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Thyroid hormones in the pathogenesis and treatment of obesity

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Abstract

Thyroid hormones (TH) are potent modulators of adaptive thermogenesis and can potentially contribute to development of obesity. The decrease of T_3 in association with reduction of calorie intake is centrally regulated via decreases in leptin and melanocortin concentrations and peripherally via a decrease in deiodinase activity, all aimed at protein and energy sparing. The use of TH in the treatment of obesity is hardly justified except in cases of elevated thyrotropin (TSH) with low/normal T_3 and T_4 and/or a low T_3 or T'_3/T_4 or a high TSH/T_3 ratio. TH treatment with small doses of T_3 can also be exceptionally applied in obese patients resistant to dietary therapy who are taking β-adrenergic blockers or with obesity developed after cessation of cigarette smoking and with hyperlipidemia and a concomitant high thryrotropin/T_3 ratio. Supplementation with Se^{2+} and Zn^{2+} may be tried along with more severe calorie restriction to prevent decline of T_3.

Keywords: Thyroid hormones; Obesity; Treatment; UCP (Uncoupling protein); Deiodinase; Thermogenesis

Obesity results when energy intake exceeds energy expenditure and even a modest, but sustained, reduction in energy dissipation is a predisposing factor for the disorder. The first publication indicating thyroid hormones (TH) to be involved in energy homeostasis was presented as early as in 1895 by Magnus Levy.

Thyroid hormones have been indicated to have at least a permissive role in adaptive thermogenesis by influencing several aspects of energy metabolism, such as substrate cycling, ion cycling and mitochondrial proton leaks (Hoch and Lipman, 1954; Maley and Lardy, 1955; Wu et al., 1999), and it is, therefore, believed that impaired thyroid function might contribute to the pathogenesis of obesity.

1. Food intake and diet-induced thermogenesis

Food intake is a potent regulator of adaptive thermogenesis. Starvation has been reported to reduce resting metabolic rate by as much as 40% (Blaxter, 1995). Even limited restriction of food intake associated with diet, even if aimed at maintaining only a 10% reduction in body weight, is associated with decreased energy expenditure. This is counterproductive during dieting and contributes to the so-called yo-yo effect and poor long-term prognosis for dietary treatment of obesity.

Feeding, on the other hand, is reported to increase energy expenditure, with both acute and chronic effects on metabolic rate. Feeding acutely increases metabolic rate by about 25–40% in humans and rodents, most probably due to the *thermic effect* of food (Sims and Danforth, 1987; Shibata and Bukowiecki, 1987).

Hyperphagia and overfeeding, even in a limited form (as one big meal), leads to increased activity of the sympathetic nervous system (SNS) and concomitant activation of monodeiodinases, responsible for deiodination of thyroxine (T_4) to triiodothyronine (T_3) and T_3 to diiodothyronine (T_2). Increased activity of the SNS and increased availability of T_3 and T_2 act synergistically to increase basal metabolic rate. Thermogenesis increases twofold after administration of norepinephrine and T_3 separately, but 20-fold when they are given together.

As mentioned above, long-term overfeeding leads (also after following periods with normal food intake) to increased energy expenditure, protecting against development of obesity and contributing to stability of body weight (Almeida et al., 1996).

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This is in harmony with the concept of so-called ''luxury consumption'' and also explains why only abnormal responses to overfeeding can contribute to the development of obesity.

2. Body weight in hypo- and hyperthyroidism

It is well established that food intake as well as the thermic effect of food is generally decreased in *hypothyroidism*. In contrast, a *hyperthyroid* state is associated with the increased amount of food eaten and increasing thermic effect of food. As a consequence of the above, body weight is on average *decreased* in *hyperthyroidism* by 15% (in comparison with the preceding euthyroid state) and an increase in body weight is generally treated as an important sign of successful therapy. In contrast, *hypothyroid patients* weigh on average *15–30% more* and lose weight during replacement therapy. It is remarkable, but well known, however, that 7% of patients still maintain their higher body weight after they reach a euthyroid state on adequate substitution therapy.

In contrast to euthyroid or hyperthyroid rats, hypothyroid rats develop obesity when fed a high-fat diet because of their inability to attain fat balance. Hypothyroid rats show an increase in the lipid gain/lipid intake ratio and a decrease in the protein gain/protein intake ratio. The increased body fat in hypothyroid rats is in line with the decrease in fatty acid oxidation and lower serum free fatty acids concentrations observed in hypothyroidism. In the absence of T_3, energy and lipid balance are not maintained despite an increased serum leptin concentration (Jossas et al., 2001). When applying T_3 treatment, oxygen consumption increases from the first day, while food intake increases after 1 week, indicating an indirect effect of T_3 on food intake, probably through progressive depletion of fat stores. It seems also probable that the inverse relationship between T_3 and leptin (leptin being high in the hypothyroid and low in the hyperthyroid state) rather reflects the changes in fat balance of body fat stores. Another possible route of action is by sensitising and amplifying the effects of adrenoceptors that suppress production of leptin in adipose tissue.

3. Target organs of TH thermogenic effects and possible involvement in development of obesity

A substantial fraction of the energy contained in food is dissipated as heat during metabolism, a phenomenon known as thermogenesis. Thermogenesis is, thus, a consequence of incomplete thermodynamic control. Two forms of thermogenesis have been distinguished, depending on the degree of adaptation to the environment: obligatory and facultative (adaptative).

This distinction may not be easy to delineate concerning the effects of TH, particularly in relation to obesity. For instance, during fasting or a very low-calorie diet (VLCD), a decline in thermogenesis reflects not only a decrease in facultative thermogenesis, but also a reduction of the basal metabolic rate. In the resting state, obligatory thermogenesis represents mostly the heat produced during resting energy expenditure, whereas in basal conditions, it is the heat produced by the basal metabolic rate.

Facultative thermogenesis is the portion of energy generated in the body dissipated as heat under physiological control. Nonshivering facultative thermogenesis is activated when additional heat production is needed to maintain body temperature, or in response to food intake; it is also called diet-induced thermogenesis. Afferent signals regarding body and ambient temperature and food intake converge in the nucleus tractus solitarius and later reach the hypothalamus where the information is integrated and from which efferent signals are sent to effector organs via the sympathetic nervous system.

A number of intracellular processes have been proposed to explain the calorigenic action of TH (obligatory thermogenesis), all of them acting in an additive manner. The overall thermogenic effect of TH, thus, involves the effects on Na^+/K^+ ATPase, the effect on the Na^+ and K^+ gradient across the membranes and on the Ca^{2+} gradient between the cytosol and sarcoplasmic reticulum, and the effects on mitochondrial oxidation. The last ones are partly long term, depending on the action of T_3 at the genomic level and partly short term, reflecting the direct action of TH, particularly diiodothyronine on mitochondria.

