasing hormone [4, 31]. Gil-Ad has suggested that an inhibition of somatostatin (also known as GH inhibiting hormone) may be involved [31], but this needs further study. The exercise-induced increase in catecholamine has been implicated but not proven [4]. Last, a possible metabolic regulator is the accumulation of hydrogen ions leading to acidosis in the blood during exercise [32].

Insulin-like growth factor-1 is the downstream hormone stimulated by GH. IGF-1 is released because of GH stimulation in the liver and also in the skeletal muscle if exercise presents a stress of sufficient magnitude. The IGF-1 response to exercise is controversial. Eliakim et al. did not find an increase in IGF-1 in children of normal weight [4]. Similarly, Pomerants et al. did not see changes in IGF-1 in boys who completed a 30-minutes cycling bout at 95% ventilatory threshold [12]. Rubin et al. also showed only a small change in IGF-1 with a similar bike protocol [8]. Nemet and Eliakim have suggested that during exercise, the IGF-1 increase is not entirely dependent on the release of GH, given that IGF-1 concentrations peak earlier than GH [33]. In support of this speculation, Rubin et al. showed an increase in IGF-1 concentration after intermittent cycling exercise in children and adolescents with Prader-Willi syndrome who present GH deficiency [8]. The cause of the release of IGF-1 during exercise is not completely understood but appears independent from GH [8, 34]. In summary, in general GH increases acutely with the stress of exercise. The increase in GH is larger with more advanced pubertal stages perhaps related to the increased sex hormones.

Sex Hormones

Surprisingly, only a few studies evaluated longitudinal changes in the concentrations of TEST with puberty and acute responses to aerobic exercise in the pediatric population. Fahey and collaborators (1979) showed the expected increase in TEST with increased pubertal stage but were unable to show a relationship between TEST responses to exercise and puberty in boys [11]. However, this early study had very low number of participants at different pubertal stages likely lacking statistical power to demonstrate differences in exercise responses based on puberty. Pomerants and collaborators, with moderate group sizes (Tanner stage I [$n = 20$], Tanner stages II and III [$n = 20$], and Tanner stages IV and V [$n = 20$]), showed in healthy boys no TEST response to a 30-minute cycling bout at 95% of the ventilatory threshold [12]. Despite increasing TEST concentrations with increasing pubertal stages, there was no relationship between TEST changes during exercise and pubertal stage [12]. In contrast, Viru and colleagues showed increased TEST and β-estradiol during aerobic exercise in girls as pubertal development increased [9]. And, in adolescent males, Hackney et al. demonstrated a higher TEST response to a graded exercise protocol when adolescents were in Tanner stage V vs. stage IV [25]. Last, in boys ages 14 years old, Killian et al. contrasted TEST changes between (1) a moderate-intensity cycling bout (90-minute long at 60% PPO) and (2) a HIIT protocol consisting of four bouts 4-minutes long separated by 3-minute rest at 90–95% PPO [22]. Both protocols showed an immediate increase in TEST after exercise, but after the HIIT protocol, TEST remained elevated 30 minutes into recovery [22]. The available data suggest that TEST concentrations may increase in response to aerobic exercise, and a larger response may be seen at increased pubertal stage.

Leptin

Leptin is a hormone produced by the adipose tissue and is involved in energy balance. A decrease in leptin results from energy imbalance or low energy availability, as well as to carbohydrate availability [35]. Leptin does not play a major role in the regulation of metabolism during excise, and, unless the exercise session leads to a large caloric imbalance, no apparent changes are seen in leptin [36]. Leptin relevance to exercise responses is related to reproductive functions: the release of luteinizing hormone [35] and its association with bone mineral density in female athletes [36]. In girls, leptin appears to be involved as a signal of energy stores in the release of sex hormones, regulating the onset of menses [37]. In boys, leptin concentrations decrease as puberty progresses [38]. Because of its role in the regulation of reproductive function, lower leptin concentrations have been related to bone mineral density problems in young athletes who present a caloric deficit [39]. However, a recent longitudinal study by Donoso and collaborators showed that although dancers had low fat mass and leptin concentrations, over time, their bone mineral density was normal when compared to controls [37].

It appears that there is no acute response of leptin to exercise, and the acute reported increases are likely related to hemoconcentration in addition to variation of the diurnal release of leptin. Kraemer and colleagues were the first to evaluate acute changes in leptin to a discontinuous graded exercise protocol in adolescent girls [40]. This study showed an acute increase in leptin with exercise, but the increase (~15%) could have been due to hemoconcentration [40]. In contrast, Souza et al. showed that a maximal exercise stress test did not influence resting levels of leptin in normal weight or obese children (6–11 years) [41]. Likewise, Pomerants et al. showed no change in leptin in boys completing a 30-minutes cycling bout at an intensity slightly above the anaerobic threshold, and this was independent of pubertal stage [38].Thus, acute exercise does not affect acutely leptin concentrations, but prolonged training can lead to diminished leptin levels perhaps indicating low energy availability [36].

Acute Hormonal Responses to Resistance Exercise

Catecholamines

Catecholamines have been proposed to be important stimulators of hormone secretion during resistance exercise [32]. Three studies,

completed by the same group, showed an increase in catecholamines in response to resistance exercise [42–44]. The first study used five sets of ten knee extensions with 40% one-repetition maximum (1RM) and two sets of knee extensions until exhaustion to evaluate plasma norepinephrine levels in adolescents (Tanner stage V), men, and women [42]. No significant differences were found among the groups for norepinephrine. However, the increase in the peak plasma epinephrine from pre-exercise was about twice as high in boys (5.0 ± 2.6 nmol·L^{-1}) as in men (2.5 ± 0.8 nmol·L^{-1}) and as in women (2.1 ± 0.6 nmol ·L^{-1}) [42]. The second study investigated plasma catecholamine responses in adolescent (15 ± 1 years) and adult male athletes at rest and after two sets of 30 half-squats at 50% 1RM with 2 minutes of recovery between sets [44]. The researchers found lower norepinephrine concentrations in the adolescents (15.7 ± 7.8 nmol·L^{-1}) compared to the adults (32.7 ± 13.2 nmol·L^{-1}), while both groups exhibited an 11-fold increase. In contrast, epinephrine changes in response to resistance exercise were similar for both groups [44]. The third study examined the effects of three sets of knee extensions under the influence of delayed onset muscle soreness (DOMS) also in adolescent boys and adults [43]. During and 15 minutes after exercise, norepinephrine and epinephrine concentrations were significantly greater than rest in both groups [43]. In this study, catecholamine responses in boys and men were similar, indicating no differences between the groups. Using [15] a step-up protocol of six sets of ten repetitions per leg while carrying 50% of lean mass, Rubin and collaborators compared catecholamine responses of children ages 8–11 years to young male adults [15]. This study showed no differences in epinephrine increases immediately postexercise but a higher norepinephrine increase in obese children and adults compared to lean [15]. The studies present different results in the degree of change of the hormones in children compared to adults. Potentially, the lower increase in norepinephrine in youth compared to adults may be related to less muscle mass activation [45].

Cortisol

The importance of cortisol response during resistance exercise is not as well studied as in endurance exercise [26]. However, the release of cortisol during resistance exercise in adolescents may be related to increasing the building blocks for protein synthesis after exercise as well as the reduction of inflammation in response to muscle damage [26]. Most certainly, measurement of cortisol concentrations as an indicator of catabolic stress can help with monitoring of training load during a training season.

Early studies were conducted in 17-year-old boys with weight lifting experience [46, 47]. These two studies used a protocol that required participants to warm up using vertical jumps once every 3 seconds and was followed by a beginning load of 50% 1RM for the snatch. Blood samples were obtained early in the morning and then before the exercise protocol (~2 pm) and 5 and 15 minutes postexercise. The study by Kraemer and collaborators demonstrated acute increases in cortisol in response to exercise at both time points [46]. In the second study, increases in cortisol at 5 and 15 minutes after the same exercise protocol were of a lower magnitude after participants completed a 1-week training program compared to before training [47]. Pullinen et al. noted that following exhaustive knee extensions, cortisol concentrations increased significantly in 14-year-old boys at the end of puberty [42]. Additionally, the change in cortisol concentration in boys (0.13 ± 0.10 μmol·L^{-1}) was greater than in men (−0.05 ± 0.06 μmol·L^{-1}). In contrast, when studying changes in cortisol under the influence of DOMS, cortisol levels were significantly lower in boys than in men during three sets of knee extensions until failure at 40% 1RM [43]. Klentrou et al. compared salivary cortisol between a plyometric lower body exercise protocol (~30-minutes long) and a lower body resistance-training protocol including 3 sets of 12 repetitions in physically active 12–14-year-old boys [48]. Participants demonstrated a decrease in cortisol after exercise following the circadian rhythm pattern but increased by 31% after 30 minutes into recovery after the plyometric protocol [48]. Additionally, Harris and collabora-

tors evaluated cortisol responses to a resistance protocol approximately 12-minute long in which adolescent boys performed four sets of three exercises completed for 30 seconds with a 30-second break in between [23]. The protocol of moderate to high effort led to a onefold increase immediately postexercise [23].

Resistance exercise protocols trigger an immediate increase or an increase during recovery in the concentration of cortisol in adolescent boys. The lower magnitude in the increase in cortisol during DOMS in youth in comparison to adults could be related to less: (1) less total muscle mass being involved thus less damage, (2) less physical pain which would elicit less a stress response, or (3) a faster recovery rate from muscle damage. Moreover, the increase in cortisol during recovery from exercise in the plyometric exercise protocol [48] could perhaps indicate a higher inflammatory response triggering the release of this hormone. This is a fertile area for study as cortisol measurements could be used to reflect changes in physiological stress during the different phases of training season in young athletes and to assess adaptations to the training load. There are no studies that have evaluated changes in cortisol with resistance exercise in girls.

Growth Hormone

GH secretion relates more closely to peak exercise intensity than exercise duration or total exercise volume. In terms of resistance exercise, as summarized by Kraemer and Ratamess (2005), many factors influence the magnitude of the response of GH including the muscle mass, the intensity, the rest period in between sets, the total volume, and the amount of work [32]. In addition, the change in GH is closely related to the increase in lactate, which reflects the effect of all factors previously presented [32]. Several studies in adults (for a complete review, see Kraemer and Ratamess) showed that during exercise the accumulation of hydrogen ions in the blood regulated the release of GH [32].

Kraemer and collaborators showed increases in GH immediately after a weight lifting session in experienced 17-year-old weight lifters [46]. Pullinen and colleagues evaluated changes in GH following exhaustive knee extensions in 14-year-old boys. GH increased tenfold from about 1.5 $\mu g \cdot L^{-1}$ at rest to about 15.0 $\mu g \cdot L^{-1}$ after exercise [42]. However, the authors indicated that after taking the change in plasma volume into consideration, the change in GH in response to exercise was not statistically significant, possibly because of the large standard deviations. In a follow-up study in 2010, Pullinen et al. observed that adolescent boys had significantly increased GH levels during and after three sets of exhaustive knee extensions with a load of 40% 1RM compared to preexercise [43]. Also, the boys displayed significantly greater GH levels than men during all stages of the exercise and recovery. Using [15] a lower body protocol, Rubin et al. showed significant increases in GH 15 minutes into recovery from exercise with no adult to children differences [15]. However, this study, as the one by Pullinen, showed large variability in the GH responses to exercise, hence increasing the difficulty to compare responses between adults and children [15]. The study by Rubin included a group of girls and boys and compared their responses to lean adult males. From the information available, it is suggested that children and adolescent respond to resistance exercise with acute increases in GH. The magnitude of the response in children is unclear at this point in terms of it being lower, higher, or similar to adults, and no information is available in females alone.

Testosterone

During childhood both girls and boys display similar levels of TEST; however, during mid-puberty (about stage III) concentrations of TEST and its free form increase significantly in boys [49]. TEST study as it relates to resistance exercise is related to its role in increasing muscle protein anabolism and its contribution to the release of GH. Heavy resistance exercises requiring the use of large muscle groups, moderate to high volumes, and short rest periods have all been shown

to increase TEST levels after an acute bout of resistance exercise, primarily in men [50].

The response of TEST to resistance exercise in children has been evaluated in a few studies. In general, all studies found an acute increase in TEST following resistance exercise [42, 44, 46]. In an early study, Kraemer et al. observed a 32% increase in TEST in junior elite weight lifters (17.3 ± 1.4 years) after a traditional weight lifting session involving high-intensity, high-speed resistance-training exercises [46]. In a subsequent study in 14-year-old boys, free TEST levels increased by 2.1 ± 2.6 pmol·L^{-1} after exhaustive knee extensions [42]. Later, Pullinen et al. showed significant increases in TEST after an exhaustive half-squatting exercise session (2 sets of 30 repetitions at 50%1RM) in adolescent male athletes [44]. The adolescent boys experienced a lesser increase in TEST levels compared to adult males. In a follow-up study, Pullinen et al. observed no changes in either free or total TEST in adolescent males during and after three sets of knee extensions under the influence of DOMS suggesting a blunting of TEST response under conditions of inflammation such as DOMS [43]. Last, Klentrou compared TEST responses between a plyometric exercise protocol and a resistance exercise protocol [48]. The increases observed in TEST in the 12–14-year-old boys during resistance exercise (~27 ± 5%) were larger than the increase shown during the plyometric protocol of ~12 ± 6%. Moreover, the increase observed in response to the resistance exercise protocol was larger than changes observed in the previous studies [42–44] perhaps due to the use of multi-segmental exercises, thus creating a larger load [48]. TEST concentrations returned to pre-exercise levels within 30 minutes into recovery [48]. In summary, TEST appears to increase in response to acute resistance exercise in adolescent boys and is normalized shortly after exercise. The magnitude of the increase in TEST is lower in adolescents than adults but appears to be related to the load and muscle mass involved. Evidence is lacking on the TEST response to resistance exercise in young children and/or girls.

The Role of Adiposity as a Modifying Factor of the Hormonal Responses to Exercise

Catecholamine release during exercise contributes to lipolysis, glycogen breakdown, and sustaining euglycemia. These effects are important for individuals who are overweight or trying to lose weight. Given the increasing rates in pediatric obesity in the last decades, researchers in pediatrics have investigated exercise-induced hormonal differences in obese children and adolescents. Eliakim et al. demonstrated a blunted catecholamine release in response to a 30-minute, high-intensity, intermittent cycling bout in obese children vs. their lean counterparts [4]. In this study, obese children responded with increased catecholamine concentrations to exercise, but the degree of increase was lower than in lean children [4]. Likewise, Rubin and collaborators also showed a lower epinephrine and borderline lower norepinephrine response to a similar protocol in obese children compared to lean children [8]. Eliakim et al. suggested that the blunted adrenergic response could be linked to a blunted GH release in obese children [4]. In contrast, a later study involving adolescent girls did not find significant differences in the catecholamine increase after about 7 minutes of maximal cycling when comparing obese, overweight, and lean participants [51]. However, in the study by Zouhal and collaborators, the lean group had fairly high levels of body fat (~30%), while the overweight group had about 37%, and the obese group had 42% of body fat. In contrast, in the two studies that showed a blunted catecholamine response, there were distinct differences in body fat between the groups of lean and obese children. Therefore, it is possible that since the participants in Zouhal and coworkers' study did not have very drastic differences in body fat content, this lack of differences may have masked possible differences in catecholamines' response [51]. Thus, some evidence suggests that in children as in adults, high levels of body fat may blunt the catecholamine response to acute exercise.

Obesity in childhood is associated with high baseline concentrations of insulin and insulin resistance [52]. Acute aerobic exercise decreases insulin concentration in children as in adults [4, 8]. And the magnitude of the decrease appears larger in the obese children compared to normal weight children [4]. In a follow-up study also using a 30-minute intermittent exercise bout at 80% VO_2max, Tran et al. showed that the decrease in insulin in children was not just only dependent on obesity per se but also on the initial (resting) concentrations of insulin pre-exercise [53]. In a step-up resistance-training protocol of low-to-moderate intensity, Rubin [15] and collaborators (2014) showed that the obese children exhibited an immediate decrease in insulin after exercise, but this was not observed in the lean children [15] supporting the previous research in response to aerobic intermittent protocols [53]. The larger decrease of insulin in obesity appears to be related to the higher resting insulin concentrations in the obese children compared to normal weight youth and not to a lower concentration at the end of exercise. Tran et al. speculated that with greater decrease in insulin in obese states with high insulin concentrations at baseline, there may be a reduced adrenergic adaptation to exercise, which may cause in turn more adrenergic outflow to the pancreas inhibiting insulin production and release [53]. In contrast, from the limited data comparing glucagon responses to acute cycling between normal weight and obese children and adolescents, no differences can be pointed out [8, 17, 28].

With regard to cortisol, two studies comparing obese and lean children did not show any differences in the response of cortisol to a similar exercise protocol between the groups [4, 8]. Eliakim et al. noted that high-intensity interval exercise resulted in a 20% increase in cortisol in normal weight children and approximately a 5% decline in obese children exercised at the same time of day; however these differences were not statistically significant. Rubin et al. also showed that normal weight and obese children responded similarly to an intermittent aerobic protocol [8]. At this point, there is not enough data to demonstrate that obesity affects cortisol response to exercise. Since cortisol increases fat utilization during exercise, which could be of benefit for obese children, it would be important to have further studies evaluating changes in cortisol in children in response to exercise perhaps conducted during the afternoon to diminish the influence of the circadian rhythm.

As mentioned earlier, GH release during exercise is affected by adiposity. In 1975, Garlaschi et al. demonstrated a decreased GH secretion in obese prepubertal children compared to their lean counterparts [17]. Similar findings were later presented by Eliakim et al., showing a larger GH response to a 30-minutes intermittent cycling bout in normal weight vs. overweight children and adolescents [4]. Similarly, Oliver and colleagues showed that the blunting of the GH response to exercise was dependent in a dose-response manner on the degree of obesity [29]; the more obese the children, the larger the blunting of the GH response. Puberty also appeared to influence this response independently of adiposity [29]. Last, in a study comparing responses in a rare form of genetic obesity, Rubin and associates also showed the blunting of GH in response to intermittent aerobic exercise in obese children ages 8–11 but not in lean children that acted as controls [8].

Eliakim and coworkers also reported a small (~15%) immediately postexercise increase in IGF-1 in obese children but not in normal weight children [4]. The increase shown in IGF-1 in obese children in this study [4] is similar to the observed increase in IGF-1 in obese and lean children from Rubin's study [8]. Based on the available evidence, obesity decreases the magnitude of the GH response to exercise. However, it does not appear to alter downstream mediators such as IGF-1.

Nemet and Eliakim have speculated that blunted GH response could be responsible for a diminished response in obese states in body composition changes because of exercise training [33]. It is possible that the combined reductions in catecholamine, GH, and perhaps cortisol could result in obese children utilizing less fat than normal children during exercise, increasing the difficulty of weight loss. The elevated insulin found

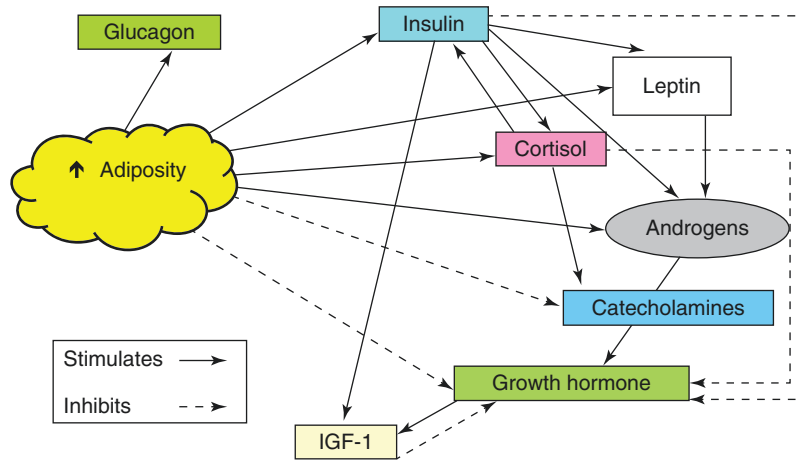

Fig. 22.1 Proposed influences of adiposity in the release of some key hormones during exercise

in obesity may be one reason behind the lack of responsiveness of the GH-IGF1 axis [54]. In terms of the blunted catecholamine response to exercise, it may be explained perhaps by the interconnection between the adrenal cortex and medulla. It is possible that the increased insulin concentration in obesity increases cortisol and that blunts the adrenal medulla release of catecholamine [55]. Figure 22.1 presents some of the demonstrated relationships and also some other possible interactions [18]. Although the mechanisms behind the alteration of some endocrine axes by excess adiposity are not fully understood, it is clear that adiposity in childhood and adolescence alters the functioning of the endocrine system not only at rest but also in response to exercise.

Chronic Changes in Hormones with Exercise Training

General Responses

Little is known about the effects of exercise training on the hormonal responses of children. Most of the studies have evaluated changes in cortisol, insulin, the GH-IGF-1 axis, or reproductive hormones. It appears data are not available on chronic changes in glucagon or catecholamines to exercise training for which a lower increase in response to exercise would be expected.

Insulin

Changes in insulin concentrations in children and adolescents in response to training protocols have been the focus of attention since the early 1990s. Improvements in insulin concentrations have been demonstrated in laboratory-controlled, school-based, and community-based studies involving aerobic exercise [56]. The largest changes in insulin concentrations in response to interventions had been observed in obese children or adolescents [57] or those who present the highest insulin at baseline [58]. Decreases in basal insulin concentrations have been associated with increased fitness [58]. Insulin may respond to exercise training independently of changes in body mass, but larger changes are observed with reduced adiposity and in a relatively short period of time (8 weeks) [56, 59].

Recently, studies have evaluated insulin changes in response to HIIT. Studies have compared HIIT to sustained low- and moderate-intensity aerobic exercise. Racil and collaborators studied obese adolescent females completing an intervention 12-week long that required three exercise sessions per week [60]. The HIIT protocol consisted on completing two sets of 6–8 bouts 30-second long at 100–110% of the speed associated with VO_2 peak separated by 30 seconds at the speed associated with 50% of the VO_2 peak. The moderate-intensity protocol used a similar approach, but the intensity was at 70–80% of the speed associated with VO_2 peak. This study showed a moderate decrease in fasting insulin in

both exercise modalities but a larger decrease in the HIIT compared to MIIT (26% vs. ~17%) [60]. Prado et al. conducted a study also in obese adolescents at Tanner stages III and IV, who completed either a HIIT protocol or a sustained low-intensity aerobic exercise matched on energy expenditure at 350 kcals. Interestingly, they did not observe any changes in fasting insulin in either group [61]. Last, Tenorio and collaborators evaluated the same protocols as Prado and coworkers but completed over a period of 24 weeks; they demonstrated a small decrease in fasting insulin 28.14 ± 15.35 vs. 27.75 ± 15.35 (μU/ml) only in the group completing the HIIT [62]. In summary, changes in insulin in response to chronic aerobic exercise have been largely studied in pediatric population as in adults, and it is currently the hormone we seem to better understand in children. If the exercise dose is sufficient (e.g., 3 days a week) and for a minimum of 20 minutes, aerobic exercise training reduces high fasting insulin as demonstrated by Davis and colleagues [59]. Additionally, in children as in adults, aerobic exercise training reduces the area under the curve for insulin in response to an OGTT in a dose-dependent manner, with larger doses being more effective (e.g., 40 minutes being more effective than 20 minutes) demonstrating the insulin-sensitive effect of exercise training [59].

Cortisol

In an early study in two subsets of mostly prepubescent female gymnasts, the concentration of cortisol was evaluated at baseline and over 3 days of intensive training (3.5–5.5 hours long) [63]. No changes in cortisol were observed in this study. Kraemer also evaluated chronic changes in cortisol in adolescent female runners over a 7-week period but did not find any changes in either resting or exercise concentrations [40]. Di Luigi et al. (2006) reported that the cortisol responses to soccer training decreased as subjects aged, suggesting that the intensity of the training program decreased or they were adapting to the training protocol [64]. A few subsequent studies in team sports evaluated changes in cortisol over the training season. Over 6 weeks of handball training, no changes were observed in adolescent boys in resting cortisol [65]. And likewise, no changes were observed after 11 weeks of soccer training in adolescent boys [66]. In contrast, in basketball players ages 12–14 years, cortisol was higher after the competitive season (14 weeks) compared to after the preparatory season (6 weeks) and the preseason [67]. In a longitudinal study, Daly and coworkers (1998) followed a group of prepubertal gymnasts and a control group of boys of similar age through the different phases of training in a year [68]. There were no differences in cortisol concentrations because of the different training phases or between the groups. However, the TEST/cortisol ratio was elevated in the phase of strength/conditioning only in the gymnast indicating a possible anabolic state [68]. And, Vänttinen et al. showed that soccer players ages 11, 13, and 15 followed over 2 years demonstrated higher cortisol than non-athletes suggesting perhaps more stress induced by the exercise training [69]. Last, in obese boys who completed a 12-week exercise intervention, cortisol concentrations decreased but not in the control group [70]. Perhaps in this later study, the children had high cortisol concentrations secondary to the excess body fat and the inflammatory state caused by the obesity. More studies are needed to clarify the chronic effect of exercise training on the baseline and exercise concentration of cortisol and their significance, but the majority of research including short-term HITT [71, 72] shows no effect or increased cortisol concentrations at rest related to training.

Growth Hormone-IGF-1 Axis

With the increased participation in competitive sports at a young age, a major concern has been that exercise training during childhood could stunt growth, because of negative influences on the GH-IGF-1 axis. This concern was partially based on the stress generated with the training and also because of the possible negative caloric balance induced by the training itself [73]. Additionally, carbohydrate availability during periods of caloric restriction may affect IGF-1 secretion [74] as well as increases in GH [73].

In an early study in prepubescent female gymnasts, IGF-1 concentrations were evaluated over 3 days of intensive training 3.5–5.5 hours long [63, 69]. The study demonstrated that IGF-1 concentrations decreased by 25% after 3 days of training with no concurrent changes in GH. No measurements of body mass or caloric balance were conducted, and a control group was not included [63]. In a follow-up study, adolescent girls went through a 5-week endurance-training period with no caloric restriction and demonstrated a decrease in IGF-1 but no change in GH or its binding protein [75]. This study included a control group not receiving the intervention [75]. Roemmich and Sinning also evaluated changes in GH, GH-binding protein (GHBP), IGF-1 in undernourished male adolescents who went through a 3–4-month wrestling season [76]. The adolescents showed increased GH concentrations and concomitant decreased GHBP and IGF-1 concentrations [76]. However, during the postseason, all hormones returned to baseline concentrations. Roemmich and Sinning speculated that GH receptors decreased, and in response, GH secretion increased to compensate for the partial GH resistance possibly triggered by the negative caloric balance [76]. In a later study, Eliakim and colleagues studied male adolescents who went through a 5-week exercise training program and a matched control group [77]. In the training group, IGF-1 and GHBP concentrations decreased similarly to the study on wrestlers (about 12% and 21%, respectively). However, the adolescents in this latter study were in eucaloric state and experienced no changes in body mass. Eliakim and coworkers speculated the existence of a transient decrease in GH sensitivity induced by exercise training [75].

This topic has been a focus of study of the group of Eliakim and coworkers, and they have proposed that there may be two phases in the response of the GH-IGF-1 axis to exercise training [33]. The initial phase is characterized by decreased IGF-1 and transient resistance to GH action with IGF-1 perhaps produced locally and not as much systemically [33]. In the second phase, if energy balance is sustained, there is a rebound of systemic IGF-1 as well as an increase in GHBP [33]. Therefore, there seems to be enough evidence to support that exercise training does not have a negative impact in the GH-IGF-1 axis if caloric balance and adequate carbohydrate are sustained. This is also seen after 2 weeks of HITT [71, 72]. Equally important, short periods of training with negative caloric balance may cause transient disruptions in GH and IGF-1.

Sex Hormones

The increased number of children and adolescents participating in competitive sports and the high level of exercise training completed by these young athletes and the possible risks associated with it have gained considerable attention. In particular, delayed menarche and alterations to the hypothalamic-pituitary-gonadal (HPG) axis have been documented in female young athletes such as runners [78]. The pediatric exercise science community undertook several studies to determine the possibility of alterations and their severity in both sexes.

In male children, Rowland and collaborators showed that an 8-week training season in cross-country male runners did not affect either total TEST or free TEST [79]. Later, the functioning of the HPG axis in undernourished wrestlers was evaluated before the season, late in the season, and after the season ended [76]. The study included a group of age-matched healthy male controls. The wrestlers' total TEST and free TEST concentrations decreased during the season and returned to baseline during the postseason. These changes were unrelated to estradiol, prolactin, or cortisol, which modulate the activity of the HPG axis. Only the levels of free TEST fell below the normal range. Gorostiaga and collaborators evaluated changes in TEST in adolescent handball players comparing 6 weeks of regular training schedule showing no effect of the training [65]. As a follow-up, this group showed no change in TEST after 11 weeks of soccer training also in adolescent players [66]. And, Brunelli and coworkers showed no change in TEST in adolescent basketball players between preseason, a preparative phase 6-week long and after the 14 weeks of competition [67]. Last, Vänttinen et al. compared TEST values of soccer players ages 11, 13,

and 15 years to age-matched youth who were not athletes over a 2-year period [69]. These results demonstrated no differences in TEST concentrations between athletes and nonathletes but the expected increase with increased age and maturation [69].

The evidence from these studies indicates that in pubertal and postpubertal males, aerobic exercise training of short and long duration may not affect the functioning of the HPG axis negatively; however, a longer training season accompanied with caloric deficit may affect the HPG axis, specifically TEST release. Nevertheless, it appears that once the athletes are in the postseason period, the levels of these male sex hormones return back to normal.

In girls, Jahreis et al. presented an increase in TEST concentration during short (3 days) and longer (7 weeks) training periods [63]. It was shown in young female gymnasts that over 3 days of intensive training (3.5–5.5 hours a day), concentrations of total TEST increased by approximately 25% with no significant change in dehydroepiandrosterone (DHEA) and its sulfate form (DHEAS) [63]. Similar findings were shown by Kraemer and colleagues during a 7-week competitive season for track and field in female adolescents [40]. Changes in the hormones were evaluated at rest and in response to a graded discontinuous exercise protocol to volitional fatigue. No effect of training was found for DHEA or DHEAS. In contrast, TEST concentrations increased over time. At the end of the season, the adolescents presented a larger exercise-induced increase in TEST compared to baseline [40], similar to data presented by Viru [9]. Overall, exercise training appears to increase TEST concentrations in girls. The data of one study in girls suggest that increases in TEST can occur after 3 days of training [63].

With regard to HITT, two studies have evaluated changes in TEST at rest and in response to exercise after 2 weeks of training. The first study included junior triathletes who completed 16 sessions of HITT over 2 weeks [72]. These athletes showed an increase in TEST at baseline after the 2 weeks [72]. A follow-up study in male junior triathletes ages 12–18 who completed 12 sessions of HITT over 2 weeks showed comparable TEST before exercise pre- and post-training but a decrease in TEST with acute exercise [71]. Thus, there appears to be no definite changes in TEST with training but changes being specific to training modality and sex with potential increases in TEST in girls and in adolescent boys in response to the stress of the exercise.

Leptin

Changes in leptin in relation to exercise training in the pediatric population has had two different scopes. One area of research has centered in understanding changes in leptin during training in female athletes (particularly those engaged in aesthetic sports), and another has been in relationship to childhood obesity.

The study of leptin changes in response to exercise training has been related to the role of leptin in the regulation of reproductive function. It has been proposed that the increased caloric expenditure induced with training and potentially the transient decrease in energy available for body functions may impair body fat development and with that induces decreases in resting concentrations of leptin [36]. In general, the concentrations of leptin have been found lower in young athletes than in nonathletes [36, 80]. However, some studies have shown no changes in basal leptin such as in prepubertal gymnasts or in runners [81]. In normal weight female adolescents showed no change in basal leptin concentrations after a 7-week running competitive season [40]. Additionally, Kraemer and colleagues also did not see differences pre-posttraining season in the acute leptin response to exercise. For an extensive discussion on this topic, please see review by Jurimae [36].

The study of changes in leptin with exercise training in obesity has centered on the fact that excess body fat increases resting levels of leptin in children [41, 57]. Studies have shown that leptin concentrations decrease in response to exercise training specifically when there are decreases in fat mass. For example, 4 months of aerobic training in obese 7–11-year-old children resulted in a decrease in baseline leptin [57]. The decrease in basal leptin concentrations was

greater in those children who had the highest concentrations at the beginning of the program, and the decrease was related to decreases in body mass and the training protocol itself [57]. When children stopped participating in the training program, their leptin concentrations increased. Since then, several studies have now followed demonstrating decreases in leptin concentrations in obese children or adolescents completing physical activity or lifestyle interventions at least 3 months long [70, 82, 83].

The new wave of studies evaluating changes in leptin with exercise intervention has involved the use of HIIT as the training modality with adolescents. Studies have compared using HIIT protocols vs. either low-intensity [61] or control conditions [60]. Studies have shown that HIIT is an effective training modality to help decrease leptin in adolescents. Additionally, Tenorio and collaborators have also showed that HIIT three times a week for 24 weeks was more effective in decreasing leptin than low-intensity aerobic exercise (−22.8% vs. −13.6%) when energy expenditure in both conditions was of 350 kcals [62]. Therefore, in obese children and adolescents, exercise training of sufficient duration that induces decrease in body fat leads to reduced leptin concentrations.

Resistance Training

Resistance training leads to increase muscular strength due to neural adaptations and muscular hypertrophy. Data in adults have shown that the main stimulus for increasing protein synthesis during resistance exercise is the mechanical deformation in the muscle. While hormones and the nutritional state can contribute to muscular enlargement, it is clear now that the positive effect on the protein synthesis cascade is initiated because of the mechanical load.

In children, the fact that significant improvements in strength with resistance training are observed in the absence of hypertrophy indicates that neurologic mechanisms likely play a large role in strength development during childhood [84]. These factors include, but are not limited to, increases in motor unit firing rate, recruitment, or conduction velocity. Strength levels increase similarly in girls and boys until the onset of puberty. Once puberty begins, anabolic hormones and growth factors play a large role in muscular enlargement in boys [50]. In this section the discussion will focus on the role of resistance training on insulin, cortisol, GH, and TEST because of their clinical relevance or their anabolic or catabolic properties.

Insulin

A few studies have evaluated chronic changes in insulin concentrations in response to resistance training in youth [85]. In a randomized controlled trial, Shaibi and colleagues demonstrated that in overweight males, two resistance-training sessions a week for 16 weeks increased insulin sensitivity by 45% in 90% of the participants [86]. Fasting insulin concentration also decreased in the intervention group, but the change was not statistically significant [86]. Van Der Heijden also showed improved hepatic insulin sensitivity in obese adolescents completing a resistance-training program but no change in peripheral insulin sensitivity or fasting insulin [87]. A follow-up randomized controlled trial in 54 obese adolescent girls and boys showed no effect of the resistance-training protocol in insulin concentrations or insulin sensitivity [88]. Davis et al. suggested that perhaps the later study lacked statistical power as the exercise dose induced gains in muscle strength but no significant change in insulin parameters [88]. There are no studies that have determined the minimal dose of resistance training that can lead to increases in muscular strength and improved insulin sensitivity in children.

Other studies have either compared the role of aerobic training to resistance training or a combined protocol. De Mello evaluated the combined effect of aerobic (30-minute) and resistance training (30-minute) to aerobic training alone (60-minute) performed 3 days per week for 1 year in obese adolescents [89]. This study showed that aerobic plus resistance training induced decreases in insulin at 6 months and 12 months compared to no decreases with aero-

bic training alone. However, this group also had lower energy intake compared to the aerobic training, so it could have been the dietary changes and not the exercise alone that induced the decrease in insulin [89]. Lee et al. reported that obese boys who completed a 3-month study assigned to either an aerobic or a resistance-training group (180 minutes/week) did not show any changes in fasting insulin compared to the control group [90]. However, boys in the resistance-training group demonstrated improved insulin sensitivity [90]. In contrasts, the same protocol in obese adolescent girls showed that was aerobic and not resistance training that led to reductions in fasting insulin concentration and increased insulin sensitivity compared to the control group [91]. Comparing also different exercise types, Racil and collaborators evaluated in obese adolescent females the potential of combining aerobic training (HIIT) with plyometric exercises [60]. The intervention group completed 3 days a week for 12 weeks of this combined protocol and showed a 29.5% decrease in insulin concentrations [60] not observed in the control group.

It appears that resistance training has the potential to be an effective modality in decreasing fasting insulin and improving insulin sensitivity in children or adolescents. Possibly, the twice a week routine may not be sufficient frequency of sessions a week given that exercise-stimulated insulin sensitivity lasts up to 48 hours postexercise [92] as studies using a 3-day a week routine were more effective. Likely, it appears the best strategy will likely include a combination of aerobic and resistance training.

Cortisol, Growth Hormone, and Testosterone

A few studies, to date, have evaluated changes in cortisol, GH, and TEST concentrations in response to resistance training. An early study by Kraemer et al. examined acute responses to a typical weight lifting training session in elite 17-year-old weight lifters and compared their responses based on having less or more than 2 years of training experience; Kraemer showed that the more trained group exhibited a larger TEST increase in response to the protocol, but this difference was not observed for cortisol or GH [46]. A very short (1 week) intervention with an increased volume of heavy resistance exercise in junior weight lifters showed that they demonstrated no change in resting cortisol or GH, lower resting TEST, and a lower magnitude in the response for cortisol, GH, and TEST to exercise. In addition, the TEST-to-cortisol ratio (T/C) was lower at rest after the training intervention [47].

Gorostiaga and collaborators evaluated changes in cortisol and TEST in adolescent handball players comparing 6 weeks of regular training schedule to 6 weeks of an added heavy-resistance-training routine twice a week [65]. The addition of the heavy resistance training showed no change in either cortisol or TEST [65]. As a follow-up, this group showed no change in cortisol but a 7.5% increase in TEST after 11 weeks of resistance training (power training) in combination with soccer training in adolescent players [66].

Two studies evaluated changes in cortisol, GH, and TEST after 1 year of resistance training. Mero et al. showed no differences in resting cortisol or GH because of training [93]. In contrast, these authors showed increases in basal TEST from 2.92 ± 1.04 to 5.81 ± 1.33 $nmol \cdot L^{-1}$ in the junior male athletes (10–12 years) participating in an athletic program but not in the nonathletic peers [93]. Tsolakis also studied the effect of long-term resistance training (12 months) on hormonal levels of 12–13 years males who practiced fencing and a control group [94]. The results of Tsolakis study indicated that the training program had no effect on serum GH or TEST concentrations. The performance variables such as handgrip strength and jump height were also similar between the fencers and the control group, which questions the stress of the training program. However, the fencers demonstrated a significant increase in leg cross-sectional area, while the control group did not. It is possible that an increase in cross-sectional leg area unaccompanied by an increase in resting TEST levels may have occurred due to acute increases in protein synthesis unrelated to TEST.

In another study Tsolakis and colleagues evaluated hormonal responses after 2 months of resistance training and 2 months of detraining in prepubertal (11–13 years) and pubertal boys (14–16 years) [95]. As expected, pre-training mean TEST levels were approximately three times greater in the older boys (14.6 ± 4.2 $nmol \cdot L^{-1}$) compared to the younger boys (4.9 ± 5.7 $nmol \cdot L^{-1}$). In response to the training program, TEST increased in the young boys (124%) and in the old boys (32%). Following the 2-month detraining period, mean TEST levels did not significantly change in either group when compared to the post-training levels. The large increase in TEST observed after training in the young boys might have been due to the effect of the resistance training coupled with the normal increase in TEST due to puberty. In a follow-up study using a similar design with a control group, Tsolakis and associates concluded that after 2 months of resistance training, boys (11–13 years) experienced a doubling of basal TEST (4.9 ± 5.7 to 10.9 ± 6.2 $nmol \cdot L^{-1}$) independent of changes in TEST because of maturation [96]. As expected, muscle strength decreased after detraining, but TEST levels were maintained throughout the detraining period.

Last, a study which examined salivary hormonal responses and performance changes during 15 weeks of aerobic and resistance training in elite wrestlers ages 17–18 years old showed 2% and 77% increases in cortisol concentrations at 12 and 15 weeks, respectively [97]. However, this study observed no significant change in TEST concentrations over the training period resulting in a decreased T/C ratio. The authors reported a negative association between the mean cortisol concentrations and the percent change in power clean and a positive association between the T/C ratio and the percent change in power clean. These authors concluded that monitoring cortisol and the T/C could be helpful to determine training loads, as potentially the increase in cortisol could be so high that could affect the anabolic/catabolic balance (assessed by the T/C ratio) and in turn affect the development of muscular strength [97].

The literature regarding resistance-training-related changes in anabolic and catabolic hormones show no effect on basal concentrations of cortisol and GH in boys or old adolescent males. Some short- and long-term studies suggest that TEST increases independent from the increases that naturally occur due to puberty, but the evidence is conflicting. Acutely it appears that in the initial phases of training, TEST may or not decrease at least in adolescent boys. Increases in muscle strength in response to resistance training during childhood and adolescence appear to mostly depend on neural adaptations [85, 96], and authors have speculated that the role of TEST or GH may be more related to their effect on the nervous system [85, 98]. Considering there are only a few studies that have evaluated changes in skeletal muscle strength and changes in hormones with puberty, it would be premature to conclude that changes in anabolic hormones such as GH or TEST are unrelated to gains in muscle mass or strength during childhood and early adolescence.

Summary and Future Directions

The studies conducted have made progress to quantify the effects of different types of exercise on the hormonal responses of children and adolescents as it is noted in Table 22.1. However, there are still hormonal responses to varying exercise conditions, for which there are no descriptive data. The majority of endocrine studies have been completed on short-term aerobic exercise with lately more studies assessing using interval training or resistance as exercise modalities. Little information exists on prolonged aerobic exercise, possibly because naturally children do not choose to participate in this form of exercise. There are some data on aerobic exercise training hormonal adaptations at rest, but very little knowledge on how the aerobically trained children respond to exercise. Data are starting to accrue on resistance exercise, but there is little information on the adaptations that occur with resistance training. Furthermore, there are very limited studies on the mechanisms causing the

Table 22.1 Summary of hormonal responses of children and/or adolescents to acute and chronic exercise

	Aerobic exercise Acute effects		Aerobic exercise Training effects			Resistance exercise Acute effects	Resistance exercise Training effects	
Hormone	GXT	Submaximal	Rest	GXT	Submaximal	During exercise	Rest	During exercise
Catecholamine	↑	↑	↓		↓	↑	?	?
Insulin[a]	NC↓	↓	↓	?	?	↓ or NC	↓ or NC	?
Glucagon	?	↑ NC ↓	?	?	?	?	?	?
Cortisol	?	↑ or NC	↓ ↑NC	?	↓ NC	↑	↑ or NC	↓ or NC
Growth Hormone	↑	↑	↑ NC	?	NC	↑	↓ or NC	↓ or NC
IGF-1	?	↑ NC	↓	?	?	?	?	?
TEST[b]	↑ ?	↑ NC	↓ ↑	?	↑	↑	↓↑ NC	↓↑
Estrogens	?	↑ ?	?	?	?	?	?	?
Leptin[a]	NC	NC	↓ NC	?	NC	?	↓ or NC	?

GXT = graded exercise test; ↑ = increase; = decrease; NC = no change;? = presently unknown or insufficient information

[a]Training effects on insulin and leptin at rest are different in normal weight showing NC and obese showing a decrease

[b]Training effects on TEST at rest are different by sex with males showing a decrease and females an increase

specific exercise responses or what is the significance of the responses. In some cases, like GH, catecholamines or cortisol, one can speculate as to the mechanisms based on the adult literature. However, a number of the hormonal responses are interactive, and there is little or no understanding of these interactions in children. For example, insulin is influenced by the catecholamine and the SNS. Both the SNS and the catecholamine responses to exercise are less in children than adults, yet the decline in insulin during exercise approximates the adult response. Resting GH concentrations differ with pubertal status, but postexercise concentrations appear similar among pubertal stages; so why the similarities?

Obesity appears to modify the hormonal responses to exercise. Could these modifications partially explain why obese children have difficulties losing weight via an exercise program? Could the modification be genetic and partially explain why the weight gain occurs? Quite interestingly, obese children typically have GH and IGF-1 levels similar or slightly lower than normal weight youth. So the question becomes, why does the obese child typically have a faster linear grow faster rate and earlier pubescence than a normal weigh child? There many important questions in need of future study. One fruitful area for expanding the current research is the study of myokine acute and chronic responses to exercise. Understanding cross talks between the skeletal muscle, the adipose tissue, and the endocrine system will help better define the adaptations in response to exercise training and the changes occurring with growth. The study of pediatrics exercise endocrinology is clearly making headway, but still there are many questions unanswered.

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Exercise in Older Adults: The Effect of Age on Exercise Endocrinology

23

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Introduction

The world's population is aging at an unprecedented rate. In 2000, 606 million people were aged 60 and over, and the number of older adults is expected to triple by the year 2050, resulting in a global population of nearly two billion senior citizens. The rate of growth for the older adult population is significantly greater than the total population growth, meaning that approximately one in five people in the world will be over the age of 60 by 2050 [1]. This dramatic demographic change will have a significant economic and social impact on society.

Characterized by a decline physical in well-being, aging is marked by changes in body composition (increase in body fat, loss of muscle mass and bone density), decreased muscular strength and power, decreased aerobic power, and increased risk of chronic disease. Sarcopenia, the loss of muscle mass with aging, is associated with diminished functional capacity and increased frailty among older adults, which threatens independence. This issue is particularly important in light of our increasing life expectancy—there are clear social, economic, and even ethical implications to continually reducing adult mortality without concomitantly striving to increase disability-free life expectancy at a similar rate. Thus, understanding the aging process is becoming an increasingly important agenda, and even more critical is understanding how best to promote "successful aging." The concept of successful aging was introduced by Rowe and Kahn [2] and is defined as a low probability of disease, high cognitive and physical function, and active engagement with life. The goal of this chapter is to examine the relationship between exercise and successful aging and, more specifically, the possible role of the endocrine system in mediating that relationship.

