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INVITED REVIEW

Circulating leptin and thyroid dysfunction

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Abstract

The identification and sequencing of the ob gene and its product, leptin, in 1994 opened new insights in the study of the mechanisms controlling body weight and led to a surge of research activity. Since its discovery, leptin has been the subject of an enormous amount of work especially within the fields of nutrition, metabolism and endocrinology. Leptin is accepted as an adipose signal, and even though the underlying mechanisms are not fully clarified, leptin, in addition to the thyroid hormones, is believed to be involved in regulation during the switch from the fed to the starved state. It is not clear whether leptin and the melanocortin pathways interact with the thyroid axis under physiological conditions other than during starvation or in response to severe illness, both states in which the hypothalamo–pituitary–thyroid axis may be severely suppressed. In addition to the suggested central relationship between leptin and thyroid hormones, there might also be a peripheral relationship although this effect is not clear. Both thyroid hormones and leptin might be involved in the adaptive thermogenesis through mitochondrial uncoupling proteins and heat production because both thyroxine and triiodothyronine are involved in the starvation-induced decrease in thermogenesis. Both rodent and human studies of leptin have failed to show any consistent relationship between thyroid function and serum leptin concentrations. However, leptin might have an important role in thyroid pathophysiology due to thyroid hormone involvement in thermogenesis and regulation of uncoupling proteins. In this review, we have focused on leptin in relation to thyroid pathophysiology.

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Introduction

In 1994, Friedman and coworkers succeeded in cloning the ob gene and demonstrated that the gene product was a 16-kDa protein subsequently named leptin (derived from the Greek term 'leptos' meaning thin), synthesized by fat cells and secreted into the blood stream (1). The human leptin gene is localized on chromosome 7q31, and contains 15 000 base pairs and 3 exons as the major coding sites driving protein synthesis (2). It was also demonstrated that correction of leptin deficiency in the ob/ob mouse caused a marked reduction in food intake and a normalization of the obesity syndrome, in agreement with the hypothesis of a circulating anorexic factor (3–5). Subsequent studies showed that the db mutation resided in the gene encoding the leptin receptor. Thus, both leptin deficiency (in ob/ob mice) and leptin resistance (in db/db mice) lead to severe obesity, strongly implying leptin as a negative feedback signal critical to the normal control of food intake, energy balance and body weight (6–8).

The function of the thyroid gland is to produce the thyroid hormones triiodothyronine (T3) and thyroxine (T4), which regulate gene transcription throughout the body (9). Unlike insulin or cortisol levels, which fluctuate widely in response to food ingestion and stress, thyroid hormones are typically maintained at a constant level that keeps the metabolic machinery running at a proper metabolic rate (9). Thyroid hormones are crucial for survival both in rodents and humans (9); yet, thyroid hormone levels are subject to major physiological regulation during the transition from the fed to the starved state, since starvation rapidly suppresses T4 and T3 levels with a subsequent reduction in the obligatory use of energy stores. Disturbance of thyroid function is associated with marked changes in both body weight and energy expenditure and it therefore seemed likely that leptin and thyroid hormones played a mutual role. The aim of this review has been to summarize the current state of knowledge of circulating leptin in relation to thyroid dysfunction.

Leptin structure, receptors and transport

Leptin is a 167 amino acid, 16-kDa, 4 α-helical protein with striking similarities to the cytokine family and

growth hormone (10). Leptin receptors (OB-R) were identified and cloned from the rat choroid plexus cDNA. They are related to class I cytokine receptor proteins like gp130, and are known to occur in at least 5 variably spliced forms, designated OB-R_{a-e} (8, 11). The receptors have an extracellular binding domain of 840 amino acids, a transmembrane domain of 34 amino acids, and a variable intracellular domain characteristic for each of the OB-R_{a-d} receptors whereas the OB-R_e lacks both the hydrophobic transmembrane and intracellular domain (8). In rodents but not in humans, leptin receptors have been identified on thyroid follicular cells (7, 12), and both in rodents and humans leptin receptors are widely expressed in the brain and peripherally in a variety of tissues (7, 12, 13). The function, if any, of leptin in many of these tissues has yet to be determined, but recently it was suggested that systematically administered leptin in rats stimulated growth and secretion of the thyroid gland through a direct mechanism involving OB-R (14).

Short-form leptin receptors (OB-$R_{a,c,d}$) in the choroid plexus epithelium may mediate the transport of circulating leptin into the cerebrospinal fluid (CSF) and, similarly, leptin receptors in the brain capillary endothelium may mediate direct transport of leptin from blood to the brain interstitium, possibly by a saturable receptor-mediated transcytosis (7, 15, 16). The existence of this saturable transport system led to the notion that there is a threshold level of serum leptin above which increases in serum leptin are not translated into proportional increases in CSF or brain leptin, a fact that may result in an apparent leptin resistance (15). Also, the balance between free and bound leptin might have an impact, since in humans the majority of leptin circulates in serum bound to macromolecules, which may modulate ligand bioactivity and bioavailability to target tissues (17). In lean individuals with relatively low adipose tissue depots, the majority of leptin exists in a bound form, whereas the proportion of free leptin is increased in serum of obese subjects (17, 18). During overnight fasting, free leptin levels decreased more in lean subjects than in obese persons, whereas bound leptin was unchanged in both groups (18). It thus seems that the ratio of free to total leptin may not be constant, but rather a dynamic equilibrium between the circulating binding proteins and free leptin, and this balance may be affected by the metabolic/nutritional state as has been demonstrated for other members of the cytokine family and haematopoietic growth factors (8). The hypothesis that free leptin selectively reflects body fat mass whereas bound leptin is closely associated with energy expenditure has been tested in several studies (19–22). In one of these studies of lean and obese healthy subjects, there was a positive correlation between both free and bound leptin and the degree of obesity but with a steeper slope for the free hormone. Serum concentrations of both leptin moieties were statistically significantly lowered by a decrease in body fat during a weight reduction program. When thyrotoxic patients increased their body fat mass by successful antithyroid drug therapy only serum free leptin concentrations reflected this increment, supporting the hypothesis that the free leptin fraction selectively represents body fat mass. In contrast, bound leptin decreased indicating a regulatory role distinct from free leptin and not related to body fat mass (19). These findings might contribute to the understanding of leptin actions related to thyroid pathophysiology.

Total plasma leptin levels showed a significant pulsatility characterized by highest leptin levels between midnight and the early-morning hours and lowest levels around noon to mid-afternoon in both adults and children (Table 1) (8, 13). The circadian pattern of leptin is similar to that of prolactin, thyrotrophin (TSH), free fatty acids and melatonin (8, 13), inversely related to that of adrenocorticotrophin and cortisol (23), delayed in relation to that of insulin (6), and synchronous to the variation of circulating luteinizing hormone and oestradiol in normal women (24). At present, the physiological significance of pulsatile leptin secretion is unknown, as is the mechanism involved in generating leptin pulses, but there is agreement that it is necessary to use appropriate sampling procedures to account for this pulsatile release in clinical studies (7, 8).

Leptin, thyroid hormones and central regulation

The thyroid system is regulated at multiple levels, one or more of which might account for nutritional adaptation (9). Thyrotrophin-releasing hormone (TRH), a neuropeptide produced in the paraventricular nucleus (PVN) of the hypothalamus, controls the release of TSH from the pituitary. TSH acts on receptors in the thyroid to promote synthesis and release of T4 and T3. In primary hypothyroidism, TRH expression increases in the PVN of the hypothalamus, and TSH production increases (9). In a state of starvation, TRH expression in the PVN is suppressed, although TRH continues to be expressed in the remainder of the central nervous system (CNS) where it does not play a role in the regulation of pituitary TSH production. Thus, TSH production falls, and the pattern of glycosylation is altered (9).

