



Thyroid hormone and leptin in the testis

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Leptin is primarily expressed in white adipose tissue; however, it is expressed in the hypothalamus and reproductive tissues as well. Leptin acts by activating the leptin receptors (Ob-Rs). Additionally, the regulation of several neuroendocrine and reproductive functions, including the inhibition of glucocorticoids and enhancement of thyroxine and sex hormone concentrations in human beings and mice are leptin functions. It has been suggested that thyroid hormones (TH) could directly regulate leptin expression. Additionally, hypothyroidism compromises the intracellular integration of leptin signaling specifically in the arcuate nucleus. Two TH receptor isoforms are expressed in the testis, TRa and TRb, with TRa being the predominant one that is present in all stages of development. The effects of TH involve the proliferation and differentiation of Sertoli and Leydig cells during development, spermatogenesis, and steroidogenesis. In this context, TH disorders are associated with sexual dysfunction. An endocrine and/or direct paracrine effect of leptin on the gonads inhibits testosterone production in Leydig cells. Further studies are necessary to clarify the effects of both hormones in the testis during hypothyroidism. The goal of this review is to highlight the current knowledge regarding leptin and TH in the testis.

Keywords: leptin, thyroid hormones, male reproductive tissue, testis, adipose tissue

INTRODUCTION

The role of thyroid hormones (TH) and leptin in testicular physiology is not fully understood. Receptors for both hormones are present in the testis (1, 2), and some reproductive functions have been described as being a result of TH or leptin actions in the testis, although the interaction between these two hormones should be further investigated.

Leptin plays a key role in body weight homeostasis and has recently emerged as a relevant neuroendocrine mediator in different cell types, including testicular cells (3). This hormone appears to act by inhibiting testicular steroidogenesis, leading to reduced levels of testosterone and modulation of gene expression (Figure 1).

The involvement of TH in the modulation of male reproductive system development and function has been neglected for several years, because of the demonstration that the adult male gonad was metabolically unresponsive to these hormones in the 1950s (4). In addition, although hyper- and hypothyroidism have no apparent clinical relevance regarding signs and symptoms related to male gonadal function, compared with the systemic effects induced by these diseases, it has been demonstrated that thyroid dysfunction might affect biochemical, morphological, and physiological aspects of testicular development (4–8). In recent decades, several studies have reported important roles for TH in modulating testicular development (2, 9–11).

The TH effects involve the proliferation and differentiation of Sertoli and Leydig cells during development, spermatogenesis, and steroidogenesis, and its disorders are correlated with sexual dysfunction. An endocrine and/or direct paracrine effect of leptin on the gonads inhibits testosterone production in Leydig cells.

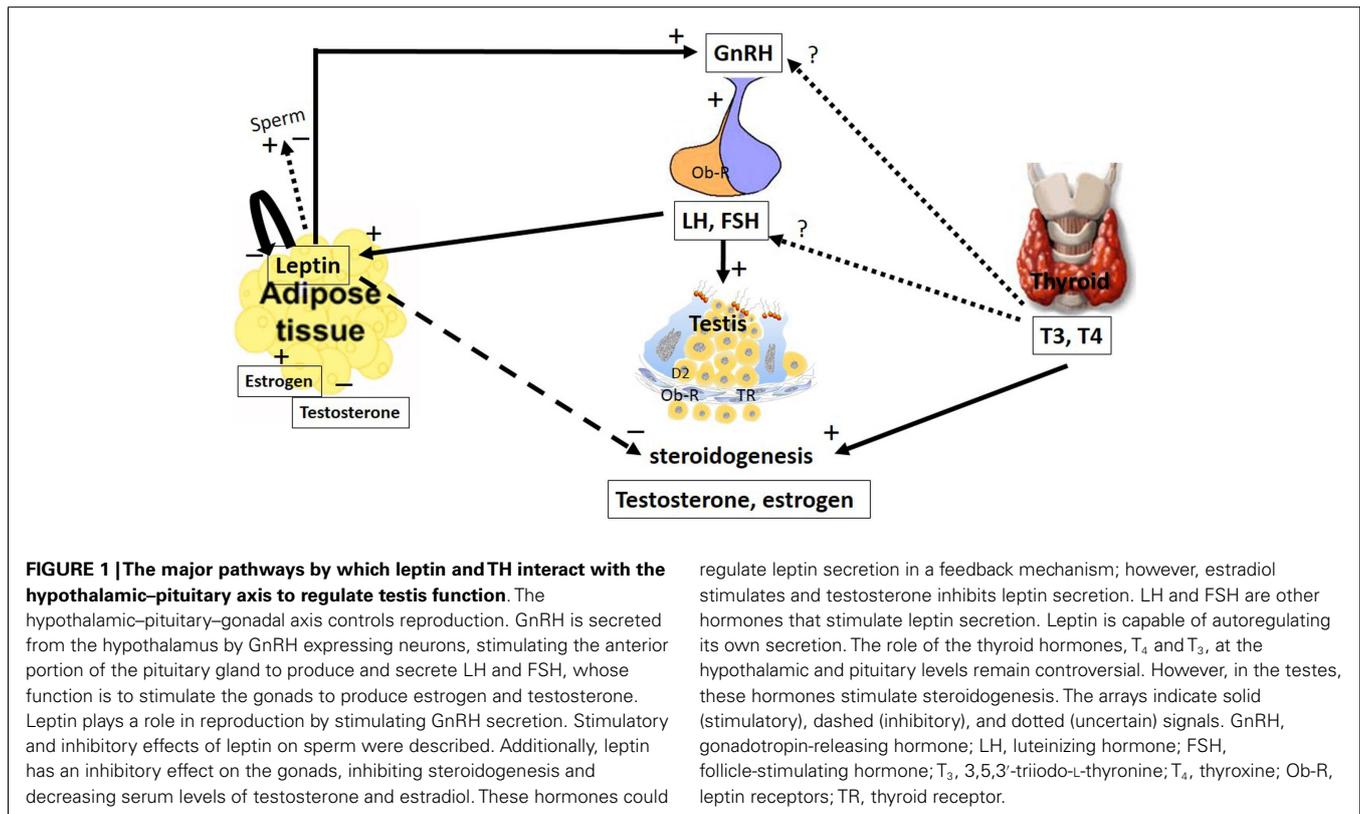
Although further studies are necessary to clarify the effects of these hormones in the testis during hypothyroidism, the goal of this review is to highlight the current knowledge regarding leptin and TH in the testis.