The Aging Endocrine System

Aging has been described as a "time dependent functional decline that converts healthy adults into frail ones" [3], an "inexorable physiological deterioration which eventually leads to the development of frailty" [4], and "the accumulation of diverse deleterious changes in the cells and tissues with advancing age that increase the risks of disease and death" [5]. Aging impacts many body systems including the endocrine system, and in the course of normal aging, several changes in endocrine function are observed. There is a progressive loss of secretory cell mass, a decline in the rate of hormone degradation, alterations in end-organ and tissue sensitivity to hormones, and changes in the modulation of endocrine feedback mechanisms

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[6]. Consequently, there are age-specific changes in various hormone levels, although any discussion of aging must distinguish between "normal" aging and the effects of age-related disease. For example, the prevalence of impaired glucose tolerance and type 2 diabetes increases among the elderly, resulting from a combination of age-related insulin resistance, genetic predisposition, and environmental factors such as poor diet [7]. Thyroid dysfunction is also more common among older adults, with overt and subclinical hypothyroidism occurring in 0.6–7.8% of elderly people, more commonly in women than men [8]. The causes of hypothyroidism in the elderly are similar to the causes among younger adults, primarily resulting from autoimmune disease [8, 9]. Both diabetes and hypothyroidism are clinically relevant conditions that require pharmacological intervention, and although prevalence is increased among the elderly, these are not universal characteristics of the aging process.

On the other hand, three key aspects of the endocrine system are altered to varying degrees throughout the course of so-called normal aging. These include the changes in sex steroids that characterize menopause and/or andropause, changes in dehydroepiandrosterone (DHEA) known as adrenopause, and changes in the growth hormone/insulin-like growth factor I (GH/IGF-I) system, known as somatopause [10].

Menopause and Andropause

Probably the most familiar age-related change in endocrine function is female menopause. The progressive loss of ovarian follicles during aging leads to the absence of follicular function resulting in the cessation of menses [11]. Three estrogens that occur endogenously are estradiol, estrone, and estriol; estradiol and estrone are interconvertible in the liver. The primary ovarian steroid hormone change that occurs as a result of menopause is a marked reduction of estradiol from the normal reproductive mean level of 257 pmol L^{-1} to a level of 40 pmol L^{-1}. Similarly, the mean circulating estrone level decreases from 211 to 100 pmol L^{-1} [12]. Estrone, produced both in the ovary and by peripheral conversion of androstenedione in adipose tissue, becomes the primary circulating estrogen after menopause [12]. Following the menopause transition, there does not appear to be any effect of increasing age on estrogen levels in women [13]. Circulating levels of progesterone, produced by the corpus luteum, do not appear to show any clear change with increasing age [11].

The loss of ovarian function at menopause also results in changes in hypothalamic and pituitary function. The pituitary gonadotropins (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) stimulate the ovarian secretion of estradiol and inhibin from follicular granulosa cells. As aging progresses, the secretion of inhibin falls, leading to a slow rise in FSH and LH [14]. As the menopause approaches, the loss of ovarian feedback leads to a profound rise in the gonadotropins, reaching a peak 2–3 years after menopause, after which they progressively decline with age [11, 15].

Men experience a decline in testosterone with age that is sometimes referred to as "andropause"; however, decreases in circulating testosterone in males are more subtle and gradual than the decline in estrogen at menopause. The Massachusetts Male Aging Study reported a 1.6% decline in total testosterone per year in men between the ages of 40 and 70 [16], and Harman [5] reported that 28% of men in their 70s in the Baltimore Longitudinal Study of Aging met the criteria for hypogonadism. Testosterone secretion is higher in the morning in young men, and this diurnal pattern appears to be blunted in older men [17]. The age-related decline in testosterone in men may result partly from changes in the hypothalamic–pituitary axis with decreased GnRH and subsequently reduced LH secretion [18]. A decrease in Leydig cell mass and function has been observed in older men which likely also plays a role in diminished testosterone levels [18]. It is important to note that unlike menopause in women, not all men experience "andropause" and there is some debate about whether the decline in testosterone observed in aging men is a true androgen deficiency. Several studies have shown an age-related decline in estradiol

and bioavailable estradiol in men, which may be a result of the decrease in testosterone, as testosterone is the main precursor to estradiol in men [19, 20]. However, this is not a consistent finding as Muller et al. [21] reported no age-related differences in estradiol among 400 men between the ages of 40 and 80.

In men, the testes are the primary source of testosterone, while in women, testosterone is secreted by both the ovaries and adrenal glands. Women also experience a decline in testosterone levels with age, independent of menopause [22, 23]. The expected testosterone concentration of a 40-year-old woman would be 0.61 nmol L^{-1}, about half the level of a 21-year-old woman at 1.3 nmol L^{-1}. Menopause itself does not result in a dramatic change, and in postmenopausal women, serum testosterone is only slightly lower than in premenopausal women [22, 24]. There is evidence to suggest the ovary is still a source of testosterone after menopause [25], although this is a somewhat controversial finding [26].

Most testosterone is transported in blood by sex hormone-binding globulin (SHBG). While SHBG increases with age in men [27, 28], the change in serum SHBG levels in women is unclear. Free testosterone, bioavailable testosterone, and the free androgen index decline dramatically with age in men as a result of a decrease in total testosterone and an increase in SHBG [29, 30]. In postmenopausal women, however, it is still unresolved whether there are systematic age-related changes in free testosterone or free androgen index, as SHBG levels in women have been reported to increase, decrease, or show no change after menopause [24, 31, 32].

Adrenopause

Dehydroepiandrosterone (DHEA) is secreted from the adrenal glands and is the most abundant steroid hormone in the body. It is a weak androgen that plays a role in many body tissues either by conversion to more potent sex steroids or by direct action on target tissues. The serum levels of DHEA and its sulfate conjugate, DHEAS, peak at 20–24 years in men and 15–19 years in women. The concentration of DHEAS in women is about 25–30% less than men, and the gender difference persists at all ages [33]. There is a progressive decline in the circulation of DHEA(S) starting in the early 20s with a decrease of 1.5% per year until levels diminish to 10–20% of the peak by the age of 70 [33, 34]. In women there is no relationship with menopausal status because DHEA and DHEAS are secreted exclusively by the adrenal glands in both premenopausal and postmenopausal women [22]. The pronounced age-related decline in DHEA(S) appears to result from a decrease in the activity of specific steroidogenic enzymes as well as diminished size of the zona reticularis in the adrenal gland [35, 36]. It is important to note that the adrenopause refers specifically to adrenal androgens as there is no consistent age-related change in cortisol concentrations [35].

Somatopause

The activity of the growth hormone/insulin-like growth factor I (GH/IGF-I) axis declines significantly with age. Iranmanesh et al. [37] demonstrated a 14% decline in growth hormone (GH) secretion per decade in men, and circulating GH over the age of 70 is approximately one-third of the level found in young adulthood [38]. GH is released in a pulsatile manner, and the older men show decreased frequency of secretory bursts and increased metabolic clearance [37]. In women, there is a threefold higher mean serum GH concentration over 24 h when compared to men that appears to result from a greater GH secretory burst mass, as opposed to a gender difference in the frequency of GH bursts [39, 40]. The age-related decline in daily GH secretion is less significant in premenopausal women compared to men [41], but menopause results in a significant decline in GH secretion in women, likely due to the fact that estrogen is a dominant regulator of GH secretion [42]. However, Lieman et al. [43] compared age-appropriate menopausal women to women with premature ovarian failure and found that age and body composition had greater effects on GH than lack of estrogen.

The age-related decline in GH may result from numerous factors including decreased sex steroids, increased somatostatin, increased body fat, and a decrease in the production of ghrelin [40, 44]. Ghrelin is a peptide secreted by the stomach that is an endogenous ligand of the GH secretagogue receptor found in the brain. Ghrelin stimulates GH secretion and also stimulates appetite [45]. Circulating concentrations of ghrelin have been shown to be lower in older adults compared to younger adults, which could contribute to both the lowered GH secretion and diminished appetite that occurs with advancing age [46]. However, several other studies have found no effect of age on ghrelin concentrations and suggest that age- and gender-related differences in ghrelin are explained by a negative correlation between ghrelin and total skeletal muscle mass [47].

Growth hormone has direct effects on metabolism and the growth of various body tissues although many of its anabolic and metabolic effects are mediated by IGF-I. The liver is the primary source of circulating IGF-I, and the actions of IGF-I are mediated by a family of IGF-binding proteins that regulate the half-life and bioavailability of IGF-I in circulation. There are at least six binding proteins, with the majority (75%) of circulating IGF-I bound in a ternary complex with IGFBP-3 and an acid-labile subunit [48]. IGFBPs mediate IGF-I actions by increasing the half-life of circulating IGF-I and by regulating IGF-I availability to bind with IGF receptors in the target tissues, in addition to exerting their own independent effects on tissues [49]. There are also at least three variants of IGF-I produced locally in skeletal muscle that have autocrine and paracrine effects on muscle tissue [50]. One of the locally produced variants of IGF-I, known as mechano growth factor (MGF), is upregulated in muscle in response to physical activity [50]. Both GH and IGF-I are important for stimulating protein synthesis and increasing muscle mass, and IGF-I stimulates satellite cell proliferation and inhibits apoptosis [51].

Serum levels of IGF-I are generally higher in men than women [52], and are inversely related to age in both men and women, likely as a result of the age-related decline in GH [52–57]. Ruiz-Torres and Kirzner [58] reported that the decline in IGF-I with age is exponential, with a much greater slope between the ages of 20 and 50 and a slower decline beyond age 55. Several studies have also reported a negative association between age and IGFBP-3, although the decline in IGFBP-3 is not as great as that of IGF-I, such that older adults typically have a lower ratio of IGF-I/IGFBP-3 [54, 55, 59] which may indicate lower IGF-I bioavailability. There is also evidence that aging results in loss of MGF expression in response to physical activity [50].

Hormones and Health in Older Adults

The potential clinical implications of diminished hormone levels among the elderly are varied. The side effects associated with the menopausal transition, such as vasomotor symptoms, and the negative impact on quality of life are well documented, although these are generally transient effects that diminish over time. A more long-term effect from loss of estrogen is reduced bone mineral density and increased risk of osteoporosis after menopause [60]. Studies have shown circulating estradiol is strongly associated with bone mineral density in men [61], thus declining estrogen levels could also have implications to the health of aging men. Sipila et al. [62] found that low serum estradiol was a significant predictor of fall-related fractures among 75-year-old women, independent of bone mineral density. They speculate this relationship could be explained by the effects of estrogen on the central nervous system, which may impact motor control and thus influence the risk of falls. Diminished testosterone levels may have negative effects on body composition, muscle mass and strength, bone mineral density, and sexual function in both men and women [17, 18, 25, 63]. Baumgartner et al. [27] examined data from the New Mexico Aging Process Study and reported that free testosterone was significantly correlated with muscle mass and grip strength in

elderly men, although the same associations were not found in elderly women.

DHEA(S) may have significant effects on many diseases and conditions of aging, including osteoporosis, atherosclerosis, diabetes, and dementia [35, 64, 65]. DHEA inhibits interleukin-6 (IL-6) production from peripheral blood mononuclear cells; thus the decrease in DHEA with age may be a significant factor for the manifestation of inflammatory and age-related diseases [66]. The apparent myriad effects of DHEA on human health and aging have led some to refer to it as the "fountain of youth" [67].

The age-related changes in the GH/IGF-I system and IGF-I bioavailability have also been associated with a plethora of health outcomes. Serum IGF-I and GH are related to strength, lean body mass, and bone mineralization in elderly men and women [27, 58, 68–70], and it has been suggested that declining levels of these hormones contribute to the musculoskeletal atrophy and pathogenesis of sarcopenia [51, 71]. Two recent cross-sectional studies have found circulating IGF-I and IGF-I bioavailability to be inversely related to metabolic risk factors and the metabolic syndrome, including dyslipidemia, obesity, and hypertension [52, 54]. Furthermore, circulating IGF-I has also been tentatively linked to cognitive decline and dementia [72]. Low IGF-I bioactivity has been associated with increased mortality among older men [73], and this is supported by studies showing a positive association between circulating IGFBP-1, IGFBP-2, and mortality among elderly men and women [74].

In contrast to the apparently positive effects of sex steroids, GH, and IGF-I on musculoskeletal health and physical function among the elderly, these hormones have been associated with increased risk of various types of cancer. Among postmenopausal women there is a positive association between circulating estrogens and development of cancer of the breast and endometrium [75–77]. Androgens, specifically circulating testosterone, have been associated with increased risk of prostate cancer [78] although other studies have found no association [79]. DHEA(S) has also been implicated in the development of breast cancer [80] and endometrial cancer [76], possibly by activating estrogen receptors [80]. IGF-I can alter cell behavior to promote unregulated cell growth and subsequently increase risk of cancer of the colon, breast, lung, and prostate [81]. IGFBP-3 seems to be inversely related to cancer risk, possibly by decreasing the availability of IGF-I to bind to the IGF-I receptor [82].

Age-related disease and dysfunction are undoubtedly the result of multiple factors. Although there is no clear consensus about the clinical implications of circulating hormone concentrations late in life, the decline in hormones corresponds noticeably with increasing risk of disease and frailty which has led to the development of "antiaging" strategies targeted at the endocrine system [71]. This is not a new concept, and in fact, interest in the relationship between hormones and the negative effects of aging dates back to the nineteenth century, when Brown–Séquard enthusiastically described the beneficial effects he experienced from self-administering canine testicular extracts, including improved physical function, stamina, and sexual performance [83]. This led to an entire industry of hormone "rejuvenation" treatments that, in many ways, continues today [84].

While pharmacological intervention may be beneficial, that is not always the case. Supplementation with growth hormone, ghrelin mimetics, and steroids may have positive effects on body composition, muscle mass, bone mineral density, and caloric intake [85], all effects which should theoretically translate to improved functional outcomes. However, changes in strength and overall quality of life as a result of hormone interventions have been inconsistent at best [85]. Furthermore, there may be risks associated with the use of exogenous hormone supplements, such as increased risk of cancer, that outweigh any potential benefits [85]. This was underscored by the highly publicized results of the Women's Health Initiative trial, which demonstrated an increased risk of breast cancer and coronary heart disease events for women using a combined estrogen/progestin therapy [86].

Physical Activity and Endocrine Function in Older Adults

The difficulty distinguishing between physical changes that result from primary aging and those that are exacerbated by environmental and lifestyle factors presents a challenge to aging research. The influence of lifestyle may partly explain why some individuals age more successfully than others [2]. For example, a significant decrease in physical activity occurs with aging, and the decline in muscle function with aging has structural and functional similarities to the loss of function observed during disuse [87]. Older adults are the least active of any group, and among Canadians, 62% of people 65 years or older are inactive [88] compared to 40% of individuals between 20 and 24 years [89]. This pattern of lower activity among older adults holds in many other nations, including the USA and Sweden [90].

Physical activity is a potent stimulus of the endocrine system; thus it seems reasonable that many of the changes in hormones associated with age may be impacted by a decline in habitual activity. Figure 23.1 illustrates the many complex interrelationships among primary age-related changes in physical and endocrine function and lifestyle factors. Numerous researchers have attempted to identify potential determinants of hormone levels among older adults. Physical activity is a variable of interest in many observational studies, based on the theory that one of the mechanisms by which physical activity promotes successful aging and reduces the risk of cancer is by altering the hormonal milieu.

Physical Activity and Sex Steroids

It has been suggested that the age-related decline in levels of DHEA is attenuated in older men who engage in long-term endurance training [91]. Ravaglia et al. [92] found that DHEAS was 80% higher among active compared to sedentary

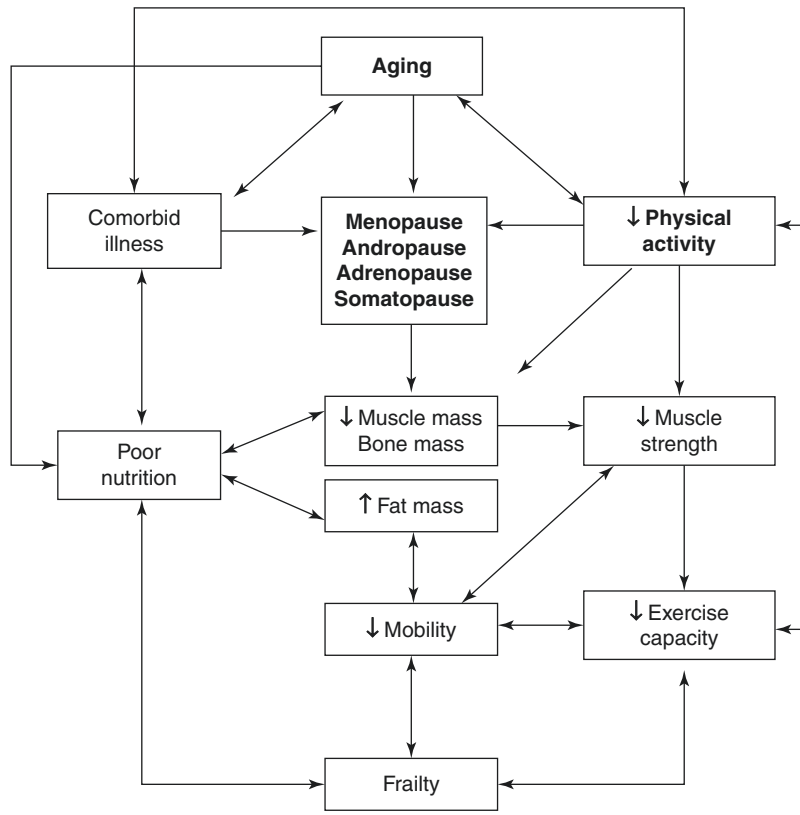

Fig. 23.1 A model of the relationships among aging, hormones, and physical function. (Adapted from Copeland [166], NRC Research Press)

elderly men. Similarly, among elderly women, Bonnefoy et al. [93] reported that circulating levels of DHEAS were positively correlated with habitual physical activity, sport participation, and aerobic power, although they did not find a similar relationship among men. These studies all employed relatively small sample sizes between 17 and 96 subjects. Larger observational studies have found no relationship between DHEA(S) and physical activity among postmenopausal women [94] or older men [21, 95].

Cauley et al. [13] studied 176 postmenopausal women and found the most active women had the lowest estrogen levels, while testosterone was not associated with physical activity. More recently, and in a much larger study, Chan et al. [96] also found an inverse relationship between physical activity and estradiol among 2082 postmenopausal women, with the most active quartile of subjects having 6% lower estradiol than the least active quartile. Unlike Cauley et al. [13], Chan et al. [96] did find an association between activity and testosterone, with the most active women having testosterone concentrations that were almost 20% lower compared to the least active women. SHBG was also significantly higher among active women [96]. Results from the Penn Ovarian Aging Study [94] confirmed the inverse association between physical activity and both estradiol and testosterone in a longitudinal study of 391 women over 10 years. Testosterone and estrogen were 15% and 19% lower, respectively, among the most active postmenopausal women [94]. They found a greater effect of physical activity on hormone levels among women in the late phase of the menopausal transition, with 54% lower estradiol and 47% lower testosterone among active women who had been amenorrheic for only 3–11 months.

Several studies have also found an association between physical activity and sex steroid hormone levels among older men. Muller et al. [21] reported a positive association between physical activity and total testosterone as well as SHBG among 40–80-year-old men. Other studies have reported a similar association among men across a broad age range [97, 98]. Ari et al. [99] compared ten male master athletes with ten sedentary age-matched controls and found the athletes had significantly higher levels of testosterone. In contrast, Ravaglia et al. [92] examined 48 active men and 48 sedentary controls between the ages of 50 and 74 years and found no difference in testosterone levels. Slowinska-Lisowska et al. [20] also found testosterone was not associated with physical activity among older males but observed that estrogen was significantly lower among older men with the highest level of activity. In contrast, Shiels et al. [98] found men who engaged in vigorous activity four times per week actually had higher estradiol, although their analyses included younger men as well.

The observed effect of activity on endogenous sex steroids among older adults is partially mediated by changes in body fat. Postmenopausal estrogen is primarily formed by the aromatization of adrenal androgens such as DHEA and androstenedione [32], and Cauley et al. [13] found a strong relationship between estrogens and obesity among postmenopausal women. It is important to note that even after controlling for obesity, the inverse relationship between physical activity and estrogen persisted [13], suggesting body fat may be only partly mediating the effects of activity. Similar to women, Muller et al. [21] found that higher levels of body fat were associated with higher estradiol levels among older men. Furthermore, Schmitz et al. [94] observed that activity-related differences in testosterone were greatest among women in the highest BMI category.

The balance of the cross-sectional evidence suggests that physical activity is associated with lower testosterone and estradiol among older women, higher testosterone among older men, and higher DHEAS and SHBG in both sexes. These results have generally been confirmed by randomized controlled trials of aerobic exercise interventions. Hawkins et al. [100] monitored circulating sex hormones in 172 older men who were assigned either an aerobic exercise program or control group. After 3 months they found a 14% increase in SHBG in exercisers and a 14.5% increase in dihydrotestosterone (DHT), with no changes in testosterone, free testosterone, or estradiol. Hawkins et al. [100] also noted the

greatest increases in DHT occurred among men (both exercisers and controls) who lost the greatest amount of body fat over the 12-month period and among exercisers who saw the greatest improvements in aerobic fitness. As insulin is known to inhibit SHBG production, it is assumed that the effect of exercise on SHBG is mediated by decreases in body fat and/or decreases in insulin. However, Hawkins et al. [100] found no effect of body fat on SHBG and saw the largest increases in SHBG among men who had the greatest improvements in fitness. Similar results in older women were reported by Friedenreich et al. [101], who found significant increases in SHBG after a year of aerobic exercise that were unrelated to insulin but appeared to be moderated by improvements in fitness. Friedenreich et al. [101] also found significant decreases in total and free estradiol in their training group compared to the control group although the differences were attenuated after adjusting for changes in body fat. Taken together, these data suggest that in older men and women, training-induced alterations in circulating estrogens are largely mediated by loss of body fat, while the increases in androgens and SHBG appear to be more strongly related to changes in fitness. The mechanism by which increasing physical fitness impacts circulating androgens and SHBG among older adults remains unclear.

Physical Activity and the GH/IGF-I System

The GH/IGF-I axis in older adults is also impacted by lifestyle factors, including physical activity [102]. Ari et al. [99] observed higher levels of GH among male masters athletes compared to sedentary controls. In contrast, Deuschle et al. [103] compared ten older male marathoners (50–78 years) and ten age-matched sedentary men and found no difference in circulating GH between the two groups. Growth hormone is secreted in a pulsatile manner from the anterior pituitary, and a single sample may be insufficient to assess the impact of physical activity on the pattern of GH release. Many more observational studies have focused on establishing the determinants of IGF-I among older adults, as it is the main downstream mediator of GH actions and is much less variable than GH with no significant diurnal variation in systemic concentrations [48].

Studies of the relationship between physical activity and IGF-I among the elderly have produced inconsistent results, with some reporting a positive relationship [91–93] and others finding no association [53, 55, 103, 104]. Orenstein and Friedenreich [105] published a systematic review of all studies of physical activity and IGF-I, and of the cross-sectional studies among older adults, there were approximately equal numbers showing a positive association between physical activity and IGF-I and those showing no association. The largest epidemiological study to date that examined lifestyle determinants of circulating IGF-I concentrations was recently reported by Parekh et al. [52]. Using the NHANES III data, Parekh et al. [52] examined the impact of diet and physical activity on IGF-I among 6058 men and women over the age of 20, and they found no relationship between IGF-I and physical activity. The apparent lack of association between habitual physical activity and circulating IGF-I is supported by the controlled trial reported by McTiernan et al. [106]. They compared the effects of 12 months of moderate intensity aerobic exercise to a stretching program in 173 postmenopausal women, and found no change in IGF-I or IGFBP-3 with the aerobic exercise, despite reporting excellent adherence to the program. Their results were not influenced by age, body mass index, or changes in aerobic power.

It is interesting to note that some of the studies that found no relationship between physical activity and IGF-I did observe significantly higher levels of IGFBP-1 among active older adults [103, 104]. This effect is possibly a result of lower insulin levels among more active older adults as insulin suppresses the production of IGFBP-1 [48]. Higher IGFBP-1 among active older adults may decrease the activity of IGF-I by forming IGF-I/IGFBP-1 complexes that cannot bind to cell surface receptors [48].

Clearly, much of the evidence describing the relationship between physical activity and

hormones among the elderly is contradictory. This is exemplified by the fact that some researchers have proposed physical activity as a way to *increase* circulating levels of steroids and IGF-I to promote musculoskeletal health and function in the elderly [107], while others suggest physical activity will *decrease* hormones and thus reduce the risk of cancer [108]. This apparent paradox requires further research.

One of the main challenges facing observational studies is the accurate measurement of physical activity. The majority of the aforementioned studies relied on self-reported physical activity, and lack of standardization is a significant problem associated with using self-report to measure a complex exposure like physical activity [109]. Furthermore, self-report questionnaires are extremely limited in their ability to objectively classify intensity and provide detailed information about the pattern of habitual physical activity. There may also be issues with the use of questionnaires specific to an older population, including vision and hearing impairments or disturbances to cognition and short- or long-term memory [109]. There can be problems with accurately reporting the intensity of exercise, as perceptions of what is "hard" activity or "light" activity depends on the tolerance and fitness level of the individual, both of which are affected by age [109]. These issues pose a significant problem to the examination of physical activity and endocrine function as it will be difficult to detect differences if activity levels among subjects are homogeneous. This is illustrated by the results of Ravaglia et al. [92], who found no relationship between physical activity and testosterone in older men, but they also found no differences in body fat or lean body mass between their most active and least active subjects, which suggests they did not vary greatly in habitual activity.

It is interesting to note the few studies that employed an objective measure of physical activity or fitness, such as a motion sensor [13] or maximal aerobic power [31, 91], found significant relationships between physical activity and hormone levels. In fact, Cauley et al. [13], who used both self-report and objective measures of activity, reported that the inverse relationship they observed between physical activity and estrogen was stronger with the motion sensor data than it was with the self-report data. As technological advances now allow more robust and detailed assessments of physical activity [110, 111], future research may be able to determine if certain patterns or intensities of activity are associated with changes in endocrine function among the elderly.

Although the measurement of hormone concentrations is, in theory, simpler than measuring a complex behavior such as physical activity, there are still many challenges to be overcome. Most studies assess hormone levels with a single blood sample, and many hormones have a circadian rhythm as well as seasonal variability that can significantly influence results [112]. Furthermore, there is often no mention of the time span between the blood draw and the most recent exercise session, and acute exercise can have an impact on hormone levels that could persist for hours or even days, depending on the intensity and duration of the exercise session. Prior exercise could be a major confounding variable when comparing hormone levels between sedentary and active older adults.

Assessing age- and activity-related changes in hormone levels is further complicated by the interactions among different hormones and by changes in binding proteins that may impact free hormone levels with no discernible effect on total hormone levels. For example, SHBG has been shown to be positively associated with both age and physical activity in older men [21], which may result in decreased free or bioavailable testosterone. Age- and exercise-related increases in IGFBP-1, IGFBP-2, and IGFBP-3 may similarly impact levels of free IGF-I and IGF-I activity [55, 104]. Furthermore, improvements in technology have allowed identification of multiple forms of protein hormones, both in circulation, such as the over 100 isoforms of GH that have been identified in serum [113], and in muscle, such as the locally produced splice variants of IGF-I [50]. The development of more advanced analytical techniques will undoubtedly yield new insights into the effect of exercise on the endocrine system.

Of course, observational studies that show a relationship between circulating hormone levels and physical activity cannot infer causation. It is certainly plausible that the relationship between hormone levels and physical activity is mediated by health. Healthy older adults with a lower "biological age" may have fewer age-related changes in endocrine function and may also be more likely engage in an active lifestyle due to higher functional capacity. One also has to consider the possibility that hormones—which have both physiological and behavioral effects—are influencing physical activity as opposed to the other way around.

Although there are no human data to support this idea, Bowen et al. [114] recently proposed the theory that gender differences in physical activity could be explained by sex hormones. Bowen and colleagues base the idea on extensive animal literature that shows a significant sex effect on patterns of physical activity. If we extend this to an aging population, perhaps individuals who maintain higher levels of anabolic hormones, due to either genetics or the impact of lifestyle factors, are more likely to engage in regular physical activity. If this were the case, then we would expect that hormone replacement among older adults might impact physical activity behavior. Although few studies have examined this question specifically, various studies of estrogen replacement therapy in women have also measured physical activity and physical function. Andersen et al. [115] examined the association between physical activity and HRT use in NHANES III data and found a higher prevalence of sedentary behavior among women who had never used HRT compared to those who had used HRT. Unfortunately this finding is limited by the cross-sectional design of the study, and experimental studies have found no impact of HRT on physical activity among women [116, 117]. Comparable studies of testosterone replacement and the subsequent effect on physical activity are scarce but perhaps warranted. Ibebunjo et al. [118] recently conducted an experiment in male mice to determine the effects of androgen depletion and androgen replacement on voluntary wheel running. They found testosterone had a significant impact on wheel running behavior as androgen-depleted mice ran more slowly and significantly less distance than mice that received testosterone replacement after orchiectomy. Ibebunjo et al. [118] therefore suggest that voluntary physical activity could be at least partially centrally mediated. If this were also true in humans, it may offer insight into the age-related decline in physical activity.

Acute Exercise-Induced Hormone Responses in Older Adults

A significant number of intervention studies have been undertaken to examine the effect of a bout of exercise on hormone levels in older adults. Typically acute exercise will stimulate significant changes in serum concentrations of many hormones in young men and women, although the nature of the response will vary depending on the mode, duration, and intensity of the exercise, as well as the training status of the individual [119, 120]. However, given the age-related changes in the endocrine system outlined at the beginning of this chapter, one might reasonably expect exercise-induced hormone responses to be different among elderly individuals.

Both acute endurance and resistance exercise have been shown to increase levels of testosterone and DHEA(S) in older men and women [121–127], although studies examining the steroid hormone response to resistance exercise are far more numerous than those examining endurance exercise. Few studies have examined the estrogen response to exercise among older adults. Kemmler et al. [124] reported a 20% increase in estradiol after 60 min of combined endurance and resistance exercise in postmenopausal women. Copeland et al. [121] also reported an increase in serum estradiol after both endurance and resistance exercise in women across a wide age range, including older adults.

The exercise-induced increases in circulating steroids range between 10% and 40% and are highly transient, with concentrations typically returning to pre-exercise values within 30–120 min following exercise. In general, steroid hormone

responses to exercise in elderly subjects are comparable to those of younger subjects, but this is not a universal finding. Aldred et al. [128] found a diminished DHEA(S) response to cycling exercise in older adults, and others have reported lower testosterone responses to resistance exercise in older men and women [125, 129]. There is evidence that blunted steroid hormone responses to exercise may relate more to exercise intensity and/or training status than age. This is supported by studies that have shown exercise-induced increases in DHEA(S) or testosterone among older women only *after* a period of regular training [122, 130], when subjects had a higher exercise intensity, as evidenced by greater postexercise lactate concentration [130]. Craig et al. [129], however, did not measure lactate or heart rate, and Aldred et al. [128] used different durations and intensities of exercise for their young and older subjects, which limits interpretation of their findings.

Exercise of at least moderate intensity will stimulate secretion of growth hormone (GH) such that plasma GH concentrations increase within 10–15 min of the start of exercise [102, 131], and this response is greater in women than men [132]. Although exercise will still induce an increase in GH among older adults [121, 125, 133, 134], the response is typically attenuated [102]. Pyka et al. [135] measured GH concentrations during a circuit of 12 resistance exercises performed by a group of old (72 ± 0.8 years) and young (27 ± 1.6 years) men and women. The young subjects showed a significant rise in GH throughout the session, while the older subjects demonstrated no significant increase. This blunted GH response to acute exercise among the elderly has been confirmed by others [122, 132, 136], and GH release during exercise is often between four- and sevenfold lower among older adults [131]. Aging also appears to eliminate the gender difference in exercise-induced GH responses with older men and women showing similar 4-h integrated GH concentrations after aerobic exercise of varying intensities [132].

It has been suggested that lower exercise intensity in the older subjects explains the blunted GH response to exercise. Indeed, many of the studies that found a lower GH response to exercise in older subjects also reported a significant age-related difference in exercise intensity [122, 135]. Weltman et al. [132] found that only exercise intensities above the individual lactate threshold increased GH output in older subjects, and even at the highest exercise intensities, the integrated GH response was significantly diminished compared to the younger subjects. Gulka et al. [137] found that the GH response to treadmill exercise was significantly lower in older versus younger women but that age differences were minimized in fit older women. It appears that a greater relative exercise intensity may be required to stimulate GH in older adults.

In addition to exercise intensity, there are other factors that may influence the GH secretion, including relative adiposity and fat distribution, sleep, and physical fitness—all factors that are influenced by increasing age [102, 138]. Vahl et al. [139] reported that abdominal fat and fitness were more important than age as determinants of GH responsiveness to multiple provocative stimuli, and Hartman et al. [102] concluded that abdominal visceral fat, fasting insulin, and IGF-I are the best predictors of GH secretion. Given this, losing weight and exercising may restore GH secretion to some degree, although there is likely a primary age effect on the hypothalamus that cannot be reversed [102].

IGF-I is the primary mediator of GH actions and can regulate GH secretion by negative feedback. While both GH and IGF-I decrease with age, peripheral responsiveness to GH remains the same [140], and thus it seems logical that if exercise impacts GH concentrations, it would also impact IGF-I concentrations. However, the IGF-I response to exercise is highly inconsistent and appears to be independent of changes in growth hormone. Several studies have shown an increase in IGF-I in elderly subjects after resistance exercise [141–143] and after a 30 s Wingate test [144], while others have found no change in the IGF-I in response to endurance or resistance exercise [121, 133]. Most of these studies measured total IGF-I, although Bermon et al. [141] also measured free IGF-I and found a 94% increase after resistance exercise, which is much greater than the ~10–15% increase that is

typically observed for total IGF-I. Orenstein and Friedenreich [105] reviewed 115 studies of exercise and IGF-I, and of the 47 studies that examined the IGF-I response to acute exercise specifically among older adults, 18 reported an increase in IGF-I, 26 reported no change, and 3 reported a decrease. This ratio of positive and negative findings was not notably different in any other age group. Thus, age does not appear to independently impact IGF-I responsiveness to exercise, as it does for GH.

Exercise may indirectly influence IGF-I activity by altering the circulating concentrations of IGF-binding proteins, but in comparison to IGF-I, a fairly limited number of studies have examined the effect of acute exercise on IGF-binding proteins in older adults. In the review by Orenstein and Friedenreich [105], 3 studies reported increased IGFBP-3 with exercise, while 13 found no change. Chadan et al. [133] found that moderate intensity activity in older women did not impact levels of IGF-I, but it did increase IGFBP-2 and IGFBP-3 and decreased levels of IGFBP-1. IGFBP-1 has been shown to inhibit IGF-I action, while IGFBP-2 and IGFBP-3 appear to potentiate the effects of IGF-I; thus Chadan et al. [133] concluded that exercise may enhance IGF-I action in elderly women. However, the decrease in IGFBP-1 is a somewhat surprising result as IGFBP-1 typically increases with exercise in a dose–response manner [145, 146], presumably as a result of decreased insulin since insulin directly inhibits hepatic IGFBP-1 synthesis [145].

Despite the large number of studies, it is difficult to draw firm conclusions about the effect of exercise on circulating hormone concentrations among the elderly. As with younger populations, the response appears dependent on the type, duration, and intensity of exercise; the age, gender, and fitness level of the subjects; the sampling interval used; whether free or total hormone concentrations are assessed; and which isoforms of various ligands are being measured. Overall, it appears that the endocrine system of older adults is still responsive to an exercise stimulus, although the response may be diminished to some degree.

The mechanism of exercise-induced changes in hormone concentrations is not clearly understood, but changes in circulating hormone levels likely result from some combination of altered secretion, decreased hepatic clearance, and hemoconcentration. Regardless of the mechanism, changes in the concentration of a given hormone will increase or decrease the probability of ligand–receptor interactions, and the biological activity of hormones is ultimately dependent on the availability of receptors in the target tissue. Bamman et al. [147] reported a 100% increase in androgen receptor (AR) mRNA in exercised muscle following an acute bout of resistance exercise in young male subjects; however, this response has yet to be demonstrated in older subjects. Roberts et al. [148] found that older men had greater AR gene expression at rest than young men, but 24 h following an acute bout of resistance exercise, there was no change in AR expression in either age group [148]. Ahtiainen et al. [149] also found no effect of either an acute heavy resistance exercise protocol or 21 weeks of resistance training on the AR protein concentration of young or old male subjects. Interestingly, they did note that individual changes in muscle AR protein concentration after exercise were predictive of increases in lean body mass and muscular strength after resistance training [149]. Increased ARs in exercised muscle may be related to exercise-induced increases in serum testosterone, as androgens are known regulators of AR protein expression [148, 149]. Thus, older adults experiencing “andropause” may subsequently have diminished responsiveness in downstream regulators of androgen activity.

Conclusion: Significance of Exercise-Induced Hormone Responses in Older Adults

As studies of the endocrine response to various exercise protocols continue to accumulate, a fundamental question remains: what is the significance of these transient changes in hormone levels to the older adult? A majority of studies cite the potential beneficial effects of anabolic

hormones on the elderly as the rationale for examining exercise-induced endocrine responses. This is based on the theory that increases in systemic anabolic hormones will facilitate training adaptations by increasing protein synthesis, lean body mass, and strength [150] and subsequently reduce the risk of sarcopenia, osteoporosis, and frailty. This theory is supported by several studies that have found a significant correlation between acute increases in GH and testosterone and increased muscle cross-sectional area with resistance training in men [151, 152]. However, Wilkinson et al. [153] challenged the view that increases in anabolic hormones stimulate muscular hypertrophy following resistance training. Ten young male subjects completed a unilateral resistance training program that resulted in significantly increased thigh muscle cross-sectional area (CSA) despite having no exercise-induced increases in circulating GH, IGF-I, or testosterone [153]. Furthermore, using an animal model, Matheny et al. [154] showed that mice with a deficiency in serum IGF-I still exhibit muscular hypertrophy following resistance training. It seems that changes in systemic hormones are not required for muscular adaptations to occur, but one could speculate that they may enhance or accelerate the adaptations. That possibility has been both refuted and supported in the literature. West et al. [155] found no difference in rates of protein synthesis after training that elicited large increases in circulating GH and testosterone compared to training with no increases in circulating endogenous hormones. However, Ronnestad et al. [156] recently demonstrated that training of the elbow flexors with elevated endogenous GH and testosterone induced superior adaptations in muscle strength and CSA when compared to similar training with no increases in these hormones. Clearly further research is needed to clarify the importance of acute increases in anabolic hormones on training adaptations. Furthermore, all of these studies used young male subjects, and it is not known if exercise-induced hormone responses might be more relevant in an elderly population with lower basal hormone levels.

West and Phillips [157] conclude that exercise-induced increases in systemic testosterone or GH are of minimal importance to the muscular hypertrophic response and suggest that locally produced androgens are more relevant to muscle anabolism. Although activation of local androgen metabolism has been shown in response to exercise in rodents [158, 159], this has yet to be confirmed in human studies. Pollanen et al. [160] compared circulating and muscle concentrations of steroids in pre- and postmenopausal women. They found the expected differences in circulating hormones, with postmenopausal women having significantly lower concentrations of estrogens and androgens, but noted that *muscle* concentrations of estradiol and testosterone were actually higher in the postmenopausal women, with no difference in muscle DHEA between the two age groups. Despite having higher muscle hormone concentrations, the postmenopausal women had lower muscle size and strength compared to the premenopausal women, and systemic hormone concentrations were positively related to muscle quality [160]. It seems that peripheral and systemic hormones may have differing roles in the regulation of skeletal muscle and further research is needed to elucidate the effects of aging and exercise on intracrine versus endocrine steroid hormone activity.

Although research on the effect of exercise on local steroid synthesis is limited, there is substantial evidence to suggest that locally produced IGF-I is upregulated in response to exercise [161]. In addition to the hepatic synthesis of systemic IGF-I, three splice variants of locally produced IGF-I have been identified in skeletal muscle, the so-called IGF-I E peptides: IGF-IEa, IGF-IEb, and IGF-IEc (also known as mechano growth factor (MGF)) [161]. The IGF-I E peptides appear to have different roles, with IGF-IEa increasing muscle protein synthesis, while MGF initiates muscle repair processes after damage [161, 162]. Animal studies have shown that muscle IGF-I expression increases in response to loading and contributes to muscle hypertrophy, even in animals with low systemic IGF-I [162]. In humans, muscle IGF-I has been shown to increase significantly after a single bout of resistance exercise in young subjects

[147]. In elderly subjects, Singh et al. [163] found that muscle IGF-I increased by almost 500% following 10 weeks of resistance training. The increases in muscle IGF-I paralleled training-induced increases in muscle damage and in developmental myosin [163]. Those results were supported by Hameed et al. [164] who found increases in MGF in both young and elderly men in response to damaging eccentric exercise. These studies suggest that age does not impair the local growth factor response to exercise, which appears to be critical for mediating training adaptations. This local response may also explain the training-induced increase in muscle size without a change in systemic hormone levels, as seen in several studies described above [153–155]. More research is needed to understand the physiological effects of exercise- or training-induced changes in systemic and peripheral hormones in order to understand the conflicting evidence regarding the health benefits and risks of increasing concentrations of hormones and growth factors. It is possible that circulating hormones and locally produced hormones have differing roles in the health and function of elderly individuals.

In conclusion, it is indisputable that exercise is beneficial to the health and quality of life of older adults [165]. Regular physical activity can minimize the age-related decline in functional capacity and associated disability and reduce the risk of many chronic diseases. It has also been clearly demonstrated that exercise has potent effects on the endocrine system and can influence circulating hormone concentrations, locally produced hormone expression, and hormone receptor expression. The balance of evidence seems to suggest that these endocrine system responses to exercise are still possible in elderly individuals if a sufficient dose of activity is achieved. Yet the link between the beneficial effects of exercise and changes in endocrine function is much more tenuous. Given the demographic shift mentioned at the beginning of this chapter, there is considerable incentive to clarify the relationships between exercise, endocrine function, and successful aging.

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24 Immune, Endocrine, and Soluble Factor Interactions During Aerobic Exercise in Cancer Survivors

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Introduction

The American Cancer Society (ACS) projected that in 2017, nearly 1.7 million Americans would be diagnosed with some form of cancer and that nearly 600,000 would die from their cancer [1]. Earlier detection and advances in newer treatments have improved disease prognosis, although cancer survivors are still faced with challenges and sequelae associated with the disease itself, the cancer treatments, or other comorbid conditions that may predate the cancer diagnosis [2, 3]. Additionally, many cancer survivors are at risk for developing a recurrence of their disease or a second malignancy [2, 3]. Therefore, interventions that may decrease the risk for developing a cancer recurrence or new malignancy while also improving survival are warranted.

Aerobic exercise may be an attractive adjunct therapy for survivors because of its potentially positive influence on biologic systems involved in disease protection and anticancer defense [4–6]. Numerous reviews have comprehensively described the effect of acute and chronic aerobic exercise on the cellular components of innate and adaptive immunity in healthy individuals [7–17]. An active lifestyle is widely considered beneficial to immune system health, leading to a reduction in communicable infections, as well as a reduction in chronic health conditions, which may include inflammation as an underlying pathophysiological mechanism [17]. Moderate and vigorous acute aerobic exercise induces a biphasic shift in circulating cell counts of lymphocyte subpopulations (e.g., natural killer [NK] cells, helper T lymphocytes, cytotoxic T lymphocytes, and B lymphocytes), in that these cell counts display marked increases during exercise and may decrease below pre-exercise values during recovery (see Fig. 24.1) [8–10, 15–17]. In contrast, circulating cell counts for other leukocyte subpopulations may exhibit a different response (e.g., cell counts for neutrophils and monocytes may increase during exercise as well as early during recovery) [8, 9, 11, 15]. When examining the impact of acute aerobic exercise on leukocyte subpopulation function, studies describe either increased immune cell function during both exercise and recovery, decreased immune cell function during both exercise and recovery, or increased immune cell function during exercise and decreased function during

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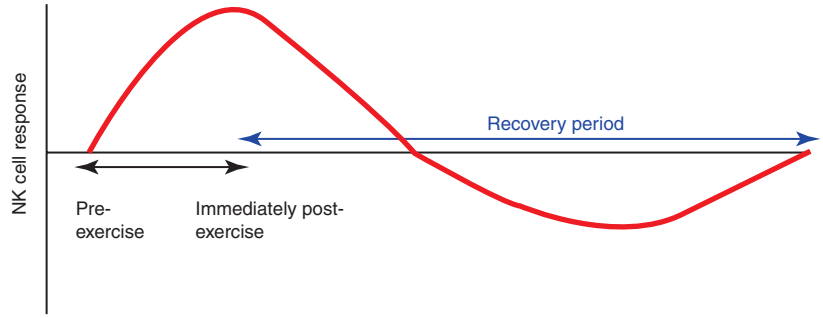

Fig. 24.1 Biphasic shift in immune cell counts, which may be seen in NK cells, helper T lymphocytes, cytotoxic T lymphocytes, and B lymphocytes. Magnitudes drawn are for illustration purposes only

recovery [8, 16, 17]. Differences among studies regarding the time points during and after exercise at which blood samples are taken and immune cell counts and function are analyzed, as well as the laboratory techniques used to determine immune cell function, may be primary factors contributing to variations in observed results across the literature [16, 17]. In general, however, the magnitude of the immune response is largely affected by exercise intensity and duration, such that moderate intensity or shorter duration exercise generally leads to smaller changes in cell counts and function and higher intensity and longer duration exercise generally leads to greater alterations in cell counts and function, relative to pre-exercise levels [8, 9, 11, 17].