In hyperthyroidism, these effects are associated with an increased ''proton leak'' through the inner mitochondrial membrane, whereas the opposite occurs in hypothyroid mitochondria. The increased proton leak is due to an increase in the surface area of the mitochondrial membrane and to changes in phospholipid composition that render the membrane more permeable to protons (Harper et al., 1993). Although the proton leak may be treated as uncoupling of oxidative phosphorylation, the process is followed by an increase in ATP synthesis so that the overall coupling efficiency remains at the euthyroid level. It is also worth remembering that the calorigenic action of TH also includes so-called substrate cycles. These include endogenous fatty acid turnover (3–10% of the overall thermogenic effect), glycerol-3-phosphate NADH shuttle (6% of the thermogenic TH effect) and Ca^{2+} cycling. Particularly in contracting muscle, excess Ca^{2+} cycling can account for 40–50% of the increased energy expenditure in the transition from the hypothyroid to the euthyroid state (Leijendekker et al., 1987). Similarly, in skeletal muscle, particularly during exercise, the switch to the α-glycerophosphate dehydrogenase (α-GPD) shuttle may also contribute to heat production by capturing the NADH generated by peroxisomal oxidation of fatty acids.

It seems probable that the target organ for TH effects on adaptative thermogenesis in humans is skeletal muscle. Although adult humans have varying numbers of brown adipose cells dispersed in white fat depots, their quantitative

contribution is much lower than in rodents. On the other hand, *skeletal muscle* represents up to 40% of total body weight and is endowed with significant mitochondrial capacity — the *putative site of TH action*.

It has also been suggested that a low metabolic rate is a predictor for risk of development of obesity (Ravussin et al., 1998; Griffiths et al., 1990) and that variations in the concentration of T_3 contribute to the observed variances in energy expenditure (600 kJ/day) (Astrup et al., 1992).

In other words, the variations in metabolic rate and the differences in body weight between humans with similar food intake can be accounted for by differences in skeletal muscle energy expenditure. Energy expenditure is regulated synergistically by both SNS and TH. Adrenalin infusion, which causes a 25% increase in whole body energy expenditure, stimulates the activity of deiodinase as well as muscle oxygen consumption by as much as 90% (Simonsen et al., 1992).

Involvement of skeletal muscle mitochondria in TH-dependent adaptive thermogenesis is indirectly indicated by the observation of substantial erosion of muscle tissue after therapeutic administration of TH in an attempt to increase energy expenditure and lower body weight.

Many studies have pointed towards the thyroid hormones as major regulators of mitochondrial biogenesis and mitochondrial function in vivo. Mitochondrial gene expression is reduced in hypothyroid animals and stimulated upon administration of TH. Several genes encoded in the cell nucleus have TH response elements, indicating that these hormones affect the genes directly through thyroid hormone receptors. It is generally accepted that TH and their receptors can translocate into mitochondria to affect transcription patterns.

The oldest concept of the mode of TH action was uncoupling of oxidative phosphorylation. However, the idea was rather rapidly abandoned in view of the nonphysiological concentrations of TH needed to demonstrate this and the compelling evidence that ATP production is increased in hyperthyroidism and that, globally, the increase in O_2 uptake induced by in vivo administration of thyroid hormone is highly coupled (Sestoft, 1980).

Current knowledge warrants the conclusion that TH at least affects the transcription of respiratory enzymes. The prevailing hypothesis on the coordination of biosynthesis of respiratory enzyme subunits encoded by both nuclear and mitochondrial genes is that TH exert their action at the level of the nuclear genes, leading to secondary signals, probably proteins acting as mitochondrial transcription modulators. Until recently, it was believed that this action was mainly exerted by T_3, which is responsible for more long-term action of TH, while T_2 directly affects mitochondrial enzymes, thereby being responsible for the more immediate short-term TH action. However, it was recently found that the mitochondrial genome possesses sequences showing strong similarity to the nuclear Thyroid Hormones Response Elements (THREs). Furthermore, TH receptors and binding of those receptors to THREs were found in mitochondria and, finally, it was quite recently shown that even T_3, as well as T_2 (which exclusively acts on the mitochondrial level), can, to some extent, regulate concomitantly both mitochondrial and nuclear genes involved in oxidative phosphorylation via the same acceptor, i.e. THRE sites.

The action of TH, although more chronic and delayed than the action of β-adrenoceptor agonists, is coupled to β-adrenoceptor stimulation by its effect, resulting in a marked increase of expression of type I and II thyroxine deiodinase (Brown, 1992; O'Brien and Block, 1996). This is most probably mediated by the increase of cyclic AMP-responsive elements binding protein (CREB), a known uncoupling protein-1 (UCP-1) enhancer (Bartha et al., 2000). Types I and II deiodinase generate active ligands for the TH receptors. T_3, in turns, increases the expressions of PGC-1 (PPAR coactivator 1), a potent coactivator of both peroxisome proliferator-activated receptor (PPAR)-γ and nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) activating the promoters of many genes responsible for the mitochondrial electron transport (Puigserver et al., 1998).

PPAR-α and PPAR-γ are known regulators of uncoupling protein-2 and uncoupling protein-3 (UCP-2 and UCP-3).

The role of the PPAR system in brown fat development and UCP expression and transcriptional activation has been demonstrated in experiments with their ligands—thiazolidinedione — for PPAR-γ and fibrates for PPAR-α. These studies showed that peroxisome proliferator-activated α and γ (PPAR-α and -γ) are positive regulators of UCP-2 and UCP-3 and that they are reacting to nutritional manipulations in a tissue-specific manner (Brun et al., 1999; Aubert et al., 1997).

PPAR-γ may be increased in obesity Vidal-Puig et al. (1997) and has been shown to compete with TH receptors for heterodimerisation with RXR (retinoid x receptor) (Meier-Heusler et al., 1995). PPAR-γ coactivator, PGC-1, is supposed to play an important role in the pathophysiology of obesity (Puigserver et al., 1998; Esterbauer et al., 1999) and also serves as a coactivator of both the T_3 receptor and PPAR-γ/RXR heterodimers included in a system of UCP enhancers. PGC-1 has recently been described as an important mediator of both TH- and catecholamine-induced thermogenesis (Puigserver et al., 1998; Boss et al., 1999).

