Functions of Thyroid Hormones

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Nishanth Dev, Jhuma Sankar, and M.V. Vinay

Abstract

Thyroid hormones (THs) play critical roles in growth, differentiation and metabolism. They are important for optimal functioning of almost all tissues with major effects on metabolic rate and oxygen consumption. The thyroid gland secretes two biologically active thyroid hormones: thyroxine (T4) and 3,5,3′-triiodothyronine (T3). TH synthesis and secretion is exquisitely regulated by a negative-feedback system that involves the hypothalamus, pituitary, and thyroid gland (the HPT axis). Some of the important functions of the thyroid hormones include- neural growth and differentiation, myocardial contractility, regulation of bone formation and resorption, development and function of brown and white adipose tissue, cholesterol metabolism and synthesis, and in-utero they are important for fetal growth and differentiation. Thus, given their pleotropic effects, thyroid hormones are critical for survival and optimal functioning of the human body.

Introduction

Thyroid hormones (THs) play critical roles in growth, differentiation and metabolism. They are important for optimal functioning of almost all tissues with major effects on metabolic rate and oxygen consumption. Therefore, it is not surprising that, thyroid gland disorders are among the most common endocrine disorders. Thyroid dysfunction affects several hundreds of people worldwide. Therefore it is important to understand its functions so as to clinically correlate with conditions causing deficiency or excess of the hormones.

N. Dev, MD (✉)
Department of Medicine, ESIC Medical College and Hospital, Faridabad, India
e-mail: devnishant@gmail.com

J. Sankar, MD • M.V. Vinay, MD
Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India

Thyroid Hormone Synthesis

Thyroid gland is a chief endocrine gland in the body situated in the anterior triangle of neck in front of thyroid cartilage. The thyroid gland

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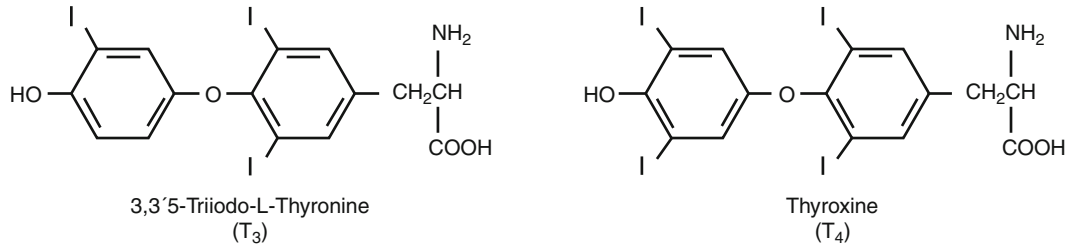

Fig. 2.1 Structure of thyroid hormones

secretes two biologically active thyroid hormones: thyroxine (T4) and 3,5,3′-triiodothyronine (T3) (Fig. 2.1). They are composed of a phenyl ring attached via an ether linkage to a tyrosine molecule. Both have two iodine atoms on their inner tyrosine ring. The difference between the two is that, T4 has two iodine atoms on its phenyl (outer) ring, whereas T3 has only one. The compound formed if an iodine atom is removed from the inner ring of T4 is 3,3′,5′-triiodothyronine (reverse T3, rT3), which has no biological activity [1].

Steps Involved in Synthesis of Thyroid Hormones

TH synthesis and secretion is exquisitely regulated by a negative-feedback system that involves the hypothalamus, pituitary, and thyroid gland [hypothalamic/pituitary/thyroid (HPT) axis] [2].

- Thyrotropin releasing hormone (TRH) is a tripeptide (PyroGlu-His-Pro) synthesized in the paraventricular nucleus of the hypothalamus. It is transported via axons to the median eminence and then to the anterior pituitary via the portal capillary plexus.
- TRH binds to TRH receptors in pituitary thyrotropes, a subpopulation of pituitary cells that secrete thyroid stimulating hormone (TSH). TRH stimulation leads to release and synthesis of new TSH in thyrotropes [3, 4].
- TSH is a 28-kDa glycoprotein composed of α- and β-subunits designated as glycoprotein hormone α- and TSH β-subunits. Both TRH and TSH secretion are negatively regulated by TH and described in detail in the section under 'central regulation of thyroid hormone function' below. An important mechanism for the negative regulation of TSH is probably the intrapituitary conversion of circulating T4 to T3 by type II deiodinase.
- TSH is the primary regulator of TH release and secretion. It also has a critical role in thyroid growth and development. TSH binds to the TSH receptor (TSHr), which also is a seven-transmembrane spanning receptor coupled to Gs. A number of thyroid genes, including Na+/I– symporter (NIS), thyroglobulin (Tg), and thyroid peroxidase (TPO), are stimulated by TSH and promote the synthesis of TH [2].
- Iodide is actively transported and concentrated into the thyroid by NIS. The trapped iodide is oxidized by TPO in the presence of hydrogen peroxide and incorporated into the tyrosine residues of a 660-kDa glycoprotein, Tg. This iodination of specific tyrosines located on Tg yields monoiodinated and diiodinated residues (MIT, monoiodo-tyrosines; DIT, diiodo-tyrosines) that are enzymatically coupled to form T4 and T3 [5].
- The iodinated Tg containing MIT, DIT, T4, and T3, then is stored as an extracellular storage polypeptide in the colloid within the lumen of thyroid follicular cells.
- The secretion of THs requires endocytosis of the stored iodinated Tg from the apical surface of the thyroid follicular cell. The internalized Tg is incorporated in phagolysosomes and undergoes proteolytic digestion, recapture of MIT and DIT, and release of T4 and T3 into the circulation via the basal surface. The majority of released TH is in the form of T4, as

total serum T4 is 40-fold higher than serum T3. Only 0.03 % of the total serum T4 is free (unbound), with the remainder bound to carrier proteins such as thyroxine binding globulin (TBG), albumin, and thyroid binding prealbumin. Approximately 0.3 % of the total serum T3 is free, with the remainder bound to TBG and albumin. It is the free TH that enters target cells and generates a biological response.

- While T4 is solely produced by thyroid gland, T3 is a product of the thyroid as well as all tissue in which it is produced by deiodination of T4. Only 20 % of circulating T3 is synthesized in the gland and rest is generated by peripheral conversion of T4 by the deiodinase present in 3 forms : type 1 deiodinase (D1), preferentially expressed in the liver and also expressed in the kidney, thyroid, and pituitary; D2, present in the CNS, anterior pituitary, brown adipose tissue, and placenta; and D3 in the CNS, placenta, skin, and fetal tissue. D1 and D2 are activating forms while D3 is the inactivating form. These are seleno enzymes and selenium deficiency is associated with decreased activity. D2 is the primary activator enzyme causing rapid increase in intracellular T3 and thereby regulate the effects in human tissues [6, 7].
- In addition to the classical thyroid hormones, thyroid gland also secretes non- classical thyroid hormones namely thyronamines, tetrac, triac, di-iodothyronine and reverse T3. The functions and actions of these molecules are still under research.