When considering the effect of aerobic exercise training on circulating immune cell counts and function, results are again variable, depending on the training volumes utilized and the immune parameter examined [8]. In the past, exercise immunology literature has typically concluded that moderate intensity/volume exercise leads to enhanced immune cell numbers and function, whereas high intensity/volume exercise leads to suppressed cell numbers and function [8, 9, 14, 17]. These observations led to the development of the "J-shaped" hypothesis, which postulates an inverse relationship between disease risk and cancer susceptibility (see Fig. 24.2) [4, 8, 9, 11, 17]. In general, the hypothesis states that regular, moderate exercise may lead to enhanced immune function and therefore a decreased risk of disease and cancer, whereas repeated bouts of exhaustive exercise and overtraining could lead

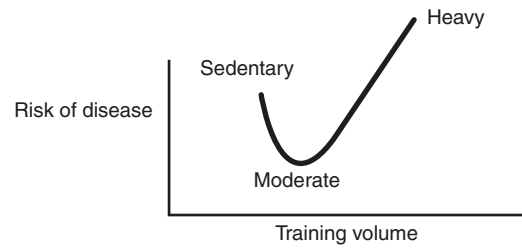

Fig. 24.2 The J-shaped curve, illustrating the hypothesis that exercise training volume and immune function are inversely related [4, 6, 7, 9]

to immunosuppression and therefore an elevated risk of disease and cancer [4, 8, 9, 11, 17]. This hypothesis is largely based on epidemiological investigations of exercise intensity and development of upper respiratory tract infections (URTI), where athletic individuals have reported higher incidences of URTI during periods of strenuous training or in the days following a major event such as a marathon [8, 10, 15, 17] and individuals participating in moderate exercise have reported fewer URTI symptoms [8, 15, 17]. More recent evidence has called into question the claim that high volumes of exercise or intense athletic competition increases the risk of acquiring an infection [17]. Instead, the increased incidence of illness observed in these cases is more likely due to the effect of other stressors on the immune system, including exposure to environmental extremes, air travel, sleep disruption, fatigue, altered or inadequate diet, dehydration, psychological stress, and the exposure to novel pathogens that can occur at any mass gathering of people [17]. The decrease in circulating immune

cell counts that have been observed after strenuous exercise more likely reflects a transient redistribution of immune cells out of systemic circulation and into peripheral tissues, where they can conduct immune surveillance of potentially infected, damaged, or malignant cells [17]. Similarly, the decrease in circulating immune cell function also reflects this shift in active immune cells from systemic circulation and into peripheral tissues [17]. Therefore, post-exercise decreases in immune cell counts and function do not suggest clinical immunodeficiency, and no conclusive evidence currently exists linking exercise intensity/training volume with immunological mechanisms and cancer risk in humans [8, 14, 15, 17].

Since aerobic exercise has been shown to influence cellular immune system responses in healthy individuals, studies regarding the effect of aerobic exercise on the immune system in cancer survivors have begun to appear in the literature. Fairey et al. [4] was the first to systematically examine the effects of exercise on immune system function in cancer survivors, at which time there were six published studies. A more recent review by Kruijsen-Jaarsma et al. [18] reveals the topic has expanded as it includes 21 published studies. The purpose of this chapter is to (1) briefly describe the role of aerobic exercise as an intervention for improving functioning in cancer survivors, (2) provide a comprehensive review of the impact of acute aerobic exercise and aerobic exercise training on cellular immune system function in cancer survivors, and (3) discuss underlying endocrine and soluble factor mechanisms that may be associated with these cellular immune responses.

Aerobic Exercise as an Intervention for Cancer Survivors

A recent review of epidemiological evidence by Moore et al. [19] shows that high levels of leisure time physical activity are associated with lower rates of 13 different cancers. Similarly, emerging epidemiological evidence is demonstrating that physical activity, along with other healthy lifestyle factors, may improve survival from breast, colorectal, and prostate cancer [20]. Proposed biological mechanisms governing the relationship between increased physical activity and decreased cancer recurrence include decreased total body fat and visceral fat, decreased exposure to estrogen and testosterone, increases in antitumor defenses, improved antioxidant defense systems, decreased inflammation, decreased insulin and glucose levels, and decreased levels of insulin-like growth factors [20]. Cancer survivors who participate in regular physical activity and exercise also tend to experience a vast array of positive outcomes including improved quality of life, cardiorespiratory fitness, muscular strength and endurance, body composition, physical function, and self-esteem, along with decreased fatigue, anxiety, and depression [21–25].

The American College of Sports Medicine (ACSM) states that exercise prescriptions goals for cancer survivors should include regaining and improving physiological and psychological parameters such as aerobic capacity, strength, flexibility, body composition, quality of life, self-esteem, and body image [2, 26]. The ACSM recommends that cancer survivors follow guidelines that are very similar to those already in place for the general population, where individuals are encouraged to exercise for at least 150 minutes/week of moderate-to-vigorous exercise, integrating aerobic, resistance, and flexibility training into the exercise prescription [2, 26]. The ACSM also gives further guidance for special considerations that may need to be taken into account when working with cancer survivors with site-specific complications including decreased bone mineral density, ostomies, central lines, feeding tubes, peripheral neuropathy, lymphedema, decreased joint mobility, pelvic floor weakness, cachexia, immunosuppression, very low blood counts (e.g., anemia, leukopenia, thrombopenia), and severe fatigue [2, 26].

Taken together, the overall statement by the ACSM is that cancer patients and survivors should be as physically active as their conditions allow and avoid inactivity and that exercise seems to be safe for most in-treatment or off-treatment

patients cleared by their physicians to participate in regular exercise [2, 26]. Just as importantly, exercise prescriptions should be individualized, taking into account the cancer survivor's goals, their pretreatment physical fitness, the presence of comorbid medical conditions, the patient's response to their cancer treatments, and any negative acute or chronical treatment side effects that they may be experiencing [2]. The improved outcomes that cancer survivors are likely to experience as a result of regular participation in physical activity would hopefully aid in mitigating long-term sequelae of cancer treatment, reduce risk of recurrence or second malignancy, as well as improve their ability to withstand any current or future cancer treatments if necessary.

As mentioned previously, aerobic exercise may be an attractive adjunct therapy for cancer patients and survivors because of the potential for positive influence on biologic factors involved in disease protection and anticancer defense [4–6]. Several cellular immune system components are associated with anticancer defense, including NK cells, neutrophils, macrophages, and T lymphocytes [4, 18]. NK cells, neutrophils, and macrophages are all part of the innate immune system, which is the body's first line of defense against pathogens. These immune cells are able to destroy tumor cells by various mechanisms including apoptosis, production of peroxides and free radicals, and production of cytokines which can act on immune cells to enhance their cytotoxic capabilities [4]. T lymphocytes are part of the adaptive immune system, which follows the innate immune response and is able to target specific pathogens. In particular, cytotoxic T lymphocytes are able to destroy tumor cells, also by apoptosis [4]. Cancer treatments may often have considerable effects on the immune system of cancer patients and survivors, particularly by decreasing cell number and function of total lymphocytes, T lymphocytes, B lymphocytes, T lymphocyte ratio ($CD4^+/CD8^+$), and NK cells [4]. Evidence suggests that decreases in immune function in cancer patients and survivors after treatment may be associated with disease relapse, poorer prognosis, and decreased survival rates [27–32]. Additionally, increased levels of pro-inflammatory and decreased levels of anti-inflammatory cytokines could be associated with treatment-related side effects such as muscle wasting [23]. Other cytokines including cutaneous T cell-attracting chemokine (CTACK) and interleukin-15 (IL-15) may affect immune responses at either peripheral or mucosal sites or could be associated with increased activity of cytotoxic T lymphocyte and lymphocyte-activated killer cells [33]. Regarding the endocrine system, cancer survivors experiencing fatigue may also experience dysregulation in adrenal hormone levels, which may affect various components of health-related quality of life (HRQOL) [34]. Therefore, research aiming to understand how aerobic exercise may modify cellular immune system responses, and how endocrine and soluble factors may mediate these immune system responses, is clinically relevant.

Aerobic Exercise, Its Influence on the Immune System in Cancer Survivors, and the Effects of Endocrine and Soluble Factor Mediators

For this chapter, a comprehensive literature search up until December 2018 identified original research articles that examined the effects of aerobic exercise on cellular immune system, endocrine system, and soluble factor responses (e.g., cytokines and C-reactive protein [CRP]) in cancer survivors. These articles were determined from online searches through PubMed. Key terms that were combined during the searches included cancer, aerobic exercise, acute aerobic exercise, aerobic exercise training, low intensity, moderate intensity, high intensity, leukocyte, lymphocyte, neutrophil, natural killer cell, monocyte, T cell, B cell, stress hormones, cortisol, insulin, estrogen, testosterone, cytokine, interleukin, and CRP. Studies were included in the review if they met the following criteria. First, studies had to be conducted using human subjects who were performing aerobic exercise during or after cancer treatment. Second, studies had to identify a cellular immune system component as an out-

come variable in the data analysis, identify an endocrine or soluble factor parameter as an additional outcome or mediating variable, and provide findings (i.e., cell counts, functional activity, blood concentrations) in text, table, or graphical form. Third, studies had to identify the methods used to measure and quantify immune, endocrine, and soluble factor responses.

Overall, 30 studies published between 1994 and 2018 met these inclusion criteria. These studies will be discussed in two major groups: the first comprising investigations using acute aerobic exercise and the second comprising studies using aerobic exercise training interventions. Where appropriate, the discussion is further subdivided so that findings among studies may be more easily compared. As described previously in the chapter objectives, this review is primarily an examination of the impact of aerobic exercise on immune responses in cancer survivors, with a discussion of underlying endocrine and soluble factor mechanisms that may be driving these cellular immune responses. Thus, the literature chosen and the structure of this chapter aim to support these objectives.

Acute Aerobic Exercise Responses

Relatively few studies have examined the influence of acute aerobic exercise on cellular immune responses, with two studies using pediatric cancer patients [35, 36] and with the remaining three being conducted in adults [37–39]. Three studies examined responses in individuals with different types of leukemia, and two studies examined responses in breast cancer survivors. Most studies were performed in cancer survivors who had completed their primary treatments. For the purpose of this discussion, acute aerobic exercise is defined as a single bout of aerobic exercise.

Shore and Shepard [35] examined the immune response to exercise at anaerobic threshold in 6 children with a history of cancer (predominantly acute lymphoblastic leukemia (ALL)) compared to 11 healthy children. The six patients had been diagnosed within 5 years of study enrollment and were either receiving chemotherapy or had completed chemotherapy. Similarly, Ladha et al. [36] studied the effect of acute moderate-to-vigorous aerobic exercise on immune function in four pediatric patients with ALL who were receiving maintenance therapy and six matched healthy controls. In both studies, the subjects performed a progressive exercise test to exhaustion to determine peak aerobic capacity ($VO_{2\,peak}$), and both tested the immune response to an acute aerobic exercise session lasting 30 minutes in duration [35, 36]. Subjects performed the acute exercise bout at an intensity corresponding to anaerobic threshold or alternated 10 minutes of running at 85% of $VO_{2\,peak}$, 10 minutes of walking at 70% of $VO_{2\,peak}$, and 10 minutes of running, again at 85% of $VO_{2\,peak}$. Immune parameters were measured from blood samples drawn pre-exercise, immediately post-exercise, and during various points in recovery [35, 36].

Shore and Shepard [35] reported an overall leukocytosis and lymphocytosis in response to exercise that followed profiles similar to those seen in healthy children. Cell counts were lower in subjects currently receiving chemotherapy, and exercise did not seem to change T cell counts ($CD3^+$, $CD4^+$, or $CD8^+$). The pre-exercise $CD4^+/CD8^+$ ratio was lower compared to healthy children, dropping to below one at 30 minutes post-exercise. Similarly, the NK cell count ($CD56^+$), cytolytic activity, and lymphocyte proliferation activity in the subjects currently receiving chemotherapy were lower compared to healthy children but not for the subjects who had completed chemotherapy. However, the authors do not describe detailed immune responses to the aerobic exercise bouts, such as the magnitude of change in cell counts and activity post-exercise relative to pre-exercise levels [35]. In the Ladha et al. study [36], a significant main effect for time was observed for neutrophil count, in that it increased significantly from pre-exercise to immediately post-exercise ($p = 0.011$). At 1 hour post-exercise, neutrophil count decreased significantly compared to neutrophil count immediately post-exercise ($p = 0.045$). At 2 hours post-exercise, neutrophil count increased significantly compared to neutrophil count at 1 hour post-exercise ($p = 0.052$). Similar changes in other immune

components were also observed. There were no significant differences in neutrophil counts between the two study groups at any time point, although significant main effects for group were observed for total lymphocyte and eosinophil cell counts ($p < 0.001$ and $p = 0.006$, respectively). Unstimulated neutrophil oxidative burst (i.e., oxidative burst measured before stimulation with phytohaemagglutinin (PMA)) was significantly higher in the control group across all time points compared to the patient group ($p = 0.029$), although no significant main effect for time was observed. When examining the ratios of PMA-stimulated neutrophils at 5, 10, and 15 minutes to unstimulated neutrophils across time (pre-exercise to 2 hour post-exercise), the patient group displayed a considerably greater increase in neutrophil oxidative burst compared to the control group ($p = 0.048–0.074$) [36].

In adults with chronic myeloid leukemia, Jonsson et al. [37] found that performing maximal exercise (~15 minutes) produced significant increases in lymphocyte and neutrophils counts (both $p < 0.001$), with lymphocyte counts returning to baseline levels during recovery and neutrophil counts remaining elevated throughout the recovery window. While immune responses were generally similar between cancer survivors and healthy controls, there was a tendency to observe lower leukocyte counts in cancer survivors [37]. In women with breast cancer who performed 45 minutes of submaximal discontinuous exercise, Evans et al. [38] observed similar results. Significant increases in all leukocyte populations occurred immediately following exercise, with granulocytes remaining elevated at 2 hours post-exercise, whereas lymphocytes had decreased but not returned to baseline levels. NK counts in both study groups followed a biphasic response to acute exercise, and in breast cancer survivors, NK counts were significantly lower than healthy controls at immediately post-exercise ($p = 0.046$) [38]. Zimmer et al. [39] compared changes in immune cell proportions following a half marathon between breast cancer survivors and age- and sex-matched controls. The authors found that leukocytes proportions varied in their responses, with neutrophil proportions showing a significant increase above baseline levels at 15 minutes following the half marathon before returning to baseline levels at 24 hours following the half marathon ($p < 0.001$). Monocyte and total lymphocyte proportions were both significantly decreased below baseline levels at 15 minutes following the half marathon ($p < 0.001$), with monocytes remaining significantly below baseline levels ($p < 0.001$) and total lymphocytes returning to baseline and remained significantly below baseline levels at 24 hours. When further examining lymphocyte subpopulations, the authors found that during the 24 hours following the half marathon, B cell, total T cell, and T helper cell proportions were significantly elevated ($p < 0.001 – p = 0.006$) [39]. Cytotoxic T cell and NK cell proportions were significantly decreased at 15 minutes following the half marathon with NK cell proportions remaining significantly decreased at 24 hours ($p < 0.001$). The decrease in NK cell proportion following the half-marathon is somewhat contradictory, as these cells are among the most rapidly mobilized cells following acute exercise. The 15-minute delay in obtaining the sample after exercise coupled with a reduced absolute intensity of the half-marathon (intensity not reported) may explain the decrease that was observed. Immune cell responses across time were generally similar between breast cancer survivors and controls, although in breast cancer survivors, T helper cell proportions were lower and cytotoxic T cell proportions were higher compared to controls ($p \leq 0.001$) [39].

All of the acute aerobic exercise studies discussed used small sample sizes (e.g., fewer than 20 participants) and primarily examined individuals with leukemia or breast tumors, thus limiting the generalizability of these trials. Moreover, not all of these studies examined changes in specific leukocyte subpopulations. Functional data from these studies is mostly lacking, with only three of these studies including a sub-analysis on some leukocyte subpopulation activity [35, 36, 38]. Recent investigations by Hanson et al. [40. 41] have examined the effect of acute exercise on mucosal-associated invariant T (MAIT) cells, finding that both maximal and submaximal acute

aerobic exercise can increase absolute MAIT cells counts, as well as preferentially mobilizing MAIT cells within T cells. Moreover, MAIT cells stimulated with PMA and ionomycin express a greater proportion of TNF-alpha immediately post-exercise, suggesting exercise increases the ability of these cells to respond to pathogens [40, 41]. While these investigations [40, 41] utilized healthy men, MAIT cells, unlike other T cell subpopulations, are resistant to chemotherapy, thus making them an interesting population to target as exercise immunology and oncology move forward [42].

Endocrine and Soluble Factor Mediators

Direct correlations between changes in immune parameters and mediating mechanisms in response to acute aerobic exercise are understudied in the cancer survivor population. However, a few studies have collectively examined changes in blood concentrations of hormones and/or cytokines using acute aerobic exercise stimuli that are the same as or similar to the ones discussed in the previous section. Although correlational analyses between changes in immune parameters, hormones, and cytokines were not largely performed, one may consider that concurrent changes in these endocrine and soluble factor parameters could potentially underlie the previously discussed immune system responses to acute aerobic exercise.

Four studies have investigated adrenal hormone and/or inflammatory cytokine responses to moderate-vigorous intensity aerobic exercise lasting approximately 30–45 minutes [43–46]. In women with breast cancer who performed 45 minutes of moderate intensity discontinuous exercise, Evans et al. [43] found that epinephrine and cortisol responses were attenuated compared to healthy controls, while norepinephrine responses were similar between breast cancer survivors and healthy controls. These attenuated epinephrine and cortisol responses in the breast cancer survivor group may have been a contributing factor to the somewhat lower NK cell responses that were observed in the breast cancer survivor group in the previously discussed study by Evans and associates, although no correlational analyses were performed among the NK cell and hormonal parameters [38, 43]. Using a similar acute aerobic exercise stimulus, Hanson et al. [44] found that in prostate cancer survivors, 45 minutes of moderate intensity discontinuous exercise also resulted in multiple changes in adrenal hormone responses when compared to healthy controls. Similar to the study by Evans et al. [43], Hanson et al. [44] observed typical exercise and recovery patterns of norepinephrine that were similar in both prostate cancer survivors and healthy controls, but that epinephrine and cortisol responses differed in some respects. Exercise-induced epinephrine responses were attenuated in prostate cancer survivors compared to healthy controls, and cortisol levels were significantly lower in the subgroup of prostate cancer survivors who received androgen deprivation therapy (ADT) compared to the subgroup of prostate cancer survivors who did not receive ADT [44]. While the authors did measure changes in leukocyte counts across time, no significant differences between study groups were observed, and leukocyte subsets appeared to follow typical acute aerobic exercise and recovery patterns [44]. Zimmer et al. [45] examined changes in IL-6, macrophage migration inhibitory factor (MIF), NK cell, and cytotoxic T cell activity in response to 30 minutes of moderate intensity exercise in non-Hodgkin's lymphoma (NHL) survivors and healthy controls. The authors observed that the exercise stimulus yielded significant increases in post-exercise IL-6 levels and cytotoxic T cell activity in the NHL survivors (p = 0.014–0.041), similar trends for which were seen in healthy controls (p = 0.075–0.076) [45]. These authors also observed significant negative correlations for MIF levels and NK cell activity (r = −0.434, p = 0.029), indicating that higher levels of MIF were associated with reduced NK cell transcriptional activity [45]. Lastly, Dethlefsen et al. [46] found that in breast cancer survivors, 30 minutes of vigorous intensity

discontinuous exercise produced increases in serum epinephrine, norepinephrine, IL-6, IL-8, and TNF-alpha, as well as a ~10% reduction in several markers of breast cancer cell viability ($p < 0.001 - p = 0.04$).

In addition to examining changes in leukocyte subsets as previously discussed, Zimmer et al. [39] also examined changes in inflammatory cytokine responses to a half marathon in breast cancer survivors and age- and sex-matched controls. The authors found that the two study groups showed similar cytokine responses across time. Serum IL-6, TNF-alpha, and MIF concentrations were significantly elevated immediately following the half marathon, with TNF-alpha concentrations decreasing significantly below baseline values at 2 hours following the half marathon ($p < 0.001 - p = 0.004$) [39].

Collectively, acute aerobic exercise seems to produce generally similar immune responses in cancer patients compared to healthy individuals. Ladha et al. [36] and Evans et al. [38] showed decreased unstimulated neutrophil function and absolute NK cell count, respectively, in cancer survivors across time compared to the healthy controls, an effect that may be related to the immunosuppressive effects of treatment (chemotherapy). Evans et al. [43] and Hanson et al. [44] suggest that 45 minutes of moderate intensity discontinuous exercise can lead to some differences in adrenal hormone response patterns in breast and prostate cancer survivors compared to healthy controls. For the breast cancer survivors in the two studies by Evans et al., [38, 43] the observed changes in epinephrine and cortisol could have been a factor mediating some of the observed NK cell responses, as NK cells are responsive to changes in catecholamine and cortisol levels [38, 43, 44]. Zimmer et al. [39] showed that a half marathon race appears to affect fit breast cancer survivors and fit healthy controls similarly, in that both tend to display typical exercise and recovery leukocyte and cytokine responses (e.g., a short-term inflammatory response followed by an anti-inflammatory response). Dethlefsen et al. [45] and Zimmer et al. [46] suggest that the changes in inflammatory cytokine levels observed in response to moderate-to-vigorous exercise may underlie significant reductions in breast cancer cell viability and increase NK cell activity, both of which indicate positive effects on mechanisms of anticancer defense. While the cancer survivors in these studies tolerated the exercise sessions appropriately and without adverse effect, the differences in immune, endocrine, and cytokine parameters that were observed between the cancer survivor participants and the control participants are likely due to physiological changes imparted by cancer treatments [46]. Thus, further research is needed to understand the effects of acute exercise on immune, endocrine, and soluble factor function in cancer survivors in order to determine optimal prescriptions of aerobic exercise, particularly for individuals whose physiology may already be weakened by chemotherapy and/or other therapies.

Aerobic Exercise Training Responses

Eighteen studies have examined the effect of aerobic exercise training on cellular immune responses [35, 47–63]. The discussion in this section will be divided into two subsections: cellular immune responses in cancer survivors who are undergoing some form of major cancer treatment such as chemotherapy, bone marrow transplant (BMT), or blood stem cell transplant (during treatment) and cellular immune responses in cancer survivors who have completed their major cancer treatments (after treatment). In this fashion, studies with similar patient characteristics will be discussed together in order to more easily compare results and conclusions.

During Cancer Treatment

Nine studies have examined the effect of aerobic exercise training on cellular immune system responses during treatment [35, 47–54]. Five studies used only adult cancer survivors [47–51]. Three studies used only pediatric cancer survivors [35, 52, 53]. One study used both adolescent and adult cancer survivors [54]. Types of cancers

included both hematological and solid tumors, and the exercise interventions were performed when patients were receiving chemotherapy, autologous peripheral blood stem cell transplant (PBSCT), allogeneic BMT, allogeneic hematopoietic stem cell transplant (HSCT), or combinations of chemotherapy and PBSCT or chemotherapy and radiation. Study designs fell into five categories: a pretest-posttest design with patients in an exercise training group and matched healthy controls also in an exercise training group [35], a single-arm exercise intervention [49], randomized controlled trial with patients divided into an exercise training group and a non-exercising control group [47, 48, 50, 51, 53], a non-randomized trial with patients divided into either an exercise training group or a non-exercising control group [54], and a non-randomized trial with patients specifically recruited into an exercise training group and a group of matched "historical" controls [52]. Exercise intervention modes varied and included cycle ergometry, relaxation and stretching exercises, walking on a treadmill, soccer, skating, cross-country skiing, and swimming. Exercise intensities were light to vigorous, ranging from 50% of cardiac reserve (defined as 220 minus age minus resting heart rate) [47] to 50–90% of maximum heart rate [35, 52, 54]. One study did not specify the exercise intensity, but the activities performed by the patients during their sessions (relaxation, stretching, and bending exercises in the supine position) were most likely of light intensity [50]. Exercise sessions were performed for durations ranging from 10 to 70 minutes and frequencies of 2 to 7 days/week. Total intervention duration ranged from 2 weeks to 3 months. Immune parameters were measured at rest from blood samples taken at the beginning and end of the exercise interventions and occasionally at time points during the interventions.

Leukemias, Peripheral Blood Stem Cell Transplant, and Bone Marrow Transplant

Dimeo et al. [47] and Kim and Kim [50] examined the effect of aerobic exercise training in adult cancer patients undergoing peripheral blood stem cell or bone marrow transplants, respectively. Changes in immune parameters were examined over the intervention period, and results were compared within and between the study groups. The immunological outcome variable in the study by Dimeo et al. [47] was duration of neutropenia during the intervention period, defined as an absolute neutrophil count of $<5 \times 10^9$ cells/L. Patients in the exercise training group experienced significantly fewer days of neutropenia compared to patients in the control group ($p = 0.01$). In the study by Kim and Kim [50], the immunological outcome variables were changes in total lymphocyte and T cell subsets before and after the intervention period. Total lymphocyte counts did not significantly differ between the two study groups or from pre-intervention to post-intervention. However, a significant group-by-time interaction effect was present where the total lymphocyte counts for the control group decreased and the total lymphocyte cell counts for the exercise training group increased ($p = 0.031$). When examining the T cell responses to the intervention (i.e., $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ ratio), the authors do not report significant differences in cell counts between the study groups or across time. Similarly, there were no significant group-by-time interaction effects for percentages of $CD3^+$ and $CD4^+$ cells or the $CD4^+/CD8^+$ ratio [50].

Shore and Shepard [35] and Chamorro-Viña et al. [52] examined the effect of aerobic exercise training in pediatric cancer patients undergoing hematopoietic stem cell treatment. The immunological outcome variables in the study by Shore and Shepard [35] were changes in cell counts (total leukocytes, total lymphocytes, monocytes, granulocytes, T cells, B cells, NK cells), cytolytic activity, and lymphocytes proliferation before and after the intervention [35]. Pre-intervention cell counts for total leukocytes, total lymphocytes, and T cells ($CD3^+$, $CD4^+$, and $CD8^+$), as well as phytohaemagglutinin (PHA)-induced lymphocyte proliferation, were significantly lower in the cancer patients compared to the healthy controls (exact p-values not reported). In the control group, total leukocyte cell count and $CD3^+$ cell count significantly decreased

pre-intervention to post-intervention (exact p-values not reported). Changes in other cell counts, cytolytic activity, and lymphocyte proliferation did not change significantly in the control group over the course of the intervention. In the patient group, there were no significant changes in any immune parameter across time, although lymphocyte proliferation and cell counts except B cell counts did decrease from pre-intervention to post-intervention. Cytolytic activity did increase over the course of the intervention in the patient group, but the change was not significant [35]. The immunological outcome variables in the study by Chamorro-Viña et al. [52] were changes in cell counts (total leukocytes, total lymphocytes, monocytes, T cells, NK cells, NK-T cells, dendritic cells) measured pre-intervention, 15 days post-HSCT, and 30 days post-HSCT [52]. No significant changes were observed for any immune parameter across time or between study groups. Cell counts were generally decreased at 15 days post-HSCT compared to pre-intervention levels and increased at 30 days post-HSCT compared to 15 days post-HSCT in both study groups. A time effect was found for total T cells ($CD3^+$), $CD4^+$, and dendritic cells ($p = 0.04$, 0.032, and 0.001, respectively). Additionally, a group-by-time interaction effect was found for dendritic cells ($p = 0.045$). However, the low p-values reported for these changes were not reported as significant because of adjustment for multiple comparisons [52].

Lastly, Hayes et al. [54] examined the effects of an aerobic exercise intervention in a sample of adolescent and adult cancer patients undergoing chemotherapy and autologous PBSCT. Type of cancer was varied and included several types of hematological cancers, breast cancer, and rhabdomyosarcoma. The immunological outcome variables in this study were cell counts for total leukocytes, total lymphocytes, and T cell subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ ratio), as well as T cell function. Results were compared across the study time points, between the two study groups, and to previous normative age- and sex-matched data. Cell counts were measured at five time points: before receiving PBSCT (PI), 17–21 days after receiving PBSCT (PII), 1 month post-PI (I1), 2 months post-PII (I2), and 3 months post-PII (PIII). From PI to PII, the effect of the PBSCT was being assessed, and all patients were considered to be in the same group for those two time points. The exercise training intervention took place between PII and PIII, and therefore immunological differences between study groups were assessed during this time. The authors found that there was no significant group-by-time interaction effect for any immunological parameter. When the results for the two study groups were pooled, significant changes across time were observed for total lymphocyte, $CD3^+$, $CD4^+$, and $CD8^+$ cell counts ($p < 0.05$); the $CD4^+/CD8^+$ ratio; and T cell proliferation index per $CD3^+$ cell ($p < 0.01$). Cell counts decreased from PI to PII (the period of time pre-PBSCT to post-PBSCT), but increased again to near- or above-PI levels at I1, I2, and PIII. Only the $CD4^+$ cell count remained significantly below PI levels at I1 and PIII ($p < 0.05$). Likewise, the $CD4^+/CD8^+$ ratio decreased over time and remained significantly below PI levels at I1, I2, and PIII ($p < 0.05$). Total T cell proliferation did not change significantly across time, although T cell proliferation per $CD3^+$ cell was significantly higher at PIII compared to PII ($p < 0.01$). Significant differences also existed for cell counts and T cell proliferation between the patients in this study and normative data. T cell counts ($CD3^+$, $CD4^+$, and $CD8^+$) were significantly lower compared to normative data at PI and PII ($p < 0.01$). $CD4^+$ cell counts remained significantly lower compared to normative data throughout I1, I2, and PIII ($p < 0.01$). Total lymphocyte and $CD3^+$ cell counts returned to normal levels at I1, but became significantly decreased again at PIII ($p < 0.01$). The $CD4^+/CD8^+$ ratio remained significantly below normative values throughout I1, I2, and PIII ($p < 0.01$). Total T cell proliferation remained significantly below normative values across all study time points, whereas T cell proliferation per $CD3^+$ cell was significantly depressed only at PII, I1, and I2 ($p < 0.01$) [54].

Solid Tumors

More recently, four studies examined aerobic exercise training during chemotherapy treatment

for solid tumors. Three of the studies were performed in adults, and one was in pediatric patients. Glass et al. [48] examined 30–45 minutes of aerobic exercise three times per week for 12 weeks compared to usual care for a cohort of adult cancer survivors undergoing treatment for a variety of solid tumors (mostly breast cancer). The authors found that training did not induce any changes in leukocyte population, although there was a trend for group differences in CD4 T helper cells (AE: 0.8%, UC: −3.2%, $p = 0.063$) and CD8 CD45 RA cytotoxic naïve T cells (AE: 2.3%, UC −4.7%, $p = 0.081$). In a single-arm study conducted by Kim et al. [49], 12 weeks of walking 30–40 minutes 5 days per week did not alter the number of leukocytes, lymphocytes, CD4 or CD8 T cells, NK cells, or NK T cells in breast cancer survivors. However, the immune cell response was rather heterogeneous (% change range: −21.1–15.6%). In contrast, Schmidt et al. [51] found that breast cancer survivors receiving chemotherapy experienced decreased T, B, and NK cell counts and that 60 minutes of exercise twice per week was unable to attenuate these changes. Lastly, Fiuza-Luces et al. [53] found that in pediatric patients undergoing neoadjuvant chemotherapy, three weekly exercise sessions lasting just over an hour saw a significant group-by time interaction ($p = 0.028$) for the proportion of NK cells expressing KIR2DS4 (a transmembrane receptor protein that inhibits NK cell activity) that was stable with exercise but increased in non-exercising controls. The authors found that treatment, independent of training, significantly decreased B, NK, and NK T cell proportions ($p < 0.05$) and was consistent with some [48, 51] but not all previous reports [49].

The general conclusions reached by the authors of these nine studies were that aerobic exercise training during cancer treatment is likely safe, does not seem to significantly hinder immune function compared to non-exercising cancer patients or healthy controls, and may even help to increase immune cell counts or reduce the number of days of immunosuppression during hospitalization. In the two studies that compared their findings to normative data or healthy controls, pre-intervention values for many immune parameters in the cancer patients were significantly depressed [35, 54], whereas treatments often lead decreases in immune counts and proportions [48, 50, 51]. Furthermore, immune cell counts and function may decrease further over the course of the exercise intervention, although differences between pre-intervention and post-intervention values were largely nonsignificant [35, 54]. General recommendations include that exercise interventions should be prescribed cautiously, on an individual basis, and with careful monitoring of immune function, especially when considering a cancer patient whose immune system has already been weakened by concurrent cancer treatments.

After Cancer Treatment

Nine studies have examined the effect of aerobic exercise training on cellular immune system responses after treatment, all but one in adult cancer survivors [55–63]. Five studies used breast cancer survivors [56, 57, 61–63], two studies used stomach cancer survivors [59, 60], and one study each was completed in thyroid survivors [58] or mixed cancer survivors [55]. The amount of time that had elapsed from completion of cancer treatment to the beginning of the exercise intervention varied among the studies, from 2 days to several years.

Fairey et al. [56], Hutnick et al. [57], Nieman et al. [61], and Peters et al. [62, 63] examined the effect of aerobic exercise training in post-treated breast cancer survivors. The amount of time that had elapsed between the completion of cancer treatment to the beginning of the exercise intervention was clearly stated in four studies as 2 weeks–2 months [57], at least 6 months [62, 63], and 3.0 ± 1.2 years [61]. One study stated that patients had completed cancer treatment between January 1999 and June 2000, but did not clearly define when the exercise intervention began [56]. Study designs fell into three categories: a one-group pretest-posttest design [62, 63], a randomized controlled trial design with subjects divided into an exercise training group and a

non-exercising control group [56, 61], and a non-randomized trial where subjects were specifically recruited into either an exercise training group or a non-exercising control group [57]. Exercise intervention modes included cycle ergometry, walking, and running (treadmill and overground). Exercise intensities were moderate to vigorous, ranging from 60% to 86% of heart rate maximum [61–63], 70% to 75% of $VO_{2\ peak}$ [56], or 60% to 75% of functional capacity [57]. Exercise sessions were performed for durations ranging from 10 to 40 minutes and frequencies of 2 to 5 days/week. Total duration of the interventions ranged from 8 to 29 weeks. Immune parameters were measured at rest from blood samples taken at the beginning and end of the exercise interventions and occasionally at time points during the intervention.

The immunological outcome variables for these five studies included cell counts for total leukocytes, total lymphocytes, granulocytes, monocytes, NK cells, neutrophils, T cells, and B cells [56, 57, 61–63]. NK cell activity (NKCA), phagocytic activity of monocytes, lymphocyte proliferation, and neutrophil function were also assessed. The effects of aerobic exercise training on immune parameters seen in these studies are similar to those previously described for cancer patients during treatment. Most studies did not observe significant changes in immune cell counts across time in the exercise training group compared to the control group [56, 57, 61, 62]. When looking at percentages of immune cell proportions rather than absolute cell counts, Peters et al. [63] did observe a significant increase in granulocytes and a significant decrease in monocytes and lymphocytes post-intervention compared to values at pre-intervention and at 5 weeks into the intervention, ($p < 0.05$). Hutnick et al. [57] found that the percentage of activated T helper cells was significantly higher in the exercise training group compared to the control group at the end of the intervention ($p < 0.05$).

When looking at the functional capacity of immune parameters, more differences were observed between study groups. The two studies by Peters et al. [62, 63] found that NKCA was significantly increased post-intervention compared to values measured pre-intervention and at 5 weeks into the intervention, and that monocyte phagocyte activity was significantly increased at both 5 weeks into the intervention and post-intervention compared to pre-intervention levels ($p < 0.05$). Nieman et al. [61] did not observe significant differences in NKCA across time between exercising and non-exercising participants, but Fairey et al. [56] observed that NKCA did increase across time in exercising participants compared to non-exercising controls ($p < 0.001$–0.39). Fairey et al. [56] did not observe significant differences in neutrophil function across time between study groups, but did observe significant increases in spontaneous lymphocyte proliferation across time in the exercise intervention group compared to the control group ($p = 0.007$). Likewise, Hutnick et al. [57] also observed significantly higher lymphocyte proliferation in the exercise training group post-intervention compared to the control group ($p < 0.05$).

Looking at other cancer types, Lee et al. [59] and Na et al. [60] examined the effect of aerobic exercise training in post-treated stomach cancer survivors. The amount of time that had elapsed between the completion of cancer treatment to the beginning of the exercise intervention was 2 days [60] and at least 2 years [59]. Lee et al. [59] used a one-group pretest-posttest design, while Na et al. [60] used a randomized controlled trial design with subjects divided into an exercise training group and a non-exercising control group. Exercise intervention modes included arm and cycle ergometry and Tai Chi. Exercise intensities were light to moderate, and Na et al. explicitly stated that the exercise intensity used in their intervention was 60% of maximum heart rate [60]. Exercise sessions were performed for 30–40 minutes and frequencies of 1 day/week [59] and twice daily for 5 days/week [60]. Total duration of the interventions was 2 weeks [60] and 24 weeks [59]. Immune parameters were measured at rest from blood samples taken at the beginning and end of the exercise intervention [59] and on days 1, 7, and 14 of the intervention [60].

Na et al. [60] examined changes in NKCA across the intervention period that immediately followed treatment and found that NKCA measured in the control group decreased from day 1 to day 7 to day 14, while NKCA increased over the three time points in the exercise training group. Additionally, NKCA measured at day 14 was significantly higher in the exercise training group compared to the control group ($p < 0.05$). Lee et al. [59] examined changes in cell counts (total leukocytes, total lymphocytes, monocytes, NK cells, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ ratio) before and after the intervention, which occurred at least 2 years post-treatment. The percentages of total leukocytes and monocytes were significantly increased post-intervention compared to pre-intervention ($p = 0.011$ and 0.02, respectively), but no other immune changes were observed across time. While both studies are from similar cancer types, the time since treatment, the exercise modes and intensities, and the different outcomes used make drawing firm conclusions difficult at this stage.

More recently, two trials have been published that provide support for some of the previous work, both of which had home-based components. Kim et al. [58] studied postsurgical thyroid cancer survivors who completed aerobic exercise 3–5 days per week, striving to attain at least 150 minutes of activity per week (along with resistance and flexibility training). Chamorro-Viña et al. [55] examined the effect of a 10-week, moderate intensity (e.g., 50–70% of age-predicted maximal heart rate) hospital-based and home exercise program in pediatric cancer survivors who had received hematopoietic stem cell transplants. Both studies showed no change in NK cell counts, although the study by Chamorro-Viña et al. [55] found that exercise led to a shift in the NK cell populations toward $CD56_{dim}$. However, NKCA was increased following training in both studies, which is consistent with the studies by Fairey et al. [56], Na et al. [60], and Peters et al. [62], but not Nieman et al. [61].

The general conclusions reached by the authors of these nine studies is similar to those described for the studies that examined the effect of aerobic exercise on immune function during cancer treatment. Specifically, aerobic exercise training either does not significantly affect immune function in cancer survivors, or it may lead to improvements in resting immune function. The specific immune function parameters that seemed to be affected by aerobic exercise training were NKCA [55, 56, 58, 60, 62], monocyte phagocytic activity [63], and lymphocyte proliferation [56, 57]. In the studies not reporting changes in immune function, training duration (8 weeks) was suggested to be too short to elicit marked changes in resting NKCA [61] or that immune markers were within the normal ranges at the beginning of the intervention [59], providing only limited room for improvement even after exercise training. Even so, maintaining or even improvements in resting immune function after exercise training following treatment are beneficial to the cancer survivor population in that it may lead to an increased capacity for clearance of infectious microorganisms and neoplastic cells, as well as improvements in immune function beyond what may be expected with normal recovery after cancer therapy [56, 57, 63].

Endocrine and Soluble Factor Mediators

Similar to what has been previously described for acute aerobic exercise, direct correlations between changes in immune parameters and mediating mechanisms in response to aerobic exercise training are also understudied in cancer survivors. However, seven studies have examined the impact of aerobic exercise training on changes in immune system parameters, hormones, cytokines, and/or CRP [46, 56, 57, 59, 64–66]. Six of these studies have focused on breast cancer survivors [46, 56, 57, 64–66], one focused on stomach cancer survivors [59], and all occurred after cancer treatment completion. Four of these studies have been previously discussed [46, 56, 57, 59].

In addition to examining changes in a wide variety of immune cell counts and function in breast cancer survivors, Fairey et al. [56, 64, 65] investigated the effect of their 15-week exercise intervention on a multitude of hormones, inflammatory cytokines, and CRP. As previously

discussed, the authors did not observe significant differences in immune cell counts or neutrophil function across the intervention period between the exercise intervention group and the non-exercise control group, although they did observe significant increases in lymphocyte proliferation and NKCA in the exercise intervention group [56]. At the same time, the authors observed that insulin growth factor-1 (IGF-1) and CRP levels decreased in the exercise group but increased in the control group across time ($p = 0.045$ and $p = 0.066$), but did not observe significant changes in any other hormone or cytokine parameter [56, 64, 65].

Dethlefsen et al. [46], Hutnick et al. [57], Saxton et al. [66], and Lee et al. [59] all examined the effects of a 6-month intervention period on their select immune, endocrine, and/or cytokine parameters. Hutnick et al. [57] and Lee et al. [59] both observed increases in immune parameters in their studies' exercise training groups across time (e.g., increased T cell activation, increased percentage of total leukocytes and monocytes). Changes in IL-6, IFN-gamma, and/or TNF-alpha were also measured, either as circulating blood levels or within cell culture supernatant. Although Hutnick et al. [57] observed an increase in T cell activation in their study's exercise training group, they did not observe changes in IL-6 or IFN-gamma levels within activated T cell culture supernatant. The authors did observe decreases in circulating blood levels of IFN-gamma in the exercise training group across time, whereas circulating blood levels of IL-6 remained similar in both the exercise training group and the control group [57]. Somewhat similarly, Lee et al. [59] also failed to observe significant changes in circulating blood levels of IL-6 or TNF-alpha across time.

Dethlefsen et al. [46] found that their 6-month exercise intervention led to significant decreases in serum IL-6 and TNF-alpha levels ($p < 0.001$ and $p = 0.003$), but did not significantly affect serum IL-8, IL-10, or breast cancer cell viability [46]. These results somewhat contrast with the results that the authors observed after a 30-minute session of vigorous intensity aerobic exercise, where breast cancer cell viability was reduced [46]. The authors propose that the transient changes in endocrine and cytokine parameters that occur with each acute exercise bout may be what drive beneficial anticancer effects, rather than changes in resting parameters over time [46]. Saxton et al. [66] observed that breast cancer survivors in their 6-month intervention group experienced significantly smaller increases in neutrophil and lymphocyte counts across time relative to the control group with lymphocyte proliferation and NKCA remaining similar from baseline to the end of the intervention period ($p = 0.02$–0.04) [66]. The authors did not observe significant changes in circulating IL-6 or TNF-alpha levels across time, although they did observe an improved diurnal cortisol pattern in the breast cancer survivors in the lifestyle intervention group at the end of the 6-month period [66].

Collectively, these studies indicate that aerobic exercise interventions in cancer survivors may lead to improvements in hormonal, cytokine, and other soluble factor levels, or these parameters may be unchanged over the course of the intervention. Several of these parameters (e.g. CRP, IL-6, IL-8, TNF-alpha, and IFN-gamma) are typically indicative of systemic inflammation, while others such as IL-10 are considered anti-inflammatory. However, the fact that not all endocrine and soluble factor biomarkers are equally affected, or that changes in these biomarkers may not always reflect the same changes in cellular immune parameters, is likely due to multiple factors. For example, different aerobic exercise training volumes may yield different magnitudes of physiological stimuli, only certain inflammatory markers may be responsive to exercise, measurements of circulating biomarkers may be very close to the assay limits of detection, or the effect of the cancer treatments may outweigh the effect of exercise [66–68]. Since the existing literature is varied regarding the types of cancer survivors studied, the characteristics of the exercise interventions utilized (e.g., mode, intensity, frequency, duration, total intervention length, aerobic exercise only vs. combined aerobic and resistance exercise, etc.), and the characteristics of the biomarkers examined (e.g., the biomarkers themselves, the time points at which biomarkers are

sampled, the analysis techniques, etc.), it is challenging to definitively correlate intervention-induced endocrine and soluble factor responses with immune responses. Even so, participating in exercise while actively receiving cancer treatment may help to lessen the negative effects of cancer treatments on endocrine and inflammatory responses while independently leading to significant improvements in aerobic fitness, muscular fitness, quality of life, stress, fatigue, sleep quality, body composition, and blood lipid levels [23, 34, 68–70].

Conclusions and Future Research Directions

Aerobic exercise is a beneficial adjunct therapy for cancer survivors as it can improve both physiological and psychological functioning. Aerobic exercise also affects immune and endocrine system functioning in cancer survivors, which may lead to potential improvements in mechanisms associated with systemic inflammation, cardiovascular disease risk, anticancer defense, cancer prognosis, recurrence, second malignancy, and overall survival. Current studies show that aerobic exercise is feasible in both pediatric and adult cancer patients during and after treatment and in some cases may lead to improvements in resting immune function, cytokine profiles, and stress and metabolic hormone levels over the course of training. However, it should be noted that cancer patients undergoing cancer therapies including chemotherapy, blood stem cell transplant, and BMT may experience decreased immune system cell counts and function compared to healthy individuals and that exercise interventions should be individualized and with careful monitoring of immune function.

Literature reviews by Fairey et al. [4] and Kruijsen-Jaarsma et al. [18] have comprehensively outlined the limitations of previous exercise immunology literature in the cancer patient/survivor population, as well as recommendations for future research. In short, future studies should continue to examine responses of cancer survivors compared to those of healthy individuals and improve their reporting of baseline participant physical characteristics, health behaviors, dietary intake, and adherence to exercise interventions. Future studies should also continue examining underlying mediating mechanisms of exercise-induced alterations in cellular immune function and relate these alterations to clinically relevant cancer outcomes (e.g., onset of long-term sequelae, treatment-related toxicities, risk of recurrence, and mortality). It is hopeful that the knowledge gained from this continuously growing field of exercise immunology will aid in developing more individualized exercise prescriptions for each cancer survivor that will capitalize on the anti-inflammatory effects of exercise and mitigate the side effects of cancer treatments. Thus, exercise continues to be a valuable clinical approach to improving cancer survivor physical functioning, quality of life, and overall survival.