Initially, leptin was viewed as a hormone designed to prevent obesity, but several studies now suggest that leptin also signals the switch from the fed to the starved state (reviewed in 6, 9) indicating an important interplay between thyroid hormones and leptin. Thus, the dominant, and perhaps sufficient, signal to the brain that suppresses TRH expression in the PVN is a drop in the level of leptin. Falling leptin levels decrease the activity of arcuate neurons expressing proopiomela-

Table 1 Characteristics of the clinical studies of circulating leptin levels in thyroid dysfunction.

Reference	Tox F/M	Type of Tox	Myx F/M	Controls F/M	Antithyroid treatment	Follow-up (months)	Body composition	Leptin assay	Pulsatility	Gender	Other factors
Zimmermann-Belsing *et al.* (51)	8/2	GD	0	12/6	MMI	12	DEXA	Linco	Fast	F/M	No
Corbetta *et al.* (63)	27/13	GD/nodular	38/36	561/393	ND	6	BMI	RIA	Fast	F(↑)	Yes
Mantzoros *et al.* (64)	0/22	Iatrogenic	0	0	T4*	0	ND	Linco	Fast	M	No
Sreenan *et al.* (65)	6/0	GD	3/3	8/3	ND	0	BMI	Other	?	F/M	No
Valcavi *et al.* (66)	12/4	Unknown	12/4	12/4	MMI	6.5	BMI	Linco	Fast	F/M	Yes
Wolthers *et al.* (67)	0/8	Iatrogenic	0	0	T3*	0.5	BIA	Linco	Fast	M	Yes
Sesmilo *et al.* (68)	12/4	GD	12/4	0	MMI	3	BIA	Linco	Fast	F/M	No
Yoshida *et al.* (69)	19/0	GD	17/0	23	ND	0	BMI	Linco	Fast	F	No
Ozata *et al.* (70)	20/0	Mixed	20/0	20/0	PTU	1	BMI	Other	Fast	F	No
Leonhardt *et al.* (71)	14/5	Mixed	19/4	13/8	ND	0	BMI	In house	?	F(↑)	No
Pinkney *et al.* (72)	17/1	GD/nodular	20/2	46/23	ND	0	Waist–hip, BIA	RIA in house	Fast	F(↑)	Yes
Diekman *et al.* (73)	21/0	Mixed	14/0	0	MMI, PTU	3	BMI	Linco	?	F	Yes
Kristensen *et al.* (74)	12/0	Iatrogenic	0	0	T4*	0.5	BMI	Linco	Fast	F	Yes
Miyakawa *et al.* (75)	28/12	GD	11/0	53/49	MMI, PTU	6	BIA	Linco	13–15 p.m.	F(↑)	No
Kautzky-Willer *et al.* (76)	16/3	GD	21/4	40/4	MMI	5	BMI	Linco	Fast	F/M	Yes
Song *et al.* (77)	39/11	GD/nodular	18/6	52/8	ND	0	BMI/BIA	Linco	10–12 a.m.	F(↑)	No
Asami *et al.* (78)	0	—	32/19	0	ND	0	BMI	Linco	?	?	No
Chen *et al.* (79)	20/0	Unknown	20/0	20/0	ND	0	BIA	Linco	Fast	F	Yes
Simo *et al.* (80)	0	—	24/6	0	ND	1	BMI	ELISA	Fast	F(↑)	Yes
Sera *et al.* (81)	15/0	GD	0	0	PTU, MMI	3	Leptin/%BF	ELISA	Fast	F	Yes
Song *et al.* (82)	11/5	GD/TAO	0	0	PTU, MMI	0.5	BIA	Linco	Fast	?	Yes
Pinkney *et al.* (83)	17/1	Unknown	20/2	46/23	ND	0	BIA	RIA in house	?	F/M	Yes
Nakamura *et al.* (84)	32/0	Unknown	0	30/0	MMI	6	BIA	Linco	Fast	F	No
Matsubara *et al.* (85)	95/0	Unknown	76/0	197/60	MMI, PTU	9	BIA	Linco	?	F(↑)	No
Ozata *et al.* (86)	19/9	GD/TAO	0	13/7	ND	0	BMI	RIA	Fast	F(↑)	Yes
Seven (87)	41/9	GD/nodular	0	12/3	PTU	1	BMI	Linco	Fast	F/M	No
Obermayer-Pietsch *et al.* (88)	23/5	Nodular	0	22/2	I^{131}	12	DEXA, BMI	Linco	Fast	F/M	Yes
Hsieh *et al.* (89)	0	Iatrogenic	26/7	30/8	T4*	6	BMI/BIA	Linco	Fast	F(↑)	No

Tox = thyrotoxicosis; Myx = myxoedema; *indicates iatrogenic hyperthyroidism with either levothyroxine or triiodothyronine; GD = Graves' disease; ND = not done; PTU = propylthiouracil; MMI = methimazole; BMI = body mass index; BIA = bioimpedance; DEXA = dual energy absorption; BF = body fat; Fast = measurements after an overnight fast/12-h fast; F(↑) = serum leptin increased in females compared with males; F or M = only female or males included in the study; F/M = both females and males included in calculations; Other factors include factors, which might be important for leptin measurements such as smokers, noradrenaline, propranolol, obesity, diabetes, insulin, glucose, prednisone, sex-steroids, cortisol, IGF-1, growth-hormone or TNFalpha.

nocortin (POMC)/α-melanocyte-stimulating hormone (α-MSH) and increases neuropeptide Y (NPY)/agouti-related-protein (AgRP). Neuronal projections of NPY/AgRP and POMC neurons to proTRH neurons in the PVN have been delineated (Fig. 1). Manipulation with both NPY/AgRP and α-MSH directly modulates TRH synthesis and processing suggesting a direct impact of leptin-induced alterations on TRH (25, 26). It is unknown whether falling leptin levels are sensed directly by the leptin receptor (the OB-R_b isoform) found in TRH neurons, or indirectly (27). A role of the melanocortin pathway in mediating the nutritional response of TRH neurons to leptin has been suggested (27, 28) since the central melanocortin system can regulate the thyroid axis and is well positioned to mediate the actions of leptin on the thyroid axis (Fig. 1), but it is not known whether the leptin and melanocortin pathways regulate the thyroid axis under normal physiological conditions and not only during starvation or in response to severe illness, another state in which the thyroid axis may be severely suppressed.