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Role of Iodine in Thyroid Hormone Synthesis and Function

Iodine is the chief elemental composition of thyroid hormones and its deficiency is a major cause of hypothyroidism in the developing world. Iodine is absorbed from the GI tract in the form of iodide which circulates in the plasma with a half-life of 24 h. About 75–80 % of the total body iodine gets concentrated in the thyroid tissue with the help of NIS (basolateral sodium iodide symporter) across a concentration gradient of 20–50 times. Iodide is excreted mainly by the kidney within 24–48 h after consumption [1, 5, 6]. There are various methods of iodine estimation namely calorimeter using spectrophotometry (most common), iodine specific electrode, neutron activation analysis and mass spectrometry.

Wolff- Chiakoff Effect

In response to increasing doses of iodine above the optimum level, the rate of thyroid hormone synthesis and release decreases due to its inhibitory action on the process of 'organification'. This acute inhibition of organification secondary to high concentration of plasma iodode levels is termed as Wolff Chiakoff effect (thyroid constipation). The underlying mechanism is probably due to inhibition of peroxidase enzyme and down regulation of NIS transporter [8]. The methods to estimate burden of iodine deficiency in field survey are :

1. Palpatory method : In areas of moderate to severe iodine deficiency, size of goiter will give an estimate of prevalence iodine deficiency.
2. Spot UI (urinary iodine) concentration: More than 90 % of iodine is eliminated by kidney and hence the median of UI in spot urine sample will give an estimate of recent intake of iodine.
3. Thyroglobulin levels: In iodine deficiency, thyroglobulin levels increase due to greater TSH stimulation and thyroid mass.

Central Regulation of Thyroid Hormone Synthesis: The 'Hypothalamic –Pituitary-Thyroid Axis'

The hypothalamic-pituitary-thyroid (HPT) axis primarily functions to maintain normal, circulating levels of thyroid hormone that is essential for

the biological function of all tissues. Important among these functions are regulation of food intake and energy expenditure among others [1, 2, 6].

Production of TRH

This regulatory system contains a group of neurons that reside in the hypothalamic paraventricular nucleus (PVN), produce TRH, and integrate a wide variety of humoral and neuronal signals to regulate the HPT axis. The TRH synthesizing neurons are present in several brain regions, but only hypophysiotropic TRH neurons located in the PVN are involved in the central regulation of the HPT axis. This nucleus is a critical vegetative center of the hypothalamus and is located symmetrically at the upper third of the third ventricle. The PVN contains a magnocellular and a parvocellular division. The magnocellular division houses oxytocin and vasopressin neurons that project to the posterior pituitary. The parvocellular division is further divided into anterior, periventricular, medial, ventral, dorsal, and lateral parvocellular subdivisions. In humans, the PVN also contains a large population of TRH neurons, especially in its medial part, but the location of hypophysiotropic TRH neurons is not yet known [9, 10].

Hypophysiotropic and Non-hypophysiotropic Neurons

Hypophysiotropic TRH neurons are functionally different from the nonhypophysiotropic TRH neurons in the PVN. Only hypophysiotropic TRH neurons project to the external zone of the median eminence, where their axon terminals release TRH into the extracellular space of this blood brain- barrier -free circumventricular organ. TRH is then conveyed to the anterior pituitary via the hypophysial portal circulation where TRH regulates the secretion of TSH from thyrotrophs and prolactin from lactotrophs. In addition to TRH, hypophysiotropic neurons also express a second neuropeptide, cocaine and amphetamine regulated transcript (CART) [2]. CART is simultaneously released into the hypophysial portal circulation and has been shown to inhibit the effect of TRH on prolactin secretion, but it has no effect on TRH induced release of TSH. Hypophysiotropic TRH neurons also express the vesicular glutamate transporter 2, establishing the glutamatergic phenotype of these cells but its physiological significance is unknown [9, 10].

In contrast to the hypophysiotropic TRH neurons, non-hypophysiotropic TRH synthesizing neurons are widely distributed in the central nervous system. However, there is little information available on the anatomy and physiologic effects of these neurons.

Role of Autonomic Nervous System in Regulation of Thyroid Function

In addition to the stimulation of TSH secretion of the anterior pituitary by TRH, the central nervous system can also regulate thyroid function via the autonomic nervous system [11, 12]. The thyroid gland is innervated by both adrenergic nerve fibers of the sympathetic nervous system and the cholinergic axons originating from the vagus nerve. Both sympathetic and parasympathetic nerves densely innervate the blood vessels of the thyroid gland, but axon terminals of these autonomic systems can also be found around the thyroid follicles, indicating that not only the blood flow, but also the activity of thyroid follicles could be under direct control of autonomic inputs. Unfortunately, relatively little data are available about how these to the thyroid gland regulate thyroid function. However, the sympathetic input seems to have an inhibitory action because electrical stimulation of the cervical sympathetic trunk decreases thyroid blood flow. Noradrenaline also inhibits the stimulatory effect of TSH on the thyroid cells in vitro and decreases thyroid hormone secretion in vivo. In contrast, electric stimulation of the thyroid nerve, which carries parasympathetic inputs to the thyroid gland, results in increased thyroid blood flow that can be prevented by atropine pretreatment. In addition to the classical transmitters, the neuropeptides, NPY and vasoactive intestinal peptide are also present in axons innervating the thyroid gland. NPY is present in the sympathetic innervation of the thyroid gland and inhibits thyroidal blood flow. In contrast, vasoactive intestinal