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Type I Diabetes and Exercise

25

Sam N. Scott, Michael C. Riddell, and Jane E. Yardley

Introduction

Type 1 diabetes (T1D) is a chronic inflammatory autoimmune disease that destroys the insulin-producing β-cells of the pancreas resulting in absolute insulin deficiency [1]. Maintaining normoglycemia (4–8 mmol/L) in individuals with T1D requires self-administration of exogenous insulin, regular glucose monitoring, and dietary control. Although T1D can be diagnosed at any age, the highest incidence is during adolescence [2]. The International Diabetes Federation estimates that over one million youth worldwide currently live with the disease [3]. T1D increases the risk of acute (e.g., ketoacidosis and hypoglycemia) and long-term complications (e.g., neuropathy, nephropathy, retinopathy, heart disease, stroke, foot ulcers) because of the lifetime exposure to fluctuating blood glucose levels. Life expectancy for those living with T1D can be shortened by 15–20 years, and risk of death is 3–4 times higher than those without diabetes [4, 5].

Regular exercise is accepted as a cornerstone for T1D management and maintenance of overall health. However, exercise represents a considerable challenge for this population. An understanding of glucose targets for safe and effective exercise and the neurohormonal responses to different forms of exercise is important. This chapter will outline the importance of regular exercise for people with T1D for maintenance of overall health and discuss the blood glucose responses to different forms of exercise in people with T1D.

The Importance of Regular Exercise for People with Type 1 Diabetes

Exercise is recommended for those with T1D because it improves cardiovascular disease risk profile [6], body composition, cardiorespiratory fitness [7], endothelial function, and blood lipid profiles [8–10]. However, most T1D patients do not maintain a healthy body mass, nor do they meet current physical activity guidelines [6, 10, 11]. The effects of physical inactivity and the benefits of regular physical activity in people with T1D are briefly reviewed below and summarized in Fig. 25.1.

Cardiovascular disease is the most common cause of mortality in people with T1D [12]. An elevated risk for cardiovascular disease in T1D is likely linked to alterations in several predisposing factors including the tendency for suboptimal glycemic control, hypertension, elevated triacylglyceride, and LDL-cholesterol levels along with

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lower HDL-cholesterol levels [12, 13]. Exercise has beneficial effects on lipid levels in individuals with T1D [14, 15] potentially independent of changes in glycemic control [8]. Endothelial dysfunction, which is common in T1D, may also be improved with regular exercise training [14]. Furthermore, higher physical activity levels over the lifespan increase longevity in T1D by about 10 years, partly due to the reduced risk of cardiovascular disease [16].

The incidence of insulin resistance in people with T1D is rising, mirroring the trend in the general population and reflecting the rising rates of obesity worldwide [17–19]. This fact is important given that insulin resistance is an additional independent risk factor for micro- and macrovascular complications in those with T1D [17, 20–24]. Regular exercise is associated with reduced insulin requirements in people with T1D [25, 26] and may even preserve β-cell function in those

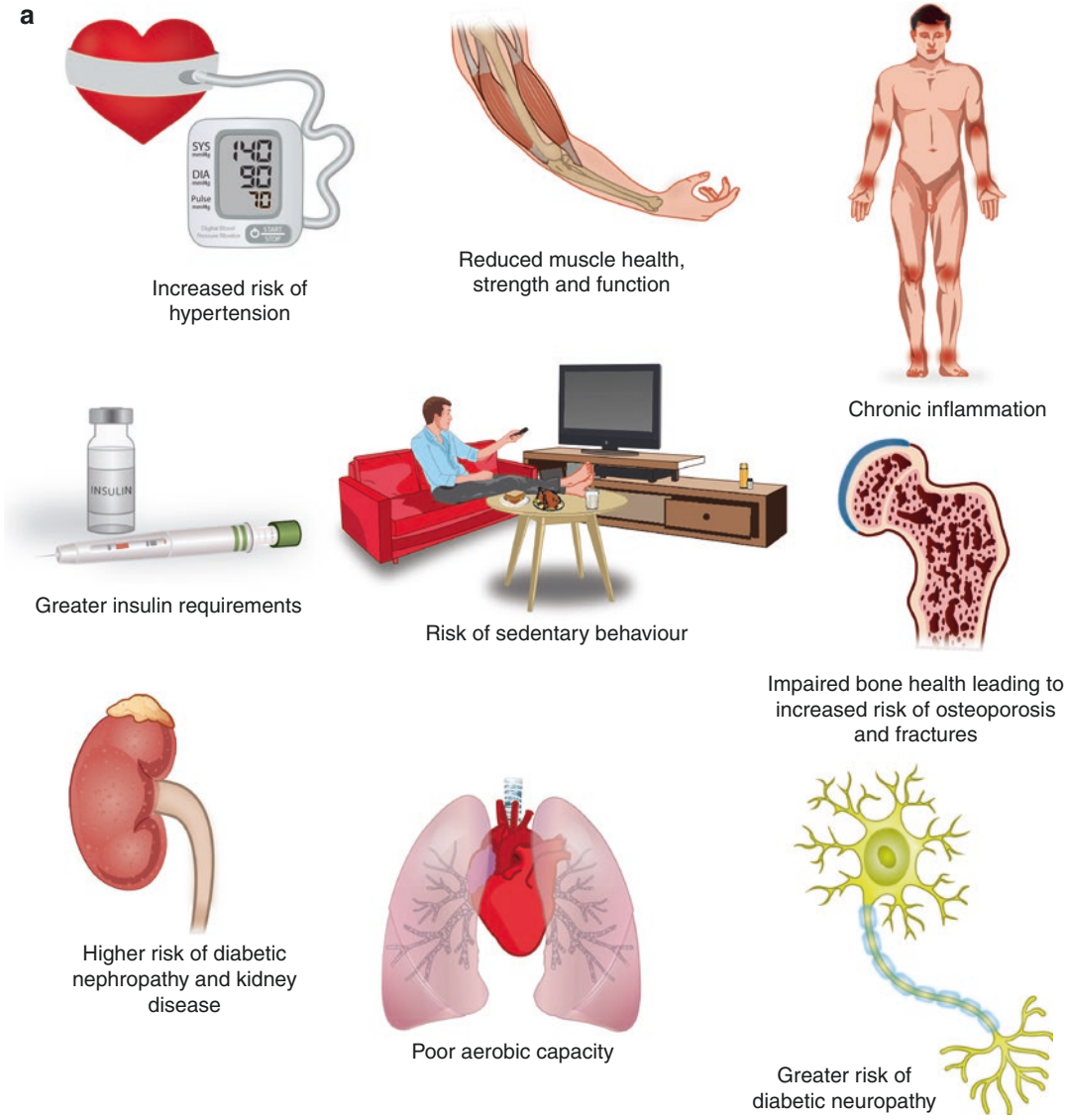

Fig. 25.1 (**a**) Summary of risk factors associated with physical inactivity in people with type 1 diabetes. (**b**) Summary of factors known to be improved with regular physical exercise in people with type 1 diabetes

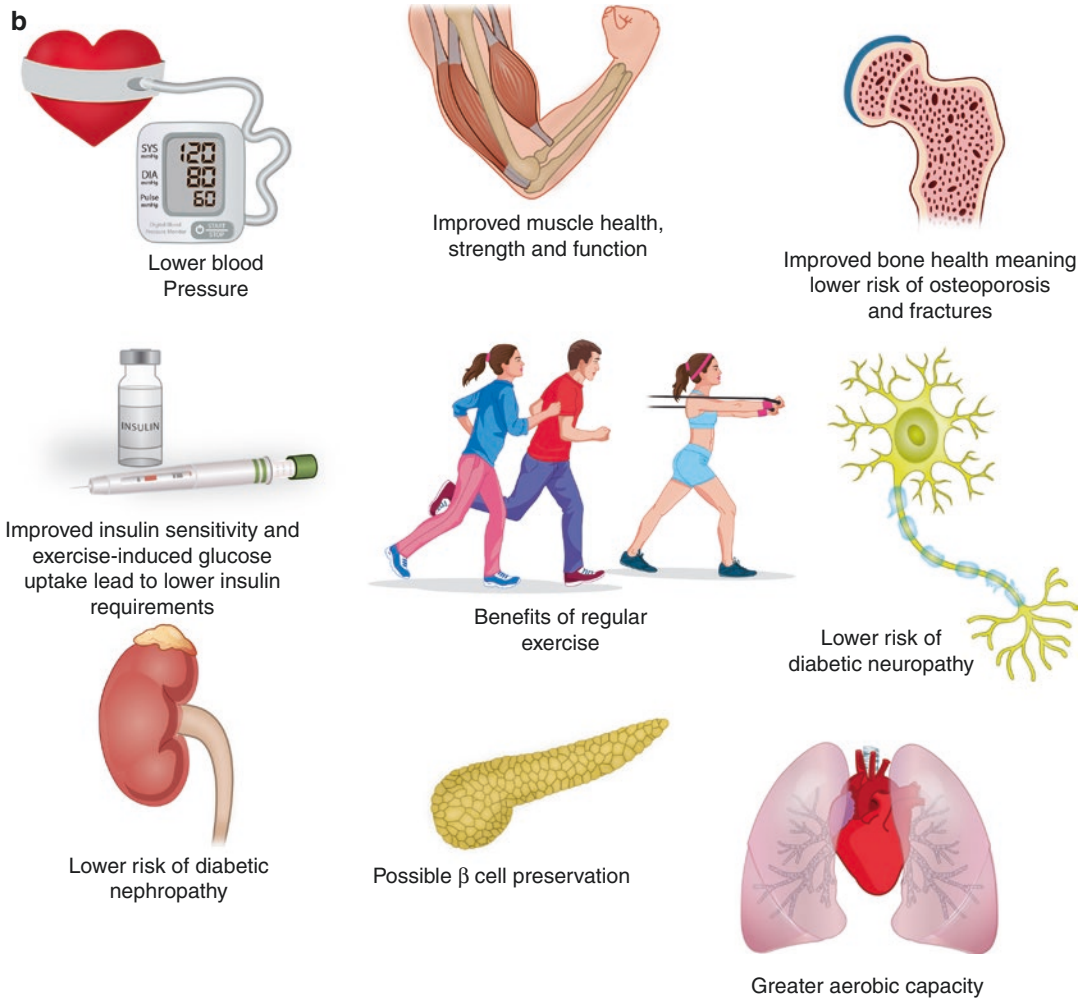

Fig. 25.1 (continued)

recently diagnosed [27, 28]. In patients who exercise regularly around the time of diagnosis, partial remission of the disease ("honeymoon" period) may increase fivefold [28]. This preservation of β-cell function has important clinical benefits: it reduces the risk of complications, improves glycemic control, and decreases insulin requirements [29]. Additional studies directly investigating the effects of exercise on β-cell mass and function in people with T1D are required [27, 30].

Physically inactive people with T1D may have lower skeletal muscle health (quantity and quality of skeletal muscle) compared to those without diabetes who are also inactive, due to increased metabolic stress, vascular impairments, and insulin resistance [31]. This impairment, along with altered mitochondrial morphology and function in young people with T1D, may lead to a vicious cycle of insulin resistance, impaired glucose and lipid disposal, and reduced basal metabolic rate. All of these factors may affect diabetes management [32, 33] as the muscle cannot respond optimally to stressors or combat elevated glycemic and lipid loads frequently experienced in T1D. In addition, impaired insulin-stimulated vasodilation can reduce blood flow and therefore glucose delivery for extraction in skeletal muscle [34, 35]. As exercise improves skeletal muscle health, it may contribute to delaying complications in those with T1D.

Increased whole-body inflammation and oxidative stress are also common in T1D [36]. Excessive plasma glucose promotes reactive oxygen species production and the expression of inflammatory cytokines [31, 37]. Oxidative stress likely contributes to diabetic myopathy through upregulation of atrophy-related genes [38]. High oxidative stress can also impact the transcription of glucose transporters, contributing to the development of insulin resistance [39]. Interleukin-6 (IL-6), an inflammatory cytokine, has been linked with numerous atrophic states and is elevated in children with T1D [40–42]. Single bouts of exercise elicit anti-inflammatory effects, and exercise training may decrease basal IL-6 levels [43]. The anti-inflammatory effects of exercise may also benefit β-cell mass due to increases in circulating growth hormone (GH), insulin-like growth factor 1, glucagon-like peptide-1, and IL-1 receptor agonist.

People with T1D have higher intramyocellular lipid (IMCL) content than weight- and activity-matched individuals without diabetes, and this is associated with impaired insulin sensitivity [44, 45]. It is assumed that as IMCL deposition increases in people with T1D, the ensuing lipotoxicity enhances stress on the tissue [46]. The chronic hyperglycemic state may be a relevant mechanism of the exaggerated IMCL accumulation in those with T1D as Perseghin et al. [45] found that patients with better (HbA1c <7.5%) metabolic control possessed a higher insulin-stimulated glucose metabolic clearance rate in association with lower IMCL. These outcomes suggest that when good glucose control is achieved, these abnormalities may be partially reversed. Studies in people without T1D demonstrate that exercise training causes intramuscular lipid droplet remodeling which is associated with improved insulin sensitivity [47].

Loss of bone mineral density, osteoporosis, and increased risk of fracture are common in inactive people with T1D, particularly in the presence of suboptimal glucose control and neuropathy [48–50]. Bone turnover is reduced, even when blood glucose levels are well-controlled. It is well established that exercise is beneficial for reducing osteoporosis risk and improving bone health in people without T1D [51, 52]. The one study to date, in children with T1D [53], would suggest that this supposition is also true for T1D patients. Resistance exercise specifically is known to have a positive impact on all of these factors and therefore may play an especially important role in people with T1D [54]. However, the impact of resistance training on long-term blood glucose control is still under some debate [25, 55, 56].

Diabetic nephropathy results from a greater incidence of hypertension in people with T1D than the general population and can lead to end-stage renal disease [57]. In addition to hypertension and elevated glucose exposure, kidney disease progression is linked to increased inflammatory markers in T1D [58]. One large cohort study [59] showed that greater leisure-time physical activity, particularly of a higher intensity, was associated with a lower risk of developing diabetic kidney disease. Similarly, cross-sectional data [60] show a strong association between exercise capacity and renal health in adolescents with T1D. Possible protective mechanisms include lowering of blood pressure, along with improved blood lipid profile, glycemic control, and endothelial function. Improved insulin sensitivity may also be important due to the association between insulin resistance and microalbuminuria [21, 61].

Barriers to Exercise in People with Type 1 Diabetes

Current guidelines suggest that people with T1D should perform at least 150 minutes of moderate-intensity or 90 minutes of vigorous-intensity physical activity per week with no more than two consecutive sedentary days. Resistance exercise is also recommended twice per week [54]. Few achieve these targets, and programs for increasing physical activity in people with T1D have failed [62]. In addition to the usual barriers cited by the general population (e.g., lack of time, work commitments, and cost) [63–65], those with T1D also list fear of hypoglycemia, loss of glycemic control, and inadequate knowledge

around exercise management [65]. The risk of hypoglycemia and the difficulties of managing blood glucose during exercise continue to pose a challenge [63, 65–69] even in the current care setting of intensive insulin therapy, hybrid closed-loop insulin pumps, and frequent glucose monitoring [70].

Hypoglycemia

Mild hypoglycemia can be defined as a blood glucose concentration of 3.0–3.9 mmol/L, serious or clinically important hypoglycemia can be defined as a blood glucose concentration ≤2.9 mmol/L, and severe hypoglycemia is defined as the patient requiring assistance from another person for recovery [71]. It is important to note, however, that symptoms of hypoglycemia can occur at blood glucose levels above 3.9 mmol/L, particularly in those who are recently diagnosed and who have been in a state of chronic hyperglycemia [72]. The symptoms of hypoglycemia can range in seriousness from mild tremor, loss of coordination, and mental confusion to convulsions, unconsciousness, brain damage, and even death [73, 74]. At the very least, an episode of hypoglycemia is a nuisance, but in extreme cases it can be fatal, potentially accounting for 2–4% of T1D deaths [75]. Reports estimate that people with T1D suffer on average 3.5–7.2 episodes of symptomatic hypoglycemia per month [76–78], although studies using continuous glucose monitors show higher unnoticed (often nocturnal) incidents [79, 80]. On average, around 12% of adults living with T1D experience at least one severe, temporarily disabling episode of hypoglycemia per year [81–84].

A bout of exercise generally increases the risk of hypoglycemia in people with T1D, either at the time of the activity or later on in recovery [85]. It is therefore not surprising that many people with T1D avoid exercise, especially if they have had a previous "bad experience" with exercise-induced hypoglycemia. Hypoglycemia is especially dangerous in sports competed at high speeds, in hazardous environments, and/or in close proximity to other competitors because aspects of visual information processing, spatial awareness, auditory function, psychomotor function, and physical performance can be impaired as hypoglycemia progresses [86, 87]. These deficits could slow judgment or reaction time leading to mistakes and injury.

Post-exercise, late-onset hypoglycemia is a common barrier to exercise for those with T1D [88–90]. The risk of exercise-induced hypoglycemia can last for up to 31 hours [89], although the first 12–24 hours may have the greatest risk due to elevated insulin sensitivity [91] and blunted glucose counter-regulation [92]. Glucose requirements following moderate-intensity exercise performed late in the day exhibit a biphasic pattern with increases occurring both immediately post and 7–11 hours post-exercise [90, 93]. If the same exercise is performed midday, the increased insulin sensitivity is more stable but is still consistently elevated for about 11 hours [91].

Hyperglycemia

While mild- to moderate-intensity exercise increases the risk of hypoglycemia, intense exercise can increase blood glucose levels, potentially leading to hyperglycemia [94–99]. In these situations, individuals may opt to correct their glucose with an insulin bolus after exercise [94]; however, care must be taken to avoid overcorrecting as this can lead to severe nocturnal hypoglycemia. In the absence of insulin (e.g., where the individual has removed their insulin pump or when insulin delivery is blocked or skipped), any level of exercise can lead to hyperglycemia and ketone formation. To reduce the risk of hyper or hypoglycemia, the Exercising for Type 1 Diabetes (EXTOD) UK guidelines [100, 101], Diabetes Canada guidelines [102], and recent international guidelines [85, 103] recommend starting exercise when ketone levels are low in blood (i.e., <1.5 mmol/L) or free/trace in urine and capillary blood glucose concentration is roughly between 7 and 14 mmol/L. When blood glucose is >14 mmol/L with low (i.e., <1.5 mmol/L) or no blood ketones, advice depends on the timing of the last meal and insulin dose. If the meal was

eaten in the last 1–2 hours, and some insulin was administered, exercise can be commenced, but blood glucose should be monitored closely. If the last meal was >2 hours ago and the individual is hyperglycemic (i.e., >14 mmol/L) with detectible blood and/or urine ketones, the patient is advised to perform a conservative insulin bolus correction (e.g., reduce their usual insulin correction dose by ~25–50% before starting exercise) to help reduce the risk of hyperglycemia and elevation in ketone bodies [103].

Hyperglycemia influences hydration status and electrolyte balance which may be particularly important during exercise. As blood glucose levels rise above 9–10 mmol/L, the renal threshold for active glucose reabsorption can be exceeded, leading to glucose loss in the urine [104, 105]. This situation leads to osmotic diuresis and dehydration if fluid intake is insufficient. High blood glucose levels in people with T1D may also influence electrolyte balance; for example, there may be greater sodium retention, which increases the risk of hypertension, or alterations in magnesium reabsorption in the kidneys [106–108]. A recent study, found that mean self-reported fluid intake was over 30% greater in T1D athletes than in age- and activity-matched controls without T1D [109]. Despite drinking fluid in line with international guidelines [110], most of those with T1D still reported post-exercise thirst. These findings [109] suggest that individuals with T1D and healthcare professionals need to take hydration and electrolyte balance into account during exercise to compensate for elevated blood glucose levels.

Normal Neuroendocrine Responses to Exercise

The pattern of neuroendocrine and autonomic nervous system counterregulatory responses to exercise, and their subsequent effects on blood glucose concentration, is related to intensity and duration of the activity [85]. In persons without diabetes, robust mechanisms help to maintain euglycemia and exercise (or sport) performance; however, with T1D, a number of these mechanisms are dysfunctional. Exercise is generally classified as "aerobic" or "anaerobic," depending on the predominant energy systems used, although many forms of exercise use a combination of the two systems. Aerobic exercise, defined here as prolonged (>30 minutes) activity performed up to 70–80% of one's maximal aerobic capacity (VO_{2max}), typically uses large muscle groups at a relatively low rate of muscular contraction in a continuous manner (e.g., running, cycling, or swimming). High-intensity or anaerobic exercise is defined here as exercise >80% VO_{2max} and cannot be maintained for long periods, especially if the individual is untrained. It should be noted, however, that activities performed at efforts >80% VO_{2max} may still have a significant "aerobic" component but with additional energy supplied from anaerobic sources (e.g., glycolysis). Very few activities have little or no aerobic component, except for burst activities like power lifting or sprinting. High-intensity interval training (HIIT) is another popular training method, which is defined as brief, intermittent periods of vigorous exercise, interspersed with periods of rest or active recovery.

During exercise, glucose utilization by working muscles must be matched equally by glucose provision by the liver and/or gut to prevent hypoglycemia. During moderate-intensity exercise, several counterregulatory mechanisms exist in people without diabetes that are activated in a stepwise and hierarchical fashion. These mechanisms ensure that glucose uptake and production are precisely matched to keep blood glucose concentration within a tight range of ~4–6 mmol/L [111]. At the onset of aerobic exercise in an individual without T1D, endogenous insulin secretion is inhibited to below fasting levels via sympathetic innervation of the islets of Langerhans [112]. As endogenous insulin has a relatively short half-life (~5 minutes), lower insulin secretion leads to a relatively rapid decrease in circulating insulin. This decrease allows an increase in glucagon secretion from the α-islets in the pancreas. Glucagon then travels via the portal vein to facilitate release of glucose from the liver [113, 114]. The decrease in insulin also sensitizes the liver to glucagon which causes a

rapid rise in cyclic AMP to stimulate glycogenolysis and gluconeogenesis [115–117]. Gluconeogenesis preserves hepatic glycogen stores and becomes increasingly important with intense, prolonged exercise. As blood glucose continues to fall, epinephrine, growth hormone, cortisol, norepinephrine, and aldosterone are released [118, 119]. These hormones stimulate hepatic glucose production and adipose tissue lipolysis. They also inhibit skeletal muscle glucose uptake to increase circulating glucose and prevent hypoglycemia. As exercise intensity increases above 50–60% VO_{2max}, lipid oxidation (percentage energy being derived from fat stores) decreases, particularly in untrained individuals, and carbohydrate becomes the predominant fuel for energy production [120].

High-intensity exercise, >80% VO_{2max}, is predominantly fueled by muscle glycogen, with minimal contributions from lipid and protein [121]. Even in those without T1D, there is a rise in glycemia during high-intensity exercise, such as a 30-second sprint on a cycle ergometer (i.e., Wingate Anaerobic Power Test) [122] or an intense ride to exhaustion that lasts only about 10–15 minutes in duration [97, 98, 123]. This rise is primarily due to a 14–18-fold increase in catecholamines compared to the 2–4-fold increase in these hormones during moderate-intensity exercise [124]. The increased catecholamines elevate hepatic glucose production 7–8-fold, to a level which exceeds skeletal muscle glucose uptake [99]. Unlike aerobic exercise, circulating insulin typically remains unchanged in such intensive activities. In addition, blood lactate concentrations increase 10–20-fold to >10 mmol/L, as the muscle is unable to oxidize all of the pyruvate generated by glycolysis and seeks to maintain acid-base balance by translocation of lactate to the blood (pyruvate → lactate dehydrogenase → lactate).

In addition to facilitating glucose production, the catecholamines associated with high-intensity exercise also limit muscle glucose uptake during exercise relative to glucose production by the liver [125]. This inhibition may be due to its stimulatory effect on muscle glycogenolysis and the subsequent increase in muscle glucose-6-phosphate, with the latter being an inhibitor of hexokinase and glucose phosphorylation in the initial actions of glycolysis [123, 124]. Following a high-intensity exercise bout in people without diabetes, there is a small rise in plasma glucose (0.5–1.0 mmol/L) that persists for at least an hour [99, 126]. Insulin secretion increases post-exercise both to lower circulating glucose concentration and to help recover muscle glycogen via glycogenesis [125, 126].

Endocrine Responses During Exercise in People with Type 1 Diabetes

The complex neurohormonal regulatory responses required for various intensities and durations of exercise are almost impossible to mimic in those with T1D because they do not secrete endogenous insulin. As a result, blood glucose responses vary considerably both between and within individuals with T1D, depending on numerous factors including type, duration, and intensity of exercise, level of circulating exogenous insulin during and after exercise, and pre-exercise blood glucose concentration. Exercise intensity is one of the key determinants of glycemia in people with T1D [85] (Fig. 25.2). A basic understanding of the hormonal responses during different types of exercise and durations is helpful in understanding the typical insulin dose and nutritional adjustments to enable glycemic control in these individuals.

In people with T1D, insulin is supplied exogenously, typically by injection or by an insulin pump. In contrast to endogenous insulin, even the fastest acting synthetic insulins have half-lives that last hours instead of minutes. Therefore, circulating insulin levels do not drop, and may in fact rise, during prolonged aerobic exercise via increased absorption rates [127–129]. The rise in circulating insulin (or perhaps the failure of insulin levels to drop in the vicinity of the α-cells) may limit the release of glucagon which reduces hepatic glucose production and promotes increased insulin-induced peripheral glucose

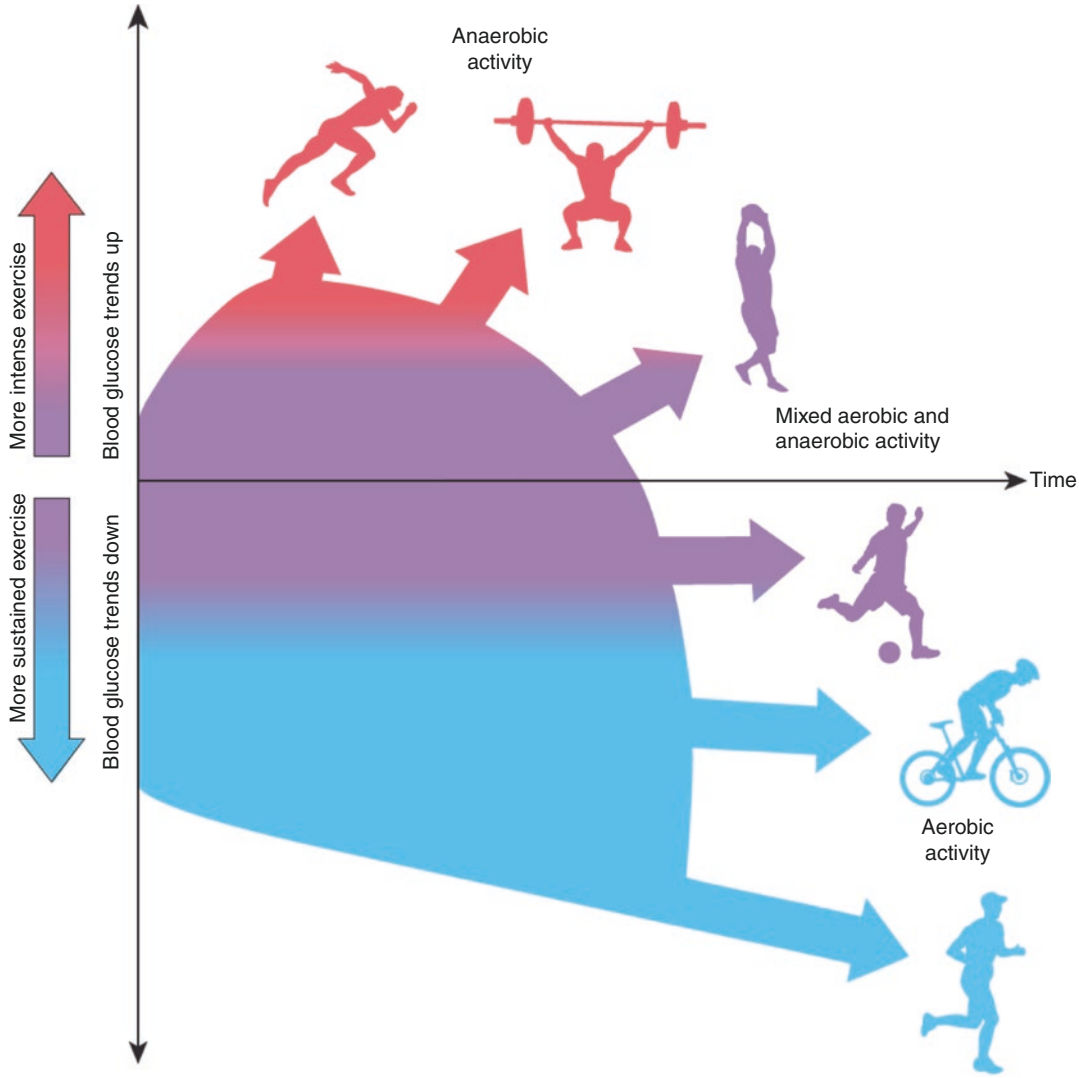

Fig. 25.2 Blood glucose trends caused by different types of exercise in individuals with type 1 diabetes. In general, aerobic exercise decreases blood glucose level, anaerobic exercise increases blood glucose level, and mixed activities are associated with glucose stability. Individual responses are dependent on various additional factors, including the duration and intensity of the activity; initial blood glucose concentrations; individual fitness; concentrations of insulin, glucagon, and other counterregulatory hormones in the circulation; and the nutritional status of the individual. (Reproduced with permission from: Riddell MC. Management of exercise for children and adolescents with type 1 diabetes mellitus. In: UpToDate, Post TW (Ed), UpToDate, Waltham, MA. (Accessed on [24 April 2019].) Copyright © 2018 UpToDate, Inc. For more information visit www.uptodate.com)

uptake [130]. Skeletal muscle perfusion is also likely to rise significantly with exercise [131], potentially increasing insulin-mediated glucose disposal into the muscle and the risk for hypoglycemia. The longer half-life of exogenous insulin compared to endogenous insulin and the location of insulin entry into the circulation (i.e., subcutaneous vs. portal circulation) are also responsible for the higher insulin levels at the onset of exercise in the individual with T1D as compared to individuals who are not using exogenous (i.e., subcutaneous) insulin [132]. Increased insulin concentration in the circulation during exercise promotes increased glucose disposal into skeletal

muscle relative to hepatic glucose production [132] and might delay lipolysis [133]. This combination results in an imbalance of glucose disposal relative to production and eventually hypoglycemia.

Beyond the first few hours of an exercise bout, the increased risk of hypoglycemia is primarily due to increased insulin sensitivity, which can vary according to the duration and intensity of exercise that was performed. The increased insulin sensitivity and continued extraction of glucose from the circulation may be due to increased glycogen synthase activity to replenish glycogen stores [134, 135]. Enhanced GLUT 4 translocation and muscle microvascular perfusion are also important for the changes in glucose uptake [136, 137]. Special care may be required to prevent post-exercise hypoglycemia following afternoon or evening exercise because there is a greater risk of nocturnal hypoglycemia [138]. The risk of nocturnal hypoglycemia following 45 minutes of moderate-intensity exercise performed in the afternoon can be as high as 30–40% [90, 139, 140].

During high-intensity exercise at workloads >80% VO_{2max}, carbohydrate (i.e., blood glucose, muscle glycogen) is the exclusive muscle fuel and is provided by an increase in glycogenolysis in the muscle and the liver. As discussed above, individuals without diabetes will experience an increase in blood glucose due to the rise in catecholamines. The resulting elevation in blood glucose is compensated for by increasing insulin secretion, usually at the end of the activity, to help normalize the transient hyperglycemia caused by the activity [126]. However, because individuals with T1D cannot increase insulin endogenously to compensate for the rise in glucose during high-intensity exercise, they tend to experience a more prolonged period of elevated glucose post-exercise [99]. During exercise, muscle glucose disposal may not be impacted to any great extent by low insulin levels [141], since contraction-mediated glucose disposal dominates [142], but hyperglycemia and ketosis can increase in individuals with T1D as hepatic glucose and ketone production rise above the level of utilization by the working muscle [143]. This situation increases the risk of hyperglycemia and ketoacidosis which may cause dehydration and decrease blood pH, resulting in impaired performance and severe illness [100]. Elevated ketone production can lead to ketoacidotic abdominal pain and vomiting and in some cases may require emergency assistance.

The rise in glycemia during high-intensity exercise led to the assumption that the addition of a short bout of high-intensity exercise would counter the glucose-lowering effects of lower-intensity exercise and stabilize blood glucose levels in individuals with T1D. It was indeed shown in a series of studies that adding a short (10-second) sprint either before [144] or after [145] a 20-minute bout of moderate-intensity (40% VO_{2max}) exercise can provide a means to counter, temporarily, the fall in post-exercise glycemia [144–147]. Where the moderate-intensity exercise on its own caused a significant drop in blood glucose in both trials, including a 10-second sprint, attenuated these declines. The stabilization of glycemia following the sprint trial was associated with elevated catecholamines, growth hormone (GH), and cortisol. The mechanism was later revealed to involve a reduction in the rate of glucose disposal rather than rate of appearance [147].

As noted, high-intensity interval training (HIIT) is a popular exercise option that consists of repeated bouts of high-intensity exercise interspersed with low-intensity recovery periods [148]. Certain forms of HIIT are a time-efficient alternative to moderate-intensity exercise resulting in similar cardio-metabolic health benefits to prolonged aerobic exercise in individuals without T1D [149–151]. Results from studies investigating the glycemic responses to HIIT in people with T1D have produced varied responses as HIIT has been shown to increase [94, 96], decrease [140, 152, 153], and help stabilize blood glucose levels [154, 155]. The varied results likely reflect the differences in exercise protocols (intensity and duration of intervals), modality of exercise (cycling vs. whole-body calisthenics) used within this training form, as well as the time of day in which the exercises were performed. It is likely that when HIIT is performed fasted, or

when insulin levels are considerably diminished, a variable rise in glucose is observed [94, 96].

The hormonal and metabolic responses to resistance exercise in T1D will most likely vary depending on the type of program utilized. Resistance may be provided in the form of weights, body weight, or elastic resistance bands. In those without T1D, high-resistance, low-repetition routines are known to elicit similar responses to anaerobic activities like high-intensity running [156], which may result in an increase in blood glucose levels. Conversely, high-repetition, low-resistance programs with short rests between sets are more aerobic in nature and may therefore result in a decrease in blood glucose. Few studies have examined glycemic responses to resistance exercise in T1D individuals and those that have consisted mostly of moderate-intensity protocols involving three sets of eight repetitions [157–160]. When performed in the afternoon, Yardley et al. [157] found that this type of protocol is associated with declining blood glucose, albeit to a smaller extent than a comparable period of moderate (60% VO_{2peak}) aerobic exercise. The resistance exercise was also associated with more stable blood glucose levels for several hours post-exercise [157]. However, an almost identical protocol performed under fasted conditions in the morning caused an increase in blood glucose and post-exercise hyperglycemia [159, 160]. This phenomenon will be discussed in more detail in the subsequent section on fasted exercise in T1D.

When combined with aerobic exercise, resistance exercise may provide some protection against hypoglycemia. One study showed that performing 45 minutes of resistance exercise before 45 minutes of treadmill running (60% VO_{2peak}) delayed declines in blood glucose during the aerobic exercise [158]. Conversely, blood glucose declines were immediate and rapid when aerobic exercise was performed first, but these were halted by the switch to resistance exercise. These authors concluded that individuals experiencing hyperglycemia during exercise should perform aerobic activities first, while those struggling with hypoglycemia during exercise should start their sessions with resistance exercise.

As mentioned previously, research in the area of resistance training and T1D is quite sparse, and studies generally have small sample sizes. More work is needed to fully understand the effect of factors such as the speed of movement, amount of weight lifted, number of repetitions and sets, and the duration of rest intervals on hormonal responses in T1D [161]. It is also uncertain whether physiological factors such as age, sex, and physical fitness level can alter these responses [162].

Additional Factors to Consider

In addition to the effect of type, duration, and intensity of exercise, there are many other inter- and intra-individual factors for people with T1D to consider (Table 25.1). These factors include the location of insulin delivery, amount of insulin in the circulation, blood glucose concentration before exercise, and composition of the last meal [163]. The site and depth of insulin injection also affect the absorption characteristics [164, 165]. Injecting insulin into the muscles used during exercise can increase the rate of absorption and cause more rapid decreases in blood glucose concentration (e.g., injecting the thigh before cycling). Higher ambient temperature increases skin temperature, which enhances subcutaneous blood flow and accelerates insulin absorption [163, 166, 167]. This increase in circulating insulin leads to lower blood glucose levels compared to cooler temperatures [129]. Collectively, these factors make it difficult for the individual with T1D to anticipate their blood glucose response to exercise.

According to one cross-sectional study [168], trained individuals with T1D have greater reductions in blood glucose during aerobic exercise than less fit individuals, possibly because their overall work rate is higher. Antecedent hypoglycemia and exercise and competition stress also affect glycemic control due to differences in hormonal responses [169–172]. In one study of T1D adolescents, change in glycemia was negatively correlated with pre-exercise blood glucose concentration, with only very high pre-exercise glu-

Table 25.1 Factors to consider around exercise and blood glucose management when living with type 1 diabetes (T1D). Readers are referred to [85] for clinical guidance

Factor	Comments
Relative intensity, duration, and type of exercise	See Fig. 25.2
Insulin	Elevations in insulin (basal or bolus) tend to increase hypoglycemia risk during prolonged aerobic activities [236, 237]
Other medications	Several other medications such as corticosteroids, oral glucose-lowering agents, inhalers, and other medications can influence glycemia [238]
Alcohol	Alcohol increases the risk of hypoglycemia [239]
Environmental conditions	Undertaking exercise at high temperatures and/or at altitude increases the risk of dysglycemia [240]. Extra consideration is needed especially if they are unaccustomed to lower temperatures
Antecedent hypoglycemia and/or moderate-intensity exercise	The counterregulatory responses may be impaired during subsequent exercise bouts and increase the risk of hypoglycemia [92, 241]
Pre-exercise blood glucose levels	Blood glucose levels tend to drop more when starting exercise with higher blood glucose concentration as long as insulin is in circulation [173]. If blood glucose is elevated, carbohydrate feeding may need to be delayed until blood glucose has lowered. However, when pre-exercise blood glucose is low, high glycemic index carbohydrate needs to be consumed
Time of day	Exercising late in the afternoon increases the risk of nocturnal hypoglycemia [242]. Early morning exercise may reduce risk of hypoglycemia due to the "dawn effect." The individual with T1D may require more vigilance after an afternoon exercise session to reduce the risk of nocturnal hypoglycemia
Hormonal factors	Menstrual cycle phase affects insulin sensitivity [243], i.e., insulin resistance during early luteal phase. Competition stress may cause blood glucose levels to rapidly rise due to an increase in cortisol and/or catecholamines [244]

Note: The above factors are only a partial list of the various factors thought to influence the individual blood glucose responses to exercise in TID

cose levels providing protection against hypoglycemia [173]. No other tested variable predicted glycemic response including age, weight, height, BMI, duration of T1D, daily insulin dose, or sex.

The time of day that exercise is performed can also alter the glycemic responses to exercise, particularly if the individual is fasting. As briefly mentioned, studies of afternoon resistance exercise in T1D observed declines in blood glucose during activity [157, 158], whereas an almost identical resistance exercise protocol performed in the morning under fasting conditions resulted in either no change [160] or a mean increase [174] in blood glucose during the exercise session. Similar outcomes were found using a repeated measures design of morning and afternoon resistance exercise, where the morning (fasting) exercise led to an increasing trend in blood glucose, while blood glucose levels declined with afternoon exercise [175]. There have also been similar findings with fasting aerobic exercise. Ruegemer et al. [176] observed declines in blood glucose following 30 minutes of aerobic exercise in the afternoon, with the same participants experiencing an increase in blood glucose when performed in the morning. A recent study also observed that T1D participants performing both moderate aerobic and HIIT in the fasted state did not experience declines in blood glucose during either exercise protocol [177]. These findings contrast with the declines in blood glucose found during later day (fed-state) aerobic exercise [140, 152–154, 157, 178–180] and HIIT [140, 152–154, 180].

There are several possible explanations for this phenomenon observed with fasted exercise. The first is that lower circulating insulin during fasted exercise sessions decreases the suppression of hepatic glycogenolysis and consequently increases blood glucose during exercise. In addition, individuals with T1D may experience the "dawn phenomenon" [181], an early morning rise in blood glucose possibly due to an increase

in circulating GH [182–184]. Previous T1D exercise studies suggest that higher GH could spare blood glucose [158, 185] by stimulating more lipolysis [186]. While these theories remain unconfirmed, it can still be suggested that those struggling with hypoglycemia during exercise, and/or those trying to avoid additional carbohydrates to aid weight management, may have greater success with early morning/fasted exercise than they would with exercise later in the day.

Using exercise diaries to carefully monitor carbohydrate intake, insulin dosage, and type of exercise along with the other factors listed above can help to improve glycemic control with practice. In a recent study, Abraham and colleagues showed that plasma glucose response to a bout of moderate-intensity aerobic exercise can be reproducible under similar glycemic and basal insulin conditions in adolescents with T1D [187]. However, more research using larger sample sizes is needed to investigate the reproducibility of blood glucose responses to different types of exercise under varying conditions. Nonetheless, this information may be useful for people with T1D who train or compete regularly, so they can devise a routine that works for them. It will also help researchers with the development of algorithms for use with the artificial pancreas.

Does Type 1 Diabetes Affect Physical Performance?

It is currently unclear how T1D can impact exercise and sport performance. Clearly, exceptional athletes live and compete with a diagnosis of T1D (see https://integrateddiabetes.com/athletes-with-type-1-diabetes/ for a partial list). Nevertheless, even those that achieve elite athlete status can struggle with glucose control around exercise which may affect their performance [188]. The athlete with T1D will recognize the importance of individualizing their insulin regime and diet according to their sporting event, training schedule, and personal experiences.

There are numerous reports that people with T1D, in general, have lower age- and activity-matched VO_{2max} scores than those without T1D [189–193]. Several cardiovascular, muscular, and metabolic impairments in T1D have been suggested to explain the potential decrement in aerobic and anaerobic performance. End diastolic volume and left ventricular ejection fraction fail to increase normally during exercise in young people with T1D [194], and there are differences in glycolytic metabolism that reflect an earlier onset of glycolysis in individuals with T1D [195]. During prolonged exercise, individuals with T1D under good glycemic control have higher glycolytic flux [195] and tend to rely more on muscle glycogen utilization [196] which might reduce endurance capacity. However, Nugent et al. [197] found no differences in VO_{2peak} in adults with long-standing T1D compared to healthy controls. Similarly, Veves et al. [198] observed that only inactive adults with neuropathic complications or a sedentary lifestyle demonstrated decreased VO_{2max}, suggesting T1D per se does not directly affect exercise capacity in trained individuals.

If disease-related impairments in physical capacity do exist in those with T1D, it is likely related to the patient's level of glycemic control over the last several days, months, and years which can have subtle effects on the muscle [32, 33] and/or performance [199]. Poortsman et al. [193] and Huttunen et al. [192] both reported that physical capacity was inversely related to the level of metabolic control as indicated by HbA1c. It is unclear however what the cause for this is, but suggestions include poorer muscle oxygenation, altered mitochondrial function [33], and/or a lower level of habitual physical activity [200–202], likely due to the challenges of living with T1D [63, 203].

Studies investigating whether there are differences in muscular strength and endurance in people with T1D show mixed results. Some studies have shown decrements in strength [204–206], muscle mass, fiber size, work capacity, and maximal force production [32]. The T1D population also displays an increased proportion of fast glycolytic fibers and glycolytic enzymes compared to controls without diabetes [195, 207], accompanied by changes in fuel oxidation and metabolic capacity. As low

insulin can reduce skeletal muscle access to carbohydrates, diabetic muscle must use other fuels. Some evidence suggests that muscle mitochondrial morphology and function is altered in T1D, even in those with reasonable metabolic control [33]. It is unclear whether exercise training can offset these potential mitochondrial disturbances.

Blood glucose concentration during a given bout of exercise may have an effect on performance; however, the degree to which acute changes in blood glucose levels influence sports performance is unclear. Exercising while in the hyperglycemic range increases reliance on muscle glycogen compared to euglycemia [208], due to impaired capacity to switch from carbohydrate to lipid metabolism. Patients are also more prone to early dehydration and acidosis when exercising with high blood glucose [209]. These factors may promote early fatigue, consequently decreasing endurance. Prolonged hypoinsulinemia/hyperglycemia would presumably lower muscle glycogen levels, reduce muscle strength, and lead to dehydration and electrolyte imbalance [210]. As such, it is likely that euglycemia promotes the best performance.

Upcoming Research in Exercise and Type 1 Diabetes

Mini-Dose Glucagon

Repeated hypoglycemia leads to reduced capacity to respond to future hypoglycemic episodes, termed hypoglycemia-associated autonomic failure [211–213]. T1D also leads to blunted or absent glucagon response to low blood glucose resulting in impaired and prolonged recovery and increased frequency/severity of hypoglycemia [214]. During an episode of severe hypoglycemia where the individual is unable to consume carbohydrate, parenteral glucagon is the only FDA-approved treatment outside of emergency medical care. Currently available glucagon kits require a solution of sterile water to be mixed with a vial of lyophilized powder before the solution is injected intramuscularly.

The use of mini-dose glucagon for treating non-severe hypoglycemia has been limited by the need to reconstitute the glucagon preparation before injection and the limited time during which it can be used once reconstituted. Regardless, Haymond and Schreiner [215] suggested that mini-dose glucagon is an effective means of treating impending episodes of hypoglycemia in children with T1D. Recently, a non-aqueous liquid form of glucagon that is stable at room temperature has been developed for use before prolonged aerobic exercise [216], which may offer a "real-world" application [217]. Rickels and colleagues [216] demonstrated that a "mini dose" of glucagon (150 μg) given subcutaneously can be used as an effective and tolerable means to prevent hypoglycemia during exercise. However, a number of limitations of mini-dose glucagon including discomfort at injection sites, inconvenience of filling disposable syringes, embarrassment of taking the injections in public, and potential nausea need to be addressed. Despite these limitations, half of the participants in the study [217] stated that they would prefer to use mini-dose glucagon rather than ingesting glucose tablets. Treatment with mini-dose glucagon may also assist in maintaining a healthy weight due to reduced calorie intake to treat mild to moderate hypoglycemia [218]. Development of this treatment will be particularly important for the development of a dual-hormone closed-loop artificial pancreas system [219].