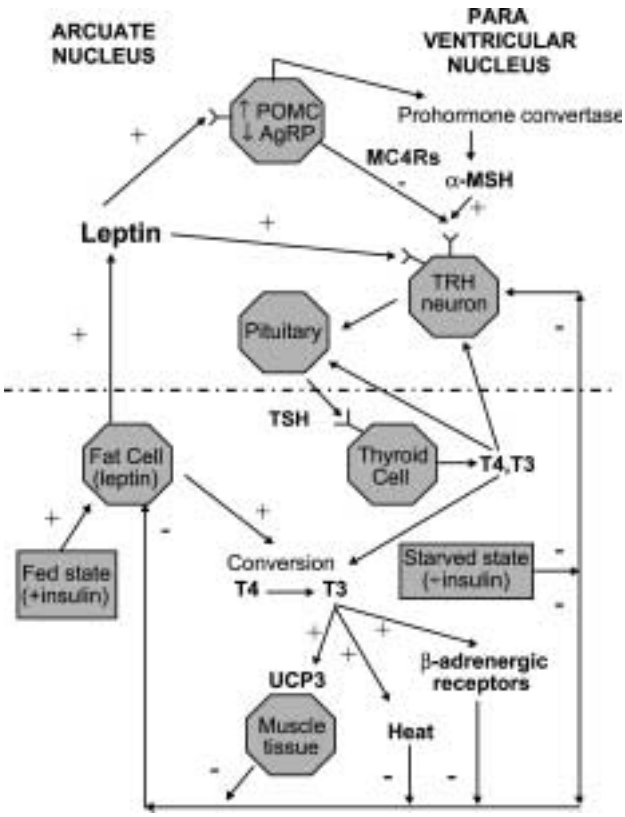

Figure 1 A possible relationship between leptin and the thyroid hormones. A sufficient level of leptin signalling is needed to maintain TRH expression in the hypothalamic paraventricular nucleus (PVN), which is necessary for normal production of TSH and production of thyroid hormones by the thyroid. Two mechanisms may be involved. Leptin regulates arcuate neurons expressing proopiomelanocortin (POMC) (induced by leptin) and agouti-related-protein (AgRP) (suppressed by leptin). These arcuate neurons project to TRH neurons, where they influence TRH expression by antagonistic actions of α-melanocyte-stimulating hormone (α-MSH) (stimulatory (+)) and AgRP (inhibitory (−)) on melanocortin 4 receptors (MC4Rs). Leptin may also act directly on TRH neurons through leptin receptors on these cells. In the absence of leptin signalling, the feedback loop between T4/T3 and the hypothalamus–pituitary–thyroid system is lost. Hence, although levels of T4/T3 may be low, TRH and TSH levels remain suppressed. Peripherally, in the starved state (absence of insulin) T3 inhibits leptin mRNA accumulation in adipocytes and the opposite effect occurs in the fed state (presence of insulin). In addition, leptin itself can stimulate T3 production via activation of the conversion of T4 to T3 and an increased T3 production stimulates heat production, uncoupling protein 3 (UCP3) expression in muscle tissue and $beta_3$-adrenergic receptors (influenced by the catecholamines). In contrast, an increase of these 3 factors inhibits leptin expression in fat tissue, which overall leads to an inverse relationship between leptin and T3 both peripherally and centrally regulated.

Leptin, thyroid hormones and thermogenesis

Total body energy expenditure represents the conversion of oxygen and food (or stored forms of energy such as fat, glycogen and protein) to carbon dioxide, water, heat and external work, and can be measured by direct calorimetry as produced heat, hence the term thermogenesis, or indirectly as the amount of oxygen consumed (indirect calorimetry). Thermogenesis can be expressed as standard metabolic rate (SMR). In contrast, adaptive thermogenesis, also referred to as facultative thermogenesis, is defined operationally as heat production in response to environmental temperature or diet, and serves the purpose of protecting the organism from cold exposure or regulating energy balance on changes in diet.

Several factors influence the adaptive thermogenesis such as environmental temperature, starvation and feeding (29). In general, humans as opposed to rodents have a broad thermoneutral zone with relatively small changes in metabolic rate occurring over relatively wide temperature changes. This difference is due, in part, to behavioral responses such as adjustments in the amount of clothing and diet (29). Starvation can decrease resting metabolic rate by as much as 40%, and food restriction sufficient to maintain a 10% reduction in body weight is associated with a decrease in energy expenditure (30). On the other hand, feeding increases energy expenditure by acutely increasing metabolic rate by 25–40% in humans and rodents (29).

The brain detects exposure to cold, leading to activation of efferent pathways controlling energy dissipation. The main effector component of this response is the sympathetic nervous system, which heavily innervates thermogenic targets such as brown adipose tissue and skeletal muscle (29). Hypothyroid rats die immediately when exposed to cold indicating that thyroid hormones are involved in thermogenesis mainly at the brown fat level. The primary molecule

involved in cold-induced thermogenesis in brown fat is uncoupling protein 1 (UCP-1), a mitochondrial inner-membrane protein that uncouples proton entry from ATP synthesis (31, 32). Two homologs of UCP-1 have been identified, UCP-2 expressed in most tissues and UCP-3 expressed predominantly in skeletal muscle and brown adipose tissue (33–35). These UCPs have proton transport activity suggested by several studies (29, 33–36). Beta-adrenergic-receptor stimulation, due to cold exposure or pharmacological agents, has both acute and chronic effects on brown adipose tissue (29). UCP-1 activity increases within seconds of acute β-adrenergic stimulation, and chronic stimulation over hours and days leads to an increased amount of UCP-1 protein, mitochondrial biogenesis, and hyperplasia and hypertrophy of brown adipose tissue. In addition, there is evidence that brown adipose tissue is also important in diet-induced thermogenesis since sympathetic nerve activity to brown adipose tissue is reduced in many models of obesity, including leptin-deficient ob/ob mice (29, 37–42).

Several studies have suggested that thyroid hormones have a permissive role in adaptive thermogenesis because their levels did not change during cold exposure or consumption of high-calorie diets (29). However, this may not be correct as thyroid hormone levels have been found to rise in some models of increased caloric intake and, importantly, to drop during starvation, an effect associated with falling leptin levels and decreased expression of hypothalamic TRH (29, 43). Falling thyroid hormone levels may thus contribute to starvation-induced decreases in thermogenesis. The mechanisms of starvation-induced thermogenesis may be a central effect on the nervous system or a direct effect on peripheral thermogenic tissues (fat or muscle tissue). Both thyroid hormone and leptin receptors are found in fat, and both leptin and thyroid hormone increase UCP-1, UCP-2 and UCP-3 expression (44). During starvation leptin decreases which, in turn, decreases UCP-1 mRNA and protein levels in brown fat. In the nucleus, UCP-1 is regulated by the enhancer element, which has binding sites for the thyroid hormone receptor. The binding of peroxisome proliferator-activated receptor-γ (PPAR-γ, a nuclear receptor expressed in white and brown fat) is essential for the function of the UCP-1 enhancer as well as PPAR-γ co-activator (PGC-1) that also binds thyroid hormone receptors and positively regulates expression of UCP-1, which, in turn, is influenced by both leptin and thyroid hormones (29). Thyroid hormones and leptin might, therefore, be involved in the adaptive thermogenesis through the mitochondrial uncoupling proteins and heat production (29).

UCP-3 is expressed in high levels in muscle tissue and brown fat in rodents and is regulated by thyroid hormone, beta$_3$-adrenergic agonists and leptin (35). Thus, the physiological variation in T3 appears to be regulating UCP-3 since UCP-3 mRNA levels are increased by elevation of T3 levels and decreased by reduction of T3 levels (35). In addition, central leptin was demonstrated to stimulate T3 via activation of the conversion of T4 to T3, and this stimulation could be responsible for the effect of leptin on muscle UCP-3, thus indicating that thyroid hormones could be important mediators of the effect of leptin on energy expenditure (35, 45). This contrasts with the role of UCP-1 in thyroid thermogenesis. Although T3 stimulates UCP-1 expression, brown fat is actually less active in hyperthyroidism probably due to a compensatory mechanism, adjusting for the extra heat production from other tissues (35, 46). Yet, there are strong implications of a relationship between leptin, body composition, thermogenesis and thyroid hormones, but the exact actions still have to be elucidated.

Leptin, thyroid hormones and body composition

Initially, in humans, it was proposed that leptin expression and circulating levels increase in parallel or are positively correlated to the amount of adipose stores estimated as total fat mass, per cent body fat or body mass index (BMI) (47–50). Recent evidence (reviewed in 7) has suggested that (i) the relationship between leptin levels and fat mass is curvilinear, rather than linear, implying that leptin secretion increases exponentially with increasing fat mass, (ii) leptin production may reflect the process of fat accumulation in the adipocytes rather than the overall amount of stored fat, (iii) adipocyte size *per se* appears to be another major determinant of leptin mRNA expression and (iv) the type of adipose tissue distribution may also be related to leptin levels, because the expression is higher in subcutaneous than in visceral fat depots.