LEPTIN

SYNTHESIS AND SECRETION

Adipose tissue is recognized as an endocrine organ that secretes steroid hormones, including glucocorticoids, growth factors, enzymes, and pro- and anti-inflammatory adipocytokines (12, 13). Leptin is the prototype adipokine that was identified as the product of the *ob* gene by its action in reducing appetite, increasing energy expenditure through action in the brain and then decreasing body weight and fat mass (14). Although anthropometric and clinical features (e.g., gender, fat mass/fat distribution, hormones, and cytokines) might influence the secretion pattern of leptin, the crucial factors that regulate serum leptin levels appear to be caloric intake and the amount of energy stored in adipocytes (15).

Leptin acts by activating leptin receptors (Ob-Rs). Several Ob-R isoforms, resulting from alternative splicing, convey differing biological activities and are involved in mediating the actions of leptin in the brain and peripheral organs. The long isoform (Ob-Rb) is expressed abundantly in the hypothalamic arcuate nucleus (ARH), ventromedial (VMN), and dorsomedial nuclei (DMN), and is the predominant signaling form of the receptor (16). Ob-Rb has the longest cytoplasmic domain, which contains the Janus Kinase (JAK) binding domain, box 1 and 2, and the consensus sequence for the signal transducers and activators of transcription (STAT) binding. Other forms have no (Ob-Re) or short (Ob-Ra, Ob-Rc–Ob-Rf) cytoplasmic domains, which contain only box 1



(17). These short isoforms of the leptin receptor are distributed in almost all the peripheral tissues, including ovary (18, 19), prostate (20–22), and testis (23) tissues, suggesting the direct effects of leptin on these organs. In addition to JAK/STAT, other pathways are involved in leptin signaling, such as mitogen-activated protein kinase (MAPK), including extracellular factor-regulated kinases 1 and 2 [ERK1 and ERK (2)] and 5'-AMP-activated protein kinase (AMPK). Additionally, leptin presents cross talk with insulin-induced pathways by stimulating insulin receptor substrates (IRS) and then initiating phosphoinositide 3-kinase activity (PI3K) (17, 24, 25). Leptin has been reported to induce suppressors of cytokine-signaling 3 (SOCS) expression, which are capable of inhibiting the JAK/STAT pathway via a feedback mechanism (26). During prolonged receptor stimulation by leptin, the inhibition of JAK phosphorylation is mediated by SOCS3, and leptin can act as a negative regulator of its own signaling.