4. Thyroid hormones and uncoupling protein (UCP)-dependent thermogenesis in relation to obesity

Calorigenic effects of TH are divided into the short-term effect, occurring within 6 h and disappearing completely after 48 h, and the long-term effects, which are observed after 30 h and lasts up to 60 days. The short-term effect involves a direct interaction of T_2 with mitochondrial enzymes and is not attenuated by actinomycin D. Long-term effects are attributable to modulation of the cellularity of different tissues and involve interaction of T_3 with nuclear receptors (thyroid

hormones receptor α1–2 and thyroid hormones receptor β1–2), which bind to regulatory regions of genes (THRE-I and -II). T_3 receptor genes are located on chromosomes 17 and 3 and are expressed in almost all tissues. Thyroid hormone receptors function by binding to specific thyroid hormone-responsive sequences in promoters of target genes and by regulating transcription. Thyroid hormone receptors often form mechanisms that together determine the specificity and flexibility of the sequence recognition. Amino-terminal regions appear to modulate thyroid hormone receptor function in an isoform-dependent manner. Unliganded thyroid hormone receptor represses transcription through recruitment of a corepressor complex. Ligand binding alters the conformation of the thyroid hormone receptor in such a way as to release the corepressor complex and recruit a coactivator complex that includes multiple protein-associated factor (PCAF) and CREB-binding protein. Structural and sequence-specific rules for coregulator interaction are beginning to be understood as are aspects of the tissue specificity of hormone action. Moreover, knockout studies suggest that the products of two thyroid hormone receptor genes mediate distinct functions in vivo (Lazar and Zhang, 2000).

The clinical findings in hypo- and hyperthyroidism are the net results of the actions of products of a variety of genes whose expression is directly or indirectly regulated by T_3.

One of the important effects of T_3 is its influence on the family of UCP. UCP is a prototypical thermogenic molecule — a mitochondrial inner membrane protein that uncouples proton entry from ATP synthesis. The UCP family, UCP-1, UCP-2, and UCP-3, has clear uncoupling activity and is widely expressed in different tissues and contributes significantly to adaptative thermogenesis. In humans, apart from UCP-1 and UCP-2, UCP-3, expressed in deep white abdominal adipose tissue and in abdominal organs (colon, gall bladder) and muscles, is equally important.

The adrenergic stimulation of brown adipose tissue (BAT) deiodinase BAT-5′ DII is mediated by α_1 adrenoceptors and causes a large increase in BAT T_3 content. If 5′ DII is blocked by iopanoic acid or its activation is prevented with the α_1 adrenoceptor antagonist prazosin, T_4 is unable to restore the response of UCP to cold in hypothyroid rats (Bianco and Silva, 1987). Thyroid status seems to have a negative effect on sympathetic stimulation of BAT and other tissues. The reduction in UCP and the response of BAT to cold is observed in T_4-toxicosis and is most probably due to the reduction of 5′ DII activity and the decrease of sympathetic stimulation caused by T_3. This is in accordance with the generally accepted idea that an increase in obligatory thermogenesis is always associated with a compensatory reduction of facultative thermogenesis (Sundin, 1981).

Although the normal increase of UCP gene transcription in reaction to cold or norepinephrine is nearly totally abolished in hypothyroidism, it is still possible to demonstrate some UCP stimulation by norepinephrine even in the complete absence of TH. This observation seems to refute the concept that T_3 acts only as a permissive factor for norepinephrine stimulation of UCP. There is, on the other hand, no doubt regarding the synergism between T_3 and norepinephrine, exerted at the gene level (Bianco et al., 1988).

T_3 directly stimulates the transcription of the UCP gene acting via its receptor on the discrete sequence of the UCP gene, high upstream in the 5′ flanking region of the gene. The number of adrenoceptors β-1 is increased in the BAT of hypothyroid rats. It is assumed that the defect in norepinephrine signaling in the hypothyroid state is related to the increased sympathetic tone of BAT (Gripois and Valans, 1982).

The increased β-3 receptor expression in BAT is reversed by administration of T_3 within 24 h in analogy to the situation in thyrotoxicosis where β-3 adrenoceptors virtually disappear as a part of a compensatory mechanism preventing overstimulation of thermogenesis (Rubio et al., 1995). In addition to the interactions taking place in the periphery, interactions at the central level may also contribute to thermoregulation. Hypothyroidism is associated with increased sympathetic activity as reflected by increased plasma norepinephrine concentration and urinary excretion, the opposite being observed in hyperthyroidism. In the last case TH, by increasing obligatory thermogenesis reduces the need for facultative thermogenesis.

As mentioned earlier, the function of the thyroid gland is to produce the thyroid hormones T_3 and T_4, which regulate gene transcription throughout the body (Hollenberg, 1998). Although the thermogenesis exerted in mitochondria by UCP activation is mainly dependent on adrenergic stimulation, recent studies indicate that there is an absolute need of TH for the adrenergic stimulation of UCP, because in hypothyroid conditions, basal and adrenergically induced UCP expression is absent.

UCP-3 levels are decreased threefold in hypothyroidism and increased sixfold in hyperthyroidism. UCP-3 RNA levels are regulated by hormonal and dietary manipulations, being decreased during starvation, and increased during overfeeding after administration of T_3, leptin, and β-3 adrenoceptors agonists.

The other mechanism of uncoupling of oxidative phosphorylation by fatty acids is also facilitated by SNS and T_3, directly through the SNS and T_3 effect on lipolysis (UCP activity is steeply modified by the increasing concentration of fatty acids) and indirectly by an effect of T_3 on blood flow in adipose tissue. T_3 treatment increases the maximal lipolytic response to β-3 and β-1 adrenoceptor agonists by 234% and 260%, respectively (Germack et al., 2000). Blood flow influences binding of fatty acids to fatty acid binding protein (FABP) and their consecutive outflow and oxidation in muscles. Responses to both norephinephrine and T_3 occur at transcriptional level and the protein synthesis is not necessary to obtain the thermogenic effect.

The role of T_3 in the stabilisation of the m-RNA transcripts is of major importance. The effects of T_3 are exerted at multiple levels at the UCP promotor, contributing to the

stabilisation of the transcripts and at other levels such as the modulation of the β-adrenoceptor population (Hernandez and Obregon, 2000). The amplifying effect of adrenergic stimulation of UCP_1 was mostly investigated in rodents' BAT. The other members of the UCP family, UCP-2 and UCP-3, are more widely expressed in humans, but show similar characteristics.

Furthermore, the syntenic region of mouse chromosome J is coincident with quantitative loci for obesity and type 2 diabetes mellitus (Warden et al., 1995). In comparison with lean controls, UCP-2 m-RNA abundance was reduced in intraperitoneal adipose tissue of morbidly obese subjects and UCP-2 m-RNA expression remained low in postobese subjects studied before and after weight reduction (Oberkofler et al., 1998).