Increased body weight contributes to insulin resistance. Patients with thyroid disturbances change their body composition dramatically during development of thyroid disease (51) and during antithyroid drug treatment; also circulating leptin is probably changed although conflicting results have appeared (Table 2). Thyrotoxic patients are resistant to insulin (52, 53). Insulin and glucocorticoids are inversely related, both increase total leptin levels *in vitro* and *in vivo*, and glucocorticoids are known to increase insulin levels and to cause insulin resistance (54). Thus, insulin resistance in thyrotoxic patients may contribute to any effects on serum leptin concentrations. However, actual leptin levels for the human studies are not comparable nor are they candidates for a meta-analysis because of the different study designs, materials and methods used; they are not, therefore, listed in the tables.

An initial observation of sexual dimorphism with higher serum leptin concentrations in women has been discarded since subsequent studies found no

Table 2 Results of the clinical studies of the relationship between circulating leptin levels and thyroid diseases. Patients with thyrotoxicosis (tox) and myxoedema (myx) were compared with controls and changes in leptin levels during treatment were compared with baseline (base). Further correlations were made between serum leptin and thyroid hormone levels (TH) and fat mass (FM). Actual leptin levels are not shown due to the different study designs, materials and methods used; the values were therefore not comparable.

Reference	Tox vs controls	Myx vs controls	Tox vs base	Myx vs base	Leptin vs TH	Leptin vs FM
Zimmermann-Belsing *et al.* (51)	↑	ND	↑	ND	ns	$P < 0.002$
Corbetta *et al.* (63)	↔	↔	ND	ND	ns	$P < 0.0001$
Mantzoros *et al.* (64)	↔	ND	↔	ND	ND	ND
Sreenan *et al.* (65)	↔	↔	ND	ND	ns	$P < 0.0002$
Valcavi *et al.* (66)	↔	↓	↔	↓	ND	ND
Wolthers *et al.* (67)	↑	ND	↔	↔	ns	ns
Sesmilo *et al.* (68)	↔	↔	↔	↔	ns	$P < 0.0001$
Yoshida *et al.* (69)	↔	↓	ND	ND	ns	$P < 0.0001$
Ozata *et al.* (70)	↑	↔	↔	↔	ns	ns
Leonhardt *et al.* (71)	↔	↑	ND	ND	$P < 0.009$	$P < 0.0001$
Pinkney *et al.* (72)	↓	↑	ND	↓	ns	$P < 0.02$
Diekman *et al.* (73)	↑	↓	↔	↔	ND	$P < 0.004$
Kristensen *et al.* (74)	↔	ND	↔	ND	ns	$P < 0.001$
Miyakawa *et al.* (75)	↓	↔	↓	↔	ns	$P < 0.0001$
Kautzky-Willer *et al.* (76)	↔	↑	↔	↔	ns	$P < 0.0005$
Song *et al.* (77)	↔	↔	ND	ND	ND	$P < 0.001$
Asami *et al.* (78)	ND	↔	ND	ND	$P < 0.05$	$P < 0.01$
Chen *et al.* (79)	↑	↑	ND	ND	ns	$P < 0.005$
Simo *et al.* (80)	ND	ND	ND	↔	ns	ND
Sera *et al.* (81)	↓	ND	↑	ND	$P < 0.008$	ND
Song *et al.* (82)	↑	ND	ND	ND	ns	$P < 0.001$
Pinkney *et al.* (83)	↓	↑	ND	↓	ND	ND
Nakamura *et al.* (84)	↔	ND	↔	ND	$P < 0.05$	$P < 0.05$
Matsubara *et al.* (85)	↔	↔	↔	↔	ns	$P < 0.001$
Ozata *et al.* (86)	↔	ND	ND	ND	ns	$P < 0.001$
Seven (87)	↔	ND	ND	ND	$P < 0.05$	ND
Obermayer-Pietsch *et al.* (88)	↓	ND	↑	ND	$P < 0.03$	ns
Hsieh *et al.* (89)	ND	ND	↑	↑	$P < 0.002$	$P < 0.0001$

ns = not significant; ND = not done; ↔/↑/↓ = unchanged/increased/decreased serum leptin compared with either controls or baseline.

differences in serum leptin when men and women with a similar percentage of body fat were compared. Twenty-six thyroid studies have taken possible gender differences into consideration (Table 1). These studies were performed with different designs, materials and methods and are not, therefore, directly comparable (Table 1). However, when the relationship between total serum leptin and fat mass was compared between female and males with thyroid diseases, total serum leptin levels were higher in females in nine studies (Table 1). The reason for these conflicting results regarding gender is not clear. The majority of the studies which found a gender difference were based on simple measurements of body composition such as BMI, bioimpedance (BIA), anthropometric index of abdominal fat distribution and/or the waist–hip ratio. However, when robust body composition techniques (Dual Energy X-ray (DEXA)) and diet control measures (isoenergetic levels) were taken into consideration, the relationship between log plasma leptin concentrations and percentage body fat was not different in men and women (55). Thus, observed gender differences might be a result of methodological differences rather than biological factors.

Leptin and thyroid function

Thyrotoxic patients are usually euthyroid after the first 3 months of antithyroid drug treatment (ATD), but many thyroid patients especially patients with Graves' disease experience several poorly defined complications such as change in body composition several months or years after obtaining euthyroidism (56). It is not clear whether these complications are related to thyrotoxicosis, autoantibodies, ATD, leptin or other factors.

Seven animal studies (44, 57–62) (Table 3) as well as 28 human studies (51, 63–89) (Tables 1 and 2) have each tried to clarify a possible relationship between leptin and thyroid dysfunction.

Animal studies

In hyperthyroid rats serum leptin was decreased in five studies (44, 57–59, 62) while in hypothyroid rats four studies found increased (57–59, 62) and three studies showed unchanged serum leptin levels (44, 60, 61) (Table 3). The reasons for these conflicting results are not clear but the conditions under which the studies were conducted differed (rat strain, weight, age,

Table 3 Serum leptin and thyroid dysfunction studied in various strains of normal rats.

Reference	Tox	Myx	Rat strain	Weight (g)	Groups (no.)	Rats F/M	Controls F/M	Age (days)	Treatment	Time (days)	Body composition	Leptin assay
Syed *et al.* (44)	↓	↔	Sprague–Dawley	180	3	0/6	0/7	20	MMI/T3	7/14	Body weight	Linco
Fain *et al.* (57)	↓	↑	Sprague–Dawley	204–275	3-11	0/2–12	0/2–12	21	PTU/T3	1–2	Body weight	Linco
Escobar-Morreale *et al.* (58)	↓	↑	Wistar	120–150	13	5–6/0	6/0	28	Tx/T4/T3	12–13	Body weight	Linco
Fain and Bahouth (59)	↓	↑	Sprague–Dawley	240–275	?	0/20	0/20	21	PTU/T3	1–2	Body weight	Linco
Curcio *et al.* (60)	ND	↔	Wistar	150–200	4	0/4	0/4	28	Tx/MMI	3–4	DEXA	Linco
Wang *et al.* (61)	ND	↔	Sprague–Dawley	250–300	7	0/8–10	0/8	28	Tx/PTU/T3	21	Body weight	Linco
Lossa *et al.* (62)	↓	↑	Wistar	70	8	0/8	0/8	25	PTU/T3	4/7/15	Body weight	Linco

Serum leptin in thyrotoxic (Tox) and myxoedema rats (Myx) respectively, compared with controls and/or euthyroid rats. ↔/↑/↓ = unchanged/increased/decreased serum leptin compared with controls. F/M, female/male; ND, not done; Tx, thyroidectomized; MMI, methimazole; PTU, propylthiouracil.

sex, different diet, measurements of mRNA leptin in rat fat tissue, administration of catecholamines or leptin, different measurements of body composition and different ways to induce hypothyroidism or hyperthyroidism) (Table 3) (57, 60–62).