Leptin secretion could be regulated by different mechanisms (Figure 2). In adipocyte tissue, leptin secretion could be stimulated by insulin, glucocorticoids, and cytokines (i.e., tumor necrosis factor α), whereas leptin release is inhibited by catecholamines, free fatty acids, cold exposure, and TH (27, 28). Estrogens induce leptin production, whereas androgens suppress it (Figure 1), thus explaining the sexual dimorphism in serum leptin levels (29, 30). A negative correlation between leptin and testosterone levels has been described in men and boys (31, 32). It has been demonstrated that leptin could control its own synthesis in adipose tissues by a negative feedback mechanism between the hormone and its receptor (Figure 1) (33). This autoregulatory mechanism was also shown in the prostate (34), however, there is no report of this mechanism in relation to the testis.

LEPTIN AND THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS

Leptin inhibits appetite and weight gain by its action in the hypothalamus by activating proopiomelanocortin (POMC)/cocaine- and amphetamine-related peptide (CART) while inhibiting the neuropeptide Y (NPY)/agouti-related protein (AgRP) neurons (35–38). In addition to this central action, leptin has been implicated in other roles, including reproductive ones where leptin stimulates gonadotropin-releasing hormone (GnRH) secretion (Figure 1). However, if leptin role is direct or not is still controversial. Some studies suggest a direct role of leptin based on the fact that the neurons secreting GnRH express Ob-R (39, 40). Watanobe (41) showed that leptin acts directly at the median eminence (ME), the anatomical structure where the axonal fibers of GnRH neurons are terminated before the neurohormone is released into the portal circulation (42); however, little or no co-expression of the leptin receptor is demonstrated at the medial preoptic area (MPOA) of the hypothalamus, which is the site where the majority of the GnRH neuronal cell bodies exist (42). This result, among others (43–45), suggests that leptin might influence GnRH secretion indirectly through interneurons and other pathways.

In the rat testis, leptin, at doses ranging from 2 to 500 ng/mL, exerted dose-dependent inhibitory effects on human chorionic gonadotropin (hCG)-stimulated testosterone production (46). Therefore, in addition to having a stimulatory effect at the hypothalamic-pituitary level, leptin appears to have an inhibitory effect at the gonadal level.

OTHER SITES THAT MEDIATE LEPTIN ACTION ON REPRODUCTION

Gonadotropin-releasing hormone is also regulated by adiponectin, which is an adipocytokine produced by adipose tissue and

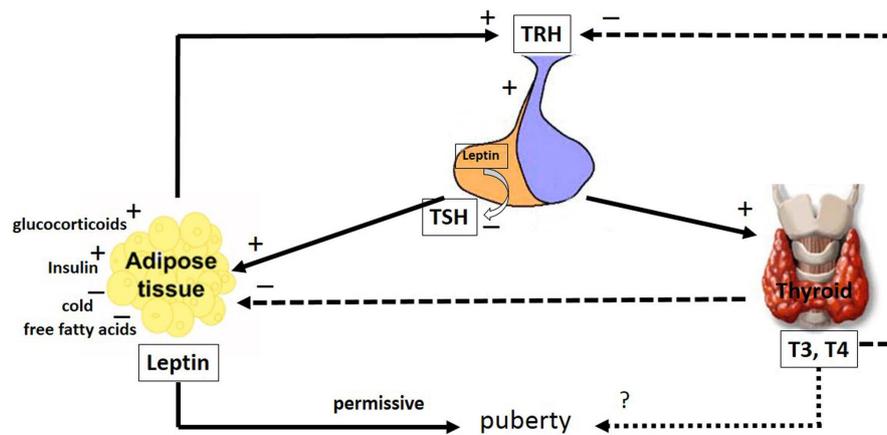


FIGURE 2 | The major pathways by which leptin interacts with the hypothalamic-pituitary-thyroid axis. The hypophysiotropic TRH neurons are metabolic integrators that are regulated by leptin and the thyroid hormones. TRH is secreted from the PVN of the hypothalamus and stimulates the anterior portion of the pituitary gland to produce and secrete TSH, whose function is to stimulate the thyroid gland to produce the thyroid hormones, T_4 and T_3 . These hormones regulate the activity of TRH neurons in the hypothalamus by a classical negative feedback pathway. Leptin stimulates TRH secretion in the hypothalamus. Leptin

produced in the pituitary is capable of inhibiting TSH secretion. Leptin secretion from adipose tissues is regulated by stimulatory factors such as TSH, glucocorticoids, T_4 and T_3 and insulin as well as inhibitory factors such as, cold and free fatty acids. Leptin and the thyroid hormones, T_4 and T_3 , have a role in puberty; however, whereas the leptin role is permissive, the T_4 and T_3 role remains uncertain. The arrays indicate: solid (stimulatory), dashed (inhibitory), dotted (uncertain) signals. TRH, thyrotrophin release hormone; TSH, pituitary thyrotropin; T_3 , 3,5,3'-triiodo-L-thyronine; T_4 , thyroxine. See text for details.

regulates metabolic function, as does leptin; the amount of GnRH in the circulation is inversely proportional to body fat (47). It has been demonstrated that adiponectin might provide a link between obesity and abnormal reproductive functioning by decreasing luteinizing hormone (LH) secretion and inhibiting GnRH receptor (GnRHR) mRNA expression in the pituitary (48, 49). At the level of the hypothalamus, adiponectin reduces GnRH expression and secretion (50), whereas GnRH decreases adiponectin expression in the pituitary (51). Additionally, adiponectin appears to mediate basal and hCG-stimulated testosterone secretion by the testis (52, 53) in a rat model.