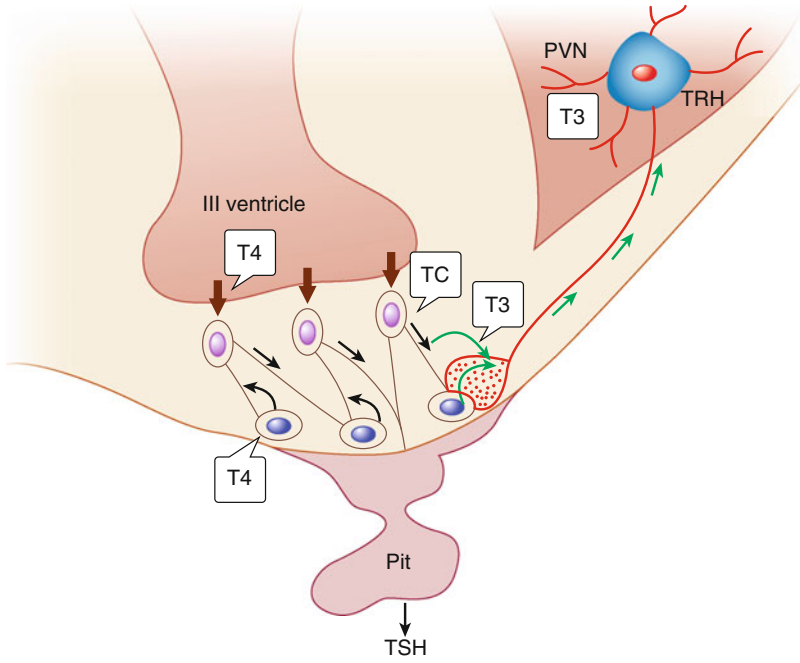

Fig. 2.2 Schematic representation of the negative feedback regulation of the Hypothalamic-pituitary-thyroid axis by thyroid hormones. *TC* Tanycyte, *TSH* thyroid stimulating hormone, *TRH* Thyrotropin releasing hormone, *PVN* paraventricular nucleus, *Pit* pituitary

peptide increases the thyroid blood flow and thyroid hormone secretion [11, 12].

Inactivation of Secreted TRH

Inactivation of secreted TRH in the brain is primarily catalyzed by a membrane bound ectoenzyme, pyroglutamyl peptidase II (PPII). PPII is a type II integral membrane protein comprised of a small, N-terminal, intracellular region and a large, extracellular domain containing the active site of the enzyme. PPII produces the dipeptide HisProNH from TRH, which is further degraded by dipeptidyl aminopeptidase IV, or spontaneously cyclizes to His-Pro diketopiperazine. PPII is primarily synthesized by neurons, but it is also produced by tanycytes, a specialized glial cell type, in the hypothalamus. Inhibition of PPII activity markedly increases the amount of TRH released from brain tissue slices, supporting the importance of thispeptidase in the metabolism of TRH. In serum, TRH is degraded by a soluble enzyme that was formerly called thyroliberinase, but was subsequently shown to be a product of the PPII gene produced in the liver by proteolytic cleavage of membrane bound PPII . Two broad specificity cytosolic peptidases, pyroglutamyl peptidase- I and prolyl endopeptidase, can also degrade TRH. However, because there is no evidence for the presence of these enzymes in the extracellular space it appears that these enzymes do not play a major role in the inactivation of released TRH [9, 11, 13].

Negative Feedback Regulation of Hypophysiotropic TRH Neurons

Negative feedback regulation of hypophysiotropic TRH neurons is an important regulatory mechanism in ensuring stable thyroid hormone levels (Fig. 2.2). When circulating thyroid hormone levels are increased, TRH gene expression is decreased in hypophysiotropic neurons and vice versa. Regulation of TRH transcription by thyroid hormone is relatively rapid because the exogenous administration of thyroid hormone can suppress transcription of the TRH gene in the PVN within 5 h [14]. This regulatory mechanism

is a unique feature of hypophysiotropic TRH neurons because thyroid hormone does not regulate TRH gene expression in nonhypophysiotropic TRH neurons. Thyroid hormone is sensed directly by the hypophysiotropic TRH neurons [14, 15].

Thyroid hormones regulate transcription of TRH gene rapidly. This is unique to hypophysiotropic TRH neurons as THs are sensed directly by these neurons.

Deiodinases and Their Role in Negative Feedback Regulation

The concept that the circulating level of T3 is solely responsible for negative feedback regulation of hypophysiotropic TRH by acting directly on these neurons has been challenged. It has been seen that restoration of circulating levels of T3 to normal levels in hypothyroid rats without the administration of T4 did not normalize TRH gene expression in the PVN. Only if very high hyperthyroid levels of T3 were achieved in the circulating blood was it possible to decrease TRH mRNA levels in the PVN into the normal, euthyroid range. These data indicate that in addition to T3, circulating T4 is also necessary for appropriate feedback control of hypophysiotropic TRH neurons. However, because T4 functions primarily as a prohormone, its conversion to T3 within the central nervous system must be an essential part of the feedback regulatory mechanism [16, 17].

Circulating T4 is as important for appropriate feedback control of TRH neurons as T3

Role of Thyroid Hormone Transporters in Secretion of Ths

Several transporters contribute to the uptake of TH into the peripheral tissue, including organic anion-transporting polypeptides (OATPs), L-type amino acid transporters, monocarboxylate transporters (MCT), and bile acid transporter. OATP1C1 has a similar high affinity for T3 and T4 and is abundantly expressed in endothelial cells of brain blood vessels, the choroid plexus, and tanycytes. The activity of the HPT axis is not affected by the lack of OATP1C1 in KO mice, however, suggesting that this transporter does not play a crucial role in feedback regulation of TRH neurons. In contrast, the MCT8 transporter, which is preferentially expressed in neurons including hypophysiotropic TRH neurons has preferential affinity for T3. In MCT8 KO mice, TRH gene expression is increased in the PVN [18].

The key points to remember about the Hypophysiotropic TRH axis are

- The Hypophysiotropic TRH neurons secrete TRH
- Under basal conditions, the activity of hypophysiotropic TRH neurons is regulated by the negative feedback effects of thyroid hormone
- This involves complex interactions between hypophysiotropic TRH neurons and the vascular system, cerebrospinal fluid, and specialized glial cells called tanycytes.
- Hypophysiotropic TRH neurons also integrate other humoral and neuronal inputs that can alter the setpoint for negative feedback regulation by thyroid hormone.
- This mechanism facilitates adaptation of the organism to changing environmental conditions, including the shortage of food and a cold environment.
- The thyroid axis is also affected by other adverse conditions such as infection, but the central mechanisms mediating suppression of hypophysiotropic TRH may be pathophysiological.

Thyroid Hormones- Mechanism of Action

The thyroid hormones mediate their actions through two mechanisms: genomic and non genomic.