Escobar-Morreale *et al.* (58) used thyroidectomized (Tx) Wistar rats treated chronically (12–13 days) with subcutaneous infusion of placebo, T4 or T3. One group (six animals/group) was treated with placebo (hypothyroid rats) and 6–7 groups were treated with increasing subcutaneous doses of T3 or T4 (thyrotoxic rats). When compared with a control group, leptin concentrations in the hypothyroid animals were unchanged. The infusion of T3 or T4 resulted in a decrease in serum leptin when compared with either hypothyroid animals or the control group, indicating high levels of serum leptin in the hypothyroid state and low levels in the thyrotoxic state. Hypothyroid animals had an elevated serum leptin relative to their body weight, which was normalized and even decreased when T3 or T4 were infused. The results were in agreement with 3 other studies (57, 59, 62) in which hypothyroid rats showed increased leptin mRNA levels and increased leptin secretion, which were acutely reversed by administration of supraphysiological doses of T3 (Table 3).

Fain *et al.* (57) induced hypothyroidism by feeding 2–12 male rats/group for 3 weeks on a low-iodine diet with the addition of 6-N-propyl-2-thiourasil (PTU), and T3 was subsequently injected intraperitoneally to induce hyperthyroidism. The different groups were compared with an age- and weight-matched euthyroid group of rats. To examine leptin and β_3-receptor expression in adipose tissue, RNA was extracted from epididymal adipose tissue (E-fat). Fain *et al.* (57) demonstrated that expression of the mRNA leptin was elevated in E-fat from hypothyroid rats compared with euthyroid and hyperthyroid rats. In contrast, the level of β_3-adrenergic receptor mRNA was lower in E-fat from hypothyroid rats compared with that from controls. Administration of T3 reversed the elevated leptin secretion, mRNA leptin and the low β_3-adrenergic receptor mRNA. In addition, a single dose of T3 increased cAMP for a specific β_3-adrenergic agonist and lipolysis. These data indicated that T3 is a potent regulator of leptin and β_3-adrenergic receptor mRNA *in vitro*. Therefore, it was suggested that the inhibitory effects of thyroid hormone on serum leptin might be a physiological phenomenon (58).

Later, Fain and Bahouth (59) confirmed their earlier results and designed a study to determine whether enhanced leptin release and mRNA content were observed in adipocytes in primary culture from hypothyroid rats, and whether T3 could reverse this enhancement by directly inhibiting or stimulating leptin formation and release by rat adipocytes (Table 3). In conditions that approximated the fed state (addition of high glucose plus glycocorticoids and either insulin or growth hormone), the concurrent presence of T3 stimulated leptin mRNA accumulation. In contrast, in conditions mimicking the fasted state (addition of glucocorticoid alone or in the presence of a β_3-adrenergic agonist) the addition of T3 enhanced the loss of leptin mRNA. Thus, T3 was found to either inhibit or stimulate the net leptin mRNA content of white adipose tissue indicating the complexity of the effects of T3 on leptin release and leptin mRNA accumulation in adipocytes (59).

Syed *et al.* (44) evaluated leptin expression and the influence of food intake, fat mass and body temperature in rats with pharmacologically altered thyroid status. They used male Sprague–Dawley rats ($n = 6$/group) treated with methimazole (MMI) (hypothyroid rats) or T3 (hyperthyroid rats), and compared them with an age-matched euthyroid group of 7 rats. Acute thyrotoxicosis was induced in rats by 24-h T3 treatment prior to death. Euthyroid rats or hypothyroid rats without T3 treatment served as controls. Fat weight was calculated as the sum of E-fat and retroperitoneal (R) fat (R-fat). In hypothyroid rats total body weight, food intake and temperature were decreased while fat weight was decreased in both chronically hypo- and hyperthyroid rats. E-fat leptin mRNA was higher in euthyroid rats compared with chronically hypo- and hyperthyroid rats. In contrast, R-fat leptin mRNA per total RNA was unchanged in the 3 thyroid states (44).

Conflicting acute responses of E-fat leptin mRNA content have been reported, with a decrease (57) or no change (44, 59) in hypothyroid rats subsequently treated with a high dose of T3. However, confirmatory data relative to thyroidal state, such as serum hormone levels, positive tissue control responses, or adiposity of the rats was not always included. The effect of thyroid hormone on body temperature was rapid and observable within 12 h of T3 treatment of hypothyroid rats (44). Thyroid hormone-associated increases in core body temperature appeared to be independent of or inhibited by leptin, because leptin levels were decreased in hyperthyroidism indicating an influence of thyroid hormones and leptin on UCP-1 to -3 and thermogenesis (44). In contrast to earlier reports (57–59, 62) serum leptin concentration was higher in the euthyroid rats but not significantly different from the hypothyroid group (Table 3). On the other hand, serum leptin was significantly lower in chronically hyperthyroid versus the euthyroid group of rats, in agreement with previous studies (57–59, 62), but in contrast to human studies (Table 3 vs Table 2). However, the differences in serum leptin disappeared when rats were corrected for fat weight, indicating that thyroid hormone administration altered fat mass, which in turn influenced the expression of leptin mRNA and serum leptin concentration (44). Although thyroid status had the expected effects on body temperature and food intake, it did not produce parallel changes in serum leptin concentrations (44).

Furthermore, it has been suggested by Wang *et al.* (61) that calorigenic actions of leptin are additive to, but not dependent on those of thyroid hormones. They hypothesized that the effects of leptin on energy metabolism may be regulated independently of the TRH/TSH/thyroid hormone axis, and they therefore studied the effect of systemic administration of leptin on body weight, food consumption, and variables of indirect calorimetry in Tx rats. They used 55 Spraque–Dawley rats, fed a balanced diet and treated with PTU for 4 weeks. They were divided into two groups (Tx + T3 or Tx − T3) and three subgroups (n = 8–10/group). The resting oxygen consumption (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER) were determined by use of an oxymax indirect calorimetric system. Serum leptin levels were not significantly affected by the hypothyroid state, in agreement with two other studies (44, 60) (Table 3). In addition, Wang *et al.* demonstrated for the first time that the effects of leptin in reducing food intake and body weight and in stimulating energy metabolism were not dependent on the presence of thyroid hormone. The thyroid gland did not constitute an integral component of the leptin action mechanism, but it was suggested that leptin and thyroid hormones might share some common downstream action sites and could act additively, although independently, to enhance calorigenic metabolism (61).

Curcio *et al.* (60) used intact or Tx adult male Wistar rats (n = 4/group), fed a standard or high calorie diet (cafeteria diet) to study diet-induced thermogenesis in hypothyroid rats. They tested the modifications of energy intake and basal metabolic rate (BMR), brown fat UCP content and thermal response, and consequent changes in body composition when the rats were fed the cafeteria diet. In intact rats, the cafeteria diet increased energy intake 5–7 times and caused BMR to increase 25%, total brown fat thermal response to noradrenalin (NE) increased two- to threefold, and total brown fat mitochondria increased 60%, UCP percentage increased 40% and total fat UCP increased 2.2-fold confirming a diet-induced thermogenesis and the activation of brown fat (29). Feeding the cafeteria diet to hypothyroid rats led to higher daily calorie intake, normal food absorption, increased R-fat, adipocyte volume, body fat, BMR, brown fat mitochondrial protein, UCP percentage and total brown fat UCP but not brown fat thermal response to NE infusion. A fourfold increase in serum leptin was demonstrated in intact rats fed the cafeteria diet in contrast to the hypothyroid rats which showed no substantial change. Thus, it was suggested that hypothyroidism caused the brown fat to become unresponsive to NE, even after 1 month on the cafeteria diet. However, these rats were able to increase basal metabolic rate and did not gain fat or serum leptin beyond that observed in intact controls kept on a similar overfeeding schedule.