Ghrelin is another orexigenic factor that acts with NPY and AgRP to influence feeding and reproduction. Whereas leptin stimulates GnRH secretion, ghrelin exerts a negative effect on this hormone secretion and on GnRH-induced gonadotropin secretion; however, it appears that at basal conditions, ghrelin presents stimulatory actions on LH and follicle-stimulating hormone (FSH) secretion (54). Relative to NPY, both stimulatory and inhibitory effects on GnRH have been observed (55–57). AgRP is expressed in leptin responsive neurons of the ARH and the ablation of such neurons in leptin or Ob-R deficient mice restores fertility (58–60).

Among the anorexigenic factors that affect the reproductive system, the α -melanocyte-stimulating hormone (α -MSH) from POMC neurons and CART from interneurons of the ARH mediate the leptin influence on GnRH secretion (61, 62). Additionally, nitric oxide (NO) released from adrenergic interneurons is capable of inducing GnRH release from GnRH neurons (23).

Another site that mediates leptin action on reproduction is the kisspeptin neurons in the ARH. Although a small amount of kisspeptin might be sufficient to trigger puberty (63), deletion of Ob-R from kisspeptin neurons (64) or ablation of more than 95% of kisspeptin neurons prior to puberty resulted in normal puberty

and fertility (65). It appears that leptin signaling in these neurons arise only after completion of sexual maturation (66). Kisspeptin neurons connect to NPY and POMC neurons (67) are another link to the integration of leptin effects on nutrition and reproduction. Kisspeptin neurons have insulin receptors that could be interesting for their effect on reproduction. Deletion of the insulin receptor from kisspeptin neurons showed a puberty delay; however, fertility was normal (68), suggesting that insulin is the potential mediator of reproduction from kisspeptin neurons.

EXPRESSION OF LEPTIN AND Ob-R IN TESTIS

In 1997, the first demonstration of Ob-R expression in the murine testis was published. The authors showed the messenger RNA (mRNA) for the common extracellular domain of Ob-R in spermatogenic and Leydig cells by *in situ* hybridization (1). Later, several studies showed that it is ubiquitous among species. Ob-R specific immunostaining was observed in the testicular interstitium of rats at an embryonic age of 19.5 days and not at an embryonic age of 14.5 days, whereas in postnatal life, it was evident only after sexual maturation (35, 60, 90 days old) and was confined to Leydig cells. No immunoreaction was observed in the seminiferous tubules. Ob-R mRNA expression was present at all ages. Ob-Ra and Ob-Rb mRNA presented a similar expression pattern, increasing from younger to older ages. The authors demonstrated that the Ob-R mRNA levels were higher in late versus early embryonic testes, as well as in adult versus immature rat testes (69). Other studies have shown a developmental Ob-R mRNA expression from 15- to 75-day old rats in constant relative levels; however, if only Ob-Rb is considered, its expression was higher near puberty, at 30 days, declining after that period (70).

Leptin expression has been detected by immunohistochemistry techniques in mouse germ cells that are cell-type and stage specific

and it was undetected in mouse Leydig cells. The authors suggest that leptin produced in testicular stem cells during the developmental stage might act on these cells in an autocrine manner to mediate their renewal (71). The developmental expression profiles of Ob-Ra, Ob-Rb, and Ob-Re show constant mRNA expression in 5-, 10-, 20-, 30-, and 60-day-old mice testis. However, in purified mouse Leydig cells, in which mRNA for leptin and Ob-Rb are not present in 14- and 60-day old mice, Ob-Ra and Ob-Re are expressed at both ages (71). Recently, mRNA for different Ob-R isoforms was detected in the adult rat testis (21).

Leptin caused STAT 3 phosphorylation after *in vitro* incubation of isolated seminiferous tubules, demonstrating that Ob-R is functional and an activator of signal transduction mechanisms in germ cells (72). Corroborating this result, recently it was shown that leptin activates STAT3 signaling in the testis and that SOCS3 expression is regulated by leptin in this tissue (73).