In obese patients, prolonged hypocaloric diet downregulates UCP-3 m-RNA expression in muscle (Esterbauer et al., 1999) and this most probably contributes to the reduced energy expenditure that has been reported after prolonged fasting (Leibel et al., 1995). However, mice with targeted disruption of the UCP-3 gene are not obese. Indirect evidence suggests anyhow that this protein contributes to the control of energy expenditure in humans and its gene is transcribed from tissue-specific promoters in humans, but not in rodents (Esterbauer et al., 2000).

Both the human UCP-2 and UCP-3 genes have been mapped to chromosome 11q13. Linkage analysis in pedigrees of the Quebec family study provided strong evidence for an association of this chromosomal region with resting metabolic rate, body mass index and body fat in adult humans (Bouchard et al., 1997).

Among the common polymorphisms identified in the coding sequence of UCP-3, the majority have no obvious effect on biological phenotypes such as body composition, resting metabolic rate, or biochemical characteristics of mitochondrial function. Furthermore, there is no direct evidence to suggest that UCP-3 uncouples muscle mitochondria. Alternatively, its role may lie in the integration of carbohydrate and fat metabolism (Samec et al., 1999), the mutations of UCP-3 being associated with impairment of fat oxidation (Argyropoulos et al., 1998; Simoneau et al., 1998). In a recent study, UCP-3 c-55t promoter polymorphism was found to be negatively associated with body mass index in a Caucasian population, but the mutations in the coding sequence of UCP-3 did not show any causal relationship to the presence of obesity (Halsall et al., 2001; Argyropoulos et al., 1998; Simoneau et al., 1998). The authors suggest an analogy to Pima Indians, where the presence of the c-55t allele is associated with a decrease of UCP message leading to increased fat oxidation and reduced body mass index (Schrauwen et al., 1999).

In view of the previous observation that the proton leak may be responsible for up to 52% of the energy expenditure in resting rat skeletal muscle (Rolfe and Briwn, 1997), it is interesting to note the observation reported by Buemann et al. (2001) on higher metabolic efficiency (increased physical activity, but normal 24-h energy expenditure and exercise efficiency at 40% VO_2 max) in val/val-55 UCP-2 subjects showing that UCP genes may affect not only metabolic rate, but also influence energy costs of exercise. It remains to be seen if the previously described (see above) effect of TH on muscle contraction is directly or indirectly associated with the possible influence on UCP transcription in muscle. As it was reported that subjects with val/ala-55 polymorphism also show increased physical activity (Astrup et al., 1999), it is probable that the associated activation of the sympathetic nervous system would stimulate deiodinase, which would produce more T_3, a contribution of TH being very likely here. Similarly, physical training leading to higher utilisation of fatty acids, as well as a high-fat diet, lead to higher expression of UCP-2 and UCP-3. It was also shown that this change is correlated to the relative percentage of more oxidative type IIA muscle fiber (Schrauwen et al., 2001). It has been previously shown that the percentage of this type of IIA and type I oxidative muscle fibers as well as muscle enzymatic oxidative capacity are inversely correlated with body mass index, respiratory quotient (RQ), physical fitness, and different measures of metabolic syndrome (Krotkiewski, 1994). A high percentage of highly glycolytic type IIB muscle fibers is characteristic both of obesity and hypothyroidism. The only exception is when energy restriction leads to an increase in UCP expression (Millet et al., 1997) and to a relative increase in the percentage of type IIA muscle fibers as well as an increase in free fatty acids concentration and availability, but to a concomitant decrease in T_3 (Krotkiewski et al., 1984). The possible explanation is that THs are not contributing to the increase in facultative thermogenesis when obligatory thermogenesis is lowered, as in the case of calorie restriction.

Also, Trp64Arg polymorphism of the β-3 adrenoceptor gene has been found to be associated with a lower metabolic rate and abdominal obesity. Polymorphism A to G of the promotor region 3826 of UCP, when combined with the polymorphism of β-3 adrenoeceptor, show an additive effect on weight gain and reduction of metabolic rate (Valve et al., 1998). The role of TH themselves and possible polymorphisms or mutations of genes coding their receptors seems very probable, but, so far, not yet adequately described in connection with obesity.

5. Obesity/overweight in different states of thyroid dysfunction

Since thyroid hormones enhance the basal metabolic rate, which is a predictor of the risk of development of obesity, it is generally held that altered thyroid function contributes to obesity. The largest study addressing this issue was conducted by Rimm et al (1975), who reported a significant association of obesity with hypothyroidism when analysing a population of 73,532 subjects.

On the other hand, overweight is also common (15–17%) among *post-hyperthyroid* patients (Jansson et al., 1993) after successful treatment of hyperthyroidism by thyroidectomy and/or radio-iodine treatment in spite of adequate substitution with thyroxine (T_4). This percentage corresponds, in fact, to the percentage of overweight people among hypothyroid patients on replacement therapy with T_4.

6. Thyroid hormone levels and thyroid and pituitary gland function in overweight and obese subjects

Thyroid hormone levels have been found to rise in animals models of increased calorie intake (Almeida et al., 1996) and it is also known that the (TSH) T_4 and T_3 levels are all frequently found to be higher in obese than in lean subjects.

Although obese subjects were reported to display higher levels of T_4 than lean controls, the ratio of T_4 to thyrotropin (TSH) and T_3 to T_4 is lower in obese than in nonobese individuals. This is consistent with the concept of lower activity of deiodinase converting in the periphery T_4 to T_3. Impaired peripheral TH metabolism, i.e. a lower deiodination rate, was also reported from kinetic studies in obese Zucker rats and reduced deiodinase activities have been implicated in lower conversion rates to T_3 in various animal models of obesity (Katzeff and Selgrad, 1993; Mc Intosh et al., 1989).

These changes are most probably adaptative in the majority of overweight patients and secondary to overfeeding and are eliminated after weight reduction.

Furthermore, it is worth emphasising that the plasma concentration of T_3, the main active TH, is not necessarily a good indicator of T_3 concentration in different tissues and organs (Hollingsworth et al., 1970). In other words, in spite of normal T_3 values, there could exist a hypometabolic state characteristic of hypothyroidism, in particular organs or tissues, like adipose tissue. Such a concept is supported by the observation that the activity of deiodinase varies in different regions of adipose tissue along with locally varying lipolytic activity of adipocytes (Nauman et al., 1989).