Iossa *et al.* (62) studied the influence of thyroid hormones on the relationship between serum leptin and fat mass, as well as on energy and macronutrient balance. Sixty-four male Wistar rats were used, divided into eight rats/group. Three groups of rats were pretreated with PTU added to the drinking water for 4 days, two groups were treated with either PTU + saline or PTU + T3, two groups were treated with daily injections of T3 for 15 days and the remaining normal rats served as controls. Measurements were performed both in the transition period from euthyroid to hypo- or hyperthyroid (0–7 days) and in the stable thyroid condition (15 days). Measurement of body composition was performed by measures of protein, fat and lipid in gut contents. Body weights and food intakes were monitored daily to calculate body weight gain and gross energy intake. Feces, urine and spilled food were collected to measure energy content in a bomb calorimeter, and, based on these data, measurements of metabolic energy (ME), gross energy efficiency, energy expenditure and total cost of storage. It was demonstrated that in the absence of T3 energy and lipid balance were not maintained despite the increased serum leptin concentrations found in hypothyroid rats (62). Thus, it appeared that T3 plays a major role in the maintenance of energy and lipid balance. As in previous studies (57–59) serum leptin concentrations were significantly increased in hypothyroid rats

and decreased in hyperthyroid rats. In addition, in altered thyroid state (i.e. the same amount of fat) it was suggested that the relationship between serum leptin and body fat changes gave significantly higher leptin concentrations in hypothyroid rats and significantly lower concentrations in hyperthyroid ones, confirming that the inverse relationship between T3 and serum leptin was not only induced by T3 (62). However, the inhibitory action of T3 on leptin production could be the result of the altered sensitivity of white fat to sympathetic stimuli. This was in agreement with earlier studies (57–59, 90) but in contrast with those of Syed *et al.* (44), although the induction of hypothyroidism was different, rats were older and fat mass increased differently during development. Thus, there might be an interaction of circulating T3 with the leptin system and the effect of thyroid hormone on the leptin system seems to be inhibitory (62).

Conflicting results have been demonstrated with increased (57–59, 62) or unchanged serum leptin (44, 60, 61) in hypothyroid rats. However, the rat studies universally show decreased serum leptin with chronic hyperthyroidism, which has failed to be demonstrated in human studies (Table 3 vs Table 2). Thyroid hormones might have physiological inhibitory effects on serum leptin because the inverse relationship between leptin and thyroid hormones was maintained over a wide range of thyroid hormone levels, from severe hypothyroidism to hyperthyroidism (58, 60–62). However, a change in fat stores is likely to contribute to the decrease in leptin as seen in chronically hyperthyroid rats (44). Although hyperthyroidism-associated decreases in leptin may mediate some of the associated changes in, e.g. food intake, the regulation of food intake with alterations of thyroid status must also involve other mechanisms such as body temperature (44). Thus, thyroid state-associated changes in body temperature did not correlate with changes in serum leptin, suggesting that thyroid-associated thermogenesis was independent of alterations in leptin concentration (44). On the other hand, it was demonstrated that the metabolic actions of leptin were not dependent on euthyroidism and that the effects of leptin and T3 treatment of thyroidectomized hypothyroid rats on oxidative metabolism were additive (61).

A possible relationship between leptin and thyroid hormones might be a physiological phenomenon (Fig. 1). The conflicting results both in animal and human studies might be due to the complexities in the regulation of the different compounds involved in the brain, in thermogenic cells and all other cells harbouring the conversion of T4 to T3.

Human studies

As in rats, studies in humans have failed to show a consistent effect of the thyroid state on serum leptin concentrations. It appears from Table 1 that the studies differed in terms of patient characteristics, length of treatment (if performed), and method for measuring serum leptin as well as for evaluation of body composition. It is therefore not surprising that extreme variation in the results was seen. In hypothyroid subjects serum leptin was found to be increased in five studies, decreased in three and unchanged in eight compared with a control group and/or euthyroid subjects (Table 2). In hyperthyroid subjects serum leptin was increased in six studies, decreased in five studies and unchanged in 14 (Table 2).

Matsabura *et al.* (85) reported the results of a large Japanese study and found leptin levels were lower in hyperthyroidism compared with a large control group. This was accounted for mainly by a decrease in BMI in overt hyperthyroidism. In other studies, BMI-matched healthy persons were used as controls (51, 70, 87, 88), and in these studies mean serum leptin concentrations in the cross-sectional comparison were unchanged in hyperthyroid patients compared with controls, irrespective of whether hyperthyroidism was due to autoimmune Graves' disease (51, 77, 87) or toxic adenoma/multinodular goitre (87). In the study by Seven (87), only the patients with Graves' disease were hyperthyroid while the patients with multinodular goitre were studied after one month of ATD. Control subjects ($n = 15$) were claimed to be matched to both groups ($n = 27$ and 23 respectively), but actual BMI data were not given nor was it stated whether the two patient groups had similar and comparable BMI values.

The first published cross-sectional study (63) divided 114 patients into primary ($n = 36$) or central ($n = 38$) hypothyroidism and hyperthyroidism ($n = 40$) respectively. The patients with hyperthyroidism comprised a mixture of Graves' disease and multinodular goitres (numbers of each not defined). Patients with central hypothyroidism had pituitary tumours, were replaced with cortisol and sex steroids, had untreated growth hormone deficiency and untreated hypothyroidism and were studied again after 6 months of T4 replacement. Body composition was measured by BMI with a defined reference range but the patients were not matched to controls. Leptin levels were adjusted for gender and BMI by calculating a standard deviation score (SDS). A correlation between serum leptin levels and BMI was seen in female patients with thyroid disorders, although the difference in correlations found in females and males could be accounted for by the different numbers in the various groups. No evidence was found in this paper for a role of thyroid hormones in leptin regulation (63). A small cross-sectional study (65) described groups of 25 each of eu-, hypo- and hyperthyroid patients. No further specification of the patients was given except in subgroups of 11 euthyroid, six hypothyroid and six thyrotoxic patients where BMI was also available. Leptin was correlated to BMI but

not to free T4 or TSH. The conclusion in this study was also that thyroid function had no influence on leptin regulation. The same conclusion was reached by Song *et al.* (77) who studied 50 hyperthyroid patients (43 Graves', seven nodular), 24 hypothyroid and 50 euthyroid patients and compared leptin levels with BMI and percentage fat as well as fat mass by BIA. No overall differences in serum leptin concentrations were found (except by gender) and no relationship was demonstrated to free T4.

Ozata *et al.* (70) further divided the patients with Graves' disease into those with and those without thyroid associated ophthalmopathy (TAO), based on the theory that human preadipocyte fibroblasts in orbital connective tissues from patients with TAO differentiate into cells resembling adipocytes and acquire expression of leptin and functional TSH receptors (91). Mean leptin levels were similar in all the groups compared both to matched controls and to each other (70).