LEPTIN ACTIONS

Considering that impaired reproductive function in the genetically obese (*ob/ob*) mouse was previously demonstrated (74, 75), leptin might be one of the hormones involved in the pathogenesis of infertility. Corroborating this hypothesis, the exogenous intraperitoneal administration of leptin in sterile *ob/ob* mice eliminated the sterility defect (76).

In the testis, it has been demonstrated that leptin might pass the blood–testis barrier by a passive, non-saturable process (77, 78). In this tissue, leptin acts as an inhibitory signal for testicular steroidogenesis (Figure 1), which might partially explain the link between decreased testosterone secretion and hyperleptinemia in obese men (79, 80). Decreased gene expression of some upstream factors in the steroidogenic pathway, such as steroidogenic factor-1 (SF-1), steroidogenic acute regulatory protein (StAR), and cytochrome P450, has been demonstrated to be involved in the molecular mechanism responsible for leptin-induced inhibition of testosterone secretion (81). Moreover, the inhibitory effects of leptin on steroidogenesis have been observed in the adrenal gland and ovaries, which are steroidogenic tissues as well (82–84).

Besides testosterone synthesis, leptin can also regulate estrogen synthesis by regulating aromatase enzyme in other tissues of the reproductive system such as prostate and mammary gland (20, 34, 85, 86). We were unable to find studies showing the effects of leptin upon aromatase in the testis.

Some other effects of leptin that can affect fertility have been already demonstrated. Leptin can alter the weight and volume of the testicles, the diameter of the seminiferous tubules, and the numbers of spermatogonia, spermatocytes, sperm, and Leydig cells (73). Also the fact that leptin is present in the human seminal plasma (87–89) and Ob-R is present at the tail region of ejaculated spermatozoa (89) as well as the relationship between leptin and sexual hormones suggests that leptin could directly or indirectly regulate sperm function.

OBESITY AND TESTIS

The effects of obesity on testis and male reproduction have been demonstrated in recent years (90). The decrease in testosterone levels as a consequence of the high leptin levels present in obese patients (91) might cause sexual dysfunction (92). However, the

mechanisms involved in the association between obesity and a decrease in male fertility are complex and not completely understood on the basis of the current literature.

A high-fat diet leads to increased leptin levels, decreased plasma testosterone level, a decline in sperm motility, Leydig cell damage, and oxidative stress, as well as a decreased testis and epididymis relative coefficient in mice. The reproductive hormone imbalance observed in obesity might affect the antioxidant status in testes. The authors suggest that the excessive oxidative stress induced by obesity affects the normal histological structures and function of testicular tissue (93). In addition, the association of testicular oxidative stress and alterations in male reproduction and fertility has been demonstrated in other conditions such as thyroid dysfunctions (94–97).

Although consensus on the effects of obesity on fertility exists, particularly in relation to sperm count, concentration and motility, there is not a complete overall agreement (98). The lack of consensus is possibly because the majority of studies involve men presenting to fertility clinics, which could clearly bias toward subfertile men, and many might have other lifestyle related comorbidities affecting spermatogenesis. Different methodologies applied in various studies could contribute to the variability of the results. Some of the fertility alterations could occur because of the effects of leptin on other tissues of the reproductive systems that express Ob-R as well, such as the epididymis (99), prostate (20–22), and seminal vesicles (22).

Testosterone is a very important hormone for maintaining reproductive function and fertility. All the reproductive tissues and spermatogenesis are testosterone-dependent. Although androgen could regulate leptin synthesis and secretion in adipose tissues (29, 30) and leptin could regulate the expression of androgen receptors in others tissues, such as the prostate (20, 34), we were unable to find studies showing any interaction between leptin and testosterone or androgen receptors in the testis.

These findings clearly demonstrate that leptin functionally regulates the male gonadal axis by acting at different levels of the hypothalamic–pituitary–testicular system.

THYROID HORMONE

THYROID HORMONE REGULATION

As major regulators of serum TH levels, the hypophysiotropic thyrotrophin release hormone (TRH) neurons of the paraventricular nuclei (PVN) play important roles in the control of energy homeostasis (100). These neurons regulate TH production through the release of TRH at the ME, which stimulates pituitary thyrotropin (TSH) release (Figure 2). TSH then stimulates the thyroid gland to produce TH. These hormones regulate the activity of TRH neurons in the hypothalamus by a classical negative feedback pathway (Figure 2) (101–103). TRH neurons receive neuronal projections from POMC and NPY containing hypothalamic neurons (104–108), brainstem catecholaminergic neurons (103, 109, 110), and neurochemically uncharacterized neurons in the DMN (111).