Genomic Pathways

Genomic pathways acts through nuclear receptors (TRs) wherein the hormones, similar to steroid hormones, after entering the cell bind to the receptors inside the nucleus which homodimerise with the transcription factors and regulate the transcription. The mechanism in detail is as follows: TRs homodimerize or interact with other nuclear receptors such as the retinoic X receptor. They belong to a large family of ligand-dependent transcription factors such as vitamins, xenobiotics, and sex steroids. They are termed as TRa and TRb and are encoded by two genes (a and b) located on two different chromosomes that express differently in developing and adult tissues. The expression of Tra1 is highest in the brain, with lower levels in the kidney, skeletal muscle, lungs, heart, and liver, whereas the expression of Trb1 is mainly in the kidneys and liver, and lower in brain, heart, thyroid, skeletal muscle, lungs, and spleen. TRb isoforms are involved in lipid metabolism as it has been found that TRb disruption in mice impairs fatty acid (FA) oxidation even in the presence of TRa over expression. TRb agonists have approximately tenfold greater affinity for TRb than TRa, with major effect on the liver and are very efficient in lowering of cholesterol [19, 20].

TRb isoforms are involved in lipid metabolism. TRb agonists such as KB141 have therefore been seen to reduce cholesterol in primates and may have future potential as a cholesterol lowering agent in humans as well

The expression of thyroid hormones and its functions also depend on co-regulators. Co-activators that facilitate thyroid hormone functions are steroid receptor co activator, p160 family, cAMP responsive elements (CREB) and PGC a1 acting through acetylation of histones and transcription upregulators. Co-repressors like NCoR and SMRT down regulate action of thyroid hormones by deacetylation. Although T3 exerts many of its actions through canonical transcriptional regulation, an increasing amount of evidence shows that many of T3 effects are initiated outside the nucleus. These effects are mediated by what are called the non-genomic pathways [21, 22].

Non-genomic Pathways

Non genomic actions are mediated through second messenger systems like Calcium-ATPase, PI3K(phosphor-inositol 3 kinase) and AMPK(AMP activated protein kinase). However, the nongenomic processes overall are poorly understood but emerge as important accessory mechanisms in TH actions. They have been observed at the plasma membrane, in the cytoplasm, cytoskeleton, and in organelles.

For example, on the cell surface through nongenomic actions, THs trigger the serine–threonine kinase (MPK/ERK) pathway via the integrin receptor initiating complex cellular events. In the cytoplasm, THs activate PI3K and thereby downstream gene transcription of specific genes. T3 also activates PI3K from the integrin avb3 hormone receptor site. Calcium is a second messenger regulated by THs through the modulation of a Ca2C-ATPase. Here again through short-term nongenomic effects THs act on intracellular calcium by modulating plasma membrane and mitochondrial pathways in rat pituitary GH3 cells. Their cellular actions involving Akt/protein kinase B and AMP-activated protein kinase (AMPK) (in mice) are well documented. In rat skeletal muscle, T3 stimulates FA and glucose metabolism through rapid activation of AMPK and Akt/protein kinase B signal transduction [21–23].

Thyroid hormones exert their action through genomic and non-genomic pathways. In genomic pathways the THs bind to thyroid hormone receptors inside the nucleus and mediate transcription. In non-genomic pathways they mediate their actions through second messenger systems such as Calcium-ATPase, PI3K and AMPK

Table 2.1 Effect of thyroid hormones on various organs and tissues of the body

Organ/tissue	Function of thyroid hormones
1. Brain	Organization and function throughout life
	Synaptogenesis, neurogenesis, migration, plasticity and myelination
	Effect cholinergic and **seretonergic activities**
	T3 is the predominant form acting on brain
2. Myocardium	Essential for aerobic metabolism and prevention lactic acidosis
	Upregulate beta adrenergic receptors and have inotropic and vasodilatory properties
	Effect intracellular homeostasis of ionized calcium
	Medical and surgical conditions may decrease T3 and T4 and increase reverse T3. This phenomenon is called 'Euthyroid sick syndrome' (ESS)
	ESS causes stunned myocardium and may cause cardiogenic shock in extreme cases.
3. Bone	Important for bone growth and development
	Involved in both bone formation and resorption
	Hyperthyroidism causes increased porosity and decreased cortical thickness
4. Adipose tissue (fat)	Important in development and function of BAT and WAT
	In WAT THs regulate basal oxygen consumption, lipogenesis, lipolysis
	TRα-1 gene is the predominantly expressed TR isoform in Ob17 cells
	Expression of these genes is modulated by high carbohydrate diet, insulin and cAMP
5. Liver	Stimulates enzymes regulating lipogenesis and lipolysis
	Regulate expression of important proteins and enzymes involved in cholesterol metabolism
	Deficiency causes hypercholesterolemia with elevated intermediate and LDL cholesterol
	TRβ-1 is the predominant isoform in liver
	Regulate gene expression of cellular pathways such as gluconeogenesis, lipogenesis, insulin signaling, cell proliferation and apoptosis
6. Pituitary	Regulate transcription of thyrotropin, prolactin mRNA
	Regulate TSH synthesis

Thyroid Hormone Functions

The Thyroid hormones are crucial for metabolism of almost all tissues in the body and play a critical role in development of CNS of fetus and infant. Thyroid hormones regulate the metabolic processes necessary for normal growth and development. The effect of thyroid hormones on various tissues and organs are described in detail here and summarized in Table 2.1.

Role in Brain Development

Thyroid hormones are vital for brain organization and function throughout life. T3 is implicated in multiple processes like neurogenesis, synaptogenesis, migration, plasticity and myelination. *Thyroid dysfunction is associated with neurological and behavioural disorders*. The subgranular zone(SGZ) of the hippocampal dentate gyrus and the subventricular zone(SVZ) are the two main neurogenic niches which produce new neurons from neural stem cells(NSC). T3 acts on SGZ and SVZ at the step where progenitor nerve cells enter the committed step in the process of forming mature neuron/neuroblast respectively influencing the progenitor proliferation and differentition. It is also hypothesised that TH may have a role on stem cells of hypothalamus [23–27].

T3 also has effect on seretonergic and cholinergic activities in the brain, contributing to psychomotor symptoms. They also have an effect on cognition and neurodegeneration. The predominant form of thyroid hormone acting in brain is the T3 and its activity is controlled by de-iodinase 2. De-iodinase 3 is the deactivating enzyme and acts as the regulating enzyme [25–27]. A detail account of role of thyroid hormone in neural development is given in Chapter 4.