Particularly the concentrations of TH are regulated quite differently in the brain tissue, with a dramatic decrease in tissue levels of T_4, unchanged levels of T_3 and deiodination to 3.3′ -T_2 and 3.5′ -T_2 diiodothyronines after 12-h fasting or calorie-reduced diet for 14 days. In contrast to other tissues, T_3 is not taken up directly from the blood, but derives from the intracellular deiodination of T_4 by 5′ DII and 5′ DIII deiodinases (Eravci et al., 2000).

The same probably applies to the syndrome of subclinical hypothyroidism contributing to the atherogenic pattern of lipoproteins in the circulation (Wiseman et al., 1993; Arem and Patsch, 1990; Steinberg, 1968) showing high similarity in lipid disturbances to that described as characteristic of obesity (Tchernof and Després, 1998). Subclinical hypothyroidism is most often defined as a condition with normal T_4 and T_3 values and slightly increased TSH levels. It is in this context remarkable that TSH concentrations are often elevated in obesity and clearly negatively correlated to resting energy expenditure. Furthermore, both the thermic effect of glucose and resting energy expenditure respond to very small doses of T_4 or T_3 (Al-Abadni et al., 1997) and this may explain the possible association between subclinical hypothyroidism and overweight.

The same line of reasoning is also confirmed by observation of the beneficial effect of small doses of T_3 on obesity-associated lipid abnormalities (Krotkiewski et al., 1997).

Furthermore, it is not improbable that sensitivity to TH can vary depending on polymorphisms of different parts of enhancers, coactivators, corepressors, and other factors involved in the transcription processes induced by TH (Weiss et al., 1999). Their concentrations (as in the case of PPAR-γ) also decrease with weight reduction, explaining the associated reduced sensitivity to TH during low-calorie diet (LCD) (Vidal-Puig et al., 1997). To explain the variability in energy expenditure and risk of development of obesity, we can assume a variety of inherited differences in interactions of TH receptors during gene transcription with corepressors and coactivators (Privalski and Yoh, 2000).

This is also exemplified by the observation that two corepressors—nuclear receptor corepressor (NCOR) and silencing mediator of retinoic and TH receptors (SMRT)—have been shown to bind to the hinge region of the TH receptors (particularly thyroid receptor β-2) in the absence of ligand (Oberste-Berghaus et al., 2000).

Taken together, the hormone profile in obese subjects is consistent with a reduced function of the thyroid gland, but is reminiscent of pituitary–thyroid hormone resistance and the varying degree of secondary impairment of TH peripheral metabolism. TSH secretion abnormalities may also be secondary, as indicated by findings that nutritional factors such as protein intake or postabsorptive protein oxidation can modulate the concentration of TSH. Therefore, the most probable explanation for the different TSH pattern in obesity is the effect of leptin and other neuropeptides on TSH-releasing hormone (TRH) (Buscemi et al., 1997).

7. Thyroid axis and central neuropeptides during starvation and very low-calorie diet treatment

Thyroid hormone metabolism in central nervous system (CNS) is subject to a highly specific regulatory mechanism that differs substantially from that described in other tissues such as liver, kidney or adipose tissue. In these latter organs, most of the active iodothyronine compound T_3 is taken directly from the blood, whereas the T_3 supply of the brain depends mainly on cellular uptake and intracellular deiodination of T_4 by mechanisms different from those described in the liver or kidney. While in peripheral tissues 5-DI deiodi-

nase catalyses deiodination of both the phenolic and thyroid ring of T_4 and T_3, in the CNS two other isoenzymes catalyse the production and metabolism of T_3. Type 5′ D-II catalyses 5 deiodination of T_4 and r-T_3 to T_3 and T_2, respectively, and type 5′ D-III catalyses thyroid ring deiodination of T_4 to r-T_3 and that of T_3 to 3,3′ T_2, thereby inactivating T_3. In contrast to other tissues, both 12 h fasting and 14 days food deprivation is associated with a dramatic decrease of T_4 concentration in the CNS. Concomitantly, there is reduced activity of 5′ D-II and unchanged 5D-III deiodinase and increased deiodination to 3,3′ and 3,5′ T_2 exerting their transcriptional effects directly on mitochondria. It was, therefore, suggested that the deiodinase activities do not ensure a stable tissue concentration of T_3, indicating the presence of additional pathways of iodothyronine metabolism in the CNS. Furthermore, whereas the thyroid receptor isoforms thyroid receptor α-1 and thyroid receptor β-1 are expressed ubiquitously, thyroid receptor β-2 is expressed almost exclusively in the hypothalamus and pituitary and probably plays an important role in central adaptation of the thyroid axis to starvation.

Only TRH produced in the paraventricular nucleus (in contrast to TRH generated in other parts of the brain) influences the secretion of TSH. Starvation and very low-calorie diet leads to a selective decrease of TRH expression in the paraventricular nucleus, which, in turn, results in a decreased production of TSH.

As it was mentioned before and recently reviewed (Krotkiewski, 2000), prolonged fasting has profound effects