Thyroid hormones play critical roles in normal pre- and post-natal growth and development. These hormones are essential for the regulation of metabolism in nearly all mammalian tissues, including the testis (112, 113).

DEIODINATION

Proper intracellular TH concentrations are required for most hormonal actions and for cellular metabolism. Thyroxine (T_4) is the major TH secreted from the thyroid gland; however, in target tissues other hormones could be produced. In peripheral tissues, there are important seleno enzymes known as deiodinases (D1, D2, and D3) that metabolize TH, regulating local TH availability (114). D1 and D2 catalyze the removal of the iodine atom in the outer ring position, generating 3,5,3'-triiodo-L-thyronine (T_3). D3 catalyzes the removal of iodine from the inner ring position, generating an inactive hormone, as reverse T_3 (3,3',5'-triiodothyronine or rT_3).

These three deiodinases, D1, D2, and D3, are expressed in the testis at different levels, from weanling to adult life (115). D3 activity predominates in the developmental period and then declines in adult life. Although D1 and D2 are present in the testis, their relative levels of activity indicate that D2 is the predominant activating enzyme in the testis (115, 116).

Thyroid hormone transport across the plasma membrane is a crucial step in TH signaling. Several TH transporter families have been identified; however, only specific TH cell-membrane transporter (MCT) 8 and 10 and organic anion-transporting polypeptide 1c1 (Oatp1c1) have been shown to be specific TH transporters (117). Inside the cell, T_3 binds to nuclear THs, which are ligand-regulated transcription factors that bind THs and DNA enhancer sequences in the promoter region of target genes and interact with co-repressor and co-activator complexes (118, 119).

SIGNALING

Thyroid hormone nuclear effects are mediated by TRa and TRb, which are members of the steroid/thyroid hormone receptor superfamily (120). The activation of these receptors modulates gene transcription and signal transduction to initiate intra-nuclear changes in cell metabolism (121, 122). Because the genomic actions of TH on gene regulation require many steps, TH-induced changes in gene expression are generally long lasting (113, 120).

The TR are encoded by two different genes, TRa and TRb. Alternative splicing leads to the production of several peptide isoforms, five of which have been described: TRa1, TRa2, TRa3, TRb1, and TRb2. TRa2 and TRa3 lack a hormone-binding domain and are hypothesized to function as TH inhibitors by competing for binding at the thyroid response elements (TREs), resulting in the suppression of transcription (123–128). Within the seminiferous epithelium, the expression of TRs is developmentally regulated, and Sertoli cells express the TRa1 and TRa2 isoforms (85).

The active TRa1 isoform is expressed in human and rat testicular Sertoli cells, with maximal expression in late fetal and early neonatal life. TRb1 is found in interstitial and germ cells during neonatal development as well as in the adult testis, although at a much lower level (129–132). The fetal and prepubertal ages are periods of maximal expression of TRa1 and TRa2 in Sertoli cells, demonstrating a critical window for TH action in the testis, in rats (2) and in human beings (133). The immature stage of sexual development coincides with the maximal TRa1 activity in response to T_3 (2). The TRa2 isoform does not bind to T_3 , and it has been suggested that TRa2 most likely exerts a dominant negative effect on the actions of other TR isoforms (134). Considering the developmental regulation of TR expression in the Sertoli cells, it was

shown that the ratio of TRa2/TRa1 increases progressively from the fetal period to adulthood. This increase reinforces the participation of TRa1 during the prepubertal period and, consequently, its involvement in testis differentiation and development (133). It could be proposed that TH play pivotal roles in testicular differentiation with maximal effects during the prepubertal period.

Although the classical mechanism of action for TH involves the modulation of gene transcription (genomic action), it has been frequently proposed that the membrane-initiated effects (non-genomic actions) of these hormones might be mediated by plasma membrane receptors. The effects triggered by cell-surface receptors are typically independent of protein synthesis (112). Although T_3 , T_4 , and rT_3 might trigger non-genomic effects, T_4 and rT_3 are more potent than T_3 in testicular cells (135–138), reinforcing a role for these hormones as physiological signals controlling reproductive functions through cell surface-initiated mechanisms.

THYROID HORMONE FUNCTION IN TESTIS

T_3 regulates the maturation and growth of the testis, controlling Sertoli and Leydig cell proliferation and differentiation during testicular development in rats and other mammalian species (5, 139). Possible mechanisms underlying the effects of TH on Sertoli cell proliferation have been recently proposed. The authors demonstrated that TH limits postnatal Sertoli cell proliferation by activation of the TRa1 present in these cells. They provided evidence that the regulation of the Cdk4/JunD/c-myc pathway might be involved in this negative control in mice (140). The testis size and sperm production are directly associated with the total Sertoli cell number during adulthood (141). The hormonal factors controlling the duration of Sertoli cell proliferation are critical determinants of male fertility (4). Although an adequate number of these cells is crucial for future male fertility, the factors controlling Sertoli cell survival, proliferation, and maturation require further investigation.