THs have important role in the critical step of maturation of neuron. T3 affects neurogenesis, synaptogenesis, migration, plasticity, myelination and also has effect on cholinergic and seretonergic activities in the brain. Thyroid dysfunction is therefore associated with neurological and behavioural disorders, psychomotor symptoms, cognitive disturbances and neurodegeneration

Effect on Myocardium

Thyroid hormones are essential for proper aerobic mitochondrial function, generation of high energy phosphates, prevention of lactic acidosis, upregulation of beta adrenergic receptors and have important role in intracellular homeostasis of ionised calcium. THs affect excitation contraction coupling, have inotropic properties and are strong vasodilators of systemic arteries including coronary arteries [28–30]. The acute changes in TH levels in the form of decreased T3 & T4 along with increased reverse T3 following acute pathogenic event(medical,surgical, traumatic) is called euthyroid sick syndrome(ESS) [31]. The ESS often depresses the myocardiun transiently and is sometimes referred to as 'stunned myocardium' [32]. The term is used in conditions where there is regional or global ischemia. The effect may vary from mild hemodynamic compromise to cardiogenic shock [33]. According to current consensus, no thyroid hormone replacement is given in patients with ESS. Recent evidences however, have shown rewarding results in 3 conditions where ESS and myocardial stunning coexist: (a) transient regional myocardial ischemia and reperfusion (b) transient global myocardial ischemia in patients undergoing cardiac surgery/bypass (c) transient inadequate global myocardial perfusion in brain dead potential organ donors. Under all these conditions, administration of T3/T4 rapid reversal of myocardial perfusion was found [28].

Effect on Bone

TH is important for normal bone growth and development. Hypothyroidism can cause short stature and delayed closure of the epiphyses. THs are involved in both bone formation and resorption [34–36]. Both osteoblast and osteoclast activities are stimulated by TH. It has been observed that there is enhanced calcification and bone formation coupled to increased bone resorption in hyperthyroid patients. There also is marked increase in porosity and decreased cortical thickness in cortical bone in hyperthyroid patients. TH may act on bone via TH stimulation of growth hormone and insulin-like growth factor I (IGF-I) or by direct effects on target genes. Recent studies have shown that T3 also can directly stimulate IGF-I production in osteoblasts and enhance T3 stimulation of [3H]proline incorporation, alkaline phosphatase, and osteocalcin [36].

Although TH increases the activities of osteoblasts and osteoclasts in vivo and in culture, little is known about its effects on the transcription of target genes in these cells. There are a number of osteoblast proteins that are stimulated by TH. These include proteins involved in matrix formation such as alkaline phosphatase, osteocalcin, and collagen. Additionally, IGF-I and IGF-binding protein-2 mRNA are stimulated by T3 in rat primary cultures. However, it is not known whether TH directly regulates transcription of these target genes. The T3stimulation of IGF and IGFBPs suggests that TH may participate in osteoblast differentiation and proliferation by regulating growth factor synthesis and action [36, 37].

Effect on Adipose Tissue

THs plays important roles in the development and function of brown and white adipose tissue (BAT and WAT) [38]. In experimental studies it has been found that T3 not only induced intracellular lipid accumulation and various adipocyte-specific markers such as malic enzyme and glycerophosphate dehydrogenase,

but also stimulated adipocyte cell proliferation and fat cell cluster formation [39].

Studies in the adult rat have shown that T3 plays important roles in regulating basal oxygen consumption, fat stores, lipogenesis, and lipolysis [40]. In WAT, T3 induces key lipogenic enzymes such as acetyl CoA carboxylase, malic enzyme, glucose-6-phosphate dehydrogenase, fatty acid synthase, and spot 14 [41]. The mechanism(s) by which T3 induces WAT differentiation currently is not known but likely involves transcriptional regulation of important target genes by TRs. Both TRα-1 and TRβ-1 are expressed in Ob17 cells, with the TRα-1 as the predominantly expressed TR isoform. The expression of these genes is also modulated by other factors such as high-carbohydrate diet, insulin, and cAMP [40]. Additionally, T3 also regulates lipolysis in a coordinate manner with lipogenesis. Thus TH stimulation of lipolysis may activate other nuclear hormone receptor systems, and thereby promote differentiation [40].

Moreover, enzymes of the lipogenic pathway, ATP-citrate lyase, malic enzyme, and fatty acid synthase, are induced by T3 in differentiating adipocytes, suggesting T3 promotes the acquisition of differentiated functions in white adipocyte tissue [40, 41].

Recently it has been shown that both TRα and TRβ-1 are differentially expressed during the development of brown adipose tissue (BAT) [42], a major contributor to facultative thermogenesis in rodents. Facultative thermogenesis occurs in response to cold exposure or overeating and depends on T3 and adrenergic stimulation of mitochondrial uncoupling protein (UCP) synthesis. It is not known whether these effects are directly mediated by T3 or via downstream signals such as free fatty acids generated by lipolysis. *The stimulation of UCP synthesis increases thermogenesis by uncoupling oxidative phosphorylation resulting in energy dissipation as heat.* Interestingly, BAT also contains a type II deiodinase whose activity increases in response to cold, thereby enabling BAT to have the important ability to regulate intracellular T3 concentration in a tissue-specific manner. This increase in T3 concentration likely saturates nuclear TRs and enhances norepinepinephrine stimulation of UCP [43, 44]. The adrenergic stimulation in BAT is predominantly, mediated by brown fat specific adrenergic β3-receptors. The dual regulation of UCP by the type II deiodinase and the adrenergic system suggests convergence of nuclear- and membrane-signaling systems in the transcriptional regulation of these important target genes in BAT, but the precise relative contributions and interplay between these regulatory systems is not yet clear [42–44].

Several human studies have shown that chronic hypo- and hyperthyroidism as well as acute T3 treatment did not affect serum leptin levels. However few have also shown that increased leptin levels correlated with adiposity and that T3 can decrease leptin levels but the mechanism is not clear [45].

Effect on Liver

TH causes stimulation of enzymes regulating lipogenesis and lipolysis as well as oxidative processes [46]. As described above (effect on fat) some of the lipogenic enzymes that are regulated are malic enzyme, glucose-6-phosphate dehydrogenase, and fatty acid synthase [46, 47]. Of these, malic enzyme has been extensively studied. Malic enzyme has been shown to be stimulated by direct action of T3 as well as secondary effect due to stimulation by other gene products that are regulated by T3. In rats it has been seen that a number of lipogenic enzymes may be regulated by growth hormone, which is induced by T3. Malic enzyme is very sensitive to T3 in the liver, but it is unresponsive in the brain, suggesting that tissue-specific factors are important in determining T3-mediated stimulation of transcription. T3 regulation of malic enzyme gene transcription also can be regulated by carbohydrate intake, insulin, and cAMP. For example, it has been seen that T3 effects on malic enzyme gene transcription are minimal in fasted animals but are most pronounced in animals fed a sucrose-containing fat-free (lipogenic) diet [46, 48]. Similar interactions between T3 and dietary carbohydrate also occur in the gene regulation of other lipogenic

enzymes. Another T3-regulated gene expressed in liver that has been studied extensively has been the one encoding S14 protein [47]. Its mRNA is rapidly induced by T3 after 20 min in hypothyroid rats and precedes the expression of lipogenic enzymes [41]. Additionally, it is coregulated by carbohydrate similar to lipogenic enzymes. Its tissue distribution is similar to those of lipogenic enzymes as it is expressed in liver, white and brown fat, and lactating mammary tissue [41].