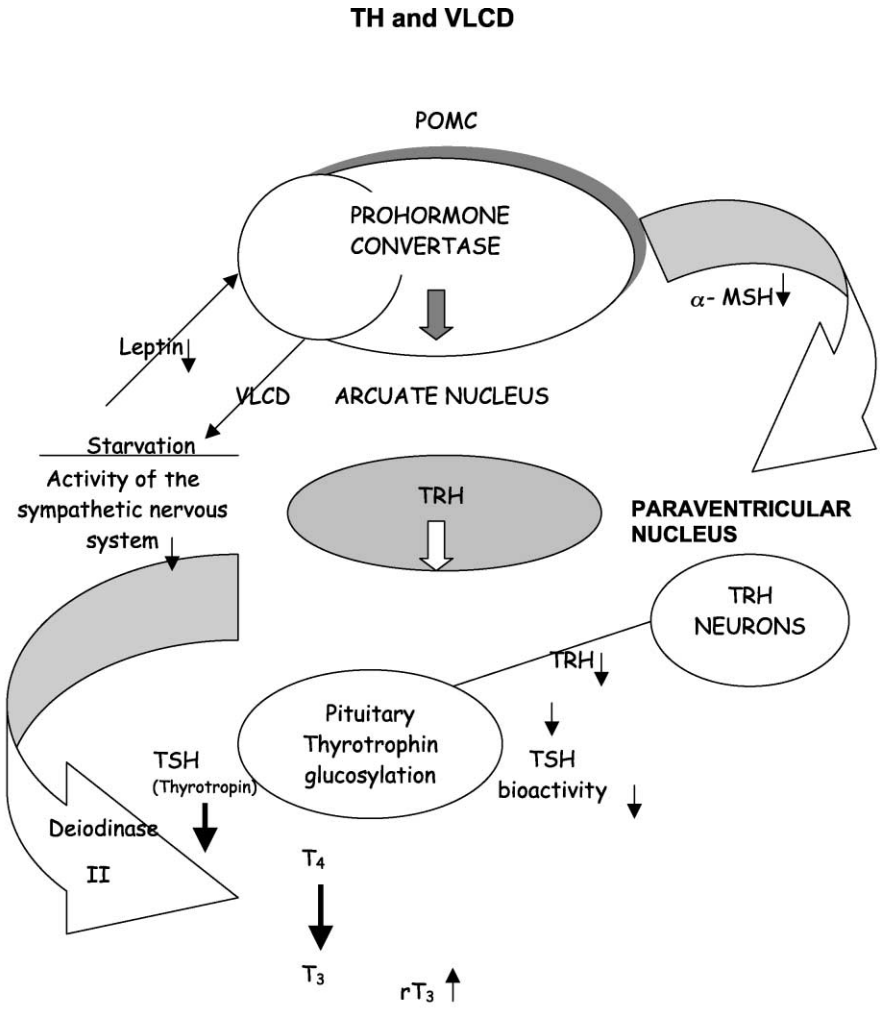

Fig. 1. Reduction of energy intake (very low calorie diet) results in a sharp decrease of leptin production. The decrease of intracerebral leptin concentration leads to a secondary decrease of melanocortin (α-MSH) production from proopiomelanocortin in the arcuate nucleus and activation of MCR-4 (melanocortin receptor-4) in the paraventricular nucleus. Both processes cause decreased firing of TRH neurons and decreased TRH expression in these hypothalamic nuclei. The decreased TRH production leads to decreased expression of TSH in the pituitary as well as its decreased glucosylation leading to lower TSH bioactivity. As a result of the central adaptation to starvation or decreased energy supply (very low calorie diet), production of TH decreases, leading to the decrease of obligatory and facultative (adaptative) thermogenesis and decrease of protein breakdown. On the periphery, decreased activity of the sympathetic nervous systems results in decreased activity of deiodinase, which further diminishes the concentration of the active thyroid hormone T_3. Both central and peripheral effects of starvation seem to act through mechanisms developed over a long period of evolution aimed at protein sparring to increase chances of survival periods of starvation and famine.

on the hypothalamic–pituitary–thyroid axis in rats, manifested by low plasma T_3, T_4, free T_3 and free T_4, and low or normal levels of TSH. In addition, fasting results in a reduced content of TSH and β-TSH messenger RNA (m-RNA) in the anterior pituitary, reduced prothyrotropin-releasing hormone (pro-TRH) m-RNA in the hypothalamic paraventricular nucleus, and a decrease in the concentration of TRH in hypophyseal portal blood. In altered states of thyroid function (hypothyroidism and hyperthyroidism), the biosyntesis of TRH neurons changes inversely with plasma concentrations of T_4 and T_3. During fasting, therefore, when circulating levels of thyroid hormone are low, the reduction of prothyrotropin-releasing hormone (pro-TRH) gene expression in the paraventricular nucleus and decreased concentration of TRH in the portal capillary system seem paradoxical, suggesting that the set point for thyroid hormone feedback regulation of TRH biosynthesis n the paraventricular nucleus is altered. Systemic administration of leptin blunts the fall in T_4 levels during fasting and blunts changes in the adrenal and gonadal axes. Because leptin levels are normally suppressed by fasting, it has been proposed that falling leptin levels provide an important signal that coordinates a number of endocrine responses to starvation including decreased thyroid thermogenesis, increased stress steroids, and inhibition of reproductive function.

Starvation influences the rate of glycosylation of newly synthesised TSH, which becomes altered and shows reduced bioactivity (Weintraub et al., 1989). The TSH decreases and its lower bioactivity leads to decreased levels of T_4 and T_3. The decreased activity of the thyroid axis together with lower activity of the sympathetic nervous system is a necessary protective mechanism for survival in famine and starvation (Ahima et al., 1996). It is believed that the acute decrease of leptin associated with starvation is responsible for the drop in TRH paraventricular nucleus production. In the rat, leptin prevented fasting-induced suppression of pro-TRH m-RNA in the paraventricular nucleus (Legradi et al., 1997). In other words, leptin can reset the sensitivity of hypophysiotropic neurons to the feedback effects of thyroid hormones. The hypothalamic arcuate nucleus mediates the responses of the thyroid axis to fasting by altering the sensitivity of prothyrotropin-releasing hormone (pro-TRH) gene expression in the paraventricular nucleus to feedback regulation by thyroid hormone.

The leptin effect is most probably mediated by the melanocortin pathway, as alpha melanocyte-stimulating hormone (MSH), which secondary to leptin falls during fasting, is also known to prevent the fasting-induced suppression of Pro-TRH gene expression when given intracerebro-ventricularly (300 mg every 6 h for 3 days during fasting) and also increase circulating levels of free thyroxine 2.5-fold over the levels in fasted controls (Fekete et al., 2000) (see Fig. 1).

It is still not known whether leptin or melanocortin can influence the thyroid axis during physiological conditions and whether leptin insensitivity and its increased concentration in serum, associated with obesity, can have any influence on the TRH–TSH–thyroid axis. The concentration of leptin in the spinal fluid (SF) is anyhow positively correlated to the serum concentration of T_4 before very low-calorie diet treatment of obese patients and the subsequent decrease of leptin concentration in SF is correlated to the decrease in concentration of TH (Krotkiewski and Erlandsson-Arbrechtsson, 2000).

8. Thyroid hormones and the protein metabolism during very low-calorie diet

The usual presentation of thyroid physiology and practice in treatment does not stress dynamic changes in hormone levels. As was mentioned before, thyroid hormone levels are subject to major physiological regulation during the transition from the fed to the starvation state. The benefit of the suppression of the T_3 level during starvation is survival from periods of famine. Starvation leads to suppression of TRH expression in the paraventricular nucleus although TRH continues to be normally expressed in the other parts of the central nervous system not engaged in the regulation of the pituitary (Blake et al., 1991).

The adaptive changes in thyroid hormone production and metabolism have been best described in connection with dietary treatment by very low-calorie diet.