The effects of TH on Sertoli cells occurs through different and perhaps interconnected mechanisms (142) including the following: the expression of cell cycle regulators p27 and p21 (143); the inhibition of aromatase at the basal conditions and after FSH stimulus (144, 145) as well as the aromatase gene transcription (146); the increase in the levels of androgen-binding proteins (ABP) (129), which increases the availability of this steroid hormone (147, 148); the increase in the androgen receptor (AR) expression (129); the increase in the LH receptor (LHR) protein expression, although the mRNA is reduced (149); and the increase in the levels of the gap junction protein connexin 43 in the testis (150). For revision, see Tarulli et al. (142).

The Sertoli cells provide physical support to germ cells and are essential for the creation of an adequate and protected microenvironment for germ cell development. These cells are responsible for the secretion of a Cl^- and K^+ -rich fluid into the seminiferous tubule lumen (151, 152). The Ca^{2+} -dependent Cl^- secretion by Sertoli cells into the extracellular fluid is under the control of extracellular nucleotide levels (153); extracellular ATP has been shown to induce intracellular Ca^{2+} uptake in Sertoli cells (154). Our group previously demonstrated that T_3 and T_4 could inhibit NTPDase activity, with a consequent decrease in extracellular ATP

hydrolysis; this reduction in hydrolysis leads to increased levels of ATP in the extracellular medium (10). It has been demonstrated that TH induces Ca^{2+} -dependent signaling pathways in testicular cells (11, 112, 136, 155). It is possible to suggest that TH action might increase the extracellular ATP levels, leading to a Ca^{2+} influx and consequent chloride-rich fluid secretion by the Sertoli cells.

The expression of specific TRs in prepubertal Sertoli cells implies the existence of an early and critical influence of TH on testicular development and function (2). Although hypothyroidism leads to a marked delay in sexual maturation and development (5, 9, 96), hyperthyroidism accelerates the appearance of the seminiferous tubule lumen (97), which marks the maturation of the Sertoli cells (156). Delayed testicular cell maturation provoked by neonatal hypothyroidism (96) is associated with reduced serum levels of the gonadotropins, FSH and LH (6).

Modifications in Sertoli cell maturation could be associated with alterations in androgen metabolism. The androgen/estrogen balance is crucial for testis development and male reproduction. The maximal expression and activity of aromatase is observed in Sertoli cells during the prepubertal period (157, 158), the period coincident with the maximal response of these cells to TH (4). Alterations in aromatase activity and expression, as well as in estrogen receptor levels, were demonstrated to be under TH control (145, 159, 160). These estrogen-mediated events might affect androgen metabolism.

Thyroidectomy in adult rats led to decreased secretion of testosterone and 17 β -hydroxy steroid dehydrogenase (HSD) activity (161). Leydig cells cultured from adult rats show an increase in testosterone and estrogen secretion after treatment with T_3 , under basal conditions and in response to LH (148). When Leydig cells are chronically stimulated with T_3 , there is an increase in the mRNA levels of the cytochrome P450 side-chain cleavage enzyme and a decrease in the cytochrome P450 17 α -hydroxylase and 3 β -HSD (162). An increase in the StAR mRNA and protein is observed after treatment of Leydig cells with T_3 , which contributes to steroid production (162, 163) (Figure 2).

Despite the important roles that THs play in the reproduction by acting in the testis, there is no consensus about how TH controls GnRH and gonadotropin synthesis and secretion. No pattern in the circulating gonadotropin hormones has been reported in hypothyroid conditions. These hormones could be unaltered, reduced, increased or LH reduced with unaltered FSH; and FSH could be increased with normal LH (147). In a recent study (149), the authors showed that rats submitted to hypothyroidism showed a reduction of the pituitary content of LH, although they presented a high level of LH in serum. They attributed this divergent result to impaired LH renal clearance, because hypothyroidism could alter clearance (164) of adrenal drug excretion (165). There was a marked decrease of total testosterone serum concentration and a reduction in the amount of testicular LHR, although the LH mRNA receptor expression was shown to be increased. Exposure of testicular cells to a high concentration of LH reduces LHR mRNA and protein (166), which could explain the results observed by Romano et al. (149). Another possibility would be the reduction of the testosterone negative feedback regulating LH and FSH.