It is well known that hypothyroidism is associated with hypercholesterolemia with elevated serum intermediate and low-density lipoprotein (LDL) cholesterol concentrations [49]. The major mechanism for these effects may be lower cholesterol clearance resulting from decreased LDL receptors. Also, the genotype of the LDL receptor gene may influence the elevation of serum LDL cholesterol concentrations in hypothyroid patients and their response to thyroxine treatment. Apart from this, it has been seen that hepatic lipase activity is decreased in hypothyroidism which decreases conversion of intermediate-density lipoproteins to LDL and high-density lipoprotein metabolism. It is not known whether these effects are mediated directly or indirectly by THs [50]. THs also have been shown to regulate the expression of several important proteins and enzymes involved in cholesterol metabolism and synthesis such as the LDL receptor, cholesterol ester hydrolase, and cholesterol acyltransferase. TRβ-1 is the predominant isoform expressed in liver, whereas TRα-1 is the major isoform expressed in heart. These differences in TR isoform expression have made it difficult to develop isoform- specific TH analogs that may have cholesterol-lowering effects but minimal cardiac toxicity [51, 52].

There is ongoing research activity in identifying various hepatic target genes. TH has been shown to regulate gene expression of a diverse range of cellular pathways and functions such as gluconeogenesis, lipogenesis, insulin signaling, adenylate cyclase signaling, cell proliferation, and apoptosis [53]. Thus gene therapy may hold promise in future in all these metabolic pathways related to thyroid.

Effect on Pituitary

THs have been shown to stimulate the transcription of GH mRNA and GH synthesis in rats. THs also can negatively regulate thyrotropin (TSH) transcription by direct and indirect mechanisms. T3 can also downregulate prolactin mRNA. TH hormone also can negatively regulate TSH by decreasing transcription of the glycoprotein hormone α-subunit (common to TSH, luteinizing hormone, follicle-stimulating hormone, and human chorionogonadotropic hormone) and the TSHβ subunit genes [54]. Recent cotransfection and knockout studies suggest that TRβ-2 isoform may be playing the predominant role in regulating TSH. In situ hybridization and immunostaining studies have shown that TRβ-2 is highly expressed in thyrotropes in the pituitary [55]. Additionally, RXRγ isoform appears to be selectively expressed in thyrotropes, suggesting that it also may play a functional role in the regulation of TSH via isoform-specific TR/RXR complexes or RXRγ homodimers [56].

Effect on Fetal Growth and Maturation

The role of thyroid hormones on fetal growth depends on various factors like

1. placental transfer of thyroid hormones
2. the maturation of hypothalamo-pituitary thyroid axis of the developing fetus
3. the peripheral conversion of T4 to more active T3 and
4. the maturation of intracellular thyroid receptors.

It has been seen that plasma T4 concentrations are correlated positively to the body weight of the fetus and newborn. The availability of these hormones in utero regulates fetal growth by acting as a signal of the nutrient and oxygen supply to the fetus. Fetal thyroid hormones are required for both accretion of fetal mass and differentiation of specific cell types. However the placental transfer of thyroid hormones are

sufficient enough and compensate for the fetal thyroid deficiency. This is attributed to their role in regulating the somatotropic axis and local tissue expression of the IGFs (insulin like growth factors) which have major role in fetal tissue accretion [57–60].

Effect on Growth

The effect of low thyroid hormones on fetal growth is evidenced by studies on thyroidectomised fetal animals. It is shown that the protein content of fetal tissues such as the heart, lung, and skeletal muscle is reduced by fetal thyroidectomy. The growth restriction is asymmetrical in the sense that greater effects are seen on the weight of soft tissues than on the length of bones. The appendicular skeleton is more adversely affected than the axial skeleton. The effect on bone metabolism is affected by reducing the osteocalcin, a marker of bone deposition rather than altering the calcium homeostasis in the body.

Effect on Fetal Metabolism (Oxygen (O_2) Consumption)

THs increase O_2 consumption by fetal tissues upto 28 % and increase umbilical blood flow. This is by inducing oxidative mechanisms by changing expression and activity of the electrogenic Na–K ATPase pump or by acting on the mitochondrial electron transport chain (ETC) and oxidative phosphorylation. These hormones are necessary for normal developmental increments in hepatic glycogen and gluconeogenic enzymes. They have an important role in maturational effects of thermogenic capacity of brown adipose tissue as has been described earlier in the text (*see adipose tissue*).

Effect on Fetal Tissue Maturation

Thyroid hormones also facilitate maturation and differentiation of fetal tissues evidenced by activation of physiological processes essential for survival immediately at birth such as pulmonary gas exchange, adaptations in cardiac function, hepatic glucogenesis and thermogenesis [60, 61].

Effect on Lung Maturation

THs tend to increase the expression of pulmonary b-adrenergic receptors and apical Na channels in the fetus thereby facilitationg lung fluid absorption at birth. It is shown that they also facilitate production of surfactant by synergistic action along with cortisol [62].

Effect on Fetal Heart and Cardiovascular System

THs promote a switch from proliferation to hypertrophy and differentiation of the cardiac myocytes by playing an important role in the perinatal switch from b- to a-myosin heavy chains in the sacromeres [63].

Other Important Effects

Effect on mitochondria*:* The mitochondria are important target for THs. They modulate mitochondrial activity through two ways: direct or indirect. In the direct pathway, the hormone enters the cell and binds to binding sites of organelles. These organelles are responsible for regulation of mitochondrial transcription apparatus. One of these binding sites, termed p43, has been identified as a bona fide TR (nuclear receptor) that binds to the D-loop region that contains the promoters of the mitochondrial genome. Thus, THs play important role in regulation of the mitochondrial transcription apparatus. In the indirect pathway, the THs act through increased, nuclear TR-dependent transcription of factors that modulate the expression of mitochondrial genes [64].