A very low-calorie diet has repeatedly been reported to be associated with a decreased concentration of T_3. There is a parallel increase in the concentration of metabolically less active triiodothyronine r T_3 and decrease in activity of types I and II deiodinase. A number of previous studies have addressed the hypothesis that the reduction in TH level during fasting may be aimed to mediate protein sparing. To ameliorate the decrease of basal metabolic rate associated with very low-calorie diets, several investigations (Carter et al., 1975) have administered supraphysiological dose of T_3 (120–150 μg/day) and concluded that T_3 plays a role in modulating protein, fat and glucose metabolism during starvation.

Other groups have administered T_3 to fasted individuals at the onset of the fast (Gardner et al., 1979). Normal volunteers fasted for 80 h with or without T_3 supplementation (5 μg every 3 h) and levels of serum T_3, rT_3, T_4, and TSH were measured as well as urinary urea nitrogen. Although the method of nitrogen measurement has recently been criticised (Kreitzman and Beeson, 2001) when the usual decrease in T_3 was prevented by the administration of synthetic T_3, urinary urea nitrogen excretion increased by 9.1%.

Vignati et al. (1978) reported that administration of T_3 during a 20-day fast resulted in restoration of serum T_3 to postabsorptive levels and increased urinary urea nitrogen and ammonia excretion. Increased release of glutamine from muscle probably accounted for most of the excess urinary

nitrogen losses. A 21% increase in urinary 3-methylhistidine excretion was interpreted as indicating increased muscle proteolysis. These findings were also considered to confirm the view that the fall of T_3 during fasting is a protective mechanism preventing excessive erosion of muscles.

The relative proportion of T_4 converted to T_3 and to reverse T_3 varies reciprocally in various conditions. T_3 levels decrease and reverse T_3 levels increase during fasting and corticosteroid administration, and enhance illness which associated with inactivation of deiodinases types I and II, and, eventually, increased activity of deiodinase type III. It has been suggested that such reciprocal alterations could represent a homeostatic mechanism to conserve energy or decrease catabolism. As it was mentioned previously, T_2 acts exclusively at the mitochondrial genome and may theoretically exert thermogenetic effect without augmenting protein catabolism.

Nair et al. (1989) supplemented obese individuals with 20 μg T_3 every 8 h for 1 week of fasting, the first week remaining without T_3 administration. Before this 3-week period, a supraphysiological dose of T_3 was given for better control of the subsequent response to T_3 administration during the actual study. T_3-treated obese patients were compared with untreated obese subjects on the same dietary treatment. During the fast, the administration of T_3 increased serum T_3 to a level similar to prefasting levels. However, these levels were twice as to those of the controls at any time during the 3-week study. Leucine kinetics did not change with T_3 treatment, indicating that T_3 does not mediate protein sparing after adaptation to fasting.

Byerley and Herber (1996) supplemented their obese patients with T_3 after 7 days of fasting to restore serum T_3 levels to normal prefasting levels to investigate the short-term effects of T_3 during very low-calorie diet. Both metabolic rate and CO_2 production decreased as expected with fasting and did not increase after T_3. Hepatic glucose appearance rates fell with fasting and increased significantly during T_3 supplementation, though not to prefasting levels. Urinary urea nitrogen excretion decreased significantly with fasting and decreased further after T_3 supplementation. As the leucine, but not the lysine, appearance rate decreased after T_3, it was concluded that the fall of T_3 rather than decreasing protein breakdown initiated the fall in hepatic glucose production. The results of this study seem also to indicate that T_3 supplementation dos not affect protein metabolism once the body has shifted from glycosysis to lipolysis to spare protein.

9. Possible effects of diiodothyronines

The rise in reverse T_3 may, to some extent, reflect a decrease in its metabolic clearance during fasting (Burman et al., 1979b). Administration of T_3 in a dose of 60 μg/day increased muscle protein catabolism during 6 days by about 72 g of muscle protein/day, while the administration of 240 μg/day of r-T_3 did not change muscle catabolism above the level observed in untreated patients. Thus, it could be assumed that r-T_3 itself and its deiodination product-T_2 would not affect protein metabolism in a way similar to T_3. Furthermore, r-T_3 was found not to influence type II deiodinase and conversion of T_4 to T_3 or the response of TSH to TRH. However, conclusive observations on the in vivo effects of r-T_3 and/or T_2 on energy expenditure and thermogenesis and their possible clinical applications are still lacking in spite of clear in vitro evidence (Papavasiliou et al., 1977) of their high resemblance to several TH effects.

10. The possible effect of TH on body weight and metabolic rate during fasting

Although some studies have reported that T_3 administration increases the rate of weight loss during fasting or semistarvation (Pasquali et al., 1984; Rozen et al., 1986; Koppeschaar et al., 1983), this is not generally confirmed. Moore (1980) and Koppeschaar et al. (1983) even reported a negative correlation between the change in serum T_3 caused by T_3 supplementation and the amount of weight lost in 10 subjects who consumed 200 kcal/day for 28 days and 50 μg T_3 three times a day during the last 14 days. A similar negative correlation was reported by Byerley (1996) with "physiological" doses of T_3 . These findings are in accordance with the lack of correlation between basal metabolic rate and serum T_3 reported by Krotkiewski et al. (1981) and Yang and Van Itallie (1984).

In a frequently quoted trial, Moore et al. (1980) reported that administration of 60 μg of T_3 daily was beneficial as regards the weight loss response, but was associated with an increase in circulating T_3 concentration; a negative correlation between final weight loss and serum T_3 was interpreted as indicating peripheral resistance to thyroid hormone action.

It has been shown that rats react with a much smaller increase in oxygen consumption when treated with triiodothyronine (T_3) during starvation than prior to starvation (Wimpfheimer et al., 1979). This insensitivity to T_3 during starvation in rats may also have its counterpart in obese patients on long-term calorie restriction, who may require much higher doses of T_3 before showing demonstrable increases of oxygen consumption (Bray et al., 1971; Carter et al., 1975; Hollingsworth et al., 1970; Meinders, 1981; Moore et al., 1980). The reduced conversion of T_4 to T_3 during fasting is associated with a proportional decrease in nuclear T_3 receptor (Moore et al., 1981). Furthermore, there are many reports of clinical tolerance to large doses of T_3 in obese patients (Danowski et al., 1967; Gelvin et al., 1978; Hollingsworth et al., 1970; Moore et al., 1980).

Although diet-induced thermogenesis is particularly apparent during ingestion of diets low in proteins (with a decrease in metabolic efficiency and ability to store ingested calories by as much as 40% in rodents) (Kevonian et al.,

1984; Rothwell and Stock, 1987), there is a strong correlation between carbohydrates and thyroid hormones. Burman et al. (1979a) fasted seven obese women for 7 days, then administered 50 g carbohydrate while continuing the fast for 5 more days, and found that dietary carbohydrate significantly increased serum T_3, decreased rT_3, and had no effect on serum T_4. Similarly, during long-term overfeeding, serum T_3 was increased when carbohydrates were isocalorically substituted for fat in the diet (Danforth et al., 1979).