Artificial Pancreas/Closed-Loop Technology

Intensive research is currently ongoing into the development of the artificial pancreas systems using closed-loop automated insulin delivery to improve glucose control while reducing the burden of dysglycemia on the patient with T1D. A closed-loop system must be able to cope with common daily challenges to glycemia including stress, illness, and exercise to predict and respond appropriately to changes in blood glucose levels. These systems combine real-time sensor glucose measurement with insulin pumps using a control

algorithm to direct insulin delivery [220–222]. To achieve autonomic control during exercise without the user informing the device, an accurate exercise wearable must be able to detect exercise onset, intensity, and duration.

Development of continuous glucose monitoring, insulin pump, and control algorithms are the key components of closed-loop systems. The MiniMed 640G (Medtronic, Inc., Northridge, CA, USA) was the first commercially available system with the ability to predictively suspend and automatically restart basal insulin based on continuous glucose monitor data [223]. Choudhary et al. [223] and Klupa et al. [224] both suggest that the system is well-tolerated and able to prevent many hypoglycemic events. The newer-generation hybrid closed-loop unit (MiniMed 670G) appears to be particularly efficacious in limiting post-exercise hypoglycemia in children with T1D [225], albeit the drop in glucose during the exercise itself is not eliminated with this newer technology [226, 227]. Other research grade artificial pancreas systems, with exercise "modes," also show some efficacy for improved exercise control [228–230].

The risk of hypoglycemia may be further reduced with bihormonal or dual-hormone closed-looped systems that deliver glucagon when hypoglycemia is detected or predicted [231]. These systems are currently limited by the need for approved stable glucagon preparations and a second pump device for glucagon [232] and a lack of safety data on prolonged glucagon use. However, the dual-hormone approach for exercise appears to have good efficacy and offers exciting future possibilities for T1D individuals who want to exercise [233–235].

Summary

Managing glycemia during and after exercise represents a considerable challenge for their individual with T1D and their healthcare provider. In addition to the effect of type, duration, and intensity of the exercise performed, people with T1D must also consider the location of insulin delivery, amount of insulin in the circulation, blood glucose concentration before exercise, and composition of the last meal. Recent advances in closed-loop technology are promising; however, until they are perfected, an understanding of the many factors that can influence glycemia is important for safe exercise participation.

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Extreme Sports and Type 1 Diabetes Mellitus in the Twenty-First Century: The Promise of Technology

Karen M. Tordjman and Anthony C. Hackney

Introduction

In less than a century, type 1 diabetes (T1DM) has turned from a rapidly fatal disease into a condition which, when adequately managed, is compatible with a normal lifestyle and a full life expectancy. Ironically, before the advent of insulin, starvation diets and extreme physical exertion were the only means to prolong the lives of the young people affected with the disease. Nowadays, however, moderate recreational physical activity (PA) is recommended as part of a healthy lifestyle for those with the disease [1], while high-frequency and high-intensity exercise has been shown to decrease cardiovascular event risk [2]. The immense popularity and high visibility of top-level sporting events, and the sizeable roster of world-class athletes with T1DM (three-time Olympian and swimming gold medal holder Gary Hall Jr., Chicago Bear quarterback Jay Cutler, and serial ironman competitor well into his fifties Bill Carson, to name a few), together with improved therapeutic tools, are now setting the stage for the growing number of individuals with T1DM engaging in extreme sports events. Media are replete with inspirational personal stories of people with T1DM achieving previously unthinkable goals and taking part in extreme endurance competitions such as iron man triathlons, ultramarathons, and exceptionally demanding endurance events [3, 4]. Likewise there is a growing number of industry-supported or not-for-profit organizations promoting organized groups of patients training for such events (http://www.teamtype1.org, https://worlddiabetestour.org, www.runsweet.com). All of these carry the message that T1DM should not be an insurmountable obstacle to such activity. This may indeed be so, however beyond fame and glitter, managing T1DM in the face of the demands of extreme PA remains a formidable challenge for an individual, but emerging technologies may bring persons with T1DM closer than ever to meet this challenge.

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Glucose Homeostasis During Physical Activity and the Hurdle of Type 1 Diabetes

Optimal muscle function during PA, and thus performance, relies critically on adequate fuel supply. During the initial part of a prolonged aerobic

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PA, muscles rapidly use their glycogen depots, and thereafter they become dependent on glucose and free fatty acid supply as their source of energy. The rapid rise of catecholamine secretion with the onset of exercise inhibits insulin secretion via α-adrenergic receptor stimulation [5, 6]. In the setting of exercise, glucose uptake by the muscle is achieved via translocation of glucose transporter type 4 (GLUT4) from the cytosol to the plasma membrane independently of insulin [7]. In addition, it has been shown that the association of GLUT4 with the plasma membrane is inversely related to the level of glycogen depletion [8]. Thus the decrease in circulating insulin does not hamper glucose influx and utilization by the muscle. At the same time, the fall in insulin alters the insulin/glucagon ratio in the portal circulation, which, combined with greater hepatic glucagon sensitivity [9], promotes augmented hepatic glucose output via glycogenolysis and later on gluconeogenesis [10]. Finally, under the rise of epinephrine and the fall in insulin, lipolysis rate intensifies, and the release of free fatty acids provides an important fuel source for the muscle [11]. These coordinated responses ensure uninterrupted energy supply to the contracting muscle while preserving normoglycemia. Upon termination of exercise, catecholamines decrease allowing renewed insulin secretion, and together with voluntary carbohydrate ingestion, muscle glycogen replenishing is gradually achieved thanks to the postexercise protracted location of GLUT4 at the plasma membrane, and heightened glycogen synthase activity [12].

As this fine regulation of energy supply to the muscle together with preservation of normoglycemia during the entire duration of the event is critically dependent on finely tuned insulin secretion, it is easy to see why this would be such a challenge in a person with T1DM (see reference 13 for a review). Indeed, not being able to reduce the prevailing insulin level achieved by exogenous administration, individuals with T1DM face the danger of hypoglycemia. This is worsened by the failure of counter-regulatory glucagon response to hypoglycemia, which characterizes individuals with long-standing T1DM [14]. Additionally, this may be compounded by the frequent occurrence of failure to mount a catecholamine response to hypoglycemia in individuals with well-controlled diabetes, the so-called hypoglycemia-associated autonomic failure [15]. A phenomenon first noticed 30 years ago is that of delayed postexercise hypoglycemia, which can occur up to 15 hours after the completion of the activity [16]. The mechanism for this is not fully elucidated and could involve protracted GLUT4 residence at the plasma membrane [17]. Finally, antecedent exercise itself has been shown within hours to blunt the response to subsequent hypoglycemia in type 1 diabetic individual [18].

Although hypoglycemia is the most dreaded derangement during endurance events in athletes with type 1 diabetes, it is by no means the only threat to glucose homeostasis. Indeed, hyperglycemia is likely to occur after short but intense anaerobic activities during which extreme catecholamine secretion drives heightened hepatic glucose output and impedes insulin action. In the individual with type 1 diabetes, the inability to respond with increased insulin secretion may result in hyperglycemia, and even in ketoacidosis, particularly amidst poor general metabolic control [19]. For further background the reader is directed to Chap. 25 – “Diabetes type 1 and Exercise” (Scott, Riddell & Yardley) in this book.

T1DM and Extreme Sports in Practice: The Evidence

Realizing the challenge, and given the growing interest in the participation of individuals with T1DM in sports in general, several professional organizations have issued guidelines for the nutritional and medical management of this condition in recreational and competitive athletes [1, 20, 21]. However recommendations for those taking part in extreme or hazardous events are still lacking. Nonetheless, a body of literature is emerging that sheds some light on the particular challenges, the distinctive issues attached to the various disciplines, and on some new avenues and technological developments that may render these activities more accessible and appealing to people with

T1DM. Additionally, this accumulating experience underscores areas of uncertainty, for which well-designed studies might evolve into strategies that could minimize the risks incurred by those involved. Unfortunately, for most of the disciplines, there are simply no data. Thus, for lack of published scientific evidence, health professionals asked to assist patients with T1DM who engage in such activities will have to apply their best judgment. The following parts will address some areas which have been the object of published research.

Extreme Endurance Events: The Marathon Paradigm

The first instance of a subject with T1DM completing a marathon run was reported in 1986 [22]. This 35-year-old man who had a 17-year history of insulin-dependent diabetes was a well-trained runner. Blood glucose concentrations were determined four times during the run and never came close to hypoglycemia; the authors mentioned a mild hypoglycemic reaction after completion of the run. They elaborated neither on the carbohydrate supply nor on the insulin dose adjustment the subject had used, but they concluded that it was possible for a patient with T1DM in reasonable control to complete a marathon. Subsequently, several small studies assessed the counter-regulatory responses of type 1 diabetic marathon runners. By and large they found very similar responses to those observed in healthy individual, with the exception of insulin levels that remained elevated in the diabetic individual despite a pre-run dose reduction of approximately 50% [23–25]. In general these studies were reassuring and helped to shape the notion that marathon running is compatible with T1DM [26]. However, there are still reports of much less favorable outcomes, highlighting the uncertainty that still casts a shadow over of the participation of type 1 diabetic individuals in marathons [27]. Among the available tools that might help alleviate the fears are new insulin analogs that may enable dose adjustments to prevent drastic glycemic excursions during or after the run [28]. In addition it has been demonstrated that continuous glucose monitoring (CGM) is feasible through the run, and that it frequently reveals periods of unsuspected hypo- or hyperglycemia during and following the race [29, 30]. It is easy to envision how the use of such a device could help characterize the individual response and plan future runs. Finally, it is to be expected that integrated systems that combine a sensing device with an insulin pump fitted with a tailor-made algorithm, which are making headway in the field, will soon become standard practice for T1DM individuals participating in such events, providing added safety and a sense of security for all. There are no good estimates of the number of individuals with T1DM who regularly engage in endurance events, but an Internet search reveals a multitude of sites and blogs with testimonials of individuals with T1DM who take part in marathons, triathlons, and extreme endurance events such as ironman and ultramarathons. It is thus obvious that such events are gaining in popularity among healthy individuals and type 1 diabetics alike, making the lack of scientific guidance for diabetic athletes all the more acute. Short of prospective studies that follow rigorous protocols, a compilation of the current experience in the area by an international consortium of exercise physiologists, sports physicians, and trainers could provide the basis for practical guidelines awaited by all those facing this challenge.

Endurance Events in Extreme Topographic and Atmospheric Conditions: The Case of Mountaineering

An interest in the participation of patients with T1DM into mountain climbing events started to make headlines over 30 years ago [31], with the early recognition that special attention needed to be given to glycemic control under these circumstances. Since then, there have been several controlled studies that have assessed the ability of individuals with T1DM to achieve extreme mountaineering summit success (above 5000 m) in comparison with nondiabetic climbers, as well as the metabolic implications of such undertaking

[32–34]. With the exception of the first Kilimanjaro Ireland expedition report [34], during which, over a rapid (5 days) ascent, a lower proportion of diabetic individuals (6/15) achieved the summit compared to controls (16/22), studies performed after sufficient acclimatization have generally shown a similar summit success rate. Likewise, the rates of acute mountain sickness (AMS) symptoms (i.e., such as high-altitude pulmonary edema, cerebral hemorrhage, or retinal hemorrhage) are no different in diabetic and in nondiabetic climbers [34]. As regards metabolic control, it also appears to be dependent on the level of antecedent training and the pace of ascent. In the rapid climbing of the Kilimanjaro by the Ireland expedition, the increased glucose utilization due to the intense effort and the low ambient temperatures mandated insulin dose reductions of approximately 50% to avoid hypoglycemia, especially during the first 2 days of trekking. The altitude-induced anorexia that accompanied climbing was another factor in decreasing the insulin doses, a fact that resulted in ketonuria in four, and mild ketoacidosis in two of the diabetic climbers [32]. However in the longer (37 days) ascent of the Himalayan Cho Oyu peak, there was no report of hypoglycemia. In fact blood glucose rose both in the diabetic and the control climbers resulting in an increase in glycated hemoglobin in both groups, and a gradual insulin dose increase was indeed required in the diabetics [33]. A general increase in blood glucose concentrations with higher altitude, along with increased insulin requirements at extreme altitude (>5000 m), appeared to parallel AMS scores [35]. Of note, in all the published studies, there was a preponderance of men; however it should be kept in mind that glucose utilization at high altitude differs between genders, as women tend to have reduced utilization rates [36]. Finally, the challenge of maintaining near normal blood glucose is compounded by the difficulty of performing finger prick-based glucose monitoring and the potential inaccuracy of glucose meters at high altitude and low temperatures [37, 38]. It has been suggested that the use of continuous blood glucose monitoring might be preferable in this setting [39, 40]. Nonetheless, despite some inconsistencies in the reported success rates and metabolic alterations with extreme mountaineering observed in T1DM climbers, there appears to be agreement that such an activity is possible in adequately trained individuals devoid of complications provided appropriate precautions are secured [41]. In contrast to marathon running, extreme mountaineering remains a fairly restricted activity practiced by limited numbers of people. Nevertheless, expeditions that include type 1 diabetic individuals should be encouraged to refer to the literature and web-based resources as they prepare for their ascent.

Scuba Diving in T1DM: Allowed but Safe?

With approximately 4 million active divers in the United States alone [42], and roughly one million diving certificates awarded annually worldwide by PADI [43], recreational diving is not usually viewed as an extreme sports activity. Nonetheless, with the threat of underwater hypoglycemia, it is widely considered as extremely hazardous for insulin-treated diabetic individuals who were banned from the field for many years. However, several surveys of diabetic individuals who defied the ban helped shape the notion that, under rigorous diving fitness selection criteria, diving could be allowed in people with insulin-treated diabetes [44, 45]. Around the same time, several investigators attempted to assess the effect of diving on glucose levels and other parameters in type 1 diabetic individual by conducting controlled studies. In a first study, the physical and biochemical responses of a small group of type 1 diabetic divers were compared to those of matched healthy divers in a hyperbaric chamber. None of the diabetic divers experienced hypoglycemia [46]. In a larger and more real life-like study, 40 T1DM individuals were compared to 43 sex- and age-matched nondiabetic divers while completing 555 dives over 5 days [47]. Blood glucose had to be maintained above 80 mg/dl prior to a dive. It was then monitored 60, 30, and 10 minutes before the dive and immediately after the dive (a now standard protocol). Although

there were no symptomatic hypoglycemic reactions during or immediately after the dive, in 7% of the dives diabetic divers surfaced with a blood glucose less than 70 mg/dl, compared to only 1% of the control divers, reaching values as low as 41 mg/dl, while the lowest value recorded in the controls was 56 mg/dl. The dives were generally accompanied by a decrease in blood glucose of approximately 50 mg/dl in the diabetic individuals and only 10 mg/dl in the controls. To avoid hypoglycemia, pre-dive carbohydrate loading was carried out – leading at times to significant hyperglycemia. The authors acknowledged this strategy could be hazardous as it theoretically increases the risk of decompression sickness.

It was just a matter of time that the continuous subcutaneous CGM technology be adapted to underwater performance and harnessed to diving with diabetes. Several studies have shown this to be technically feasible. In a study by Swedish researchers [48], 12 diabetic divers and 12 healthy controls performed 5 dives over 3 days while connected to a CGM system proven to resist pressure conditions up to a depth of 24 meters. There were no symptomatic or serious hypoglycemic episodes. The few low blood glucose values (<70 mg/dl) recorded immediately before or after the dive were found to be related to the mean blood glucose documented in the individuals in the 2 weeks preceding the study, and to the duration of diabetes. The authors concluded that such systems could provide useful information prior to and during dives, and could add an element of safety to diving with diabetes. The Italian "Diabete Sommerso" project assessed the feasibility of a combination of intensified group training with emphasis on diabetes management, pre-diving acclimatization, and the use of a continuous blood glucose monitoring device during immersion [49]. This study of 12 T1DM individuals confirmed previous observations that, when appropriate precautions are taken, underwater hypoglycemia could be almost eliminated. In this study, there were no hypoglycemic episodes during the dives, and only four mild episodes following the dive. Despite reaching hyperglycemia after the dive on a number of occasions, blood ketones did not rise. Finally, there was no evidence for greater blood bubble formation, suggesting that hyperglycemia was not associated with a greater risk for decompression sickness. Both these studies analyzed the CGM data retrospectively with the aim of studying the feasibility of recording reliable interstitial fluid glucose determinations from the device under the hyperbaric conditions of the dive, and documenting safe glucose levels in the divers. In a later study, investigators studied the practicality of real-time use of the data generated by the CGM system by the divers themselves [50]. The specially modified device, hosted in a waterproof case, had been previously shown to yield accurate interstitial glucose levels in a hyperbaric chamber simulating the conditions prevailing in a dive of 40 meter. In this study, two divers accessed their CGM data during repeated dives (28 in total) that took place over a week, with not one system failure. One diver even treated herself with sweet cranberry juice on repeated occasions when her glucose levels were approaching 70 mg/dl, and she could witness the rise in glucose concentration while continuing the dive. Although the authors called for further studies to confirm their preliminary experience, they suggested that the CGM data could be simultaneously captured by a diver buddy, adding even more safety to diving with type 1 diabetes. Recently, it has been suggested that even the new, less cumbersome, CGM flash-based technology device might be suitable for diving even though the developers recommend the device should not be submerged deeper than 1 meter [51].

Continuous glucose monitoring during diving as a proof of concept has been shown to be feasible. Further development of dedicated devices should be the next step in making diving safer and more acceptable to people with T1DM. In this regard efforts will have to be invested in closing the loop underwater as well. For the time being, even though a growing number of T1DM individuals turn to pump for insulin delivery, and more of those benefit from sensor-augmented devices, there is essentially no experience with insulin pumps during diving. So far simulation of hyperbaric conditions in a variety of insulin pumps, demonstrated unreliable functioning in

all [52]. For now, the use of such advanced devices underwater remains an unfulfilled goal and a technological challenge.

As there does not appear to be an overrepresentation of diabetic individuals among diving-accident victims, along with the findings of the observational and prospective studies, the overall picture that emerges provides reassurance that diving is compatible with T1DM if strict precautions are followed. Some of these are included in the proceedings of the 2005 joint UHMS/DAN (Underwater Hyperbaric Medicine Society/Divers Alert Network) meeting that provided a framework for diving professionals and potential divers with diabetes [53]. All these have contributed to create a permissive atmosphere, which gradually succeeded in lifting decade-long bans on diving with T1DM. There are still unanswered questions such as the effect of depth, and particularly that of varying temperatures on insulin kinetics and glucose absorption, or the performance of evolving technologies in these conditions. Answers to these will require more research, which should ultimately lead to long awaited updated protocols and guidelines for the growing body of divers with T1DM worldwide.

Closing the Loop: Emerging Technologies – The "Holy Grail" for the Type 1 Diabetic Athlete?

Summarizing the early attempts at creating an artificial pancreas that would eliminate the dread of hypoglycemia and free patients from injections, an anonymous article published 45 years ago in the *British Medical Journal* concluded by saying that "the artificial pancreas brings us one step nearer to the diabetics' dream of eliminating injections and hypoglycemia. However, years of development will certainly be needed for its miniaturization and implantation, and even then it seems unlikely to have universal application" [54]. Short of finding a cure for T1DM, the goal of developing such a system appears within reach today more than ever. In the early days of the concept of an artificial pancreas, continuous glucose monitoring implied venous blood glucose determinations. This has now been replaced by interstitial glucose concentration measurements which, in most existing systems, maintain an excellent correlation with reference venous or capillary blood glucose determinations expressed as MARD (mean absolute relative difference) of about 10% [55]. Some of these systems are now paired with insulin pumps in sensor-augmented pumps (SAP) or sensor-integrated systems. Other than emitting alarms when undesired (hyper or hypo) thresholds have been reached, and signaling glucose trends (upward or downward), integrated systems are capable of suspending basal insulin delivery in anticipation of hypoglycemia (based on glucose trend, insulin on board, and absolute glucose values). More advanced hybrid systems (one already US FDA approved) are capable of not only suspending delivery but also modifying the rate of basal insulin delivery in a closed-loop fashion. Data emitted by these devices are received and managed through smartphone or computer applications and can be received not only by the patient and the treating health care team but also by family members, adding another dimension to safety. In the close future, improved closed-loop devices will have insulin infusion algorithms that will incorporate data from sensors of physical cues such as heart rate, blood pressure, oxygen tension, sweating, and degree of PA. Although there is no published research on the use of the existing sensor-integrated pumps in elite athletes with type 1 diabetes, there is a growing body of evidence that in the context of PA, these systems can not only provide retrospective data to better plan for future activity, but they are also capable of curbing the danger of hypoglycemia, particularly that of delayed postexercise hypoglycemia [56]. In the ASPIRE study, 50 individuals with T1DM were required to exercise to induce hypoglycemia below 70 mg/dl. An exercise session consisted of up to six cycles of mild-to-moderate intensity exercise on a stationary bicycle or treadmill lasting 15–30 min each, with a rest period of 5–15 min between each cycle. Each subject underwent the exercise session twice in random order, once with the regular pump program running, and the second time with a low glucose

suspend (LGS) option turned on to shut off basal insulin delivery for 2 hours. Individuals were followed for about 4 hours after crossing the hypoglycemic threshold (the protocol had a provision for terminating the session and treating the individualism case of glucose values below 50 mg/dl or above 300 mg/dl). The LGS session resulted in a significantly less severe (nadir 59.5 vs. 57 mg/dl) hypoglycemia that lasted 32 minutes less than during the LGS off session, without rebound hyperglycemia [57]. In a 2013 study, 12 pump users type 1 diabetic adolescents and young adults were admitted twice for a 48-hour period, which included a sedentary control day followed by a day when they were required to exercise in the afternoon on a treadmill for 4 consecutive 15 minute periods designed so as to reach 65–70% maximum heart rate. Each subject underwent the evaluation in random order, once under CGM while under an open loop mode, and the second time under a hybrid closed-loop system. The day spent under the closed-loop system resulted in overall mildly but significantly lower 24-hour glucose levels. The most notable achievement was a highly significant reduction in the percentage of overnight hypoglycemic (below 70 mg/dl) values under the closed-loop, which went down from 4% to 1.5% on the sedentary days, and from 11% to 5% on the exercise days, together with a significant reduction in the percentage of values spent above the target range (above 180 mg/dl) [58]. In a somewhat similar in-clinic design, a later study compared the efficacy of a SAP with a LGS in response to a fixed threshold, to that of an algorithm that suspended insulin delivery according to anticipated low glucose at various thresholds, in 19 patients required to do moderate intensity exercise (cycling twice 30 min with a 30 min rest, at 55% VO_2max). The predictive algorithm significantly reduced the need to treat hypoglycemia within 4 hours from the start of the exercise, suggesting added benefit over the LGS modality. Of note, this study did not assess the effect on delayed nocturnal hypoglycemia [59]. Finally, in a real-life setting observational study of 20 children (aged 10–13 year) at camp during a week of intense PA, the 7 children who wore the predictive low glucose management system had a lesser need for carbohydrate ingestion to treat or prevent nocturnal hypoglycemia (3.5 g vs. 10 g), and the duration of time spent in hypoglycemia at night was shorter (38 min vs. 64 min) than in the children who only had the SAP (8/13 of those also had a LGS option). This suggested added benefit for the low glucose predictive option in preventing postexercise delayed hypoglycemia in real life too [60].

Extreme Sports and Type 2 Diabetes Mellitus

This chapter was written to specifically address the issue of the challenge extreme sports pose to athletes affected with T1DM. Almost by definition, these individuals are likely to belong to the age range typically represented in these activities. For many of these elite athletes, engaging in sports that represent an incredible challenge is part of the appeal. For others, the diagnosis of T1DM is a hitch in the course of their competitive career, and they make a point not to surrender to it. However of the 415 million people affected by diabetes worldwide, about 90% of them suffer from type 2 diabetes (T2DM). There are only anecdotal reports of active top athletes with T2DM, such as Arizona Cardinals (American professional football [National Football League – NFL]) cornerback Patrick Peterson [61]. In fact, intense PA appears to protect from T2DM, not only while the athlete is active but also later in life [62]. A fairly recent phenomenon, which parallels the epidemic of obesity, is the growing number of youths with T2DM, and the numbers are staggering. According to a recent survey, 17.4% of US adolescents 12–19 years of age have prediabetes [63], many of whom will go on to develop T2DM. Moderate regular physical activity is recommended as part of the therapeutic approach to diabetes in adults. Given the understandable reservation to resorting to lifelong pharmacological solutions, even more emphasis is placed on PA as a way to prevent or treat diabetes in children. It is reasonable to expect that a fraction of these individuals might comply to the point of

embracing strenuous PA as part of their lifestyle. While there is a moderate amount of literature showing that exercise improves the metabolic control in youths with T2DM, there is essentially no research done on the challenges that lie ahead of individuals with T2DM who aim to go beyond recreational PA.

Conceptually, at the level of counter-regulation and glucose homeostasis, the problem of not being able to reduce prevailing insulin levels at the time of exercise faced by individuals with T1DM is obviously less of a concern in the majority of patients with T2DM who do not use insulin. Likewise, failure to mount a glucagon response to hypoglycemia, so common in type 1 diabetic patients, is also probably a minor concern in T2DM. On the other hand, the potential hyperglycemic effect of the hormonal response to strenuous exercise in individuals with T2DM who may augment insulin secretion to cope with the demand, but who also suffer from insulin resistance, has not been adequately studied. Although the long-term beneficial effects of habitual PA in individuals with T2DM are not questioned, there have been conflicting findings on the effects of acute exercise on same or next day glycemic profile in T2DM individual. These were probably the result of using study designs that differed in types and length of exercise, whether postprandial or fasting, the inclusion or exclusion of insulin-treated individual, the amount of previous conditioning, and the individual genders. Even the recent introduction of CGM was unable to resolve these differences. At this stage it is unclear which type of exercise, at what time of the day, and for what duration has the most significant impact on subsequent short-term glycaemia in T2DM patients [64–67].

Other than occasional anecdotal reports, there are essentially no studies that have systematically looked at the responses of individuals with T2DM to extreme endurance sporting challenges. In the absence of professional answers, T2DM patients training for endurance events are now turning to forums on the Internet with a multitude of questions ranging from appropriate nutrition, medication adjustment, and precautions needed in the presence of complications. Although the Internet contains some success stories of people with T2DM participating in triathlons and marathons [68–70], these are few and far between. Amidst these, the lack of evidence-based professional guidance is all the more acute. CGM is now possible not only throughout endurance events but also days after their completion. This technology should be urgently implemented to systematically assess the glycemic profiles of individuals with T2DM who take part in such activities. Until this information is available, health professionals will only be able to rely on very limited data and common sense when providing recommendations to their T2DM patients who wish to engage in intense physical activity.

Conclusions

The purpose of this chapter was to show that participation in extreme endurance sporting events is no longer beyond imagination for individuals with T1DM. As the popularity of such events is soaring, the number of amateur sportspeople with T1DM who engage in such activities is expected to keep growing. Practitioners will therefore be increasingly solicited to provide relevant, sports-specific advice to their patients. Fortunately, thanks to a better understanding of the challenges posed by intense PA on metabolism and glucose homeostasis, and with the promise of the remarkable technological progress of the last decade, particularly the emergence of closed-loop management of T1DM, a brighter and safer future is now in sight for diabetic patients eager to engage in extreme sports challenges in the twenty-first century.

Along with the accumulating and reassuring body of scientific evidence, this chapter also highlighted areas where data are lacking, particularly with regard to T2DM.

As the existing official recommendations are somewhat outdated [21, 53, 71], it is suggested that newer and updated guidelines and protocols that incorporate the most recent advances be issued by the relevant bodies to provide a framework for patients and clinicians alike.

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The Endocrine System in Overtraining

27

David R. Hooper, Ann C. Snyder, and Anthony C. Hackney

Introduction

The development of athletic performance requires a delicate balance between an adequate stimulus that drives adaptation and the provision of sufficient recovery time to allow these adaptations to take place. When this balance is disrupted, outcomes such as overreaching "OR" or overtraining "OT" can develop. Definitions for these terms were initially developed by Kreider et al. [1] and have been adopted in other seminal papers in the area, including the joint consensus statement on overtraining syndrome provided by the European College of Sport Science and the American College of Sports Medicine [2]. These definitions are as follows:

Overreaching—an accumulation of training and/or nontraining stress resulting in short-term decrement in performance capacity with or without related physiological and psychological signs and symptoms of maladaptation in which restoration of performance capacity may take from several days to several weeks.

Overtraining—an accumulation of training and/or nontraining stress resulting in long-term decrement in performance capacity with or without related physiological and psychological signs and symptoms of maladaptation in which restoration of performance capacity may take several weeks or months.

Overreaching can be further subdivided into functional overreaching "FOR" and nonfunctional overreaching "NFOR." In the case of FOR, an enhanced training stimulus such as an increase in training volume or intensity is applied by the coaching staff in an effort to provide a substantial stress, which will ultimately temporarily reduce performance. However, when followed by adequate recovery, it will result in a "supercompensated" state, where the athlete demonstrates an improved level of performance when compared to their level prior to the beginning of the new training stimulus, hence the term "functional." On the other hand, when the recovery period following the training stimulus is insufficient, performance does not increase beyond the level prior to the new training stimulus and in fact may decrease; thus this process is "nonfunctional." The primary difference between NFOR and OT is the length of time that performance level is diminished. In the case of NFOR, the deleterious effects may last weeks or months, versus OT where the effects may last months or longer. The

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term "overtraining" has also been extended to "overtraining syndrome," or "OTS," where "syndrome" is used to signify that many factors may contribute to its development and that exercise may not be the only cause [2].

As the primary sign of OTS is a long-term reduction in physical performance level, other factors that could be contributing to this underperformance other than simply inadequate recovery from training must be ruled out. These contributing factors that must be ruled out include, but are not limited to, endocrine disorders (thyroid and adrenal glands, diabetes), anemia, mineral deficiency (such as iron and magnesium), eating disorders, caloric deficit, allergies, infections (i.e., Lyme, EBV, CMV), and many others. Thus, OTS is a *diagnosis of exclusion*. In addition, as the primary difference between OTS and NFOR is recovery time, by definition OTS cannot be determined until months after the performance decrement began.

Due to the difficulty in defining OTS as previously described, it is challenging to provide clear data on the incidence of OTS. However, the incidence of overtraining that has been reported has ranged from approximately two-thirds of elite distance runners [3–6] to 20–50% of swimmers, basketball, soccer, individual and team sport players [3, 7–11], and military personnel [12]. With regard to these reported incidence rates, it is worth noting that those on the higher side may not be distinguishing between OTS and NFOR.

While scientists and sports medicine professionals know that overtraining occurs in athletes, most of the knowledge and/or information concerning it comes primarily from personal experience, case studies, and short-term experimental investigations, none of which because of methodological limitations have added substantially to the scientific literature as will be seen during this chapter. When an athlete becomes overtrained, it will usually take months if not years to recover [13–15]; thus it would be highly unethical to deliberately overtrain an athlete just for science sake; therefore, scientists have very little long-term training information [15]. In spite of this lack of direct scientific information, much work has been performed to examine, predict, and prevent overtraining in athletes, and this chapter will examine some of this work especially as it relates to the endocrine system and the current proposed mechanisms. Also, since few truly experimental studies concerning overtraining and the overtraining syndrome are available in the literature, this chapter uses more review articles than might be a normal practice to examine the current state of the body of knowledge on the topic.

Endocrine Function and the Overtraining Syndrome

As the endocrine system is very involved in physiological adaptations and recovery to stress, much effort has been placed in examining hormone levels during different periods of training. As a result, a litany of blood hormone concentrations, as well as other blood biomarkers, have been suggested as worthy of measurement during training [16]. Before we discuss this literature, it is important to keep the interpretation of blood hormone concentrations in their proper context.

Firstly, a hormone is a messenger molecule that initiates a response from its target tissue upon interaction with its receptor. The measurement of a blood hormone concentration may provide some idea of what is happening in that particular system (i.e., increased circulating testosterone suggestive of an increase in hypothalamic-pituitary-gonadal axis activity), but it does not provide the complete picture. For example, if a hormone is elevated in the blood, it is not exerting its effect. This does not occur until it binds with its receptor, at which point it is no longer in circulation. Therefore, a blood sample may reveal baseline concentrations that would indicate no changes in that particular axis, but the hormone may absolutely still be initiating a response.

Secondly, for hormone concentrations to be compared either within a single study or comparisons made between studies, multiple factors must be controlled for, or at least considered. For example, fed vs. fasted, time of day, during exercise or at rest, time since last activity (some hormones, such as cortisol may take more than

24 hours to return to baseline), men vs. women, and hydration status, to name a few. These factors do not have minor impacts on hormone concentrations but rather very substantial effects and should not be taken lightly (see Chap. 1 – Methodological Considerations in Exercise Endocrinology, Hackney et al.).

Finally, regular measurement of blood hormone concentrations or other biomarkers may provide valuable insight into the stress or recovery status of the individual, but ultimately access to these data requires expertise in phlebotomy as well as the necessary equipment. Thus, attaining these data is not a simple process and is also expensive.

Due to the factors already mentioned above, in addition to the details discussed throughout this section, a single blood hormone concentration will likely never in and of itself be capable of identifying an overtrained state.

While there are multiple hormones and biomarkers that have been studied in the area of overtraining, two areas in particular have received a great deal of attention: testosterone/cortisol and sympathetic/parasympathetic imbalance.

Testosterone/Cortisol

Under appropriate conditions, exercise training leads to adaptations which stabilize the pituitary-adrenocortical system by lowering resting levels of the stress hormones [17, 18]. However, excessive exercise training has been hypothesized to lead to neuroendocrine overload, which may result in any number of the overtraining syndrome symptoms [17]. Thus, hypothalamic-pituitary dysfunction has been hypothesized with overtraining to disrupt the balance between anabolic (i.e., testosterone) and catabolic (i.e., cortisol) hormones and therefore may affect/prolong recovery [18–20].

An initial challenge in measuring resting testosterone and/or cortisol concentrations, particularly in athletic populations, is attaining a true resting sample that is not confounded by prior activity. In an albeit extreme example, neither testosterone (reduced) nor cortisol (elevated) returns to baseline levels on the day following an ultramarathon and in fact were not back to baseline until 2 days following the event [21]. In a less extreme example, professional Portuguese soccer players were tracked for 72 hours following a match, during which time only minimally intensive power, speed, agility, and strength testing were performed, and yet cortisol concentrations were remained significantly increased at 24 and 48 hours following the match [22]. Thus, before we even discuss the results of testosterone and cortisol-related research in the area of overtraining, it is important to point out the practicality of requiring 72 hours of rest for an athletic population in order to adequately interpret these hormones is extremely impractical. Nevertheless, testosterone and cortisol have been extensively studied in athletic populations.

Testosterone

With regard to testosterone, early research in this area sought out to assess whether men exhibit the same reduction in basal sex hormone concentrations as had already been demonstrated in women, as evidenced by a strong correlation between running mileage and incidence of amenorrhea [23]. Wheeler et al. [24], followed by many others [25–34], confirmed this was indeed the case. Possible negative effects of this reduced testosterone concentration may be similar to those seen in what was previously termed "the female athlete triad," such as reduced bone mineral density [32], and, in further support of the analogy with the triad, may be influenced by inadequate calorie intake [32]. In fact, this analogy has been previously suggested [35], and the International Olympic Medical Committee has suggested an umbrella term of relative energy deficiency in sports [36] that encompasses the symptoms and etiology of the condition in both males and females.

It is important to note, however, that reduced basal testosterone concentrations are not necessarily indicative of reduced performance, as is evidenced by the high rates of reduced testosterone in the world's most elite endurance athletes

that participated in the Kona Ironman World Championships and the lack of correlation between baseline testosterone and race performance in that event [37]. Thus, although reduced testosterone is seen in endurance athletes, and at times of intense training even in strength/power sports such as American football [38], it cannot itself be used to diagnose overtraining, as it does not necessarily lead to low performance levels or have any measureable effect on performance at all.

With regard to cortisol levels in relation to the overtraining syndrome, these have been shown to be both variable [39] and equivocal, with investigations showing no change [20, 40–49], increases [43, 50–53], and decreases [39, 54–56] in the values, and thus cortisol by itself seems to be a poor marker of overtraining [2].

Adlercreutz et al. [57] suggested monitoring the ratio of testosterone to cortisol as a means of examining this anabolic/catabolic balance in the athlete. Levels used to indicate an overtrained state were a decrease of 30% in the testosterone/cortisol ratio or values <0.035 [57]. While some athletes have been identified with this technique [12, 43, 51, 52, 57], many others have been observed to be overtrained by having met the specified criteria, yet with no change in the testosterone/cortisol ratio [20, 40, 42, 44–47, 58]. Banfi and Dolci [59] observed that the ratio of free testosterone/cortisol was very useful in monitoring professional soccer players, but the criterion values used, while decreased from the control period, were much greater than the 0.035 proposed by Adlercreutz et al. [57]. Likewise, Hoogeveen and Zonderland [50] showed decreased levels of the testosterone/cortisol ratio in heavily trained cyclists, but not to the levels proposed by Adlercreutz et al. [57]. Interestingly, Lane et al. [43] showed in cyclists during heavy training that low daily dietary carbohydrate intake can induce a significant and substantial (>40%) reduction in the free testosterone/cortisol ratio in as little as 4 days, suggesting dietary factors can strongly influence these hormones.

Ultimately, considering true basal testosterone and cortisol concentrations are hard to attain in athletic population due to the length period of rest required, in addition to the lack of a clear association between these hormone concentrations and performance level, neither testosterone, cortisol, nor their ratio with each other appears to be reliable markers of overtraining.

Sympathetic/Parasympathetic Imbalance

Since 1959, Israel [60] and later others [3, 4, 18, 19, 61, 62] have discussed two forms of overtraining: the basedowoid and the addisonoid forms. While strict interpretation of these terms would indicate that thyroid hyperfunction (Morbus Basedow) and adrenal hypofunction (Morbus Addison) would occur, neither the thyroid nor the adrenal glands have been solely shown to be directly involved in the overtraining syndrome. Rather, it is hypothesized that the sympathetic system (basedowoid) is activated during the early stages of overtraining, while in later stages of overtraining, the sympathetic system is inhibited, and the parasympathetic system (addisonoid) predominates [18, 63]. The sympathetic form has also been associated more with high-intensity explosive type sports such as sprinting, jumping, and throwing, while the parasympathetic form has been thought to be more prevalent in distance athletes [4, 8, 13, 18, 19, 62, 64]. While initially presented as two separate syndromes, current thinking is that they are both part of a continuum which extends from training to overreaching to the overtraining syndrome [4, 8, 17–19, 61], with the sympathetic form occurring initially, then the sympathetic system is inhibited, leading to the parasympathetic form [13, 19]. As such, catecholamine levels have been examined in overtrained athletes with decreases [4, 42, 65] and increases [3, 7, 8, 13, 66] or no change [67] observed. Methodological problems (capillary vs. venous blood, half-life time, time of day, relationship to exercise, etc.) and intraindividual variability seem to cause this conflict in the results and leave the use of catecholamine levels, and quite possibly other hormone levels as well, problematic for the determination of the overtraining syndrome [15, 42, 50, 55]. Similarly,

as free catecholamine levels have been used as an indicator of overtraining, especially the sympathetic nervous system activity, plasma levels typically only reflect an acute level (e.g., response to exercise), while 24-h urine levels would give an indication of average activity for the day [64]; thus the collection period greatly affects the results and their interpretation. Finally, the type of exercise training may affect the analysis as it has been suggested that volume-related overtraining may affect hormone levels more than intensity-related overtraining [67, 68].

Many other hormones have been examined in the hopes of providing a diagnostic tool for determining the overtraining syndrome. As many of the symptoms of the overtraining syndrome are similar to central fatigue, 5-hydroxytryptamine (5-HT) has been examined and/or indirectly assessed through a related hormone—prolactin [69]. Hackney et al. [28, 30, 70] and Budgett et al. [69] observed that plasma prolactin release was higher in overtrained athletes than in well-trained athletes, and thus these athletes may have greater 5-HT receptor sensitivity. Resting and acute post-high-intensity resistance exercise overtraining show no changes in growth hormone levels [61]. The hormone leptin is known to regulate energy balance and suppress appetite [55, 71, 72]; thus it could also be a component of the overtraining syndrome. However, plasma leptin levels were not changed with short-term (8 days) strenuous training nor were changes in leptin related to changes in plasma cortisol (which increased) and changes in the testosterone to cortisol ratio (which decreased) [52]. Changes in plasma leptin, however, were significantly related (r = 0.596) to decreases in serum testosterone [52]. Baylor and Hackney [73] did report reduced leptin levels in some female rowers undergoing functional overreaching, and this change was related to reductions in triiodothyronine (T3). Since the testosterone/cortisol ratio has not been shown to be extremely reliable, Atlaoui et al. [39] examined the 24-h urinary cortisol/cortisone ratio in elite swimmers and observed that the ratio was related to exercise performance and tracked changes in the training program. More research with this ratio may be warranted.

The exercise performance implications of the endocrine system changes that occur with overtraining are incompletely understood, as discussed above. Some of the problems presented could be methodological. Others could be that the hormone levels of each athlete are so individualized as is the athlete's response/adaptations to their exercise program that consensus information is not likely to occur. Food intake and composition can also alter the resting concentrations of a number of hormones as do stress and diurnal and seasonal variations. The resultant conclusion from examining the literature relative to the overtraining syndrome is that no one hormone can be tested to confirm or refute the occurrence of the overtraining syndrome; thus single blood tests are not of value in determining if the overtraining syndrome has occurred or not.

While single blood tests for hormone levels have not been found to be diagnostically beneficial, Meeusen and colleagues [14, 74] have tested a two-bout exercise protocol that has shown significant differences between control, overreached, and athletes with the overtraining syndrome. As one of the symptoms of overreaching and the overtraining syndrome is fatigue and affected athletes can typically start a performance or practice at normal pace but then have to reduce the pace due to the fatigue, Meeusen and colleagues [14, 74] hypothesized that having athletes perform two maximal exercise tests would distinguish between the different groups of athletes. To this end, a testing protocol was devised which involved the subjects performing two incremental exercise tests to exhaustion 4 hours apart as might occur when subjects perform two workouts in a day. Blood samples were then collected before and after each exercise bout and analyzed for hormone levels. During the first exercise test, the appropriately training athletes and the overreached athletes had similar results both in respect to exercise performance and hormonal responses [74]. However, exercise performance during the second exercise bout was decreased 3% in the appropriately trained subjects, 6% in the overreached subjects (following a training camp where training volume was increased 58%), and 11% in the subject who

Classification	Recovery time	Performance
Acute fatigue	1 day to multiple days	Increase
Functional overreaching	Days to weeks	Temporary decrement
Non-function overreaching	Weeks to months	Stagnation or decrease
Overtraining syndrome	Months to years	Decrease

Fig. 27.1 Possible presentation of the different stages of training, overreaching, and overtraining syndrome. (Adapted from Meeusen et al. [2])

reported to the laboratory with the overtraining syndrome [74]. Hypothalamic-pituitary hormone levels during the second test were likewise different between all three groups, with the overtrained athletes' hormone levels not increasing [74]. During a second study, ten athletes who had decreased exercise ability performed the two-bout exercise protocol [14]. Retrospectively, five of the athletes were classified as nonfunctional overreaching, and five of the athletes were classified with the overtraining syndrome [14]. While exercise performance was not different between the two groups of subjects, following the second exercise bout, ACTH and prolactin levels were much higher for the nonfunctional overreaching athletes than for the athletes with overtraining syndrome (who showed small or no increases in these hormone levels) [14]. No appropriately trained athletes were included in this second investigation. Collectively, the results suggest that due to the fatigue associated with overreaching and the overtraining syndrome, the two-bout exercise protocol may be useful as a monitor of the two. Unfortunately, the group differences were only distinguishable after the second exercise bout to exhaustion, and performance in just one bout of exercise was not beneficial in classifying the athletes. Thus, both exercise bouts seem to be needed for the protocol to be diagnostic. However, since the two tests to exhaustion were performed within a very short period of time (i.e., 4 hours), repeated performance of this test throughout a training season would need to be built into the training program regime, or if not it could add excessively to the exercise training performed (Fig. 27.1).

Other Hypothesized Mechanisms of the Overtraining Syndrome

Due to its role in mediating metabolic and anabolic cellular responses during altered energy states [75], insulin-like growth factor-1 (IGF1) has been identified as an appropriate marker for OTS [76]. IGF1 is a part of the highly complex GH/IGF1 axis. In short, IGF1 is released by the liver but can also be released locally (such as by skeletal muscle) which operates through autocrine and paracrine mechanisms. To add to the complexity and difficulty gauging the GH/IGF1 axis, IGF1 is circulated bound to 1 of 6 possible binding proteins, which are not merely a transport mechanism, but can actually either stimulate or inhibit the biological action of IGF1 [77]. Thus, it is recommended that IGF1 not be measured in isolation, but rather in conjunction with the binding proteins [78].

Due to high energy expenditure and energy intake restriction combined with sleep deprivation, Henning et al. [79] state Army Ranger School induces symptoms similar to OTS. However, due to the relatively short-term recovery required following the Army Ranger School (2–6 weeks), in reality the consequences of the training are actually more similar to NFOR. Following this 8-week-long intense physical and mental exertion, Henning et al. [79] reported drastic changes in testosterone (−70%) in addition to several aspects of the GH/IGF1 axis, including reductions in total IGF1 (−38.7), free IGF1 (−41%), and IGFBP-6 (−23.4%) and increases in IGFBP-1 (+534.4%), IGFBP-2 (+98.3%), and IGFBP-3 (14.7%). Thus, biomarkers related to the GH/IGF1 axis are sensitive to changes related to NFOR. The authors suggest that future research in this area should assess whether the recovery of these markers is associated with the recovery of FFM and physical performance following their reduction in response to this training period.