INTERACTION BETWEEN LEPTIN AND THYROID HORMONES

The hypophysiotropic TRH neurons are metabolic integrators, fixing the set point of the hypothalamic–pituitary–thyroid axis. Several studies have demonstrated that leptin modulates thyroid function, acting in the hypothalamus, pituitary and thyroid. Additionally, leptin modulates the activity of deiodinase enzymes (167–171). This hormone stimulates the hypothalamic production of TRH directly at the PVN (106, 172–174) and indirectly via the ARH, which act as energy sensors. At the ARH, leptin upregulates the activity of the α -MSH neurons and down-regulates the activity of the NPY/AgRP neurons. The α -MSH neurons and NPY/AgRP neurons have stimulatory and inhibitory projections, respectively, to the TRH neurons (100, 106, 174–178).

Systemic administration of leptin increased the serum TSH concentration in rats, potentially because of the leptin action at the hypothalamus (Figure 2). Because the direct pituitary effect of leptin on TSH release was inhibitory, this TSH release might result from an autocrine–paracrine effect exerted by locally produced leptin (Figure 2) (170, 171). TSH significantly stimulated the leptin secretion by human adipose tissue *in vitro*, suggesting a new mechanism in the inter-relationship between adipose tissue and the thyroid axis (Figure 2) (179). It has been demonstrated that food deprivation, associated with low leptin levels, leads to decreased TSH synthesis in the pituitary and TRH in the hypothalamus (171, 180). The mechanisms underlying the secretion of tropic hormones from pituitary cells might involve the modulation of NO levels. Coiro and colleagues suggested that TRH stimulates TSH release via NO in humans (181). Corroborating these findings, Radwanska and Kosior-Korzecka (182) recently demonstrated that leptin-stimulated TSH secretion is dependent on NO release from the anterior pituitary cells of ewe lambs. In this context, the effect of leptin on the thyroid axis might modulate the effects of THs on the male reproductive system.

It has been proposed that leptin might be a trigger for the onset of puberty in children, because leptin levels increase in both girls and boys prior to pubertal gonadal activation (183). In girls, however, leptin concentrations continue to rise, likely due to stimulatory effects of estrogen, while leptin concentrations decrease in boys, due to the inhibitory effects of testosterone (29, 30, 183, 184). However, data of Mann and colleagues (185) do not support the concept that a transient rise in leptin triggers the onset of puberty. They demonstrated that circulating leptin levels decline throughout puberty, and there is no transient rise of leptin prior or in association with the onset of puberty in male monkeys. Leptin, however, is currently thought to have a more permissive role in pubertal maturation, as the administration of exogenous leptin alone could not trigger early puberty in patients with congenital leptin deficiency (186). Although leptin probably is not the primary factor for puberty initiation, lower levels of this hormone could be associated with a delay in the onset of puberty (187). It has been shown that some developmental changes in the T_4 levels parallel those of leptin. Whether the peripubertal changes observed in T_4 levels suggest thyroid involvement in initiating pubertal events remains to be elucidated.

The relationship between TH and leptin serum levels is not completely understood; however, it has been demonstrated that leptin levels might be increased in the hyperthyroid state in

humans (188). In addition, Asami and coworkers (189) have demonstrated a relationship between the serum levels of leptin and TH in children. A direct effect of leptin stimulating hepatic TH activation by modulation of deiodinase activity and expression was shown in chicken embryo hepatocytes (190). The authors demonstrated that the expression and activity of D1 were increased, whereas those of D3 were decreased, in leptin-treated cells. A D1-increased gene expression and activity in the adipose tissues of obese humans was documented, suggesting a role for T₃, formed from T₄ by D1 activation in response to leptin, in the modulation of adipose tissue metabolism (191). These studies support that TH and leptin might act in conjunction in the modulation of several cell functions.

Corroborating the relationship between TH and leptin, it has been shown that hypothyroidism reduces the expression of members of the Ob-Rb–STAT3 signaling pathway in the basomedial hypothalamus and pituitary of rats; in addition, hypothyroid rats are resistant to the acute anorectic action of leptin (192). Additionally, hypothyroid mice exhibited decreased circulating leptin levels because of a decrease in the fat mass and reduced leptin expression in white adipose tissue. In neurons of the ARH, hypothyroid mice showed increased leptin receptor Ob-R expression and decreased suppression of the cytokine-signaling 3 transcript levels (193).

The main pathways by which leptin and TH interact with the hypothalamic–pituitary axis to regulate testis function are summarized in **Figures 1** and **2**. This review is a first demonstration proposing explanations for the possible relationship between TH and leptin activity in the testis. Further studies are needed to clarify how these two important hormones might act together in modulating male reproduction.

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