The changes in plasma glucose parallel changes in serum T_3. During severe calorie restriction and prolonged fasting, plasma glucose concentration and serum T_3 decreases parallel. In contrast, increasing serum T_3, by treatment during fasting, significantly increases the plasma glucose concentration through the increase in hepatic glucose production (Nair et al., 1989).

11. Evidence for therapeutic benefits of giving thyroid medication in association with moderate calorie restriction

Although the practice of giving thyroid medications to obese patient has been common for more than half a century, few controlled therapeutic studies have been done (for review, see Krotkiewski, 2000).

Aldersberg and Mayer (1949) and Edwards and Swyer (1950) found no improvement in weight loss in obese outpatients when doses of 60–100 μg/day of desiccated thyroid were supplemented with diet. Sabeh et al. (1967), on the other hand, reported that desiccated thyroid given to starving patients accelerated weight loss by 40%. Corman and Alexander (1965) reported that obese patients tolerated 200–300 μg L-triiodothyronine a day and that weight loss was only 0.5 to 2 lb/month on an unrestricted diet.

Danowski et al. (1967) suggested that pharmacological doses of desiccated thyroid with partial caloric restriction might enhance weight loss in some patients, but the data were not more convincing than their previous studies of thyroid administration during starvation.

Hollingsworth et al. (1970) reported a greater mean weight loss (29.1 lb) in the T_3-treated patients (75 μg three times a day) compared with placebo (17.2 lb) after 8 weeks on an 800 kcal diet, with no significant difference after 12 and 24 weeks. The authors reported a higher pulse rate and tachycardia and even atrial fibrillation in one of the participants, but not the increase of systolic blood pressure described by Danowski et al. (1964).

Later attempts to eliminate these side effects by simultaneous administration of β-adrenergic blockers did not prevent the protein breakdown and excessive decrease of lean body mass.

In summary, although administration of TH results in *increased weight loss* and basal metabolic rate, most of the studies used supraphysiological doses of T_3, varying from 150 to 2000 μg T_3/day. High doses of T_3 caused several side effects, such as serious cardiac problems, muscle weakness, and excessive erosion of lean body mass (see also the review by Krotkiewski, 2000).

Recently, much smaller doses of T_3, varying between 5 and 20 μg, have been used as a supplement to low-calorie diet (and/or very low-calorie diet). The aim of supplementation was rather prevention of hypoglycemia during a very low-calorie diet (Pasquali et al., 1984) or amelioration of hyperlipemia, increase of the concentration of sex hormone-binding globulin (SHBG), and improvement of the hormonal profile without major influence on either the rate of body weight loss or basal metabolic rate during a low-calorie diet (Krotkiewski et al., 1997).

It is important to note that the lowering of T_3 associated with low-calorie diet fasting and a very low-calorie diet can be partly prevented by supplementation with zinc and selenium due to their ability to increase (by 67% and 47%, respectively) deiodinase activity (Kralik et al., 1996).

Supplementation with potassium is also recommended to prevent the TH-induced depletion of potassium in muscles, and supplementation with calcium to prevent the TH-induced osteopenia (Uzzan et al., 1996).

12. In summary

THs belong to the oldest arsenal of anti-obesity preparations. Desiccated thyroid, thyroglobulin, and the colloid protein of the thyroid gland were very popular during the 1940s. They were used together with diuretics during the 1950s and together with amphetamine and amphetamine derivatives during the 1960s. TH therapy continued later with synthetic T_4 and, more recently, with T_3 and even experimentally with rT_3. Thyroid hormone is one of the most commonly prescribed medications in Western countries. In the USA, synthroid (L-thyroxin, Boots Paracy) was ranked fifth among new and repeat prescriptions in the national prescription audit.

In spite of still being commonly in used, TH can hardly be recommended as a safe adjunct in the treatment of obesity.

Although TH by different mechanisms act synergistically with the sympathetic nervous system as thermogenetic agents, they have several other effects that are potentially harmful to treated obese patients. The main adverse effect of TH is an accelerated breakdown of proteins and influence on the cardiovascular system.

The decrease of the concentration of TH during fasting and very low-calorie diet is an expression of adaptation to starvation conditions to ameliorate the excessive erosion of muscle tissue. The diminution of the concentration of the main active thyroid hormone T_3 and the reduced sensitivity of its receptor contribute to the decrease of metabolic rate, contributing to the poor long-term prognosis for the treatment of obesity and so-called yo-yo effect. The decrease of the pituitary–thyroid axis and TSH activity during starvation is

also a consequence of a dramatic decrease in leptin concentration and subsequent decrease in production of melanocortin, leading to a decreased synthesis of TRH and decreased sensitivity of TSH.

Attempts to increase the rate of energy expenditure were either unsuccessful, when supplementation with TH was done with low doses, or associated with side effects, when TH were given in supraphysiological doses. Providing the administration of TH is motivated, it seems reasonable to try treatment with small doses of T_3 rather than T_4. The administration of r-T_3 and/or T_2, although possibly safer by not causing protein breakdown, should first be clinically verified in terms of effectiveness, particularly with respect to eventual acceleration of body weight loss and increased thermogenesis, before it can be recommended as an alternative treatment modality.

Administration of TH, with some reservations, can be taken into the arsenal of possible remedies used in the treatment of obesity and overweigh, in case of:

1. Subclinical hypothyroidism with elevated TSH, but low T_3 and T_4 and some degree of hyperlipemia;
2. Adequately substituted hypothyroidism with concomitant overweight persistent and resistant to dietary therapy;
3. Hyperthyroidism treated by thyroidectomy or radioiodine in patients with adequate substitution, but persisting overweight resistant to standard dietary treatments;
4. Other cases where some degree of peripheral resistance to TH, for instance, due to decrease of deiodinase activity, can be suspected;
5. Patients receiving β-adrenoceptor blockers showing verified resistance to dietary treatment and a low T_3/T_4 ratio.

Patients showing a tendency to weight increase after cessation of cigarette smoking in spite of unchanged dietary and physical exercise habits with demonstrated low T_3/T_4 ratio.

In patients with a low T_3 and/or low T_3/T_4 ratio during very low-calorie diet or prolonged low-calorie diet, it seems worth to try supplementation with Se^{2+} and Z^{2+} to increase deiodinase activity before considering supplementation with TH.

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