Recently, two other mechanisms have been proposed related to the overtraining syndrome; one of these is the tissue trauma theory proposed by Smith [80, 81]. Smith [80, 81] reviewed the current hypothesized mechanisms of the overtraining syndrome (such as was done above) and then proposed that since none of these mechanisms totally explained the overtraining syndrome, possibly these mechanisms function in response to trauma to the tissue. Hence, trauma to the muscle, skeletal, and/or joint systems could be the initiator of the overtraining syndrome. Exercise training and competition are known to result in stress/injury of the tissue as part of the adaptive process [80, 81] as put forth by Selye [82] many years ago. This trauma could be due to the eccentric nature of the exercise, the increased energy needs, ischemia, etc., which would result in mild inflammation as adaptation occurred. With insufficient recovery any of these initial adaptive occurrences could lead to a decrement in performance, the single most recognized sign of overtraining. Thus, Smith [80, 81] hypothesized that exercise-induced microtrauma leads to local acute inflammation which results in the release of cytokines. With increased training (high-volume and/or high-intensity) and insufficient recovery, the local acute inflammation is perpetuated to local chronic inflammation, and the enhanced level of cytokines released results in activation of the circulating myocytes. The activated myocytes then produce large quantities of pro-inflammatory cytokines, which leads to a systemic immune/inflammatory response. The systemic inflammation is then proposed as the central cause of the overtraining syndrome. The primary cytokines proposed to be involved with the overtraining syndrome are IL-1β, TNF-α, and IL-6, though little research has been performed with the overtraining syndrome and the cytokine levels [80, 81]. The results of the functions of these cytokines (e.g., mood change, loss of appetite, altered hormone levels—decreased testosterone and increased cortisol—fatigue, decreased muscle mass, and increased infection) are for the most part very similar to the symptoms of overreaching and the overtraining syndrome, thus the proposed mechanism. More work needs to be performed in this area to solidify this hypothesis, but it is interesting to note that elevated prolactin is associated with pro-inflammatory mediators and that previously mentioned studies have reported increased prolactin in overtraining athletes [28, 51, 67, 69, 83].

Morgan et al. [6] initially drew the parallel between altered mood state and overreaching/the overtraining syndrome; however, Armstrong and VanHeest [84] recently connected the two more thoroughly. Armstrong and VanHeest [84], in examining both the overtraining syndrome and major depressive disorder literature, observed that both share common brain structures, immune responses, and endocrine pathways. Hence, they proposed that the two shared a very similar mechanism. Morgan et al. [6] previously had observed that up to 80% of athletes with the overtraining syndrome had significant mood changes and elevated levels of psychological depression. Part of this mechanism could be that both depression and the overtraining syndrome have a dose-response relationship with stressful events. Likewise, hypothalamic-pituitary dysfunction and an enhanced parasympathetic activity have

been observed in depressed patients [84]. While many similarities exist, Armstrong and VanHeest [84] strongly recommend that further research be performed in this area before antidepressant medications are used with athletes who have the overtraining syndrome.

Although not a direct measurement of the endocrine system, heart rate variability (HRV) has been used extensively in the field of athlete monitoring as an indicator of whether sympathetic or parasympathetic activity is prevailing, with an increase in HRV being suggestive of an increase in parasympathetic relative to sympathetic activity [85]. The "variability" in HRV is a reference to the consistency of the time period between heart beats, more specifically ventricular depolarizations, often referred to as the "R-R interval" on an electrocardiograph. While multiple ways to evaluate HRV have been suggested, according to a review by Buchheit [86], the most useful resting measure of HRV is the time domain index RSMMD (squat root of the mean of the sum of the squares of differences between adjacent normal R-R intervals) measured during short (5-minute) recordings in supine position upon awakening in the morning.

With regard to the studies of HRV in overreached or overtrained athletes, research has been equivocal, with studies showing no change, inconsistent changes, or changes in parasympathetic modulation [2]. For example, a recent study by Coates et al. [87] induced overreaching in a group of endurance athletes over a 3-week period, which was confirmed by decreases in performance and maximal heart rate, as well as an increase in heart rate recovery (difference between heart rate at 60s into a maximal fitness test and the heart rate at termination of the test), which was suggestive of greater fatigue. Despite these clear indications of fatigue, no changes were seen in HRV. In addition, it has been suggested that HRV may be capable of identifying "global fatigue," but it cannot discriminate between different levels of fatigue [88] and thus would not be able to distinguish between FOR, NFOR, and OTS. Therefore, the joint consensus statement of the European College of Sport Science and the American College of Sports Medicine states that while a good tool in theory, HRV does not provide consistent results [2].

Training Considerations to Prevent the Overtraining Syndrome

In order to diagnose NFOR or OTS, by definition a period of performance decrement of weeks or months has occurred, and a full recovery from OTS could take years [2]. Thus, it is clear that preventing NFOR or OTS, rather than treating them, is the primary goal. This can be achieved through regular monitoring of fatigue and performance, perhaps even as frequent as daily.

Research in the area of monitoring training loads has significantly increased in recent years which led to a consensus statement from many experts in the field [89]. A central theme in this area of study is the differentiation between internal and external training loads. Internal loads are defined as the relative biological (physiological and psychological) stressors placed on the athlete [89]. These could be heart rate, oxygen consumption, or rating of perceived exertion (RPE). Alternatively, measures of external load quantify the work performed by the body, which can be assessed by power output, speed, acceleration or distance covered, among others [89].

Neither internal nor external loads are preferred, but rather an integrated approach that considers both provides the greatest insight [89]. For example, an internal load may be high (characterized by a high average percentage of maximal heart rate), but alone that single factor does not have sufficient context. If in this case the external load was low (evidenced by low speed, low power output), the high internal load would not have been expected and is thus indicative of fatigue and possibly an early sign of overreaching. On the other hand, a low internal load combined with a high external load could be indicative of positive training adaptations. In addition, measures of perception can be utilized in combination with workload to guide decisions regarding training prescription. For example, if the athlete is perceiving the workload as high, when measures of

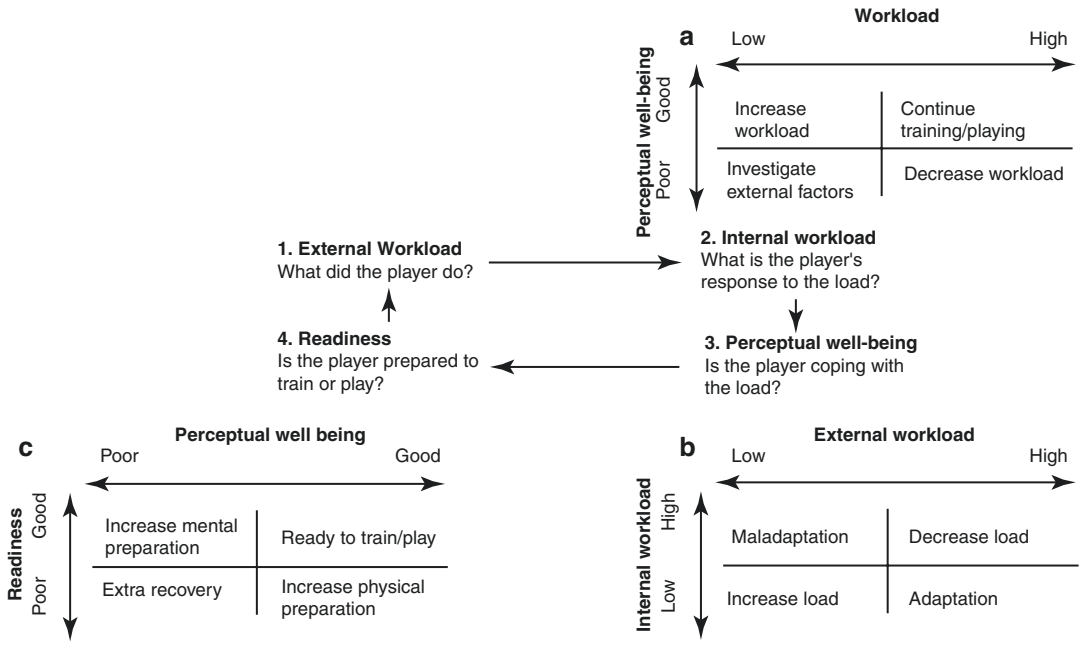

Fig. 27.2 Athlete monitoring cycle. (Modified from [90])

internal or external load are low, this is again suggestive of accumulating fatigue. This interaction of internal and external loads has been previously described as a key part of the athlete monitoring cycle [90] (Fig. 27.2).

One recent example of integrating both internal and external loads was illustrated by Lacome et al. [91]. In this study, the authors made an assessment of players' fitness at the beginning of a training cycle using a submaximal fitness test. Then, small-sided games were assessed utilizing global positioning system (GPS) (for external load) and heart rate (HR) (for internal load) variables. During these initial small-sided games, the authors produced a prediction model to estimate heart rate responses from future workouts based on the respective GPS variables for that particular workout. If successful, this predicted HR could be compared to the measured HR of future workouts to provide an assessment of how well the athlete tolerated the training session that day. For example, a higher measured HR than predicted HR would indicate fatigue, whereas a lower measured HR than predicted HR would be suggestive of positive adaptation.

After demonstrating that their prediction model was accurate, the authors showed that the deviation between measured and predicted heart rate was largely correlated with changes observed from submaximal fitness testing [91]. Thus, these prediction equations can be conducted daily and provide accurate assessments of fitness development without the need for constant formal fitness testing and perhaps quickly identify cases of overreaching before they become NFOR or OTS.

These types of regular assessments of internal and external training loads are a much better choice than monitoring hormone levels of athletes during training which is expensive, impractical, and probably of questionable benefit [7, 18, 42, 58, 59, 69, 92, 93]. Also, as hormone values generally are different between athletes, obtaining a single diagnostic value is very difficult [93]. Hence long-term tracking of the athlete would be a necessity, which in itself can be problematic.

Conclusion

As the difference in performance time between a gold medal and not medaling is very small in most cases, most athletes are willing to train excessively to the point of overtraining in order

to prepare for competition, as most would rather become overtrained and fail in their competition due to that than go into the competition not sufficiently prepared. Thus coaches and athletes have to learn to adjust training when increased physical and/or emotional stress is apparent to maximize the training adaptations while reducing the probability of overreaching and/or the overtraining syndrome occurring.

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28 Hormones as Performance-Enhancing Agents

Erick J. Richmond and Alan D. Rogol

Introduction

The word doping was introduced into the English language in the late nineteenth century. It is likely derived from the Dutch (Afrikaans) word *doop (or dop)*, the name of an alcoholic beverage made of grape skins used by Zulu warriors to enhance their prowess in battle. The term became current around the turn of the twentieth century, originally referring to the illegal drugging of racehorses. The practice of enhancing performance through foreign substances or other artificial means, however, is as old as competitive sport itself. Organ "therapies," stimulants, alcohol, and hallucinogenic mushrooms have been used since the ancient Olympics (776 BC to 394 AD).

Today, the use of doping agents is no longer restricted to competing athletes; young adolescents in schools and noncompeting amateurs also use them. Recent studies show that approximately 1% of the entire population in the United States of America (USA) and Sweden use androgens [2]. Bodybuilders and nonathletes use androgens to increase muscle mass and "to look better" [3]. One study about androgen users in the USA demonstrated that approximately 80% were recreational athletes and bodybuilders [4].

It is apparent that many athletes take a "cocktail" of drugs making it virtually impossible to denote any single agent as causing a specific outcome or adverse event. Despite very little evidence of beneficial effect of most doping agents and significant potential adverse consequences, many athletes and coaches continue to search for ways to mask their use to avoid detection or to start using new agents that might be more difficult to detect or not tested currently.

Athlete's Biological Passport (ABP)

The fundamental principle of the ABP is to monitor selected biological variables over time that may *indirectly* reveal the effects of doping, rather than attempting to directly detect the doping substance or method itself (considered an adverse analytical finding). The ABP infers the use of a prohibited substance (or method) by the monitoring of discriminant biomarkers over time—likened to the longitudinal record of disease-related biomarkers for *personalized* medicine. Its purpose is to identify and target individual athletes for specific analytical testing by intelligent and timely interpretation of passport data [5].

The ABP is operationally defined as the longitudinal profile for an athlete and is generated

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based on an adaptive model using Bayesian statistical tools that utilize data from an athlete's previous samples *to predict* likely individual limits or "reference range" for future samples. It may also be used to target conventional anti-doping tests or athletes with "abnormal" profiles and is thus complementary to traditional anti-doping testing.

The rationale for the longitudinal record is that doping substances trigger physiologic changes that provide physiologic enhancements. That is, doping can be detected specifically with selected biomarkers, and like the differences between pharmacokinetics and pharmacodynamics, the biological effects of the drug remain detectable much longer than the substance (chemical) itself. Thus, there is a paradigm shift in doping control: from the direct identification of the banned substance to detection of *abnormalities* in biomarkers. The first module was constructed to detect blood manipulation by the use of erythropoietin-stimulating agents or via blood transfusion. The second is a steroid module used to identify exogenous anabolic steroids or other indirect steroid doping substances or methods. A third module to detect use of prohibited peptides and protein hormones (e.g., recombinant human growth hormone [rhGH], insulin-like growth factor I [IGF-I], insulin, and rhGH-releasing peptides) is under development.

The goal is to have one passport for each athlete with *all* data points included. Those data can come from any of the WADA-accredited and WADA-approved laboratories and are entered into a central database, whose acronym is *ADAMS*, under a single identification number for each athlete. It contains WADA-approved lab results and doping control forms with a link to the therapeutic use exemption (TUE) forms, should that athlete obtained one.

Therapeutic Use Exemption (TUE)

Some athletes will have conditions (or diseases) for which a WADA-banned substance (or method) is required to return an athlete to "physiologic" function [1, 5]. The substance in question is required to treat an acute or chronic medical condition, such that the athlete would experience a significant impairment to health if the prohibited substance or prohibited method were to be withheld in the course of treating an acute or chronic medical condition. In addition any previous (non-banned) therapies were ineffective, and the impairment is not due to the previous administration of a banned substance. Such athletes are required to apply for an international standard (IS) TUE, absolving them from the situation of the finding of "the presence of a prohibited substance or its metabolites or markers, and/or the use or attempted use, possession or administration or attempted administration of a prohibited substance or prohibited method" [1].

A common example in endocrinology would be insulin therapy for an athlete with type 1 diabetes mellitus. For these athletes a therapeutic use exemption (TUE) is available and necessary because there are no other, non-banned alternatives.

The components of a TUE are the following:

- Complete medical details including history, clinical findings, and laboratory and imaging results.
- The *necessity* to administer prohibited medications includes dosage, route, and frequency of administration. These must be certified by a suitably qualified *medical specialist*.
- The medical necessity cannot be the result, wholly or partially, of prior use of a drug (or method) from banned classes or methods.
- Under no circumstances will permission be given to use any *synthetic* anabolic steroid.

For rhGH, anabolic steroids, and insulin, the suitably qualified medical specialist is usually a board-certified medical or pediatric endocrinologist.

Insulin

Physiology

Insulin is a 51-amino acid peptide hormone synthesized and secreted in a pulsatile fashion from the beta cells of the islets of Langerhans in the

pancreas into the portal vein. Pancreatic β-cells secrete 0.25–1.5 units of insulin per hour during the fasting state, accounting for over 50% of total daily insulin secretion, with the remainder being meal-related.

Serum insulin levels normally begin to rise within 10 min after food ingestion and reach a peak in 30–45 min.

Glucose is the principal stimulus for insulin secretion, although other macronutrients and hormonal and neuronal factors also may alter this response.

Insulin stimulates the uptake of glucose into muscle and fat by making available an increased number of glucose transporters (Glut-*4*) at the cell membrane, thus increasing the flux of glucose to the interior of the cell. However, its main effect is inhibitory to lipolysis, glycolysis, gluconeogenesis, ketogenesis, and proteolysis [6, 7].

Insulin regulates hepatic glucose output by inhibiting gluconeogenesis and promoting glycogen storage. Similarly, in muscle cells, insulin-mediated glucose uptake enables glycogen to be synthesized and stored and for carbohydrates, rather than fatty acids or amino acids, to be utilized as the immediately available energy source for muscle contraction. The use of insulin following a bout of exercise may replenish glycogen and ATP stores more quickly than rest or feeding alone.

Although insulin stimulates the uptake of amino acid into cells and promotes protein synthesis in a range of tissues at high insulin concentrations, the major action of insulin is to inhibit the breakdown of proteins (proteolysis), which occurs at lower insulin concentrations.

Rationale

The primary source of carbohydrate during exercise is derived from muscle glycogen stores. The greater the amount of glycogen stored, the longer one should be able to exercise. In addition, insulin leads to the accumulation of amino acids in muscle and theoretically additional substrate for protein synthesis and increase in muscle mass.

Performance Enhancement

The theoretical performance benefits of insulin are mediated by an increase in muscle glycogen storage and the inhibition of proteolysis but have not been demonstrated in clinical or scientific trials. That does not deter athletes from injecting insulin and its analogues, likely because insulin is but one of a "cocktail" or drugs along with training to enhance anabolic activity. In addition one must consider more rapid recovery from training and competition, but again there are no data to show this effect.

Adverse Events

The most common adverse effect of insulin use is hypoglycemia. Most athletes who abuse insulin are likely adept at balancing the ingestion of carbohydrate when injecting rapidly acting insulin analogues.

Another problem associated with insulin is weight gain, although most competitive athletes are accustomed to diet and follow training regimens that allow them to have a strict control over weight gain.

Detection

Regular and some short-acting insulins are very difficult to detect, because of the very short time one can note a high concentration of insulin (half-life approximately 4 min) or a high ratio of insulin to C-peptide, as has been used to distinguish subcutaneous regular insulin use from insulin-stimulating medications such as the sulfonylureas. These agents drive the endogenous secretion of proinsulin, an equimolar compound composed of insulin and its connecting C-peptide. Both of these analytes may be detected with commercially available immunoassays from blood samples [8].

Insulin analogues may be detected by chromatography followed by mass spectrometry given their sensitivity and specificity to determine precise molecular weights and amino acid "tags" of

peptides and proteins. These have proved useful to detect and identify synthetic insulins or their degradation products [9].

Human Growth Hormone

Physiology

The main isoform of human growth hormone (hGH or GH)is a 191-amino acid, 22-kDa peptide with a significant amount of a 20-kDa splice variant form. GH functions as a major metabolic hormone in the adult by optimizing body composition and physical function and regulating energy and substrate metabolism. Metabolic actions of GH also closely interact with those of insulin in the control of fat, glucose, and protein metabolism during the fasted and fed states. GH promotes fat metabolism by enhancing lipolysis and fatty acid oxidation [10, 11]. This function is particularly important during the fasted state, when GH secretion is enhanced, resulting in the partitioning of fuel utilization toward fat and the sparing of protein. GH exerts profound effects on glucose metabolism both directly and indirectly by antagonizing insulin action. GH enhances glucose uptake and utilization in cells: this is referred to as its insulin-like effect. At the whole-body level, GH suppresses glucose oxidation and utilization while enhancing hepatic glucose production, potentially for increased use of glucose that is non-oxidative in nature [12, 13]. Protein anabolism is a signature property of GH that reduces urea synthesis, blood urea concentration, and urinary urea excretion. GH also acutely stimulates amino acid uptake and incorporation into protein in vivo [14].

GH is secreted in a pulsatile fashion every hour or 2 and has significant peaks approximately 90 min after the onset of deep sleep and within minutes of completing a bout of exercise. Different stimuli affect the frequency and magnitude of the GH pulses.

Exercise and physical stress increase GH levels [15]. Emotional deprivation is associated with suppressed GH secretion, and attenuated GH responses to provocative stimuli occur in endogenous depression [16]. Nutrition plays a major role in GH regulation. Chronic malnutrition and prolonged fasting increase GH pulse frequency and amplitude [17]. Obesity decreases basal and stimulated GH secretion [18].

Rationale

rhGH is a popular drug of abuse in athletes because of its anabolic and lipolytic properties. Its detection is difficult, and it is prevalent in the sports environment. Abusers believe that rhGH will increase their muscle mass, muscle strength, and aerobic and anaerobic exercise capacity and also may help to recover faster after connective tissue sports injuries. All are considered by the athlete to enhance his/her performance.

Performance Enhancement

rhGH is quite actively being abused by athletes; up to 5% of US high school students have tried growth hormone as an anabolic agent, although that proportion seems quite high given the expense and that it is administered by injection [19, 20].

Despite its popularity, there is no conclusive evidence that rhGH improves athletic performance [21].

A study in recreational athletes demonstrated that in the short term, growth hormone significantly increased lean body mass, reduced fat mass, and marginally increased sprint capacity, but not strength, power, or endurance [22].

The mechanisms through which GH acts on exercise performance are more complex than the simple increase in lean body mass. For instance, GH stimulates erythropoiesis under various conditions and exerts significant cardiovascular effects, increasing plasma volume and peripheral blood flow and enhancing left ventricular stroke volume and cardiac output [23, 24]. All these factors may contribute to increased aerobic capacity. Evidence suggests that GH therapy alone, in the absence of some form of exercise program, may increase lean body mass, but not functional capacity, indicating that training may have to be

combined with GH replacement in these patients to increase physical performance.

It is unlikely that an athlete uses rhGH in isolation (or in the usual therapeutic dose), thus making it quite difficult to define its exact role to affect athletic performance. There is however one very well-controlled study that shows an effect on strength in a group of well-defined abstinent steroid abusers using rhGH [25]. That effect may well be transient given that the subjects were steroid-abstinent for a very short time and likely in a catabolic state. Other studies also show a small increase in muscle strength [26–28].

Adverse Events

The most common side effects of rhGH replacement include edema, arthralgia, and myalgia. Although GH antagonizes insulin action, the risk of developing hyperglycemia is very low. Other adverse effects including sweating, fatigue, and dizziness have been reported after rhGH administration in healthy individuals. The severity of these side effects may be augmented in those athletes who use "cocktails" combining rhGH with anabolic steroids, which could have synergistic effects, such as fluid retention and interaction with cardiac function [29, 30].

Detection

Detection of rhGH may be accomplished in blood samples by two different strategies—the isoform and the marker methods. The former is capable of detecting the use of rhGH for approximately 24–36 h and the latter approach for up to approximately 2 weeks [31].

(a) The GH-isoform method is based on the ability of certain assay antibodies (monoclonal) to detect only the most common isoform (22 kDa) which is identical to the recombinant molecule (also noted as "rec") and others to detect most of the common isoforms of pituitary hGH which is composed of 45–55% 22 kDa and the rest multiple different isoforms (antibodies also noted as "pit"). Each burst of hGH secretion from the pituitary contains multiple isoforms, but administered rhGH contains only the 22-kDa form. The pharmacologic precept is that a large dose of rhGH will dampen or shut down pituitary hGH release and what will be measured is virtually all 22 kDa. One submits samples to both the rec and pit assays and forms a ratio between the two. Any ratio of the 22-kDa form to the pit forms above 1 indicates administration of rhGH within the recent past [32].

(b) The GH-marker method is based on the precept that the administration of rhGH will produce increases in certain circulating analytes (markers) (GH-responsive proteins—IGF-I axis) and those of bone and connective tissue turnover (anabolism and catabolism). Markers of the IGF-I system remain elevated for several days following cessation of rhGH administration (although IGF-I itself may remain raised for more than 1 week); however, markers of collagen synthesis and/or breakdown may remain elevated for up to 8 weeks. The test for rhGH, and perhaps IGF-I, based on this marker approach relies on a combination of markers leading to the derivation of specific algorithms which differ for the various combination of biomarkers and for men and women [33].

Newer and likely more robust methods are being evaluated to detect the administration of rhGH (see below).

Doping Control, the Future

Given the inadequacies of the extant tests, others are being devised to broaden the duration of detection. These include a series of collagen-dependent peptides and proteomic, nucleic acid, and genomic-based strategies. Some of the limitations of the marker method include age of the athlete and gender dependency; therefore, new techniques have been sought including a new set of biomarkers that would be reliable, robust, and

sensitive to permit longer-term detection of rhGH (and perhaps IGF-I) doping.

Further approaches that are being developed include use of hydrogel nanoparticles in a preprocessing step to capture rhGH in the urine followed by isoform differential immunoassays as noted above [34]. Another approach in the developmental stage is to use nanobodies, tiny antigen-binding molecules derived from camel heavy-chain antibodies and made into the usual sandwich ELISA configuration on the tip of M13-phage [35]. Using a strategy similar to that for proteomics, Kelly and co-workers evaluated specific inhibitory RNAs comparing pre- and posttreatment cell-free samples following administration of rhGH [36]. They identified (microarray) and confirmed (RT-qPCR) four miRNAs that were differentially expressed in individuals receiving *therapeutic* doses of rhGH.

Theoretically, these changes should also apply to some of the alterations induced by administration of IGF-I. A different approach might be appropriate for IGF-I and GHRH analogues because each would have amino acids that differ from the native protein. The GH secretagogues might be traceable in the urine and would present many new compounds for a urinary "doping" library using liquid chromatography followed by mass spectrometry [37].

Insulin-Like Growth Factor I

Physiology

IGF-I is the main effector for the action of hGH. Systemic IGF-I is synthesized primarily in the liver, where its production is GH-dependent; IGF-I is also produced in multiple extrahepatic tissues, where it acts locally as an autocrine/paracrine growth factor under the control of multiple hormones, including hGH [38]. Most biological actions of IGF-I are mediated through the type I IGF-I receptor (IGF1R), which is structurally and functionally related to the insulin receptor, whereas hGH is insulin antagonistic several hours after ingesting a meal, the main effect of hIGF-I is to reduce glucose levels (insulin-like). It is strongly anabolic in muscle but has a very much diminished effect on lipids, in comparison to hGH. In fact, children with virtually no IGF-I (growth hormone receptor deficiency, Laron type) gain a disproportionate amount of fat when treated for many years with rhIGF-I [39].

Rationale

The rationale for using rhIGF-I as an ergogenic aid differs little from that of rhGH. The potential benefits include increased muscle protein synthesis and the sparing of glycogenolysis. It stimulates glycogen synthesis and increased fatty acid availability.

There are many sites in the Internet advertising the sale of IGF-I as a more powerful drug than rhGH, and its purported benefits include improvements in energy and endurance, tissue repair, muscle growth, rebuilding of cartilage, and ligament repair.

Performance Enhancement

The prevalence of IGF-I abuse is probably much lower than that for GH because, unlike GH, there is no readily available natural source, and therefore all IGF-I is obtained through recombinant DNA technology. At present there are no clinical or scientific trials that have shown a performance benefit for rhIGF-I use.

rhIGF-I has been used as a growth-promoting agent in children with both primary IGF-I deficiency and in a few genetic conditions which are associated with short stature [40].

Adverse Events

The most common adverse effect of rhIGF-I is hypoglycemia. Other reported side effects after rhIGF-I include jaw pain, headache, seizures, altered liver function, fluid retention, and myalgia [41].

In the longer term, there is the theoretical aspect of tumorigenesis, although not enough data exist. There are some associative data on patients

with certain cancers (e.g., breast, prostate, and colon) who have had higher IGF-I levels in the years before their cancers became detected [42].

Patients with GH insensitivity treated with rhIGF-I have also experienced lymphoid tissue hypertrophy, encompassing tonsillar/adenoidal growth and associated snoring and sleep apnea and thymic and splenic enlargement [43].

Detection

rhIGF-I is available as a commercial product and may have a similar rationale to rhGH for its use as an ergogenic agent to improve sport performance. It is clear that the hGH isoform test would not detect doping with rhIGF-I. Theoretically, the GH-responsive marker approach should work given that many, but not all, of the metabolic effects or hGH are mediated by IGF-I.

Erythropoietin

Physiology

Erythropoietin (EPO) is a 30.4-kDa glycoprotein hormone that is mainly produced by the kidney and is a key regulator of red blood cell production [44]. EPO stimulates the proliferation and differentiation of bone marrow erythroid precursors [45]. Its production is inversely related to the partial pressure of O_2 in the blood. Following administration, there is a direct relationship between hemoglobin level and increased performance of rhuEPO in rats and humans [46].

Successful cloning of the human EPO gene [47] permitted the production of recombinant human erythropoietin (rhuEPO) and later the approval to treat patients with anemia. More recently several newer generations of EPO analogues have been produced [48].

Rationale

EPO leads to the production of red blood cells. Since these carry oxygen to active muscles, one should expect enhanced endurance performance because of the additional flux of oxygen.

Performance Enhancement

Due to its effect of increasing hemoglobin (Hgb)-bearing erythrocytes responsible for the oxygen-carrying capacity of the blood, EPO has been used extensively as a performance-enhancing aid in sports, particularly in endurance disciplines requiring an adequate supply of oxygen to the heart and the muscles.

An increase in aerobic capacity of up to 5–10% was estimated in humans due to increased maximum capacity to transport and utilize oxygen ($VO_{2\ max}$), velocity at $VO_{2\ max}$, and maximal aerobic power [49, 50].

Adverse Events

Serious side effects may occur with EPO abuse, including hypertension, headaches, antibody-mediated anemia, and an increased rate of thrombotic events as a result of an EPO-induced rise in the hematocrit and thickening (increased viscosity) of the blood [51]. In addition, EPO withdrawal may be implicated in neocytolysis, that is, the hemolysis of young red blood cells in the presence of increased hematocrit [52]. Ultimately, EPO abuse may cause death [53].

Detection

EPO may be detected in urine by electrophoretic methods. All forms have a common protein backbone but different carbohydrate moieties because they are engineered in Chinese hamster ovary (CHO) or baby hamster kidney (BHK) cells. The carbohydrate linkages differ significantly in structure and in electric charge, producing easily detected differences in electrophoretic mobility [48].

More recently other forms of rhuEPO have become available—darbepoetin-α or novel erythropoiesis-stimulating protein (NESP). It is

a glycoprotein that has five amino acids that differ from the natural human protein, permitting additional carbohydrate to be attached and conferring a different electrical charge from the physiological molecule. A PEGylated EPO called continuous erythropoiesis receptor activator (CERA) is the newest generation of rhuEPO whose plasma half-life is extended considerably by the attached polyethylene glycol molecule. Acute use of one of the recombinant molecules can be detected for 3–7 days only (except for CERA whose detection window is longer), although it is probable that the pharmacodynamic effect on red cells lasts longer. The newer tests can detect use of any of the first three generations of recombinant products [48].

Other, still investigational products denoted as "synthetic erythropoietin-stimulating agents," for example, peginesatide (Hematide) are EPO receptor agonists. They are able to stimulate erythropoiesis but do not have the amino acid backbone of EPO. Thus, they would not be detected in the usual doping assays. However, novel tests are being developed to detect their potential abuse [54]. However, recently peginesatide has been recalled from the market due to serious life-threatening reactions [55].

HIF stabilizers are new compounds that mimic the hypoxia-driven expression of endogenous EPO in the kidney. This experimental drug is administered orally and intended to treat anemia in patients with chronic kidney disease. This drug stimulates erythropoiesis with EPO at physiological concentrations and has been used for doping purposes [55].

Anabolic Steroids

Physiology

Androgens are sex steroid hormones that promote the development and maintenance of the male sex characteristics. Testosterone is the principal secreted androgen in men. Androgens have both virilizing and anabolic effects. For decades, pharmaceutical companies have attempted to develop androgens that have preferential anabolic activity and reduced or no androgenic activity; these compounds have been referred to as anabolic steroids. In males, more than 95% of testosterone is secreted by the Leydig cells under the control of luteinizing hormone (LH). The remainder is produced via conversion of weakly androgenic precursors in the adrenal cortex.

The principal androgenic testosterone metabolite, dihydrotestosterone, mainly induces development of the primary male sex organs, while testosterone (along with dihydrotestosterone) is mainly responsible for the secondary sexual (male) characteristics. Testosterone enhances muscle hypertrophy, strength, endurance, and power [56]. In addition, testosterone has been suggested to lead to muscle anabolism via antiglucocorticoid actions, potentiation of muscle IGF-I activity, and attenuation of myostatin action and signaling [57–59]. This metabolic action follows from the binding of testosterone to the androgen receptor.

Rationale

Testosterone is the drug of abuse most often detected in sports doping control in recent years and is considered as a serious global public health problem. Athletes continue to use it because of its anabolic effects and cosmetic purposes.

Performance Enhancement

The positive effects of steroids on body composition including increased fat-free mass, muscle size, strength, and power are highly dose-dependent and correlated with serum testosterone concentrations [60]. The anabolic effect of testosterone is dose-dependent, and significant increases in muscle size and strength occur with doses of 300 mg per week or higher [61]. Anabolic androgens have improved exercise tolerance and the adaptability of muscle to overload by protecting against muscle fiber damage and increasing the rate of protein synthesis during recovery [62].

Adverse Events

Many side effects associated with anabolic androgens use involve multiple organ systems [3, 63], including acne, gynecomastia (in men), mood and psychiatric disorders [64], increased risk of suicidal or homicidal death [65], dyslipidemia [66], suppression of the hypothalamic-pituitary-testicular axis and spermatogenesis resulting in infertility, testicular atrophy, increase in liver enzymes, cutaneous striae, and injection-site pain [67]. In adolescents with growth potential, there will be an acceleration of epiphyseal maturation of the long bones and shorter than predicted adult stature. In girls and women, hirsutism and disordered ovarian and menstrual cycles can occur with quite small doses of anabolic steroids.

Detection

Analytical techniques that have been applied to anabolic-androgenic steroids (screening and confirmation) have included sample preparations based on liquid-liquid extraction of urine samples, concentration of the extracts, and separation of the analytes by gas-liquid or thin layer chromatography [68].

Over the years there have been improvements and innovation in analytical techniques permitting increased sensitivity and specificity in the compounds (including multiple metabolites) and fragments detected. Most modern techniques include derivatization, followed by a chromatographic step, and one or more mass spectrographic analyses to detect ions and fragments. For anabolic-androgenic steroids (and many other small molecules), the identification is noted by its retention time for the chromatographic step and its relative abundance of characteristic ions compared to a stored library of such fragments and ions [68, 69].

Further analysis of suspected exogenous anabolic steroid administration involves a stable isotope ($^{13}C/^{12}C$) ratio [70].

Selective androgen receptor modulators (SARMS) are a novel class of androgen receptor ligands that show improved pharmacokinetic characteristics and tissue-selective pharmacologic activities. The goal is to find compounds to improve physical function and bone health without adverse effects on the prostate or cardiovascular outcomes. These properties are likely to affect athletic performance, and the class of compounds has been banned by WADA since 2008, despite few data and few approved agents [1, 71, 72].

Epilogue

Gene Doping

Somatic gene therapy involves the manipulation of expression of specific genes or specific tissues. When done for the purpose of enhancing athletic performance (gene doping), it is considered a threat to sports and competition. World Anti-Doping Agency's (WADA) definition of gene doping is "1-The transfer of cells or genetic elements (e.g., DNA, RNA); 2-The use of pharmacologic or biologic agents that alter gene expression… with the potential to enhance athletic performance" [1].

Studies in humans have shown that it is possible to introduce new genetic functions in forms sufficient and stable enough to modify traits that produce serious disease and thus to ameliorate life-threatening illness and ease suffering [73]. However, it is a short theoretical leap of logic, but likely a very high practical hurdle, to alter athletic performance in individual athletes.

Prime examples are IGF-I with studies in mice showing muscle hypertrophy especially in response to resistance training [74] and myostatin based on a single boy with remarkable muscle hypertrophy caused by an inactivating mutation of the myostatin gene [75]. There are a number of others (e.g., EPO and various other growth factors) that are of great interest and have been part of laboratory experimentation [76–78]. More intriguing are those targeted to energy generation, peroxisome proliferator-activated receptor-δ (PPAR-δ, a protein to regulate the oxidation of fatty acids, and

cytosolic phosphoenolpyruvate carboxykinase (PEPCK) to regulate gluconeogenesis) [77].

Risks are mainly unknown and in the short term include gene silencing, immune reaction, and infection of the germ cells. They are especially unknown in the longer term.

More recently techniques to detect gene transfer, whether systemic or local (e.g., a single muscle), are effective under laboratory conditions. These methods are based on the polymerase chain reaction [79], using a direct-detection approach based on the presence or absence of transgenic DNA in peripheral blood [80], a mass spectrometric detection of small inhibitory RNAs (siRNAs) [81], and identification of exon/exon junctions which do not exist in the natural gene [82].

Summary and Future

Of the agents noted above, EPO (and other agents in this family) and the anabolic-androgenic steroids are unequivocally performance-enhancing. rhGH under unusual circumstances can be performance-enhancing, but the preponderance of data do not show enhanced athletic performance. Data simply do not exist for the performance-enhancing qualities of insulin or IGF-I, although athletes have used both but especially insulin itself.

What about the future? Part of that is outlined in the Epilogue and likely with a myriad of additional genes. Those trying to enhance performance pharmacologically (laboratories and athletes) are often (always) ahead of those trying to detect new compounds that may have ergogenic qualities. It is analogous to an international "arms race."

There are anecdotes concerning the use of growth hormone releasers—ghrelin, growth hormone-releasing hormone, and formulations of amino acids. However, data do not exist concerning their effect on athletic performance, and the physiologic precept is that if growth hormone is released endogenously or administered exogenously, the hypothalamus and pituitary become sub-responsive to further natural stimuli.

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Metabolic Syndrome, Hormones, and Exercise

29

Konstantina Dipla, Andreas Zafeiridis, and Karen M. Tordjman

Introduction

The metabolic syndrome (MetS) is a cluster of cardiometabolic risk factors that considerably increase the risk of developing type 2 diabetes and cardiovascular disease. The intent of this chapter is to present the physiological mechanisms by which exercise can prevent and counterbalance the detrimental effects of MetS. To that end the content initially addresses the definition, epidemiology, and consequences associated with MetS. Next, the physiological impact of acute and chronic exercise training on blood glucose and blood pressure regulation is presented. Current literature data concerning the preventive and therapeutic effects of exercise in individuals with MetS are analyzed and summarized. The final topic addresses the exercise prescription characteristics for optimizing glycemic and blood pressure control and minimizing the chronic complications of the metabolic syndrome.

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History and Definition of the Metabolic Syndrome

The recognition that a number of metabolic conditions cluster together, a condition that eventually came to be known as the metabolic syndrome, dates back almost a century ago [1]. The first components noted to associate together were hypertension and obesity. Later, several descriptions of such clusters, which included, in addition to obesity or abdominal obesity, hyperuricemia, gout, and hyperlipidemia, in different combinations, were described as discrete entities by a series of investigators. Moreover, as noted by Phillips, the clustering of these conditions was also noted to impart an increased risk for myocardial infarction [2]. The modern era of the MetS investigation, which ignited a large volume of research, came about in 1988, when Gerald Reaven coined the term Syndrome X to describe this atherogenic cluster of impaired glucose tolerance, hypertension, hyperlipidemia, and insulin resistance (inferred from hyperinsulinemia). Reaven suggested that insulin resistance was the common pathogenetic mechanism linking the components of the syndrome [3]. Notably, Reaven did not include obesity as an obligatory component of this syndrome; however a year later, Kaplan added obesity and renamed this cluster the deadly quartet [4], while Zimmet emphasized the role of physical activity as a protective factor in the development of this syndrome

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[5]. In spite of the wide adoption of this concept, particularly as a tool for detection and early intervention, as well as a common ground for epidemiologic studies, the last two decades have seen repeated attempts at setting criteria to diagnose the syndrome. Indeed, various medical organization bodies differed on issues such as the mandatory presence of obesity and the criteria best defining obesity, given ethnic differences, exclusion of diabetes, and others. Recognizing these difficulties, the latest effort at harmonizing diagnostic criteria for this condition culminated in the 2009 joint statement of six such organizations (International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity), each of which had been involved in the publication of previous criteria. According to this statement, the diagnosis of MetS relies on the presence of at least three out of the five criteria listed in Table 29.1. Notably, the presence of abdominal obesity was not mandatory; it was also acknowledged that the threshold for waist circumference, as an index of abdominal obesity, should be viewed as more ethnic and gender-specific. Notably also, hyperglycemia in the diabetes range was not an exclusion criteria [6].

Table 29.1 Joint interim statement criteria for the diagnosis of the metabolic syndrome (adapted from reference [6]). Any combination of three out of five criteria defines the presence of the metabolic syndrome (MetS)

Measure	Categorical cut points
Elevated waist circumference	Population- and country-specific cut points
Hypertriglyceridemia (or treated)	≥150 mg/dl
Reduced HDL- cholesterol (or treated)	≤40 mg/dl (men) ≤50 mg/dl (women)
Elevated blood pressure (or treated hypertension)	Systolic blood pressure ≥ 130 mm Hg and/or Diastolic blood pressure ≥ 85 mm Hg
Elevated fasting glucose (or treated dysglycemia)	≥100 mg/dl

Despite this achievement, within just a few months, a World Health Organization (WHO) expert consultation report came out, deconstructing MetS as a diagnostic entity with no clinical usefulness, and suggested its sole value was educational in nature [7]. However, the concept with its limitations was here to stay. Indeed, since the WHO statement, there have been roughly 45,000 publications dealing with various aspects of the MetS issue, attesting to its wide acceptance.

Epidemiology of the Metabolic Syndrome

One line of critique in the WHO document focused on the notion that MetS is a premorbid condition that increases the relative risk of developing cardiovascular disease (CVD) about twofold [8] and the incident of type 2 diabetes mellitus (T2DM) by roughly fivefold [9] and that includes epidemiologic studies that comprise subjects who already had T2DM and/or CVD, which renders the comparison between them devoid of true value. Nevertheless, data regarding the prevalence of MetS continue to accumulate, mostly from cross-sectional studies. With the growing global epidemic of obesity, it comes as no surprise that the prevalence of MetS has reached staggering levels. Figures vary according to the criteria used to define the syndrome; the country; the type of population, whether rural or urban; the ethnic composition; and the age of the subjects in the sample studied. In general, the prevalence is higher in urban regions and increases with age. In the USA, using the most current joint interim statement criteria [6] on the NHANES sample, MetS is present in above a third of adults over the age of 18 and has increased by 35% in the last 30 years [10]. The most current data for the years 2011–2012 indicate that MetS is present in almost 35% of adults and in almost 50% in those aged 60 years and older, while in all age groups, Hispanics are more affected [11]. In Asia, figures vary between a prevalence of approximately 25% in mainland China [12] and 9.1–22.2% in working Japanese men, while it

was found in only 4.4% of working Japanese women [13, 14]. Across ten European countries, MetS was present in 24.3% of adults over 20 years of age; however there was a great variation from a prevalence of over 60% in the Lithuanian cohort as opposed to less than 10% in the Sardinian one. In the cumulative sample, the prevalence was over 30% for individual's ≥70 years [15]. The frequency of MetS is also experiencing a dramatic increase in developing countries too, such as the sub-Saharan African region, ranging between 30% and over 40%, and clearly more pronounced in women [16]. Finally, the Middle East countries, particularly Saudi Arabia, are experiencing a record prevalence rate of MetS with about 40% of adults affected [17, 18].

Pathophysiology of the Metabolic Syndrome

It is beyond the scope of this chapter to provide an extensive, in-depth review of the mechanisms that have been put forth to explain the pathogenesis of the syndrome. Several sequences of events have been suggested; however it is also clear that like any of its components in isolation, MetS requires a permissive genetic makeup, which then colludes with an unhealthy lifestyle and environmental factors. It is generally believed that a modern sedentary lifestyle and an obesogenic environment, combined with a calorie-dense diet, are the triggers for the mechanisms that culminate in the emergence of the various components of MetS [for extensive reviews [19, 20]].

A proposed simplified scenario (Fig. 29.1) starts with a genetic propensity, most likely also augmented by epigenetic modifications [21], i.e., to store excess energy in visceral adipose depots that starts in motion a range of intricate, interrelated, and eventually detrimental processes. The excess visceral adipose tissue energy deposition creates a state of chronic tissue inflammation typically ascribed to adipose tissue macrophages (M1) and mediated by an array of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin-6 (IL6), interleukin-1-beta (IL1β), plasminogen activator inhibitor-1 (PAI-1), and resistin, while adiponectin production and action are reduced [22].These inflammatory cytokines play a major role in the adipose tissue dysmetabolism characterized by insulin resistance development. Impaired insulin signaling in the adipose tissue leads to unopposed lipolysis, which results in an increased efflux of free fatty acids that are responsible for the lipotoxicity seen in a variety of tissues.

In the skeletal muscle, the excessive intramyocellular free fatty acid accumulation impairs insulin action and reduces overall glucose disposal [23, 24]. The free fatty acid flux to the liver leads to hepatic triglyceride accumulation and hepatic steatosis; it stimulates the output of VLDL, hypertriglyceridemia, and the generation of atherogenic small dense LDL cholesterol particles, while the simultaneous increased clearance of HDL cholesterol contributes to the typical dyslipidemia pattern of MetS [25]. Insulin resistance in the liver eventually results in increased hepatic glucose output, which, combined with reduced skeletal muscle glucose disposal, creates additional secretory demand on the pancreatic beta cell. When this demand cannot be met, hyperglycemia ensues, first as impaired fasting glucose or impaired glucose tolerance, ultimately culminating in T2DM.