Conclusion

Thyroid hormones (THs) play critical roles in growth, differentiation and metabolism. They are important for optimal functioning of almost all tissues with major effects on metabolic rate and oxygen consumption. The thyroid gland secretes two biologically active thyroid hormones: thyroxine (T4) and 3,5,3′-triiodothyronine (T3). TH synthesis and secretion is exquisitely regulated by a

negative-feedback system that involves the hypothalamus, pituitary, and thyroid gland (the HPT axis). Iodine is the chief elemental composition of thyroid hormones and its deficiency is a major cause of hypothyroidism in the developing world. Thyroid hormones exert their action through genomic and non-genomic pathways. Thyroid hormones have important functions in regulating neuronal differentiation, maturation, migration, in cholinergic pathways in the brain. In the heart and peripheral vessels they are essential for aerobic mitochondrial function, prevention of lactic acidosis, have inotropic and vasodilatory properties. THs are involved in both bone formation and resorption. They have important role in the development and function of brown and white adipose tissue and T3 plays important role in regulating lipogenesis and lipolysis. They have important role in cholesterol metabolism and synthesis and their deficiency is known to be associated with hypercholesterolemia. THs regulate transcription of thyrotropin, prolactin mRNA and TSH synthesis in the pituitary. In the developing fetus, THs are required for both accretion of fetal mass and differentiation of specific cell types. They have important roles in maturation and differentiation of vital organs such as lungs and heart. Thus, given their pleotropic effects, thyroid hormone functions are critical for survival and optimal functioning of the human body.

References

1. Kopp P. Thyroid hormone synthesis. In: Braverman LE, Utiger RD, editors. The thyroid: fundamental and clinical text. 9th ed. Philadelphia: Lippincott Williams and Wilkins; 2005. p. 52.
2. Fekete C, Lechan RM. Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions. Endocr Rev. 2014;35:159–94.
3. Persani L. Hypothalamic thyrotropin releasing hormone and thyrotropin biological activity. Thyroid. 1998;8:941–6.
4. Harris AR, Christianson D, Smith MS, et al. The physiological role of thyrotropin releasing hormone in the regulation of thyroid stimulating hormone and prolactin secretion in the rat. J Clin Invest. 1978;61:441–8.
5. Spitzweg C, Heufelder AE, Morris JC. Thyroid iodine transport. Thyroid. 2000;10:321–30. Review.
6. Taurog A. Hormone synthesis. In: Braverman L, Utiger R, editors. Werner and Ingbar's the thyroid. Philadelphia: Lippincott-Raven; 1996. p. 47–81.
7. Kohrle J. The selenoenzyme family of deiodinase isozymes controls local thyroid hormone availability. Rev Endocr Metab Disord. 2000;1:49–58.
8. Wolff J, Chaikoff IL. Plasma inorganic iodide as a homeostatic regulator of thyroid function. J Biol Chem. 1948;174:555–64.
9. Lechan RM, Fekete C. The TRH neuron: a hypothalamic integrator of energy metabolism. Prog Brain Res. 2006;153:209–35.
10. Kádár A, Sánchez E, Wittmann G, et al. Distribution of hypophysiotropic thyrotropin releasing hormone (TRH) synthesizing neurons in the hypothalamic paraventricular nucleus of the mouse. J Comp Neurol. 2010;518:3948–61.
11. Amenta F, Caporuscio D, Ferrante F, et al. Cholinergic nerves in the thyroid gland. Cell Tissue Res. 1978;195:367–70.
12. Melander A, Sundler F, Westgren U. Sympathetic innervation of the thyroid: variation with species and with age. Endocrinology. 1975;96:102–6.
13. Schmitmeier S, Thole H, Bader A, et al. Purification and characterization of the thyrotropin releasing hormone (TRH) degrading serum enzyme and its identification as a product of liver origin. Eur J Biochem. 2002;269:1278–86.
14. Sugrue ML, Vella KR, Morales C, Lopez ME, Hollenberg AN. The thyrotropinreleasing hormone gene is regulated by thyroid hormone at the level of transcription in vivo. Endocrinology. 2010;151:793–801.
15. Segerson TP, Kauer J, Wolfe HC, et al. Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. Science. 1987;238:78–80.
16. Kakucska I, Rand W, Lechan RM. Thyrotropin releasing hormone gene expression in the hypothalamic paraventricular nucleus is dependent upon feedback regulation by both triiodothyronine and thyroxine. Endocrinology. 1992;130:2845–50.
17. Fekete C, Mihály E, Herscovici S, et al. DARPP32 and CREB are present in type 2 iodothyronine deiodinase producing tanycytes: implications for the regulation of type 2 deiodinase activity. Brain Res. 2000;862:154–61.
18. Jansen J, Friesema EC, Milici C, Visser TJ. Thyroid hormone transporters in health and disease. Thyroid. 2005;15:757–68.
19. Pascual A, Aranda A. Thyroid hormone receptors, cell growth and differentiation. Biochim Biophys Acta. 2013;1830:3908–16.