Chen *et al.* (79) studied premenopausal women cross-sectionally. They investigated 20 patients with hypothyroidism, 20 with hyperthyroidism and 20 controls that were not BMI matched to the patients. The hypothyroid patients had a higher BMI and also a higher serum leptin level compared with euthyroid controls. Hyperthyroid patients had similar BMI compared with the euthyroid controls (20.9 ± 0.6 vs 21 ± 0.5), but with higher leptin concentrations (10.1 ± 1.1 vs 6.5 ± 0.9). These authors provided indirect evidence for the influence of thyroid function across the range from hyper- via eu- to hypothyroidism on leptin regulation by finding a correlation (albeit weak) between leptin concentrations and the zinc/copper ratio in plasma, the bioavailability of which is altered by thyroid hormones. A later study (89) confirmed the evidence for the influence of thyroid function across a range from hypo- via eu- to hyperthyroidism on leptin regulation.

In the Japanese study (85), comprising a total of 368 Japanese females, only 27 had pre-treatment hyperthyroidism and 19 had pre-treatment hypothyroidism, but no classification of the hyper- or hypothyroidism was performed. Leptin levels did not correlate with either thyroid function or plasma lipids respectively. This study also reported few hyper- and hypothyroid patients before and after therapy with ATD and levothyroxine respectively (85). Unfortunately, the patients were studied only during a short follow-up period, which may account for the lack of significant change in serum leptin concentrations. In contrast, when two large cross-sectional groups of female patients with either pre-treatment hyperthyroidism or hyperthyroidism during treatment were compared a difference was indeed found, with low serum leptin at the time of newly diagnosed hyperthyroidism and normal serum leptin in euthyroid patients treated with ATD. Thus, an effect of ATD to increase serum leptin could be suggested, which was later confirmed in a prospective study of ATD in Graves' disease (51) and in another study of patients with nodular toxic goiter before and up to one year after radioiodine therapy (88). In both these studies (51, 88) serum leptin changes were dissociated from body weight changes as measured by BMI and from proportional or actual fat mass as measured by DEXA, indicating different rates of lean and adipose mass weight gains during normalization of thyroid function, irrespective of whether this was achieved by radioiodine therapy (88) or ATD (51).

Pinkney *et al.* (72) divided their cross-sectional hyper- and hypothyroid patients into lean and obese subjects and compared their leptin levels to thyroid function, BMI, percentage body fat by BIA, and plasma noradrenalin and found evidence for a modification of leptin secretion by thyroid status independent of adiposity and noradrenalin (72). The study was presented as a short communication with relatively small numbers in each group. Also, 11 initially hypothyroid patients were studied when euthyroid after T4 treatment and a decrease in serum leptin without changes in BMI was demonstrated.

A different approach was used by Simo *et al.* (80) who studied 30 patients previously thyroidectomised for thyroid carcinoma, 4 weeks after discontinuation of T4 therapy (in connection with whole body scintigraphy with I^{131}). Profound short-term hypothyroidism was obtained without any noticeable change in body weight and no difference whatsoever could be observed in plasma leptin levels. This strongly suggested that serum leptin was unrelated to thyroid function when body composition did not change. Similar to Pinkney *et al.* (72), Kautzky-Willer *et al.* (76) divided their patients into lean and obese subjects and compared them to BMI-, age- and sex-matched controls. Their hypothesis was related to thyroid hormones being permissive for adrenergic activation, which, in turn, has been shown to decrease leptin expression in adipocytes and thus plasma leptin (58). By this subdivision of patients each study group consisted of about 10 members. Serum leptin levels were only elevated in obese subjects and were unrelated to thyroid function or lipid status. The patients were studied again at euthyroidism (12–20 weeks) after either ATD or T4. The former hyperthyroid patients displayed a fall in plasma glucose, insulin and free fatty acids, but not in plasma leptin concentrations. Neither was there a significant change in plasma leptin in the formerly hypothyroid patients, so even after restoration of normal thyroid function plasma leptin and insulin remained higher in obese than in lean patients with former thyroid dysfunction. However, a direct comparison of changes between pre-treatment and post-treatment leptin values in individual patients cannot be found in the paper – only mean values of pre- and post-treatment levels respectively are given. A type 2 error due to small numbers may also play a role. Their main conclusions, although not directly related

to thyroid function, were (i) the correlation of leptin with body fat mass was preserved in thyroid dysfunction, (ii) plasma leptin was markedly increased in obese hypothyroid hyperinsulinaemic patients and (iii) plasma leptin was not affected by oral glucose loading.

Kristensen *et al.* (74) performed both *in vivo* and *in vitro* studies. Twelve healthy premenopausal women were given 60 μg T3 daily for 7 days and were investigated before, and at days 8 and 14 (7 days after stopping T3) using indirect calorimetry (resting energy expenditure (REE)) in seven of the patients and plasma samples in all. T3 induced a 1 kg weight loss which was not regained the week after. Leptin levels did not change significantly during this short-term hyperthyroidism while both REE and oxygen comsumption increased. The *in vitro* study in this paper demonstrated an approximately 50% reduction in basal leptin and the dexamethasone-stimulated release of leptin from cultured human adipocytes as well as an approximately 50% reduction in basal and stimulated leptin mRNA expression within the cells. The results of the *in vitro* study were in accordance with another study by Fain and Bahouth (59) in which leptin mRNA expression decreased in isolated rat adipocytes and rat adipose tissue fragments after T3 addition, whereas the reverse was found when thyroid hormone was added together with insulin. Yoshida *et al.* (92) studied the mouse preadipocyte cell line 3T3-L1 and found stimulation by thyroid hormones. However, in preadipocytes, leptin expression was only a small fraction (1%) of that in mature adipocytes, and, in addition, the 3T3-L1 cell line is a clonal mouse preadipocyte cell line making the study less relevant to human leptin physiology. The authors concluded that T3 at very high concentrations might possibly be a negative physiological regulator of leptin levels in humans.

Nakamura *et al.* (84) found no difference in serum leptin concentrations between 32 women with Graves' disease compared with 30 controls with similar percentage body fat measured by BIA. The correlation between serum leptin and percentage body fat was strongest in the controls but was also present in the patients with hyperthyroidism, and there was some correlation to free T4 levels. Treatment with ATD of a subgroup of these patients demonstrated no change in leptin levels but a significant change in the anthropometric indices and thus a reduction of percentage leptin (from prediction). Two other short-term studies in healthy males treated for a short period with T3 did not demonstrate any changes in leptin levels. In the paper by Mantzoros *et al.* (64) 22 males were given T3 in order to induce a hyperthyroid state verified by a drop in cholesterol and an increase in osteocalcin. No mention of BMI was made and no leptin changes were seen. Wolthers *et al.* (67) used a randomized, placebo-controlled cross-over design in 8 healthy males receiving T3 and/or growth hormone and/or placebo. REE was assessed by indirect calorimetry and body fat by BIA. Serum leptin levels did not change under the influence of either growth hormone or T3.

Valcavi *et al.* (66) found lower leptin levels in 16 untreated hypothyroid patients and an increase after 6–30 weeks of T4 therapy. In 16 hyperthyroid patients leptin levels were similar in the untreated state and after 12–28 weeks of methimazole. In the cross sectional part of this study an age-, sex-, and BMI-matched control group was used. Diekman *et al.* (73) also looked at 21 hyperthyroid and 14 hypothyroid patients before and after 3 months of ATD and thyroxine respectively. They compared with BMI and found that serum leptin concentrations were lower in hypothyroid compared with hyperthyroid women. Also when leptin concentrations were expressed as Z-scores from the mean values of female controls matched for BMI and age, Z-scores were lower in the hypothyroid than in the thyrotoxic women (15 Graves' disease) (-0.63 ± 0.21 vs 0.53 ± 0.18, $P < 0.001$). After treatment, Z-scores did not deviate from the expected values (0.05 ± 0.28 vs 0.08 ± 0.16; $P = 0.98$) and their study thus supported the concept that the thyroid state modulates serum leptin concentrations independent of BMI, with a small decrease in hypothyroidism and a small increase in hyperthyroidism. Restoration to euthyroidism was accompanied by an increase in serum leptin in both cases, which is in keeping with the previously mentioned longer-term prospective study of Graves' disease (51). Sesmilo *et al.* (68) studied 16 patients with hypo- and 17 patients with hyperthyroidism, all of autoimmune origin, before therapy and every 6–8 weeks until euthyroidism was reached. Body composition was measured by BIA and BMI which both correlated with serum leptin concentrations. No correlation was found with thyroid hormones. Leptin levels in the patients were not compared with healthy controls but individual changes were looked for, and although an increase was seen both in hypothyroid patients after treatment (14.5 ± 2.6 vs 16.9 ± 2.7) and in hyperthyroid patients after treatment (10.7 ± 1.8 vs 12.4 ± 2.2) these increases were not significant. No change was observed in BMI or body fat. This was unlike the significant increase in mean serum leptin observed in a longer study (51) of Graves' disease. Mean BMI also increased in these patients but the increase was less relative to the increase in total leptin.