The pathogenesis of hypertension in MetS is complex. Again, impaired insulin action appears to play a central role. Indeed insulin-mediated activation of endothelial nitric oxide synthase (eNOS) is reduced in the metabolic syndrome, leading to a drop in circulating and local nitric oxide, a potent vasodilator, and to the reciprocal increase in endothelin-1. There is also evidence for an increased expression and activation of both the systemic and the local adipose tissue renin angiotensin systems, resulting in elevated levels of angiotensin II, a potent vasoconstrictor. Furthermore, angiotensin II inhibits eNOS and stimulates NADPH oxidase, which has been implicated in the generation of hypertension and is also a major system for the generation of reactive oxygen

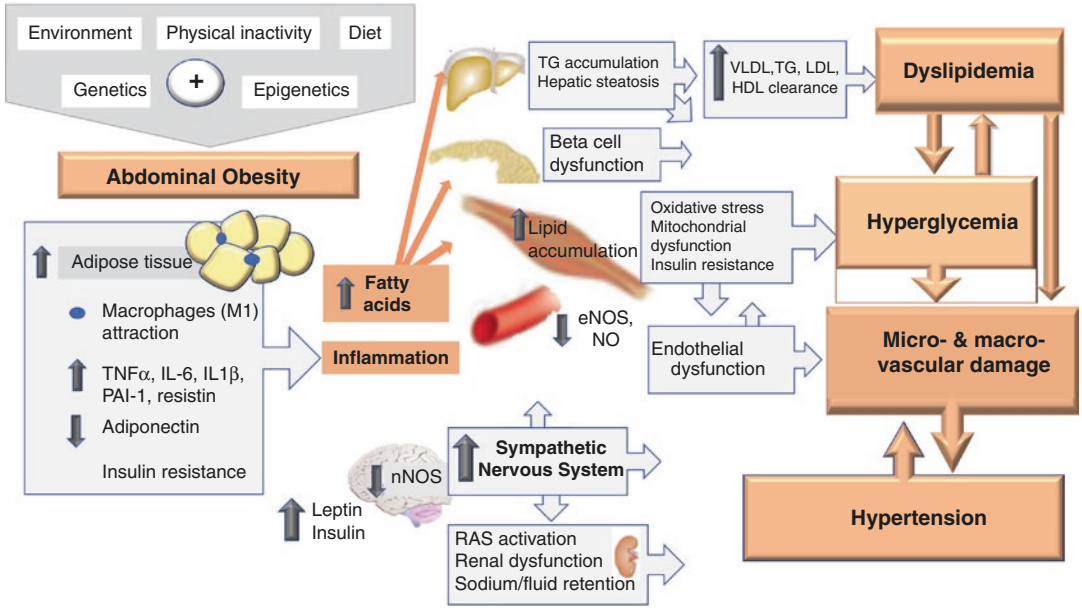

Fig. 29.1 Proposed mechanisms for the pathogenesis of the metabolic syndrome. Genetic predisposition augmented by epigenetic modifications leads to increased adipose tissue. The excess visceral adipose tissue creates a state of chronic tissue inflammation; attraction of adipose tissue macrophages (M1), mediated by an increase of pro-inflammatory cytokines (TNFα, IL6, IL1β, PAI-1, and resistin); and a reduction of anti-inflammatory adipokines (adiponectin). These alterations result in local insulin resistance, unopposed lipolysis, and an increased efflux of fatty acids and lipotoxicity in various tissues. In the skeletal muscle, excessive fatty acid accumulation impairs insulin action and reduces glucose uptake. In the liver, it leads to hepatic triglyceride (TG) accumulation and steatosis and contributes to dyslipidemia. In addition, reductions in eNOS and reduced NO, along with alterations in RAS and sympathetic nervous system overactivity, provide a mechanism for elevation in blood pressure and hypertension. Abbreviations: TNFα tumor necrosis factor alpha, IL6 interleukin-6, IL1β interleukin-1-beta, PAI-1 plasminogen activator inhibitor-1, eNOS endothelial nitric oxide synthase, nNOS neuronal nitric oxide synthase, NO nitric oxide, TG triglyceride, RAS renin angiotensin systems

species. Finally, sympathetic nervous system (SNS) overactivity has been documented in the metabolic syndrome, providing yet another mechanism for elevated blood pressure (BP) in this condition [26] (Fig. 29.1).

Many of the processes involved in the pathogenesis of MetS such as the pro-inflammatory and oxidative stress state, endothelial dysfunction, atherogenic lipid profile, diabetes, and hypertension are also instrumental in the accelerated atherosclerosis, a complication of the syndrome. Accumulating evidence, however, strongly suggests that adoption of a healthy lifestyle (healthy diet, high levels of physical activity, and regular participation in exercise programs) can offset part of the genetically mediated cardiovascular risk and reduce the development of T2DM [27].

Exercise is one of the major links between the hormonal modulators of energy intake and output and has been considered a cornerstone treatment for obesity-related metabolic complications. Regular exercise training can effectively improve body composition in obese individuals; can improve BP and lipid profile and glycemic control; and thus can prevent further development of obesity-related comorbidities [28–31]. To understand how exercise can improve the metabolic profile and offset the cardiovascular risk in individuals with MetS, a brief review on the mechanisms involved in glucose and BP control is presented next.

How Exercise Can Improve Glycemic and Blood Pressure Control in the Metabolic Syndrome

How acute exercise can improve blood glucose control Even a single exercise session can improve glycemic control by inducing adaptations at the extracellular and at the skeletal myocyte level. In more details, during exercise, there is a dramatic increase in skeletal muscle blood flow, in order to supply nutrients and oxygen to the exercising muscle (see Chap. 25). The synergetic action of SNS and secretion of hormones, such as insulin, facilitates capillary recruitment and promotes vasodilation in terminal arterioles, increasing microvascular perfusion [32–34]. Even though during acute exercise plasma insulin concentrations decline, the increase in BP and muscle blood flow ensures insulin delivery. Consequently, insulin-stimulated microvascular perfusion increases, securing improved glucose delivery [35]. At the skeletal myocyte level, insulin and exercise stimulate glucose uptake through two distinct signaling mechanisms: an insulin-dependent pathway and a non-insulin-dependent pathway. The *insulin-dependent pathway* begins with insulin binding to its receptor inducing phosphorylation of the insulin receptor substrate-1 (IRS-1). These actions activate the PI3K/Ser/Thr/Akt pathway and result in the translocation of glucose transporters (GLUT4) from an intracellular location to the cell membrane, promoting glucose uptake into the myocyte [36–38]. Besides the insulin-dependent pathway, exercise induces a greater GLUT4 translocation in a *non-insulin-dependent pathway*, promoting further muscle glucose uptake [39–42]. Briefly, during myocyte contraction, Ca^{2+}-dependent signaling and the increase in the AMP/ATP ratio stimulate the Ca^{2+} calmodulin complex (CaMKII) and the AMP-activated protein kinase (AMPK), signaling GLUT4 translocation and promoting further increase in muscle glucose uptake [43–46]. Other possible mechanisms that have been described to stimulate exercise induced-glucose uptake involve p38-mitogen-activated protein kinase (p38-MAPK) and nitric oxide synthase (NOs) [47–50]. The potential role of the latter (NOs) in the contraction-induced glucose uptake seems to be fiber type-dependent (observed mainly in fast-twitch fibers); however, the exact pathway is still under investigation [50–53]. A role of hexokinase II in GLUT4 translocation during exercise has also been reported [54–56]. However, when GLUT4 were overexpressed in mice, in the absence of hexokinase II overexpression, muscle glucose uptake during exercise was not enhanced [55], suggesting a synergistic action of multiple mechanisms to facilitate glucose uptake during muscle contraction. The non-insulin-dependent pathway is important because it remains intact even when the insulin-dependent pathway is dysfunctional, as in insulin resistance states, and can be used to lower blood glucose concentrations [57–59].

How chronic training can improve glucose homeostasis and muscle metabolism Besides the acute improvements in skeletal muscle glucose uptake during exercise, chronic exercise training further assists in glucose homeostasis by promoting alterations in genes' expression (such as increases GLUT4 expression) and encouraging mitochondrial biogenesis and fiber type transformation [43, 60–63]. More specifically, during muscle contractions, the acute mechanical/metabolic stress and the increase in cytosolic Ca^{2+}-related signaling mechanisms (CaMKII, AMPK and MEF2, p38 MAPK, and calcineurin) described above induce increases in peroxisome-proliferator-activated receptor-γ-coactivator 1α (PGC-1α) expression [43, 44, 61, 64]. Studies, conducted mainly in vitro, showed that PGC-1α activates two transcription factors, nuclear respiratory factor 1 and 2 (NRF-1 and NRF-2, respectively), which in turn promote expression of nuclear genes (such TFAM) that stimulate mitochondrial DNA transcription and replication [62, 65–67]. Gradually, superimposition of repeated transient mRNA bursts during and following each exercise bout can contribute to increases in respiratory chain and oxidative enzymes' content and/or activity, resulting in greater fatty acid oxi-

dation, less accumulation of fatty acid intermediates, and, thus, less inactivation of the insulin receptor and better insulin signaling [60, 68]. In addition to these improvements in the skeletal muscle, regular exercise training promotes angiogenesis (by upregulating angiogenic factors, such as VEGF and miRNA-126) which is of great importance, as obesity and hypertension have been associated with structural and functional capillary rarefaction [69–71].

How acute exercise can improve blood pressure A single bout of moderate−or high-intensity exercise can lower BP levels below the pre-exercise levels. This postexercise BP lowering effect, termed "post exercise hypotension", can last up to a few hours and can be an effective non-pharmacological strategy for prehypertensive and especially for hypertensive individuals [72–74]. The mechanisms underlying postexercise hypotension point to centrally mediated decreases in SNS and peripheral vasodilatory mechanisms that work independently and synergistically lowering BP after exercise [75–77]. Briefly, during exercise, muscle afferents' activation promotes the release of substance P, which in turn stimulates the GABA interneurons of the nucleus tractus solitarius (NTS) and activates the neurokinin-1 receptor (NK1-R) [78]. Prolonged activation of the NK1-R during exercise results in its internalization. At the cessation of exercise, the reduction in substance P, the diminished NTS GABA interneurons input, and NK1-R internalization contribute to a reduction in baroreflex function and postexercise hypotension [79]. In addition, in the early postexercise phase, local vasodilatory mechanisms induced by histamine binding to H1 and H2 receptors of the vascular endothelial and smooth muscle cells, respectively, also contribute to postexercise hypotension [76, 77]. A role of nitric oxide synthase (NOs) and NO in the postexercise vasodilation was shown in early animal studies; however, recent studies in humans did not find a direct involvement of NOs in postexercise hypotension [80].

Besides the beneficial short-term effects of exercise in BP control, numerous randomized control trials have shown antihypertensive effects of exercise after long-term participation in exercise programs in normotensive adults and in those with prehypertension/hypertension. Aerobic and resistance exercise training can effectively reduce high BP (by 5–7 and 2–3 mmHg, respectively), resulting in a 20–30% reduction in CVD risk [72, 74, 81, 82]. For these reasons, professional associations (such as the American Heart Association, American College of Sports Medicine, and European Society of Hypertension) recommend exercise for the prevention and treatment of hypertension [72, 74, 83].

How chronic training can improve blood pressure levels Regular exercise training can counterbalance the detrimental effects of MetS by inducing neurohumoral and vascular (structural and functional) modifications: reductions in SNS activity in the muscle vasculature and improvements in endothelial function and arterial elasticity, with favorable adaptations in both macro- and microcirculation (as assessed by pulse wave velocity, augmentation index, central BP, and small artery compliance) [72, 74]. Decreases in circulating catecholamines and vasoconstrictors, improvements in insulin sensitivity, increases in vasodilatory substances (such as an upregulation of nitric oxide synthesis/bioavailability), and restoration of baroreceptor control are postulated mechanisms explaining the antihypertensive effects of exercise [84, 85]. In individuals with obesity, endurance exercise training coordinately upregulates skeletal muscle oxidative capacity, reduces intramuscular triglyceride content, and upregulates adipose triglyceride lipase [31, 86]. These physiological adaptations probably favor fat oxidation and may alleviate the toxic lipid accumulation in skeletal muscle in obesity. Furthermore, aerobic training can improve hemodynamics during submaximal exercise, that is, lower exercise BP, systemic vascular resistance, and the double product (i.e., decrease myocardial oxygen demand for the same exercise load), which could prevent the occurrence of adverse cardiovascular events during exercise in the MetS population [87].

The majority of individuals respond with marked changes in metabolic risk factors following a long-term lifestyle/exercise intervention

(responders); however, a few individuals might not exhibit significant changes (non-responders) [88]. Stuckey et al. (2015) showed that following a lifestyle intervention, participants exhibiting clinically important changes in systolic BP at 6 months of intervention were the ones with the greater metabolic improvements across 12 months of intervention, compared with those without clinically important systolic BP changes. This finding is in agreement with data showing that chronic vascular stress (induced by hyperglycemia and/or hypertension) can decrease the sensitivity of the vasculature to the adaptive responses normally induced by exercise [89, 90]. Large amounts of adipose tissue and the alterations in the hormonal and metabolic milieu in individuals with MetS might blunt the neurohumoral adaptations to exercise.

Endocrine/Neural Dysfunctions That Can Alter the Adaptations to Exercise in Individuals with the Metabolic Syndrome

Individuals with obesity/MetS often display a suboptimal physiological response to acute exercise stimuli due to, at least in part, hormonal disturbances (such as altered secretion of adipocytokines affecting insulin signaling in the endothelium and altered endocrine secretion affecting lipolysis) and associated macro- and microvascular dysfunction (i.e., alterations in endothelial cells phenotype and impaired dilatory capacity) [91]. Experiments in both animals and humans with obesity/hypertension/MetS have shown that during exercise, capillary rarefaction and lower recruitment (i.e., reduced capillary density or less functional capillaries), muscle microvascular dysfunction, and reduced functional sympatholysis (due to lower NO bioavailability) can impede perfusion, oxygen and nutrients' delivery to the exercising muscles and brain [33, 92–99]. These disturbances along with an overactivity of the exercise pressor reflex and alterations in baroreceptor sensitivity result in higher sympathetic outflow and exaggerated vasoconstriction and can cause exaggerated exercise BP responses and/or exercise intolerance [95, 100–102]. In fact, Gaudreault et al. reported that about half of normotensive men with MetS undergoing a maximal symptom-limited treadmill test had an exaggerated response to exercise [95]. These men presented greater abnormalities in the autonomic nervous system and greater insulin resistance. Moreover, alterations in central command, a reflex originating from higher brain regions, can also influence the acute responses to exercise and exercise perception/tolerance. Although this area is still under investigation, supporting evidence showed that chronic consumption of a high-fat diet, genetically induced obesity, and visceral obesity in combination with diabetes, were associated with brain insulin resistance and structural changes in the hypothalamic/prefrontal cortex, hippocampus, and higher cortical brain regions that possibly contribute to the presentation of cognitive deficits [92, 103–106]. The above dysfunctions could also contribute to modifications in cerebral hemodynamics and reduced cerebral oxygenation during exercise and exercise intolerance reported in obese individuals with metabolic disturbances [63, 107, 108].

Furthermore, hormonal disturbances directly related to lipolysis and/or skeletal muscle protein synthesis ([nor]epinephrine, insulin, cortisol, growth hormone, testosterone, triiodothyronine, atrial natriuretic peptide, insulin-like growth factor-1) during exercise in obesity can also contribute to a suppressed lipolytic response and/or lower protein synthesis in the MetS population (see references [109, 110] for reviews of the topic). Although muscle SNS activity is found higher in obesity, the exercise-induced metabolic response to epinephrine and growth hormone appears to be blunted, while cortisol levels are higher. Elevated leptin levels in obese individuals downregulate β-adrenoreceptors in white adipose tissue and, thus, contribute to a suppressed lipolytic response during acute endurance exercise in MetS. The extent to which different exercise programs can modify cardiovascular risk and the progression to T2DM in MetS depends on the participant's individual characteristics and appropriate patient-tailored exercise programs which should be prescribed to achieve optimal benefits.

Characteristics of the Exercise Program for Improving Glucose and Blood Pressure Control in the Metabolic Syndrome

The exercise program characteristics (frequency, intensity, type, time) can play a key role in improving the cardiometabolic risk in MetS (Table 29.2).

Exercise frequency During a single bout of exercise, GLUT4 mRNA concentrations in skeletal muscle increase and result in higher GLUT4 protein content within 16–24 h after the exercise bout [111]. However, the effects of exercise on glucose uptake are short-lived (last up to 24 hours) [112–114]. Similarly, postexercise hypotension only lasts a few hours [72, 74]. The above highlights the importance of the frequency of exercise training, in order to achieve the optimal exercise-induced benefits on GLUT4 and BP. Individuals with MetS should exercise most days of the week, with no more than two consecutive days without physical activity [115].

Intensity and exercise type Endurance/aerobic training, involving large muscle groups (i.e., activities such as fast walking, running, biking, or swimming), is important for optimal glucose and BP control. During aerobic exercise, oxidative fibers are recruited. These fibers have a greater abundance of GLUT4 proteins [116, 117], higher protein levels of hexokinase II and of electron transport chain complex II than glycolytic fibers, and thus, their ability to transport, phosphorylate, and oxidize glucose, respectively, is greater than in glycolytic [118]. Therefore, aerobic training can assist in an enhancement of oxidative fibers and contribute to greater blood glucose uptake. Aerobic training can also reduce waist circumference (by ~2 cm), increase high-density lipoprotein cholesterol (by ~3.2 mg/dL), reduce triglycerides (by ~7.5 mg/dL) and resting systolic and diastolic BP (by approximately ~5 and 3 mmHg, respectively) [119]. These improvements can lessen the risk of stroke mortality and mortality from heart disease in people with MetS. The extent, however, to which these responses are exercise dose−/intensity-dependent is still under investigation.

A number of studies suggested that higher-intensity exercise (at 75–80% VO_{2peak}) compared with lower intensity enhances acutely hexokinase concentration/activity and results in greater glucose uptake and promotes greater improvements in insulin sensitivity and glycosylated hemoglobin (HbA1c) [54, 120–124]. However, a recent meta-analysis revealed similar cardiometabolic

Table 29.2 Exercise prescription summary incorporating recommendations for individuals with insulin resistance and prehypertension/hypertension (based on current guidelines by the American Diabetes Association, American Heart Association, American College of Sports Medicine, and European Society of Hypertension) [72, 74, 115]

	Aerobic exercise	Muscle strengthening	Flexibility/balance
Frequency	Most days of the week (>5 days)	3 days per week	2–3 times per week
Intensity	At least moderate intensity (≥40–60% of heart rate reserve or VO_2 reserve, or 55–76% of maximal heart rate, or "12–13" on Borg's scale)	1–3 sets moderate to vigorous intensity [70–75% of a 1-RM or 10–12 repetitions to fatigue] Muscle endurance exercises (i.e., up to 20 reps while decreasing the load and the rest interval between sets) for patients unable to perform higher intensity (due to musculoskeletal pain, high blood pressure, or other comorbidities)	
Type	Continuous or interval	6–8 exercises involving large muscle groups.	Core strengthening and lower body exercises for improving balance
Time	>150 min/week, with long-term target >210 min/week	30–60 min per session	

adaptations between moderate- and high-intensity exercise in patients with insulin resistance [125]. Furthermore, in inactive individuals with low insulin sensitivity and autonomic dysfunction, even a low exercise dose (400 kcal/week) was shown effective in improving insulin sensitivity; however, it may take a longer time (>12 weeks) to observe improvements after low-volume training [126–128].

The overall training volume of the exercise programs is an important component not only for reducing HbA1c levels [124, 129] but also for better BP control. In individuals with stage I hypertension, the duration of postexercise hypotension was linked to aerobic exercise intensity in a "dose response" fashion [73, 130].

Interval training High-intensity interval training (HIIT) has been proposed as an alternative approach to moderate-intensity continuous training to improve glycemic control in healthy, in MetS, and in diabetic individuals. HITT is defined as repeated high-intensity exercise bouts (≥90% VO_{2peak} or 80–100% of maximal heart rate) of relatively short duration (usually from 30 s to 2 min), alternated with equal periods of active (30% of VO_{2peak}) or passive rest. This type of exercise promotes a high degree of muscle fiber recruitment, activation of AMPK, and depletion of glycogen, while it increases GLUT4 abundance and skeletal muscle mitochondria content in younger and in older adults [131–135]. Greater improvements in postprandial glycaemia and micro−/macro-vascular function with HITT than continuous exercise have also been reported in overweight/obese adults and in patients with T2DM [136, 137]. However, there are only a few studies examining the effects of exercise intensity on the improvement of glycemic control or the mode of aerobic exercise (continuous vs. HITT), in which the overall physiological stress was matched [28, 138]. Using NMR-based metabonomics, Zafeiridis et al. showed that acute moderate/high continuous (80% VO_{2peak}) and HIIT (95–110% VO_{2peak}) exercise sessions, when performed with similar overall physiological stress, resulted in comparable global metabolic responses in healthy individuals [139]. Similarly, another study showed that moderate- and high-intensity exercise (40% vs. 80% VO_{2peak}, respectively), when performed with equal total work, results in equivalent increases in GLUT4 mRNA and protein [140]. In agreement with the above studies, no differences in various body fat measurements were revealed between interval (either as HIIT or sprint interval training) and moderate-intensity continuous training [141]. In addition, a systematic review comparing the efficacy of the two modes of exercise (HIIT vs. continuous) for reducing BP, in adults with preestablished hypertension, showed that although HIIT improved VO_{2peak} to a greater magnitude than moderate-continuous, both exercise modes provided comparable reductions in resting BP. Thus, both types of exercise can be used in individuals with MetS, if tolerated and executed in a safe fashion.

Time (duration) The duration of the exercise program is another important component of the exercise prescription for improving glycemic and BP control. Weekly exercise duration >150 min was associated with greater HbA_{1c} declines than of <150 min regardless of exercise intensity [142, 143].

Besides participation in structured exercise programs, increasing overall spontaneous physical activity and reducing sitting time are important for individuals with fully developed MetS or those in risk of MetS. In fact, Suliga et al. showed that in individuals declaring low physical activity levels, the risk of MetS and abnormal triglycerides concentration was higher compared to those declaring high physical activity, regardless of BMI [144]. On the other hand, reducing sedentary time is another key component for optimal benefits, as longer sitting time has been associated with abdominal obesity [144]. Thus, sedentary adults and individuals with insulin resistance are encouraged to interrupt prolonged sitting with short walks and engage in light walking after meals in order to improve overall glycemic control [115] and to prevent acute leg vascular dysfunction associated with excessive time spent in the sitting position [145].

Muscle-strengthening exercise Inactivity favors loss of muscle mass and muscle strength.

Individuals then become even less active, have more muscle weakness, and progressively have a reduced quality of life. In middle-aged and older adults, low muscle mass has been linked to an increased risk of T2DM, independent of general obesity [146]. Increasing muscle mass results in greater glucose uptake; thus, resistance and strengthening exercises should be an integral part of the exercise program. In higher-intensity resistance exercise [70–75% of one-repetition maximum (1-RM) or 10–12 repetitions to fatigue per set], 3–4 sets are proposed for individuals with impaired fasting glucose without other cardiovascular complications [147]. Alternatively, for individuals with comorbidities unable to perform muscle hypertrophy and higher-intensity resistance training, muscle endurance exercises are preferred choices (see Table 29.2).

Concurrent training Combining aerobic with resistance exercise results in greater improvements in insulin sensitivity than aerobic or resistance exercise alone [148, 149]. In the elderly, even a low-intensity concurrent exercise program for 6 weeks was effective in improving insulin resistance, independently of improvements in body weight [150]. However, whether the greater improvements with concurrent exercise are due to synergistic effects of the two exercise modes or whether the effects are due to the greater amount of exercise performed requires further investigation [151].

Summary and Conclusions

Increasing physical activity is recommended for improving glycemic control and lowering BP and should be encouraged in all people with obesity, hypertension, diabetes, or overt MetS [152]. Chronic aerobic exercise training enhances glucose uptake by the skeletal muscles and promotes mitochondrial biogenesis and improvements in mitochondrial function. Aerobic exercise also improves in micro- and macro-endothelial vascular function and promotes angiogenesis. On the other hand, resistance exercise training is effective for increasing skeletal muscle mass and, thus, enhancing glucose uptake. Both types of exercise are effective in improving blood pressure levels. The combination of aerobic with resistance training can have additive effects, resulting in significant improvements in glycemic and blood pressure control and dyslipidemia. Even increasing leisure time activities (e.g., walking, jogging, swimming, tai chi, yoga) can significantly reduce HbA1c. However, special considerations are required for individuals with comorbidities (such as CVD, uncontrolled retinopathy, or nephropathy) when designing the exercise program. Based upon the current evidence, exercise should be performed preferably in all days of the week, at moderate intensity (at 40–60% of VO_2 reserve), with 30–60 minutes of continuous or accumulated exercise per day. Endurance exercise should be supplemented by resistance-strengthening exercises. Furthermore, the combination of dietary modifications that promote weight reduction and exercise improves hyperglycemia and reduces cardiovascular risk factors more than either dietary interventions or physical activity alone [153]. Individualized exercise prescription and patient-tailored selection of activities and diet are key components for a more successful management of MetS. Finally, it is recommended, to curb the escalating MetS epidemic, that primary prevention through promotion of a healthy lifestyle should be a global priority.

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30 Exercise and Training Effects on Appetite-Regulating Hormones in Individuals with Obesity

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Introduction

The purpose of this chapter is to review the role of acute and chronic exercise on appetite regulation in normal and obese individuals. This is a critical topic as the epidemic of overweight and obesity is one of the major health concerns of the twenty-first century. Specifically, it has been transformed from relatively minor public health issue to an important globalized threat to public health.

Linked to complex interactions between biological, psychological, behavioral, and environmental determinants, obesity is considered as a pathological condition by the World Health Organization (WHO). Furthermore, the scientific literature confirms that the condition leads to an increase in the prevalence of many comorbidities, such as (*a*) cardiovascular diseases [1], (*b*) osteoarticular complications [2], (*c*) metabolic dysfunctions [3], and (*d*) a risk for some types of cancer [4].

The pathology of obesity is accompanied by alteration in the secretion of the appetite related hormones which can lead to disturbances in food intake [5]. These hormones can be divided into two categories: that being anorectic (appetite-suppressing) and orexigenic (appetite-stimulating). The main hormones regulating appetite and satiety are ghrelin, glucagon-like peptide-1 (GLP-1), peptide YY (PYY), cholecystokinin (CCK), leptin, and oxyntomodulin (OXN). Moreover, physical activity (PA) and regular exercise play a leading role in the management of the energy balance of the normal-weight or obese individual [6–10]. Despite the clear role of PA in the management of obesity, few studies have examined the modulatory effects of PA and regular exercise on hormones controlling and stimulating food intake. This chapter presents an overview on this topic and provides a consensus on the available literature.

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Obesity and Food Intake

Obesity is the product of imbalance between energy intake and expenditure; in this case, energy intake via food intake overcomes energy expenditure [11]. Energy expenditure consists of three components, namely, basal metabolism, postprandial thermogenesis, and physical activity (PA). The latter is considered the most valuable and is known to be decreased in obese subjects [11], while food intake is typically increased [12, 13].

How food intake is defined and characterized is an important and complex question in this issue. Specifically, food intake is comprised of three phases. The first is called the ingestive phase and is characterized by the sensation of hunger. The second, the prandial phase, corresponds to the food intake where the satiation process takes place. The third and last phase, called the postprandial phase, is characterized by the state of satiety. The regulation of food intake is part of a multifaceted system involving hormonal signaling from the entire body including the gastrointestinal system and adipocytes (i.e., fat or adipose cells). In general, food intake is mainly under the control of the hypothalamus. It integrates the nervous and hormonal signals of eating behavior and caloric intake. Hormones affecting the brain centers are synthesized and released from peripheral tissues including the intestine and adipose cells. Thus changes in adipose tissue brought on by exercise training or dietary restriction may affect appetite-regulating hormones. The effectiveness of this non-pharmacological intervention for weight management is mainly due to the possible alteration in gut hormones which regulating appetite and food intake. For example, it has been shown that exercise training reduces central obesity and inflammation in obese individuals [14]. These results may reflect changes in adipocytes size and in turn decreases in secreted appetite-regulating hormones by adipocytes such as leptin. To clarify this speculation, our intent in the following sections is to address the main hormones controlling/associated with food intake in healthy and obese subjects, as well as the various alterations caused by obesity on the secretions of these appetite hormones. Moreover, we attempt to identify (a) the effects of PA and acute and chronic exercise on the concentrations of these hormones in healthy as well as obese individuals and (*b*) the underlying mechanisms of acute and chronic exercise on appetite regulation.

Anorectic Hormones

GLP-1

GLP-1 (glucagon-like peptide-1) is an intestinal hormone, secreted in response to food intake. It stimulates insulin secretion by the β cells and reduces glucagon secretion by α cells in response to a meal, resulting in a decrease in hepatic glucose production. Hence, this physiological action of endogenous GLP-1 is glucose-dependent.

According to several studies [15–17], obesity is associated with an attenuated postprandial response of GLP-1 to stimuli. GLP-1 30 min after the consumption of a control meal is significantly attenuated in individuals with obesity compared to the individuals with the normal body weight, delaying satiety and leading to excessive food intake. It has been reported in normal subjects, GLP-1 levels were increased 10 minutes after ingestion of a standard liquid meal and then decreased and leveled of within 60 minutes, while in obese subjects GLP-1 levels were decreased at 20 minutes postprandial (Table 30.1) [16] and gradually returned to the baseline within 60 minutes. Conversely, in the preprandial period, Adam et al. [15] reported similar levels of GLP-1 between obese and normal adults.

Collectively, these findings suggest that individuals suffering from obesity have an inhibition of the feeling of satiety 20 minutes following the ingestion of a meal and would possibly explain an uncontrolled increase in food intake that would favor an imbalance of the energy balance in favor of inputs. However, the available studies are limited, and further research is necessary to test this assumption.

Table 30.1 Studies examining the effect of acute exercise on GLP-1

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Findings
Studies in exercise-trained females				
Hallworth et al. [24]	Healthy, active BMI = 23.5 ± 2.8 $\dot{V}O_{2max}$ = 40.7 ± 5.4	*N* = 9 Age = 22–39	(1) moderate-intensity continuous training (MICT; 30 min; 65% $\dot{V}O_{2max}$); (2) sprint interval training (SIT; 6 × 30 sec "all-out" cycling sprints with 4-min recovery)	↑ GLP-1
Howe et al. [23]	Highly trained BW = 58.4 ± 6.4 kg $\dot{V}O_{2max}$ = 55.2 ± 4.3	*N* = 15 Age = 18–40	Moderate-intensity (MIE, 60% $\dot{V}O_{2max}$) and high-intensity (HIE, 85% $\dot{V}O_{2max}$) treadmill running	↑ GLP-1
Ueda et al. [22]	Healthy middle-aged BMI = 27.6 ± 0.4 VO_{2peak} = 23.5 ± 0.9	*N* = 20 Age = not reported	Aerobic exercise with 65% of HRmax	↑ GLP-1
Larson-Meyer et al. [28]	Runners BMI = 19.8 ± 1.0 $\dot{V}O_{2max}$ = 49.7 ± 3.0	*N* = 9 Age = 21–27	60-min 70% $\dot{V}O_{2max}$ run or walk	↑ GLP-1 post-exercise vs. rest
Unick et al. [19]	BMI = 32.5 ± 4.8	*N* = 19 Age = 20–39	Walking at 70–75% age predicted HRmax until 3.0 kcal·kg^{-1} of body weight is expended (average energy expenditure, 354 ± 72 kcal; average duration, 42 ± 8 min)	↔ GLP-1
Studies in exercise-trained males				
Yang et al. [29]	Adolescents BMI > 30	*N* = 35 Age = 13–16	"Living high-training low" (LHTL)	↑ GLP-1
Tom J. Hazell et al. [30]	Active	*N* = 10 Age = not reported	30-min cycling at 65–85% $\dot{V}O_{2max}$)	↔ GLP-1
Hunschede et al. [27]	Normal weight (*n* = 11) overweight/obese (*n* = 11)	*N* = 22 Age = 10–18	High-intensity exercise (HIEX) at 70% peak oxygen consumption (VO_{2peak})	↔ GLP-1
Daniel P. Bailey [26]	BMI = 23.5 ± 2.0	*N* = 12 Age = 19–27	(1) MIE-normoxia, (2) MIE-hypoxia, (3) HIIE-normoxia, and (4) HIIE-hypoxia	↔ GLP-1
Beaulieu et al. [25]	BM = 76.9 ± 9.7	*N* = 8 Age = 22–28	Sprint interval training (SIT) exercise	↔ GLP-1
Ueda et al. [31]	BMI = 22.5 ± 1.0 $\dot{V}O_{2max}$ = 45.9 ± 8.5	*N* = 10 Age = 19–28	Cycling 30 min at 75% or 50% $\dot{V}O_{2max}$ or rest	↑ GLP-1
Ueda et al. [32]	7 obese and 7 age-matched subjects of normal weight	*N* = 14 Age = 18–27	Cycling exercise at 50% $\dot{V}O_{2max}$	↑ GLP-1
Combined studies with exercise-trained males and females				
Holliday et al. [18]	Overweight (4 males, 4 females) BMI = 27.7 ± 1.7	*N* = 8 Age = 22–46	Very low volume sprint interval exercise (4 30 s "flat-out" cycling on an ergometer)	↑ GLP-1
Martins et al. [33]	Overweight/obese volunteers (5 males and 7 females) BMI = 32.3 ± 2.7 $\dot{V}O_{2max}$ = 30.5 ± 4.9	*N* = 12 Age = 24–44	Acute isocaloric bouts of high-intensity intermittent cycling (HIIC) and moderate-intensity continuous cycling (MICC) or short-duration HIIC (S-HIIC)	↑ GLP-1
Catia Martins et al. [34]	Healthy, normal-weight volunteers (6 males and 6 females)	*N* = 12 Age = 21–31	Cycled for 60 min at 65% of their maximal heart rate or rested	↑ GLP-1
O'Connor et al. [35]	Marathon runners (23 males and 3 females)	N = 26 Age = 19–61	Marathon running: Average time = 239 min	↑ fasting GLP-1

Abbreviations: *BMI* body mass index (kg/m^2), $\dot{V}O_{2max}$ maximal oxygen uptake in mL/kg/min, *BM* body mass (kg), *TDEE* total daily energy expenditure, *DEF* deficit, *BAL* balance, *NR* not reported, ↑ increase, ↓ decrease, ↔ no change, *wk* week, *min* minute

Acute Exercise and GLP-1

Studies investigating the effects of acute exercise on GLP-1 in obese subjects are thus fare rare, and there are only few studies which have been carried out mostly on persons with normal body weight or overweight individuals. For example, very low volume sprint interval exercise resulted in increased GLP-1 in overweight men and women [18]. On the other hand, acute exercise at moderate intensity (~60% $\dot{V}O_{2max}$ (maximal oxygen uptake)) resulted in higher GLP-1 in female runners [15, 19]. In addition, a recent meta-analysis [20] concluded that exercise would increase GLP-1 levels in normal-weighted subjects. Therefore, it can be assumed that normal healthy subjects would have a reduced desire to eat following this type of exercise. These results, however, remain to be confirmed in individuals with obesity and within different modalities of exercise.

Chronic Exercise and GLP-1

It is well-known that weight loss induced by calorie restriction reduces GLP-1 levels, but weight loss induced by PA has been reported to induce a reverse effect. To this end, Martins et al. [21] were the first to examine the effect of 12 weeks of aerobic training on fasting GLP-1 levels and postprandial phase in individuals with obesity (energy expenditure = 500 kcal on the treadmill 5 times per week). This type of training had no impact on the fasting GLP-1 concentration, but tended to increase it in the postprandial phase. This trend would help to explain why aerobic training results in reduced body mass. In fact, in some studies [22–24], moderate-intensity training and aerobic training resulted in increased levels of GLP-1 in trained men or women. Interestingly, studies that investigated the effects of high-intensity interval training (HIIT) (intermittent interval type) were not able to detect any adaptations in these hormones [25–27]. To shed further light on this issue substantially, more research is warranted on this topic.

PYY

PYY is an anorectic hormone that is co-secreted with GLP-1. The secretion of this hormone is proportional to the quantity of dietary fats ingested during food intake [36]. The highest postprandial PYY concentration occurs approximately 2 hours after meal ingestion and is correlated with the size and type of the meal [37, 38]. Dietary fat intake is the most potent stimulant of PPY secretion, while carbohydrate intake has a limited effect in individuals with or without obesity [39]. Previous studies [40–42] have shown that an individual suffering from obesity has an attenuated postprandial PYY response (Table 30.2). These alterations could lead to an uncontrolled food intake, i.e., always favoring an imbalance of the energy balance equation to a positive status. Zwirska-Korczala et al. support this point, as they have reported that PYY secretion was decreased in women who were obese compared to normal-weighted subjects [42].

Acute Exercise and PYY

It appears no previous studies have investigated the impact of acute exercise on PYY in individuals with obesity or overweight subjects. In contrast to studies involving dietary intervention in obese individuals, studies on acute exercise in healthy subjects reported an increase in PYY levels in both men and women [22–24, 26, 28, 30, 43–48]. These findings, however, are not supported by others because some studies have reported no changes in the PYY levels immediately after exercise (Table 30.2) [49–52]. The increases in PYY levels are important because they could induce the suppression of hunger and potentially reduce the compensation (i.e., increased food intake) for exercise energy expended during the recovery from exercise. This point remains to be verified in individuals with obesity.

Chronic Exercise and PYY

Most research studies examining the effect of chronic exercise (moderate intensity) on PYY found no changes in its concentrations [21, 22, 56–61], while only few studies showed an increase in PYY levels after long-term exercise intervention (>32 weeks) in overweight or obese individuals (Table 30.3) [60, 62].

Regarding the effect of exercise training, an increase in fasting PYY concentrations and a significant decrease in body fat were observed after 32 weeks of training in overweight adolescents

Table 30.2 Studies examining the effect of acute exercise on PYY

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Findings
Studies in exercise-trained female				
Hallworth et al. [24]	Healthy-trained BMI = 23.5 ± 2.8 $\dot{V}O_{2max}$ = 40.7 ± 5.4	*N* = 9 Age = 22–39	(1) moderate-intensity continuous training (MICT; 30 min; 65% $\dot{V}O_{2max}$); (2) sprint interval training (SIT; 6 × 30 sec "all-out" cycling sprints with 4-min recovery)	↑ PYY
Howe et al. [23]	Highly trained Body weight = 58.4 ± 6.4 kg, $\dot{V}O_{2max}$ = 55.2 ± 4.3	*N* = 15 Age = 18–40	Moderate-intensity (MIE, 60% $\dot{V}O_{2max}$) and high-intensity (HIE, 85% $\dot{V}O_{2max}$) treadmill running	↑ $PYY_{3–36}$
Ueda et al. [22]	Healthy middle-aged BMI = 27.6 ± 0.4 VO_{2peak} = 23.5 ± 0.9	*N* = 20 Age = 49.1 ± 0.8	Aerobic exercise with 65% of maximal heart rate	↑ PYY
Larson et al. [28]	Runners BMI = 19.8 ± 1.0 $\dot{V}O_{2max}$ = 49.7 ± 3.0	*N* = 9 Age = 21–29	60-min 70% $\dot{V}O_{2max}$ run or walk	↑ PYY post-exercise vs. rest
Studies in exercise-trained males				
Hazell et al. [30]	Active	*N* = 10 Age = not reported (young healthy)	(1) moderate-intensity 8 continuous training (MICT; 30-min cycling at 65% $\dot{V}O_{2max}$); (2) high-intensity continuous 9 training (HICT; 30-min cycling at 85% $\dot{V}O_{2max}$); (3) sprint interval training (SIT; 6 × 30 s 10 "all-out" cycling bouts with 4-min recovery periods); (4) control (CTRL; no exercise)	↑ Total PYY only after HICT
Kojima et al. [50]	College endurance runners BMI = 19.3 ± 0.4 $\dot{V}O_{2max}$ = 67.1 ± 1.0	*N* = 23 Age = 19–21	20 km outdoor run (EX) or a control trial with an identical period of rest (CON)	↔ $PYY_{3–36}$
Bailey et al. [26]	BMI = 23.5 ± 2.0	*N* = 12 Age = 19–24	(1) MIE-normoxia, (2) MIE-hypoxia, (3) HIIE-normoxia, and (4) HIIE-hypoxia	PYY concentrations were higher in HIIE than MIE under hypoxic conditions during exercise
Douglas et al. [43]	BMI = 23.0 ± 1.9	*N* = 15 Age = 19–23	60 min of continuous moderate-high-intensity treadmill running	↑ PYY
Beaulieu et al. [25]	BMI = 76.9 ± 9.7	*N* = 8 Age = 22–28	Sprint interval training (SIT) exercise	↑ PYY
King et al. [49]	BMI = 22.6 ± 1.8	*N* = 9 Age = 22 ± 1.2	90 min of moderate-intensity treadmill running	↔ PYY
Sim et al. [51]	Overweight BMI = 27.7 ± 1.6; body mass = 89.8 ± 10.1 kg)	N = 17 Age = 30 ± 8	Acute effects of high-intensity intermittent exercise (HIIE)	↔ PYY
Kawano et al. [44]	BMI = 22.1 ± 2.0 $\dot{V}O_{2max}$ = 47.0 ± 6.2	*N* = 15 Age = 24.4 ± 1.7	Rope skipping 3 times for 10 min with 5-min rest at 64.8% ± 6.9% $\dot{V}O_{2max}$; cycling 3 times for 10 min with 5-min rest at 63.9% ± 7.5% $\dot{V}O_{2max}$	↑ PYY3–36 immediately post-exercise

(continued)

Table 30.2 (continued)

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Findings
Dieghton et al. [45]	BMI = 23.7 ± 3 $\dot{V}O_{2max}$ = 52.4 ± 7.1	*N* = 12 Age = 22 ± 3	Cycling: Steady-state (SS) 60 min at 59.5% ± 1.6% $\dot{V}O_{2max}$. High-intensity (HI): 10 times for 4-min intervals at 85.8% ± 4% $\dot{V}O_{2max}$ with 2-min rests	↑ PYY3–36 post-exercise in SS and HI
Deighton et al. [46]	BMI = 24.2 ± 2.9 $\dot{V}O_{2max}$ = 46.3 ± 10.2	*N* = 12 Age = 23 ± 3	Control (CON), endurance exercise (END), sprint interval exercise (SIE)	↑ PYY During exercise but most consistently during END
King et al. [53]	BMI = 22.8 ± 0.4 $\dot{V}O_{2max}$ = 57.3 ± 1.2	*N* = 12 Age = 23.4 ± 1.0	Treadmill running at 70% $\dot{V}O_{2max}$ for 90 min in exercise energy deficit (ED), food deficit (FD), or control	↑ PYY3–36 post-exercise in ED
King JA [52]	Body weight = 76.2 ± 1.0 kg	*N* = 69 Age = 22.4 ± 0.3	90 min of resistance exercise and 60 min of swimming, 60 min of brisk walking, 90 min of treadmill running	↔ $PYY_{3–36}$
Broom et al. [47]	BMI = 23.1 ± 0.4 $\dot{V}O_{2max}$ = 62.1 ± 1.8	*N* = 11 Age = 21.1 ± 0.3	Treadmill running 60 min at 70% $\dot{V}O_{2max}$	↑ PYY post-exercise
Shorten et al. [48]	BMI = 24.1 ± 2.3 VO_{2peak} = 53.8 ± 8.9	*N* = 11 Age = 20.8 ± 2.1	Treadmill running at 70% VO_{2peak} for 40 min at neutral (25 °C) or in heat (36 °C)	↑ PYY post-exercise
Ueda et al. [31]	BMI = 22.5 ± 1.0 $\dot{V}O_{2max}$ = 45.9 ± 8.5	*N* = 10 Age = 23.4 ± 4.3	Cycling 30 min at 75% or 50% $\dot{V}O_{2max}$ or rest	↑ PYY3–36 in 75% vs. 50% $\dot{V}O_{2max}$ at 60-min post-exercise
Ueda et al. [32]	Obese and normal weight BMI = 19.1–24.7 BMI = 26.0–34.6	*N* = 14 Age = 26–34	Cycling exercise at 50% $\dot{V}O_{2max}$	↑ PYY
Combined studies with exercise-trained males and females				
Douglaset al. [54]	Healthy, overweight/obese (25 males and 22 females) BMI = 22.4 ± 1.5 BMI = 29.2 ± 2.9	*N* = 47 Age = 22–58	60-min treadmill exercise (59 ± 4%) peak oxygen uptake	↑ PYY
Martins et al. [33]	Overweight/obese volunteers (5 males and 7 females) BMI = 32.3 ± 2.7 $\dot{V}O_{2max}$ = 30.5 ± 4.9	*N* = 12 Age = 24–44	Acute isocaloric bouts of high-intensity intermittent cycling (HIIC) and moderate-intensity continuous cycling (MICC), or short-duration HIIC (S-HIIC)	↔ $PYY_{3–36}$
Russell et al. [55]	Endurance runners Males (*n* = 11), BMI = 21.9 ± 1.5 $\dot{V}O_{2max}$ = 63.7 ± 6.3 Females (n = 11), BMI = 21.0 ± 1.1 $\dot{V}O_{2max}$ = 53.2 ± 5.4	*N* = 21 Age = 18–36	8-day session: 7-day running 90 min at 63% $\dot{V}O_{2max}$ + 1-day 10-kilometer time trial	Total PYY ↑ immediately post-exercise ($p < 0.0001$)
Martins et al. [34]	Healthy, normal-weight volunteers (6 males and 6 females)	*N* = 12 Age = 21–31	Cycled for 60 min at 65% of their maximal heart rate or rested	↑ PYY
O'Connor et al. [35]	Marathon runners (23 males and 3 females)	*N* = 26 Age = 19–61	Marathon running: Average time = 239 min	↑ PPY post- and 30-min post-race

See Table 30.1 for abbreviation definitions

Table 30.3 Studies examining the effect of chronic exercise on PYY

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Findings
Gibbons et al. [56]	Overweight/obese individuals (13 males and 19 females)	*N* = 32 Age = 37–49	12-wk exercise intervention – 5 exercise sessions per wk	↔ PYY
Martins et al. [57]	Sedentary obese individuals (30 females, 16 males) BMI = 33.3 ± 2.9	*N* = 46 Age = 25–44	12 wk of isocaloric programs of moderate-intensity continuous training (MICT) or high-intensity interval training (HIIT) or a short-duration HIIT (1/2HIIT)	↔ PYY
Guelfi et al. [58]	Overweight/obese males BMI = 30.8 ± 4.2	*N* = 33 Age = 42–56	12-wk training (3 day/wk), 3 groups: Aerobic (*n* = 12) 40–60 min at 70%–80% HRmax; resistance (*n* = 13) 3–4 sets, 8–10 reps at 75%–85% 1RM; control (*n* = 8)	↔ PYY
Ueda et al. [22]	Healthy middle-aged females BMI = 27.6 ± 0.4 VO_{2Peak} = 23.5 ± 0.9	*N* = 20 Age = 49.1 ± 0.8	12 wk of exercise training with 65% of HRmax	↔ PYY
Gueugnon et al. [59]	Obese adolescents (10 boys and 22 girls) (BMI z score = 4.1)	*N* = 32 Age = 14–15	Physical exercise 5 times/wk, during the following 7 months	↔ PYY
Martins et al. [21]	Sedentary overweight males and females BMI = 31.3 ± 2.3 $\dot{V}O_{2max}$ = 32.9 ± 6.6	*N* = 15 Age = 28–46	12-wk exercise intervention	↑ Postprandial PYY
Jones et al. [60]	Overweight male and female adolescents BMI = 31.8 ± 5.2	*N* = 12 Age = 14–16	32 wk of exercise training	↑ Fasting plasma PYY
Kelly et al. [61]	Older obese males and females with impaired glucose tolerance BMI = 34.4 ± 1.7	*N* = 19 Age = 65–69	12 wk of moderate-intensity aerobic exercise (treadmill/cycle ergometer) at ~75% of maximal oxygen uptake capacity combined with a eucaloric or hypocaloric diet	↔ PYY
Roth et al. [62]	73 obese children and 45 age-matched normal-weight children	N = 118 Age = 9–13	1-year diet and exercise intervention	↑ Fasting plasma PYY

See Table 30.1 for abbreviation definitions. *HRmax* heart rate maximum

[60]. In addition, Martins et al. [21] reported a trend toward increased postprandial PYY levels in obese men and women after 12 weeks of training (aerobic training at 75% of HRmax). More recently, Guelfi et al. [58] indicated that in obese individuals, aerobic or resistance training did not significantly alter fasting and postprandial PYY levels. Thus, evidence of PYY's contribution to improve satiety following exercise and/or training in the individuals with obesity remains controversial.