20. Tata JR. The road to nuclear receptors of thyroid hormone. Biochim Biophys Acta. 1830;2013:3860–6.
21. DelViscovo A, Secondo A, Esposito A, et al. Intracellular and plasma membrane-initiated pathways involved in the [Ca2C]i elevations induced by iodothyronines (T3 and T2) in pituitary GH3 cells. American Journal of Physiology. Endocrinol Metab. 2011;302:E1419–30.
22. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. Endocr Rev. 2010;31: 139–70.
23. Senese R, Cioffi F, de Lange P. Thyroid: biological actions of 'nonclassical' thyroid hormones. J Endocrinol. 2014;221:R1–12.
24. Remaud S, Gothié JD, Morvan-Dubois G, et al. Thyroid hormone signaling and adult neurogenesis in mammals. Front Endocrinol (Lausanne). 2014;5:62.
25. Oppenheimer JH, Schwartz HL. Molecular basis of thyroid hormone-dependent brain development. Endocr Rev. 1997;18:462–75.
26. Pop VJ, Kuijpens JL, van Baar AL, et al. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clin Endocrinol (Ofx). 1999;50:149–55.
27. Chan S, Kilby MD. Thyroid hormone and central nervous system development. J Endocrinol. 2000;165: 1–8.
28. Novitzky D, Cooper DK. Thyroid hormone and the stunned myocardium. J Endocrinol. 2014;223:R1–8.
29. Ririe DG, Butterworth JF, Royster RL, et al. Triiodothyronine increases contractility independent of b-adrenergic receptors or stimulation of cyclic-30,50-adenosine monophosphate. Anesthesiology. 1995;82:1004–12.
30. Klein I. Clinical, metabolic, and organ-specific indices of thyroid function. Endocrinol Metab Clin North Am. 2001;30:415–27.
31. Warner MH, Beckett GJ. Mechanisms behind the non-thyroidal illness syndrome: an update. J Endocrinol. 2010;205:1–13.
32. Luo Y, Cha DG, Liu YL, Zhou SF. Coronary microcirculation changes during myocardial stunning in dogs. Cardiology. 2010;117:68–74.
33. Heusch G. The regional myocardial flow-function relationship: a framework for an understanding of acute ischemia, hibernation, stunning and coronary microembolization. Circ Res. 1980;112:1535–7.
34. Allain TJ, McGregor AM. Thyroid hormones and bone. J Endocrinol. 1993;139:9–18.
35. Mosekilde L, Eriksen EF, Charles P. Effects of thyroid hormones on bone and mineral metabolism. Endocrinol Metab Clin North Am. 1990;19:35–63.
36. Huang BK, Golden LA, Tarjan G, et al. Insulin-like growth factor I production is essential for anabolic effects of thyroid hormone in osteoblasts. J Bone Miner Res. 2000;15:188–97.
37. Glautchnig H, Varga F, Klaushofer K. Thyroid hormone and retinoic acid induce the synthesis of insulin-like growth factor-binding protein 4 in mouse osteoblastic cells. Endocrinology. 1996;137:281–6.
38. Ailhaud G, Grimaldi P, Negrel R. Cellular and molecular aspects of adipose tissue development. Annu Rev Nutr. 1992;12:207–33.
39. Flores-Delgado G, Marsch-Moreno M, Kuri-Harcuch W. Thyroid hormone stimulates adipocyte differentiation of 3 T3 cells. Mol Cell Biochem. 1987;76: 35–43.
40. Oppenheimer JH, Schwartz HL, Lane JT, et al. Functional relationship of thyroid hormone-induced lipogenesis, lipolysis, and thermogenesis in the rat. J Clin Invest. 1991;87:125–32.
41. Kinlaw WB, Church JL, Harmon J, et al. Direct evidence for a role of the "spot 14" protein in the regulation of lipid synthesis. J Biol Chem. 1995;270: 16615–8.
42. Tuca A, Giralt M, Villarroya F, et al. Ontogeny of thyroid hormone receptors and c-erbA expression during brown adipose tissue development: evidence of fetal acquisition of the mature thyroid status. Endocrinology. 1993;132:1913–20.
43. Silva JE, Larsen PR. Adrenergic activation of triiodothyronine production in brown adipose tissue. Nature. 1983;305:712–3.
44. Lowell BB, Susulic VS, Hamann A, et al. Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. Nature. 1993;366:740–2.
45. Leonhardt U, Gerdes E, Ritzel U, et al. Immunoreactive leptin and leptin mRNA expression are increased in rat hypo- but not hyperthyroidism. J Endocrinol. 1999;163:115–21.
46. Oppenheimer JH, Schwartz HL, Mariash CN, et al. Advances in our understanding of thyroid hormone action at the cellular level. Endocr Rev. 1987;8: 288–308.
47. Oppenheimer JH, Schwartz HL, Strait KA. An integrated view of thyroid hormone actions in vivo. In: Weintraub B, editor. Molecular endocrinology: basic concepts and clinical correlations. New York: Raven; 1995. p. 249–68.
48. Petty KJ, Desvergne B, Mitsuhashi T, et al. Identification of a thyroid hormone response element in the malic enzyme gene. J Biol Chem. 1990;265: 7395–400.
49. Brent GA. The molecular basis of thyroid hormone action. N Engl J Med. 1994;331:847–53.
50. Tan KC, Shiu SW, Kung AW. Effect of thyroid dysfunction on high-density lipoprotein subfraction metabolism: roles of hepatic lipase and cholesteryl ester transfer protein. J Clin Endocrinol Metab. 1998;83:2921–4.
51. Underwood AH, Emmett JC, Ellis D, et al. A thyromimetic that decreases plasma cholesterol levels without increasing cardiac activity. Nature. 1986;324:425–9.
52. Falcone M, Miyamoto T, Fierro-Renoy F, et al. Antipeptide polyclonal antibodies specifically recognize each human thyroid hormone receptor isoform. Endocrinology. 1992;131:2419–29.

53. Feng X, Jiang Y, Meltzer P, Yen PM. Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray. Mol Endocrinol. 2000;14:947–55.
54. Samuels HH, Forman BM, Horowitz ZD, et al. Regulation of gene expression by thyroid hormone. J Clin Invest. 1988;81:957–67.
55. Wood DF, Docherty K, Ramsden DB, et al. Thyroid status affects the regulation of prolactin mRNA accumulation by tri-iodothyronine and thyrotrophin-releasing hormone in cultured rat anterior pituitary cells. J Endocrinol. 1987;115:497–503.
56. Sugawara A, Yen PM, Qi YP, et al. Isoform-specific retinoid X receptor (RXR) antibodies detect differential expression of RXR proteins in the pituitary gland. Endocrinology. 1995;136:1766–74.
57. Chung HR. Adrenal and thyroid function in the fetus and preterm infant. Korean J Pediatr. 2014;57:425–33.
58. Ng PC. The fetal and neonatal hypothalamic-pituitary-adrenal axis. Arch Dis Child Fetal Neonatal Ed. 2000;82:F250–4.
59. Kester MH, Martinez de Mena R. Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. J Clin Endocrinol Metab. 2004;89:3117–28.
60. Thorpe-Beeston JG, Nicolaides KH, et al. Maturation of the secretion of thyroid hormone and thyroid-stimulating hormone in the fetus. N Engl J Med. 1991;324:532–6.
61. Feingold SB, Brown RS. Neonatal thyroid function. NeoReviews. 2010;11:e640–6.
62. Biswas S, Buffery J, Enoch H, et al. Pulmonary effects of triiodothyronine (T3) and hydrocortisone (HC) supplementation in preterm infants less than 30 weeks gestation: results of the THORN trial: thyroid hormone replacement in neonates. Pediatr Res. 2003;53:48–56.
63. Seri I, Tan R, Evans J. Cardiovascular effects of hydrocortisone in preterm infants with pressor-resistant hypotension. Pediatrics. 2001;107:1070–4.
64. Yen PM. Physiological and molecular basis of thyroid hormone action. Physiol Rev. 2001;81:1097–142.