In children ($n = 51$) a study was performed during ongoing T4 replacement of hypothyroidism. Serum leptin concentrations correlated only in the group of older children ($n = 16$) with serum T4, but not with TSH or free T4. The numbers were, however, too small for a significant correlation to be expected (78).

Miyakawa *et al.* (75) used a cross-sectional study of patients with Graves' disease (12 males and 28 females) and hypothyroidism (11 females). Body composition was determined by BIA, and they also measured TSH-receptor antibodies. They found a correlation between

percentage fat mass and leptin only in female patients with Graves' disease, with hypothyroidism and in female controls, but not in male patients with Graves' disease, despite the fact that they had lower leptin levels and percentage fat mass. This was in keeping with the notion that testosterone possibly inhibits leptin levels (93). A subgroup of female patients were studied approximately 5 months after reaching euthyroidism with either ATD ($n = 9$) or levothyroxine ($n = 11$) treatment. No change in leptin concentrations of patients with hypothyroidism, but a small significant decrease in the mean leptin level of patients with Graves' disease (10.4 ± 8.2 μg/l vs 8.2 ± 6.4 μg/l) was demonstrated. No correlation was found to thyroid function or presence or absence of thyroid autoantibodies in the patients, but in 66 healthy controls log-transformed serum leptin levels significantly correlated with serum TSH levels and, concomitantly, a negative correlation was observed between free T4/TSH ratios and log transformed leptin levels (75).

Sera *et al.* (81) studied 15 thyrotoxic patients under the influence of either beta adrenergic blockers alone (only 11 patients), or ATD +/− beta adrenergic blockers. No significant changes in serum leptin/percentage body fat (by BIA) were seen in hyperthyroidism during beta adrenergic blockade alone. They found a decrease in plasma leptin/percentage body fat during beta adrenergic blockade and normalization of thyroid function during ATD therapy. There was a further significant correlation between the change in leptin/percentage body fat and the change in free T4. After normalization of thyroid function leptin/percentage body fat did not change further when the beta adrenergic antagonist was withdrawn. The authors concluded that thyroid hormone increased serum leptin levels during suppression of beta adrenergic receptors in hyperthyroid patients. They also hypothesized that the thermogenic effect of thyroid hormone might be partly mediated through leptin secretion and they advocated taking into account the adrenergic receptors when the functional relationship between thyroid hormone and the leptin system is investigated in clinical studies. This has not been done in any of the other studies.

Their hypothesis on thermogenesis was supported by a recent demonstration of divergent serum leptin concentrations depending on whether free and bound leptin were measured instead of only total leptin; free leptin and total fat mass increased while bound leptin and REE decreased during correction of thyrotoxicosis, indicating that the free leptin fraction selectively represented the fat mass (19). This study might explain the conflicting results obtained when measuring total leptin in the majority of the studies of thyroid patients (Table 2).

As previously mentioned, one of the problems with the human studies is the fact that BMI may not reflect body fat (70) and the majority of the studies were based on such measurements. Two studies used measurement of body composition by DEXA scans, which allows better correlations to fat measures (51, 88), and found that although serum leptin levels increased during the 12-month ATD treatment of thyrotoxic patients compared with baseline and in accordance with what was found in rats (57, 58, 62), there was no relationship between changes in serum leptin and total fat mass.

Leptin and thyroid autoimmunity

Leptin is capable of modulating the immune response. Proinflammatory cytokines induce leptin production and leptin has a direct effect on the generation of an inflammatory response (94). Graves' disease and Hashimoto's thyroiditis are autoimmune diseases of the thyroid gland, which best fit a polygenic, multifactorial model of disease in which genetically susceptible individuals are exposed to a constitutional or environmental insult resulting in immune system activation. Autoantibodies directed against the TSH receptor are responsible for the hyperthyroidism of Graves' disease.

Seven human studies of the relationship between leptin and autoimmune thyroid disease included Graves' disease with or without TAO and Hashimoto's thyroiditis (Table 1) (51, 65, 69, 75, 81, 82, 86). One study demonstrated an increase in serum leptin before and after pulse therapy with methyl-prednisone but no significant changes were found when the Graves' patients with TAO were treated with oral methyl-prednisone indicating that acute immune suppression with methyl-prednisone may influence serum leptin while a more chronic suppression might not (82). Serum leptin levels were similar in Graves' patients with or without TAO. Based on the studies summarized in the previous section there is not enough evidence for a relationship between autoimmune thyroid disease and leptin. None of the studies found any correlation between serum leptin and thyroid autoantibodies, and four of the studies found evidence that thyroid function is more important than autoimmunity in determining serum leptin concentrations (78, 81, 84, 88).

Conclusions

As mentioned earlier, the results of the clinical studies of leptin and thyroid disease are very confusing. Different factors such as autoimmunity, leptin binding proteins, leptin pulsatility, fertility, gender, oestrogens, insulin, cortisol, $beta_3$-adrenergic agonists and thermogenesis have been taken into account, but even then, no explanations were found for the conflicting results (Table 2). However, it is likely that there is a relationship between leptin and the thyroid gland probably via an influence of leptin on the negative feedback regulation of thyroid hormones as

well as an influence on thermogenesis (Fig. 1). When influenced by insulin, leptin has been suggested to regulate the secretion of TRH in the hypothalamus. Peripherally, in the starved state in the absence of insulin, T3 inhibits leptin mRNA accumulation in adipocytes while the opposite is likely to be the case in the fed state in the presence of insulin (54, 59, 92). In addition, leptin itself can stimulate T3 production via an activation of T4 to T3 conversion in rats (35, 45), and increased T3 production, such as in thyrotoxicosis, stimulates heat production, UCP-3 expression in muscle tissue (44, 46) and beta$_3$-adrenergic receptors (58, 81, 94). An increase in these three factors inhibits leptin expression in fat tissue (6, 9, 44), which overall leads to an inverse relationship between leptin and T3 (44, 57, 58, 60–62) regulated both peripherally and centrally (Fig. 1) (6, 9). In contrast, the low level of T3 in hypothyroid patients may lead to decreased leptin expression, decreased conversion of T4 to T3, a decrease in beta$_3$-adrenergic receptors, and decreased heat production and UCP-3 in muscle tissue. The central regulation of thyroid hormones by leptin in hyper- or hypothyroidism is probably overruled by the regulation of the thyroid hormones themselves. The many factors influencing the leptin level and the thyroid hormones could explain the different results of the thyroid studies in patients (Table 1). In conclusion, several studies have suggested that thyroid hormones are possible important mediators of the effect of leptin on energy expenditure but further studies of the influence of thyroid function on the various forms of leptin (free, bound, total) are still required to gain more insight into the relationship between leptin and thyroid dysfunction.

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