PP

Another anorectic hormone, pancreatic polypeptide (PP), is secreted following meal consumption and affects dietary caloric intake [63]. Studies on PP levels in individuals with obesity have shown conflicting results. Some investigators found no differences between normal-weighted and obese subjects [64], while others have demonstrated lower levels of PP in individuals with obesity [65]. If PP levels are decreased in

obese individuals, this might be considered as a physiological condition or consequence of obesity.

Acute Exercise and PP

The literature on the impact of acute exercise on PP in healthy or obese individuals is limited. Previous research studies examining the effect of acute exercise on PP found increases in PP postprandial levels [34, 66], fasting PP levels [35, 67, 68], and responses to exercise [69]. Furthermore, a meta-analysis [20] of studies in healthy individuals found that a single session of exercise induces an increase in PP concentrations. These increases in PP levels could potentially explain the reductions in appetite reported during the hours following exercise (see Table 30.4 for summary of study findings).

Chronic Exercise and PP

There is a discrepancy among the findings of studies examining the training effect on PP, where some studies reported increased levels of PP after moderate chronic exercise [20, 71], while others found no changes whatsoever (Table 30.5) [72, 73].

Leptin

Leptin is perhaps the most highly studied adipocyte hormone. Its secretion increases proportionally with the lipid content of a consumed meal. This hormone signals the hypothalamus receptors to reduce appetite and increase energy expenditure [75]. To that end, leptin potentiates the effects of CKK on the inhibition of food

Table 30.4 The effect of acute exercise on PP

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Results
Hilsted et al. [70]	Marathon runners (males)	*N* = 6 Age = not reported	3 h of exercise (cycle ergometer) at 40% $\dot{V}O_{2max}$ versus resting	↑ Fasting PP
Greenberg et al. [66]	Normal-weight volunteers (males)	*N* = 7 Age = 23–39	45 min of exercise (cycle ergometer) at 50% $\dot{V}O_{2max}$, 30 min after breakfast versus resting	↑ Postprandial plasma levels of PP
O'Connor et al. [35]	Marathon runners (23 males and 3 females)	*N* = 26 Age = 19–61	Marathon running	↑ PP
Sliwowski et al. [67]	Normal-weight males	*N* = 19 Age = 20–24	Treadmill run to exhaustion in fasting or fed state (5 min after a liquid meal)	↑ PP plasma levels after exercise, independently of feeding
Martins et al. [34]	Healthy, normal-weight volunteers (6 males and 6 females)	*N* = 12 Age = 21–31	1 h or intermittent cycling at 65% of HRmax (1 h after breakfast) versus resting	↑ Postprandial levels of PP
Mackelvie et al. [69]	Normal weight, BMI = 20.7 ± 0.5; overweight, BMI = 32.4 ± 1.7 (male adolescents)	*N* = 17 Age = 15–16	One hour after a standardized breakfast, subjects either cycled for 60 min at 65% of their HRmax or rested	↑ PP
Feurle et al. [68]	Long-distance runners or cyclists (males)	*N* = 18 Age = 25–35	Maximal bicycle ergometer test includes 150 and 75 W in turn for athletes and non-athletes. After 8 min the workload was gradually increased every 2 min until exhaustion. The maximal workload achieved 379 W for athletes and 240 W for non-athletes	↑ PP

See Table 30.1 for abbreviation definitions

Table 30.5 The effect of exercise training on PP

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Results
Hurley et al. [72]	Normal-weight sedentary males	*N* = 7 Age = college age	10 weeks exercise program (20 min of jogging at 70% V̇O2 max, three times/week)	↑ Fasting and peak PP levels
Howarth et al. [73]	Obese male	*N* = 40 Age: 12 weeks	Included five 60-min sessions per week, speed 18 m/min with a running belt at a 5% incline and began 1 week after the streptozotocin treatment and continued for 12 weeks	↔ PP
Kanaley et al. [74]	Healthy obese males and females BMI = 30–45	*N* = 13 Age = 40–44	Short-term aerobic exercise training (15 days)	↑ PP
Øktedalen et al. [71]	Young males	*N* = 24 Age = 21–26	5-day training course with long-term physical exercise (35% of $\dot{V}O_{2\,max}$), calorie supply deficiency	↑ Fasting PP during the course in the low-caloric ↓ PP to pre-course levels after 8 h of rest ↑ In the low-caloric subjects more than in the subjects with calorie balance

See Table 30.1 for abbreviation definitions

intake, but this mechanism is disrupted by obesity [76]. For example, researchers investigated the leptin levels after 12 hours of fasting and after ingestion of a standardized meal over a 2-hour period in normal-weighted and women with obesity [77]. Basal leptin levels were significantly higher in the obese group than in the normal-weighted group and then progressively decreased at 15-, 60-, and 120-min postprandial. These data are in contrast with those of the normal healthy individuals, whose leptin levels remained relatively constant during the postprandial period. Thus while leptin promotes satiety, for individuals with obesity, there is a tendency to develop resistance to leptin, thereby inducing a regulatory disruption that can potentially lead to uncontrolled food intake.

Acute Exercise and Leptin

Vatansever-Ozen et al. found that fasting leptin concentrations are not affected by long-term aerobic exercise (105 min at 50% of $\dot{V}O_{2max}$ followed by 15 min at 70% of $\dot{V}O_{2max}$) in healthy participants. However, it significantly decreases hunger that may be related to the other appetite-regulating hormones such as ghrelin [78]. Indeed, in individuals with obesity, Martins et al. point out that exercise-induced weight loss results not only in fasting but also in postprandial leptin level changes actually favoring food intake. Regarding aerobic training in obese individuals, after 12 weeks, the leptin levels after exercise were significantly decreased on an empty stomach and after the ingestion of a standardized meal, leading to a weight loss of approximately 3.5 kg from 96.1 ± 11.0 to 92.6 ± 11.7 kg. Thus, the reduction of leptin concentrations after chronic aerobic exercise may reflect an improved action of leptin, i.e., sensitivity within individuals [79] (Table 30.6).

Chronic Exercise and Leptin

With regard to exercise training effect, most studies found decreased levels of leptin following low- or moderate-intensity exercise training [79, 96–112], while some other studies found no change in its levels (Table 30.7) [113–117]. Most

Table 30.6 The effect of acute exercise on leptin

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Results
Yi et al. [80]	15-month-old male SD rats with type 2 diabetes (450–470 g)	N = not reported Age = 15 months	Acute (two 90-minute sessions with a 45-min interval between each session) exercise	Activation of leptin-AMPK-ACC signaling (leptin signaling pathway)
Guerra et al. [81]	Young healthy males	*N* = 15 Age = 21–25	30-s sprint exercise (Wingate test)	Sprint leptin signaling mimetics in human skeletal muscle
Landt et al. [82]	Healthy males	*N* = 26 Age = 28–32	Stationary ergometer for 2 hours and prolonged exercise of an ultramarathon	↓ Leptin
Weltman et al. [83]	Healthy young males	*N* = 7 Age = 25–29	30 min of exercise at various intensities and caloric expenditures	↔ Leptin levels during the exercise and during the recovery (3.5 hours)
Bouassida et al. [84]	Physically active (5 males and 12 females)	*N* = 17 Age = 21–25	45 seconds of supramaximal exercise at 120% of peak aerobic power	↔ Leptin
Torjman et al. [85]	Healthy untrained males	*N* = 6 Age = 18–45	60 minutes of treadmill exercise at 50% of $\dot{V}O_{2max}$	↔ During a 4-hour recovery period
Essig et al. [86]	Trained males	*N* = 11 Age = 21–44	2 separate exercise tests, an 800 and 1500 kcals treadmill run	↓ Leptin
Olive et al. [87]	Trained males	*N* = 9 Age = 22–33	60 min of running at 70% of $\dot{V}O_{2max}$	↓ Leptin after exercise, 24 and 48 hours during recovery
Kraemer et al. [88]	Postmenopausal females	*N* = 30 Age = 46–56	30 minutes of exercise at 80% of $\dot{V}O_{2max}$	↓ Leptin
Nindl et al. [89]	Males	*N* = 10 Age = 20–22	50 sets of resistance exercise: 15 sets squat, 15 sets bench press, 10 sets leg press, 10 lat pull-down exercise	↓ Leptin at 9, 12, and 13 hours following the exercise
Zaccaria et al. [90]	Males	*N* = 45 Age = 32–50	Three competitive endurance races: a half marathon run, a ski-alpinism race, and an ultramarathon race	↓ Leptin – only after prolonged endurance exercises like the ski-alpinism and the ultramarathon races
Sliwowski et al. [67]	Normal-weight males	*N* = 19 Age = 20–24	Treadmill run to exhaustion in fasting or fed state (5 min after a liquid meal)	↓ Leptin level after meal
Ferguson et al. [91]	Normal-weight males and females	*N* = 16 Age = 25–31	60-minute exercise (cycle ergometer) at 65% of $\dot{V}O_{2max}$ versus resting	↔ Leptin
Jürimäe et al. [92]	College level rowers	*N* = 8 Age = 17–26	Rowing over a distance of 6.5 km at the individual anaerobic threshold	↔ Leptin immediately after exercise and decreased after 30 min of recovery
Kyriazis et al. [93]	Young healthy obese males	*N* = 15 Age = 23–27	Single exercise session of moderate intensity (58.4% of $\dot{V}O_{2max}$) for 60 min	↔ Leptin

Table 30.6 (continued)

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Results
Racette et al. [94]	Sedentary volunteers, normal and obese males	*N* = 5 Age = 36–41	Moderate-intensity cycle ergometer exercise (50% of maximal heart rate)	↔ Leptin and leptin production
Tuominen et al. [95]	Healthy males	*N* = 26 Age = 30–34	Glycogen depletion, insulin clamp 44 h after a 2-h treadmill exercise at an intensity of 75% $\dot{V}O_{2max}$	Leptin correlated directly with serum insulin, cortisol, and triglyceride and inversely with GH concentrations. They are decreased by glycogen-depleting exercise and increase during hyperinsulinemic clamp

See Table 30.1 for abbreviation definitions. *AMPK* AMP-activated protein kinase, *ACC* acetyl-COA carboxylase

Table 30.7 The effect of exercise training on leptin

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Results
Carter et al. [113]	Overweight or obese, insulin-resistant horses	*N* = 12 Age = 9–21	4 wk at low intensity and 4 wk at higher intensity, followed by 2 wk of detraining	↔ Leptin
Pérusse et al. [118]	Sedentary adult (51 males and 46 females)	*N* = 97 Age = 17–40	20-wk endurance training on a computer-controlled cycle ergometer	↔ Leptin levels after acute exercise ↓ Leptin levels in males but not in females after completing the endurance training
Dede et al. [96]	Patients with type 2 diabetes	*N* = 60 Age = not seen	Aerobic exercise	↓ Leptin
Murakami et al. [97]	Obese non-diabetic individuals	*N* = 42 Age = 46–51	Weight reduction by a 12-wk calorie-restricted diet with or without aerobic exercise	↓ Leptin
Sari et al. [98]	Obese females	*N* = 23 Age = 30–52	Exercise program (45-minute walking sessions at 60–80% of HRmax) every day for 4 wk (total 20 exercise sessions)	↔ Leptin levels after acute exercise ↓ Leptin levels after chronic exercise training
Koutsari et al. [99]	Healthy, postmenopausal females	*N* = 8 Age = 56–64	Daily moderate-intensity exercise (walking on the treadmill for 60 min each afternoon) with a short-term high-carbohydrate diet	↓ Fasting and postprandial circulating leptin concentrations after daily moderate-intensity exercise and consumption of a short-term high-carbohydrate diet
Ordonez et al. [100]	Obese young females with down syndrome	*N* = 20 Age = 21–29	10-wk aerobic-training program on a treadmill (30–40 min) at a work intensity of 55–65% of peak heart rate	↓ Plasma leptin levels

(continued)

Table 30.7 (continued)

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Results
Reseland et al. [119]	Males with metabolic syndrome	*N* = 186 Age = 42–48	Long-term reductions in food intake Increasing physical activity (60-min workouts of aerobics, circuit training, and fast walking and jogging, 3 times/wk)	↓ Plasma leptin concentration after both the diet and the exercise interventions
Houmard et al. [114]	Healthy young individuals(9 females and 7 males) Older individuals with relatively more adipose tissue (8 females and 6 males)	*N* = 30 Age: 21–22 Older = 57–60	Short-term aerobic training (60 minutes at 75% of $\dot{V}O_{2max}$ during 7 successive days)	↔ Leptin
Gippini et al. [115]	(1) 25 non-professional body builders (2) 21 mild overweight sedentary subjects (3) 19 normal-weight sedentary controls	*N* = 65 Age = 26–30	Resistance exercise	↔ Leptin production
Kraemer et al. [116]	Adolescent female distance runners	*N* = 7 Age = 14–16	Intense exercise for 7 wk	↔ Leptin concentration of resting and post-maximal exercise
Kraemer et al. [117]	Middle-age obese females	*N* = 16 Age = 41–44	9-week training program (3–4 days of exercise including 20–30 min of step aerobics 2 days/week and treadmill running or stationary cycling on additional days)	↔ Leptin concentration
Gomez-Merino et al. [101]	Soldiers, males	*N* = 26 Age = 19–23	3 wk of a military training	↓ Leptin
Unal et al. [102]	24 trained young male athletes and 22 healthy sedentary males	*N* = 46 Age = 16–23	Regular exercise	↓ Leptin
Unal et al. [120]	10 professional football players and 17 healthy sedentary males	*N* = 27 Age = 17–20	Regular specificity exercising	↓ Leptin levels in the football players than healthy males
Fatouros et al. [103]	Inactive males BMI = 28.7–30.2	*N* = 50 Age = 65–78	Resistance training (6 months, 3 days/week, 10 exercises/3 sets)	↓ Leptin
Ishii et al. [104]	Sedentary subjects with type 2 diabetes	*N* = 50 Age = 50–66	6 wk of an aerobic training	↓ Leptin
Hickey et al. [105]	Sedentary middle-aged males (n = 9) and females (n = 9)	*N* = 18 Age = not reported	12 wk of an aerobic training	↓ Leptin
Okazaki et al. [106]	15 obese and 26 nonobese middle-aged sedentary females	*N* = 41 Age = 47–59	Mild aerobic exercise (50% of $\dot{V}O_{2max}$) and personal diet counseling for 12 wk	↓ Leptin

Table 30.7 (continued)

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Results
Herrick et al. [107]	3 males and 7 females	*N* = 10 Age = 22–60	6 months of a diet/exercise weight loss program	↓ Free leptin index at 3 M and 6 M for males and 6 M for females
Ackel-D'Elia et al. [108]	Adolescents	*N* = 132 Age = 15–19	Leisure physical activity, aerobic training, and aerobic plus resistance training as 6-month interdisciplinary therapy	↓ Leptin after aerobic training and aerobic plus resistance training
Lau et al. [121]	Overweight adolescents (5 girls, 13 boys)	*N* = 18 Age = 10–15	Resistance training (3 times a week on alternate days for 6 wk)	↔ Leptin
Ko et al. [109]	Sturdy males	*N* = 36 Age = 21–26	Aerobic exercise using a treadmill (60% of heart rate reserve) and weight training (nine different exercises for the large muscles) 5 days/wk for 8 wk	↓ Leptin
Martins et al. [79]	Overweight and obese healthy sedentary individuals BMI = 31.3 ± 3.3	*N* = 22 Age = 29–46	2-wk supervised exercise	↓ Fasting and postprandial leptin concentrations
Kohrt et al. [110]	Older females	*N* = 61 Age = 60–72	2-month flexibility-exercise program followed by a 9-month exercise program included walking, jogging, and stair climbing	↓ Serum leptin levels
Pasman et al. [111]	Obese males	*N* = 15 Age = 32–43	Weight loss and endurance exercise training (3–4 times weekly, 1 h/sessions, moderate intensity) for 4 months	↓ Plasma leptin levels independently of changes in plasma insulin levels and body fat percentage
Thong et al. [122]	Obese males	*N* = 52 Age = 42–47	12 wk of weight loss and exercise	↔ Circulating leptin without weight loss ↓ Circulating leptin with weight loss
Hayase et al. [112]	Premenopausal (*N* = 9) postmenopausal females (*N* = 9)	*N* = 18 Age = 36–58	Aqua exercise (2 times a week) plus resistance exercise (1 time a wk) for 10 wk	↓ Plasma levels of leptin in both groups

See Table 30.1 for abbreviation definitions. *TDEE* total daily energy expenditure

available research has focused on aerobic/endurance exercise training with or without concurrent or in combination with resistance training. Interestingly, the only study that investigated the effect of intensive training (endurance and interval training) found no changes in leptin levels at resting and post-maximal exercise. However, this study is limited in generalizability as it looked at adolescent female runners as a population [116].

CCK

Cholecystokinin (CCK) is an anorexigenic hormone secreted by the duodenal and jejuna mucosa when highly acid food enters the small intestine. Studies have shown that reduced level of CCK may contribute to reduced feelings of fullness and add to the difficulty in losing weight in some obese people [123]. This hormone is found to be

Table 30.8 The effect of acute exercise on CCK

Reference(s)	Population	Number of participants (N) and age (yrs.)	Intervention	Results
Bailey et al. [126]	Physically active males divided to normoxic = 14 or hypoxic = 18 BMI = 23.6 ± 1.6 $\dot{V}O_{2max}$ = 50 ± 9	*N* = 32 Age = 19–25	Cycling to exhaustion in normobaric normoxia and normobaric hypoxia	↑ CCK in normoxic condition ↓ CCK in hypoxia condition
Philipp et al. [127]	11 male and 8 female marathon runners	*N* = 19 Age = 20–58	Long-distance running (marathon run)	↑ CCK in pre-run highest concentration after the run
Ströhle et al. [128]	Healthy untrained subjects (2 females and 8 males)	*N* = 10 Age = 23–29	The anti-panic effects (behavioral) of aerobic exercise (30 min at 70% of $\dot{V}O_{2max}$)	↓ CCK-4
Sliwowski et al. [67]	Normal-weight males	*N* = 19 Age = 20–24	Treadmill run to exhaustion in fasting or fed state (5 min after a liquid meal)	↑ CCK

See Table 30.1 for abbreviation definitions. *CCK* cholecystokinin

impaired (reduced) in individuals with obesity who are experiencing body weight reduction. Indeed, according to Sumithran et al. [124], CCK concentrations are reduced in individuals with obesity who lost 14% of their initial weight, after 8 weeks of a hypocaloric diet and 2 weeks of stabilization. Similarly, in men with obesity who underwent a low-calorie dietary intervention for 8 weeks, with a weight loss of approximately 15%, postprandial CCK concentrations decreased significantly compared to baseline values [125]. Both of these studies suggest that CCK is reduced as a result of rapid weight loss and, unfortunately, may serve as a "rebound" mechanism that promotes appetite and ultimately increases weight after such rapid weight losses.

Acute Exercise and CKK

So far, data on the impact of acute exercise on CKK levels seems to have focused only on normal-weighted individuals (Table 30.8). These studies reported an increase in CKK levels immediately after exercise and for up to 2 hours after the exercise [67, 126]. These significant increases in CKK levels are consistent with suppressed feelings of hunger, reported during the hours following exercise [20]. The limited data in this area do not allow for the generalization of the effect of exercise and training on CKK levels in individuals with obesity. Future studies are urgently needed in this topic.

Chronic Exercise and CKK

Studies of the effect of exercise training on CKK levels are very limited and controversial. For example, in several studies, plasma CCK levels [126, 129] and CCK content of the intestine [130] increased significantly following intensive training or hypoxia condition. Conversely, CCK was decreased in female runners after high-intensity training [131], but no changes in its levels were found in another exercise study (Table 30.9) [130]. Interestingly, for this latter study, Martins et al. indicated that a 12-week training program in individuals with obesity induced a mean decrease in body weight of 3.5 kg (from 96.1 ± 11.0 to 92.6 ± 11.7 kg); but it had no significant effect on fasting or postprandial CKK concentrations [79].

OXN

Oxyntomodulin (OXN) is a peptide hormone that is produced by the L cells in the small intestine; it functions to reduce food intake and likely increase energy expenditure in humans [132, 133]. Animal research supports it is one of the gut

Table 30.9 The effect of exercise training on CCK

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Results
Bailey et al. [126]	Physically active males divided to normoxic = 14 or hypoxic = 18 BMI = 23.6 ± 1.6 $\dot{V}O_{2max}$ = 50 ± 9	*N* = 32 Age = 19–25	Cycle training for 4 wk in normobaric normoxia and normobaric hypoxia	↔ Training effect on the resting or exercise plasma CCK response in normoxia
Bailey et al. [129]	Male mountaineers	*N* = 19 Age = 26–50	20 days stay at 5100 m (high altitude) to investigate its possible role in the pathophysiology of anorexia, cachexia, and acute mountain sickness (AMS); examined at rest and during a maximal exercise test at sea level before/after the expedition	↑ Plasma CCK during the second day in rest ↑ Plasma CCK after maximal exercise ↔ CCK response in five subjects with anorexia on day 2 compared with those with a normal appetite There was no relationship between the increases in CCK and AMS score ↑ resting CCK in subjects with AMS
Martins et al. [79]	Overweight and obese healthy sedentary individuals BMI = 31.3 ± 3.3	*N* = 22 Age = 29–46	2-wk supervised exercise	↔ Plasma CCK
Hirschberg et al. [131]	Long-distance runners	*N* = 14 Age = 25–29	High-intensity training period consisting of 11 hour of training/wk	↓ Postprandial CCK

See Table 30.1 for abbreviation definitions. *CCK* cholecystokinin

hormones that decreases stomach acid and alters emptying of stomach contents [134]. The effect of OXN on energy expenditure in human has not been fully understood. Nevertheless, Wynne et al. suggest that OXN does not alter resting energy expenditure; but increases activity-related energy expenditure. This assumption needs to be investigated further [135].

It is reported that OXN elevations can cause body weight loss in human [136, 137]. For example, a weight loss of 2.3 kg in overweight and obese individuals following receiving subcutaneous OXM administration three times daily (400 nmol pre-prandial) over a 4-week period was observed [136].

Interestingly, OXN was recently shown to alter glucose metabolism in human too. Improvements in insulin secretion and glucose-lowering levels in type 2 diabetes mellitus (T2DM) patient were found following OXM infusion (3 pmol/kg/min) [138].

Liu et al. explored the chronic effect of OXN infusion and its anti-obesity potential in rats. They found improvement in energy expenditure in obese rodents, suggesting that long-acting OXM analogues may be utilized as novel therapy to prevent and treat obesity in human. Nevertheless, far more work is necessary before these pharmaceutical interventions could be pursued on a regular clinical basis [139].

To date, there is no study in the literature appears to address the acute and chronic effects of exercise on OXM in obese or normal healthy individuals.

Orexigenic Hormones

Ghrelin

Ghrelin, also known as the hormone of hunger, is a peptide and a stomach-derived orexigenic hormone essentially produced by endocrine cells of the gastric mucosa and recognized as the endogenous ligand of the orphan growth hormone (GH) secretagogues receptor. It is involved significantly in food intake and appetite regulation.

The circulating concentration of ghrelin is inversely associated with body mass index (BMI) [140]. In fact, in individuals with obesity and high BMIs, ghrelin levels are reduced [141]. This effect is proposed to be caused by a deficiency of insulin sensitivity and hyperinsulinemia [142, 143]. In the postprandial period, suppression of ghrelin is reduced in obese individuals compared to normal healthy subjects, resulting in a higher energy expenditure [144]. This may contribute to an alteration of satiety signaling in obese patients and the establishment of a positive energy balance and weight gain.

Acute Exercise and Ghrelin

Several researchers [145, 146] observed an interaction between exercise and ghrelin as a regulator of appetite and energy homeostasis. So far only two studies [34, 147] have systematically focused on the concentration of ghrelin in healthy subjects after aerobic exercise (60 min at 74% $\dot{V}O_{2max}$ and 60 min at 65% of HRmax), and both groups reported no changes in ghrelin following moderate-intensity exercise. However, other studies have reported decreases in ghrelin concentrations in response to aerobic [148–150] or resistance [151–153] exercise (Table 30.10). In a study by

Table 30.10 Studies examining the effect of acute exercise on ghrelin

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Findings
Studies in exercise-trained females				
Howe et al. [23]	Highly trained BW = 58.4 ± 6.4 kg, $\dot{V}O_{2max}$ = 55.2 ± 4.3	*N* = 15 Age = 18–40	Moderate-intensity (MIE, 60% $\dot{V}O_{2max}$) and high-intensity (HIE, 85% $\dot{V}O_{2max}$) treadmill running	↓ Acylated ghrelin
Tiryaki et al. [154]	Runners BMI = 28.3 ± 1.8	*N* = 9 Age = 20–24	60-min running at 53% $\dot{V}O_{2max}$	↔ Acylated ghrelin
Larson-Meyer et al. [28]	Runners BMI = 32.7 ± 0.8	*N* = 9 Age = 18–40	60-min 70% $\dot{V}O_{2max}$ run or walk	↑ Acylated ghrelin post-exercise vs. rest
Unick et al. [19]	BMI = 32.5 ± 4.8	*N* = 19 Age = 20–37	Walking at 70–75% age predicted HRmax until 3.0 kcal·kg^{-1} of body weight is expended (average energy expenditure: 354 ± 72 kcal; average duration: 42 ± 8 min)	↔ Acylated ghrelin
Studies in exercise-trained males				
Kojima et al. [50]	College endurance runners BMI = 19.3 ± 0.4 $\dot{V}O_{2max}$ = 67.1 ± 1.0	*N* = 23 Age = 17–23	20 km outdoor run (EX) or a control trial with an identical period of rest (CON)	↓ Acylated ghrelin
Bailey et al. [26]	BMI = 23.5 ± 2.0	*N* = 12 Age = 19–24	(1) MIE-normoxia, (2) MIE-hypoxia, (3) HIIE-normoxia, and (4) HIIE-hypoxia.	Plasma acylated ghrelin concentrations were lower in hypoxia than normoxia post-exercise
Douglas et al. [43]	BMI = 23.0 ± 1.9	*N* = 15 Age = 19–23	60 min of continuous moderate-high-intensity treadmill running	↔ Acylated ghrelin

Table 30.10 (continued)

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Findings
King et al. [49]	BMI = 22.6 ± 1.8	*N* = 9 Age = 20–24	90 min of moderate-intensity treadmill running	↔ Acylated ghrelin
Sim et al. [51]	Overweight BMI = 27.7 ± 1.6	*N* = 17 Age = 22–38	Acute effects of high-intensity intermittent exercise (HIIE)	↔ Acylated ghrelin
Kawano et al. [44]	BMI = 22.1 ± 2.0 $\dot{V}O_{2max}$ = 47.0 ± 6.2	*N* = 15 Age = 22–27	Rope skipping 3 times for 10 min with 5-min rest at 64.8% ± 6.9% $\dot{V}O_{2max}$; cycling 3 times for 10 min with 5-min rest at 63.9% ± 7.5% $\dot{V}O_{2max}$	↓ Acylated ghrelin up to 30-min post-exercise ($p < 0.02$)
Deighton et al. [46]	BMI = 24.2 ± 2.9 $\dot{V}O_{2max}$ = 46.3 ± 10.2	*N* = 12 Age = 20–26	(Control (CON), endurance exercise (END), sprint interval exercise (SIE))	Acylated ghrelin was suppressed during exercise but more so during SIE
King et al. [53]	BMI = 22.8 ± 0.4 $\dot{V}O_{2max}$ = 57.3 ± 1.2	*N* = 12 Age = 22–25	Treadmill running at 70% $\dot{V}O_{2max}$ for 90 min in exercise energy deficit (ED), food deficit (FD), or control	↓ Acylated ghrelin post-exercise ($p < 0.05$)
Vatansever et al. [78]	Elite soccer players BMI = 22.0 ± 0.4 $\dot{V}O_{2max}$ = 62.74 ± 5	*N* = 10 Age = 19–21	Treadmill running 105 min at 50% $\dot{V}O_{2max}$ and then 15-min 70% $\dot{V}O_{2max}$	↓ Acylated ghrelin 120-, 180-, 240-min post-exercise ($p < 0.05$)
Broom et al. [47]	BMI = 23.1 ± 0.4 $\dot{V}O_{2max}$ = 62.1 ± 1.8	*N* = 11 Age = 19–22	Treadmill running 60 min at 70% $\dot{V}O_{2max}$	↓ Acylated ghrelin post-exercise ($p < 0.05$)
Ueda et al. [32]	Obese and age-matched subjects of normal weight	*N* = 14 Age = 18–27	Cycling exercise at 50% $\dot{V}O_{2max}$	↔ Plasma ghrelin
Combined studies with exercise-trained males and females				
Holliday et al. [18]	Eight overweight (4 males and 4 females) BMI = 27.7 ± 1.7	*N* = 8 Age = 22–46	Very low volume sprint interval exercise (430 s "flat-out" cycling on an ergometer)	↓ Acylated ghrelin
Douglas et al. [54]	Healthy, overweight (25 males and 22 females) BMI = 22.4 ± 1.5 BMI = 29.2 ± 2.9	*N* = 47 Age = 22–58	60-min treadmill exercise (59 ± 4%) peak oxygen uptake	↔ Total ghrelin
Metcalfe et al. [155]	5 males & 6 females BMI = 23 ± 3 $\dot{V}O_{2max}$ = 51 ± 11	*N* = 11 Age = 20–26	10-min reduced-exertion high-intensity interval cycle training session	↓ Acylated ghrelin
Russell et al. [55]	Endurance runners Males, BMI = 21.9 ± 1.5 $\dot{V}O_{2max}$ = 63.7 ± 6.3 Females, BMI = 21.0 ± 1.1 $\dot{V}O_{2max}$ = 53.2 ± 5.4	*N* = 21 Age = 18–36	8-day session: 7-day running 90 min at 63% $\dot{V}O_{2max}$ + 1-day 10 km time trial	↑ Total ghrelin immediately post-exercise ($p < 0.0001$)
Burns et al. [147]	Males BMI = 23.4 ± 1 $\dot{V}O_{2max}$ = 63.2 ± 2.5 females BMI = 22.5 ± 0.8 $\dot{V}O_{2max}$ = 52.1 ± 2.4	*N* = 18 Age = 23–27	Treadmill running for 60 min at 73.5% $\dot{V}O_{2max}$	↔ Total ghrelin post-exercise compared to control trail

Abbreviations: See Table 30.1 for some abbreviation definitions. *BW* body weight (kg), *HRmax* heart rate maximum, *TDEE* total daily energy expenditure, *DEF* deficit, *BAL* balance, *NR* not reported, *HIIE* high-intensity intermittent exercise, *MIE* moderate-intensity exercise, *HIE* high-intensity exercise, *END* endurance exercise, *SIE* sprint interval exercise, *1RM* one-repetition maximum, *ED* energy deficit, *FD* food deficit, ↑ increase, ↓ decrease, ↔ no change

Toshinai et al., ghrelin was suppressed in an intensity-related manner after 40-min exercise at a progressive intensity (4 stages of 10 min of progressive intensity) in healthy men. In this study, changes in ghrelin levels were correlated with changes in adrenaline (r = 0.533) and norepinephrine (r = 0.603). The reduction in ghrelin was attributed to reductions in the gastric blood supply induced by the sympathetic nervous system, which in turn causing a decrease in the release of ghrelin into the bloodstream [149]. Therefore, it appears that maximal and progressive-intensity aerobic exercise would have an impact on the secretion of ghrelin via catecholamine (epinephrine and norepinephrine)-mediated mechanisms.

Chronic Exercise and Ghrelin

With regard to the training and its impact on ghrelin levels, Leidy et al. performed 24-hour blood sampling in a normal-weighted group of women, before and after regular exercise involving 12 weeks of moderate exercise training (45 min, 5 times/week) accompanied by a dietary intervention. The intervention resulted in a 4% reduction in body weight and an increase in ghrelin concentrations during daytime [156] (Table 30.11). However, the design of this study did not allow determination of whether the observed changes came from training, dietary intervention, or the associated weight loss.

In morbidly obese men and women, following aerobic exercise training combined with dietary restriction, Morpurgo et al. [157] observed that, despite a 5% weight loss, circulating levels of ghrelin (either on an empty stomach or after meal) remained unchanged. Numerous studies report increased levels of ghrelin concentration after moderate-intensity exercise training [21, 34, 156, 158–163], while others have found no changes [52, 56–58, 164] (Table 30.11). These findings lead to the question of whether the magnitude of changes in this hormone is dependent to exercise training intensity and/or volume, a research question that needs to be pursued. Additionally, while the ghrelin adaptations to aerobic training have been extensively investigated, no data are reported concerning anaerobic training adaptations.

Underlying Mechanism of Appetite Regulation by Acute and Chronic Exercise

As mentioned before, the intensity of exercise could play a role in ghrelin secretions via catecholamine-related mechanisms [10]; but, to date, no study has investigated the responses of ghrelin following vigorous exercise or training which are known to increase catecholamine secretions in both normal healthy and individuals with obesity. In fact, Larson-Meyer et al. [28] found increased levels of acylated ghrelin after moderate-intensity training in trained women, while, in some other studies [10, 167], such training resulted in decreased levels of acylated ghrelin or even no changes in the hormone (Table 30.11) [19, 154]. These discrepancies perhaps can mainly be explained by different training protocols employed in these studies (e.g., training duration, type activity, and intensity).

Moderate exercise intensity had no effect on ghrelin and PYY concentration after moderate carbohydrate supplementation [55]. But hormones increased after 2 hours of intense exercise [55]. Furthermore, increased ghrelin after exercise is correlated with observed increased growth hormone levels [55]. These changes suggest that ghrelin concentration following acute exercise is not related to fuel consumption during exercise; thus, it seems that higher negative energy expenditure (> 1000 kcal) is needed to suppress acylated ghrelin in response to acute exercise [78, 93].

Another mechanism that might justify responses to exercise is the amount of changes in body weight. It has been reported that weight loss induced by exercise training is associated with the gradual increases in plasma levels of ghrelin in obese individuals. However, these changes appear to occur if the body weight decreases by more than 3 kg. These findings suggest that an increase in ghrelin following exercise training may be a compensatory mechanism for losing weight but not hyperphagia [158]. In addition, it has been noted that such increases induced by exercise training or diet intervention are associated with decreasing in central obesity [162] and

Table 30.11 Studies examining the effect of chronic exercise on ghrelin

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Findings
Kang et al. [165]	Obese middle-aged females	*N* = 26 Age = 46–54	5 times per wk for a span of 12 wk	↑ Ghrelin
Gibbons et al. [56]	Overweight/obese individuals	*N* = 32 Age = 37–49	12-wk exercise intervention, 5 exercise sessions per wk	↔ Acylated ghrelin
Martins et al. [57]	Sedentary obese individuals (30 females and 16 males) BMI = 33.3 ± 2.9	*N* = 46 Age = 25–44	12 wk of isocaloric programs of moderate-intensity continuous training or high-intensity interval training (HIIT) or a short-duration HIIT (1/2HIIT)	↔ Acylated ghrelin
Arikan et al. [164]	Females BMI = 22.0 ± 0.6 Males BMI = 22.6 ± 0.8	*N* = 35 Age = 18–24	60-min cycling exercises in 4 days of the wk for 8 wk at 50–70% of previously determined heart rate	↔ Ghrelin
Ueda et al. [22]	Healthy middle-aged females BMI = 27.6 ± 0.4 VO_{2Peak} = 23.5 ± 0.9	*N* = 20 Age = not seen	12 wk of exercise training with 65% of HRmax	↑ Ghrelin
Guelfi et al. [58]	Overweight/obese males BMI = 30.8 ± 4.2	*N* = 33 Age = 42–56	12-wk training (3 days/week); 3 groups: Aerobic (*n* = 12) 40–60 min at 70%–80% HRmax; resistance (*n* = 13) 3–4 sets, 8–10 reps at 75%–85% 1RM; control (*n* = 8)	↔ Acylated ghrelin
Gueugnon et al. [59]	Obese adolescents (10 boys and 22 girls) (BMI z score = 4.1)	*N* = 32 Age = 14–15	Physical exercise 5 times per wk during the following 7 months	↑ Ghrelin
Martins et al. [21]	Sedentary overweight males and females BMI = 31.3 ± 2.3 $\dot{V}O_{2max}$ = 32.9 ± 6.6	*N* = 15 Age = 28–46	12-wk training (5 days/week); treadmill walking or running at 75% HRmax until 500 kcal energy deficit	↑ Ghrelin
King et al. [52]	Males BMI = 76.2 ± 1.0	*N* = 69 Age = 22–23	90 min of resistance exercise and 60 min of swimming. 60 min of brisk walking. 90 min of treadmill running	↔ Acylated ghrelin
Hagobian et al. [160]	Overweight: Males BMI = 25.7 ± 2.3 VO_{2peak} = 44.9 ± 4.8 Females BMI = 28.0 ± 3.5 VO_{2peak} = 34.9 ± 5.2	*N* = 18 Age = 15–29	Treadmill running 50%–65% VO_{2peak} until 30% of TDEE in DEF or BAL conditions (crossover)	Females: ↑ acylated ghrelin
Konopko et al. [161]	Obese patients BMI ≥ 40, of both sexes	*N* = 33 Age = 20–60	6-month physical exercise (a 45-min walk, five times a week)	↑ Ghrelin
Kelishadi et al. [162]	Obese children	*N* = 100 Age = 7–9	Physical training for 6 months	↑ Ghrelin
Martins et al. [34]	Healthy, normal-weight volunteers (6 males and 6 females)	*N* = 12 Age = 21–31	Cycled for 60 min at 65% of their HRmax or rested	↔ Acylated ghrelin
Santosa et al. [163]	Hyperlipidemic females BMI = 28–39	*N* = 35 Age = 35–60	6-month weight loss trial	↑ Ghrelin
Foster et al. [158]	Females BMI = 24–25	*N* = 173 Age = 50–75	12-month, moderate-intensity aerobic exercise intervention. A minimum of 45 min of moderate-intensity aerobic exercise, 5 d/wk for 12 months	↑ Ghrelin

(continued)

Table 30.11 (continued)

Reference(s)	Population	Number of participants (N) and age (yr)	Intervention	Findings
Leidy et al. [166]	Normal-weight young females BMI = 20.9 ± 1.5	*N* = 22 Age = 17–25	3-month energy deficit-imposing diet and exercise intervention, aerobic exercise, 4 times/wk at 70–80% of HRmax	↑ Ghrelin

Abbreviations: See Table 30.1 for some abbreviation definitions. *BW* body weight (kg), *HRmax* heart rate maximum, *TDEE* total daily energy expenditure, *DEF* deficit, *BAL* balance, *NR* not reported, *HIIE* high-intensity intermittent exercise, *MIE* moderate-intensity exercise, *HIE* high-intensity exercise, *END* endurance exercise, *SIE* sprint interval exercise, *1RM* one-repetition maximum, *ED* energy deficit, *FD* food deficit, ↑ increase, ↓ decrease, ↔ no change

subsequently return to the baseline in the overweight or obese subjects after stabilizing weight [162, 168]. To confirm this, one study reported that exercise training- or diet-induced weight loss is associated with ghrelin elevation even in normal-weight subjects, which is negatively correlated with body weight loss [166]. Thus, we can propose that changes in this body weight regulatory hormone serves as a compensatory mechanism to stabilizing body weight. That is, it decreases in obese subjects to suppress the appetite and eventually results in losing weight. In contrast, it increases in normal-weight subjects to increase appetite and subsequently leads to weight gain [53]. More research is needed to address this point and provides evidence to support or refute this inference.

In another study, it has been shown that diet restriction increased acylated ghrelin and decreased PYY3–36 as a compensatory mechanism, whereas exercise with the same energy deficits had no effects on these hormones [53]. Thus, these findings strengthen the hypothesis that ghrelin may be affected by some metabolites such as glucose or other hormones such as insulin [169] and leptin.

Considering the role of leptin in obesity, it has been reported that exercise training reduces leptin level in obese individuals [79]. The mechanisms behind this reduction might be related to increases in leptin sensitivity or its turnover (i.e., hormonal removal) [170], which lowers fat mass [97, 102]. In addition, changes in leptin levels following exercise training are not related to body mass index, but it is more significantly correlated with changes in body fat percent [115]. Furthermore, evidence indicates exercise intensity is a key exercise element influencing the adaptive responses of leptin to exercise training [103].

Although the underlying mechanism of appetite-suppressing effects of leptin is not clear, it has been shown that inactivating AMPK in the hypothalamus by leptin [171] leads to acetyl-COA carboxylase (ACC) inactivation [172]. In support of this finding, it has been reported that inactivation of malonyl-COA decarboxylase increased body weight [173]. Malonyl-COA is the substrate of ACC that inhibits fat oxidation via carnitine palmitoyltransferase (CPT) suppression [174, 175]. These findings show the significant role of malonyl-COA in appetite regulation. Moreover, leptin specifically increases malonyl-COA concentration through ACC activation [176] and in turn possibly impedes CPT1-a in the hypothalamus [177]. However, a hypothesis exists that CPT1-c may mediate leptin anorectic signaling pathway in the hypothalamus [177]. As mentioned, intense exercise is associated with suppressing appetite during recovery. And, there is evidence that sprint interval exercise and moderate continuous exercise equally decrease hunger feeling and increase GLP-1, but have no effect on PYY. Furthermore, suppressing of hunger feelings remains longer after the sprint interval exercise [24] which might be related to the observed increased plasma glucose levels after intensive exercise. In support of this speculation, it has been shown that increases in plasma glucose levels are associated with malonyl-COA in the hypothalamus. This effect may be due to increased glucose influx to the brain that leads to higher malonyl-COA, a lower AMP/ATP ratio,

AMPK inactivation, and finally increases in ACC activity [178].

The mechanism of leptin reduction and appetite suppression by exercise training is controversial. It has been well documented that exercise training, by decreasing malonyl-COA concentration and ACC activity, increases the capacity of lipid (fat) oxidation in skeletal muscles [179, 180]. Exercise training may have an opposite effect in the hypothalamus, because malonyl-COA has a significant role in appetite-regulating signals in the brain. Since there is no existing study to address this in humans, though, we can only speculate that exercise training by increasing fat oxidation in peripheral tissues and preserving plasma glucose increases glucose influx into the brain results in increasing malonyl-COA and untimely suppresses food intake. These assumptions need to be clarified by future research.

Table 30.12 Assessment of the strength of the evidence for an effect of physical training on each hormone (e.g., strong, limited, weak, indeterminate, or insufficient data)

	Effect of physical training	
Hormones	Aerobic training[a]	Anaerobic training[b]
Ghrelin	Indeterminate	Insufficient data
Glucagon-like peptide-1 (GLP-1)	Insufficient data	Insufficient data
Peptide YY (PYY)	Indeterminate	Insufficient data
Pancreatic polypeptide (PP)	Insufficient data	Insufficient data
Cholecystokinin (CCK)	Insufficient data	Insufficient data
Leptin	Indeterminate	Insufficient data
Oxyntomodulin (OXN)	Insufficient data	Insufficient data

[a]Aerobic training includes endurance training (low to moderate intensity)
[b]Anaerobic training includes high-intensity interval training, strength, and sprint training

Summary

In summary, studies have reported decreases in ghrelin concentrations in response to aerobic exercise, while aerobic or resistance exercise training associated with weight loss increases or has no effect on levels of ghrelin in obese individuals. GLP-1 is found to increase in response to a single bout of exercise in normal-weight individual, while in response to training, results are meager and contradictory. For example, aerobic or moderate-intensity training is found to induce slightly elevated levels of this hormone, whereas some others reported no changes in overweight or obese individuals. However, low volume sprint interval exercise increased GLP-1 in overweight men and women. For PYY, similar results exist suggesting that low- to moderate-intensity training resulted in slightly higher or no changes in this hormone, while no findings exist in regard to high-intensity training in obese or overweight individuals. Similarly, changes in PP and CKK reported following exercise training are controversial, and they only include normal or overweight individuals. Table 30.12 provides a summary of the research consensus on the effects of exercise and training on different obesity- and appetite-regulating hormones.

Most of these hormones are influenced by exercise intensity in healthy and overweight individuals; but findings on obese individuals are limited and to some extent contradictory. A number of these hormones have been studied extensively. For example, for leptin roles and effects of stimulating factors have been investigated properly, while others have been investigated to a lesser extent and not systematically. Therefore, further research is required to better understand the effects of these hormones on appetite and hunger suppression in individuals with obesity or morbidly obese individuals to confirm exercise training effectiveness. In addition, further research is needed to investigate the effects of new exercise training protocols such as high-intensity interval training (HIIT), concurrent exercise training, functional training, CrossFit® training, and different combinations of these exercise modalities – are these training modes influential and effective in bringing about changes in these hormones? The definitive answer to this current question is unknown. Finally, because of

the differences in obesity-related hormones between men and women, investigating the effect of gender on acute responses and adaptations to exercise training would be highly useful and provide valuable information for exercise prescription in different populations [181].

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