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REVIEW

Thyroid hormones as molecular determinants of thermogenesis

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Abstract

Thyroid hormones (TH) are major modulators of energy metabolism and thermogenesis. It is generally believed that 3,5,3′-triiodo-L-thyronine (T3) is the only active form of TH, and that most of its effects are mediated by nuclear T3 receptors, which chiefly affect the transcription of target genes. Some of these genes encode for the proteins involved in energy metabolism. However, a growing volume of evidence now indicates that other iodothyronines may be biologically active. Several mechanisms have been proposed to explain the calorigenic effect of TH, but none has received universal acceptance. Cold acclimation/exposure and altered nutritional status are physiological conditions in which a modulation of energy expenditure is particularly important. TH seem to be deeply involved in this modulation, and this article will review some aspects of their possible influence in these conditions.

Keywords brown adipose tissue, cold, energy expenditure, iodothyronine, mitochondria, nutrition, thermogenesis, thyroid hormone, uncoupling protein.

Basic notions about thyroid hormones

The thyroid gland, present in all vertebrates, efficiently concentrates iodine (Hetzel & Maberly 1986) and couples it to tyrosine residues from thyroglobulin protein, forming monoiodothyrosines and diiodothyrosines. These are in turn condensed to produce iodothyronines, and hence thyroid hormones (TH) (for major details, see Braverman & Utiger 2004). The thyroid gland produces two main iodothyronines: 3,5,3′,5′-tetraiodothyronine (nowadays commonly called thyroxine or T4) and 3,5,3′-triiodo-L-thyronine, also called T3. However, it also produces small amounts of other iodothyronines [i.e. reverse-T3 (r-T3) and 3,5-diiodo-L-thyronine (3,5-T2)]. The thyroid gland is part of the hypothalamic–pituitary–thyroid axis. The control of TH secretion is exerted by a classic negative feedback loop: in brief, thyroid-releasing hormone (TRH), produced predominantly by the neurones of the paraventricular nucleus (PVN) of the hypothalamus, stimulates the release of thyroid-stimulating hormone (TSH) from the pituitary, and this in turns stimulates TH synthesis and release. The increase in the blood concentration of TH inhibits the production of both TSH and TRH, leading to a downregulation of thyreocyte function. The consequent decrease in the blood level of TH removes the negative feedback signal, and the system 'wakes up' again (Braverman & Utiger 2004).

Until a few years ago, it was a common assumption in the literature that T4 was a precursor, and that T3 was the only active TH, but a growing body of evidence seems to suggest that other iodothyronines (such as T4 itself, as well as rT3 and 3,5-T2) could be of biological relevance (Hulbert 2000, Lanni *et al.* 2001, Goglia 2005). The bulk of the circulating T3 is not produced centrally by the thyroid, but instead by the action of the deiodinase enzymes present in various tissues.

These catalyse the sequential monodeiodination cascade leading to the formation of T3 and rT3 from T4, and of diiodothyronines from T3, and so on. Briefly, three separate deiodinating enzymes have been described: type 1, type 2 and type 3 iodothyronine deiodinases (D1, D2 and D3, respectively) (Bianco *et al.* 2002). In the rat, D1 is expressed preferentially in peripheral tissues such as liver and kidney, but it is also expressed in many other tissues such as pituitary, thyroid gland, central nervous system (CNS), intestine and placenta (St. Germain & Galton 1997, Bianco *et al.* 2002). It performs both outer-ring deiodination and inner-ring deiodination of T4 (producing T3 and rT3, respectively) and is inhibited by 6-propylthiouracil (PTU). D2 is predominantly expressed in the pituitary, in several regions of the brain, such as in the hypothalamic glial cells (Diano *et al.* 2003), and in brown adipose tissue (BAT) (Bianco *et al.* 2002), where it catalyses the outer-ring deiodination of T4 to T3. It is insensitive to PTU, but it is inhibited by the polyiodinated radiocontrast dye iopanoic acid (IOP). Unlike D1, D2 is positively regulated in hypothyroidism, and serves to generate T3 from T4 in this condition. D3, which is present within the CNS, placenta, skin and fetal tissues (St. Germain & Galton 1997), performs inner-ring deiodination, with T3 and T4 as the principal substrates, and is potently inhibited by IOP.

Basic notions about thermogenesis

The control of body weight depends on a balance being achieved between energy intake and energy expenditure. When an imbalance exists it can lead to a decrease or increase in body mass. A major component of energy expenditure, especially in homeothermic organisms (endotherms), is the so-called thermogenesis, the term used by scientists to describe the mechanisms by which warm-blooded species generate heat. Endotherms are able to produce heat and to maintain their body temperature independently of the surrounding environment, and it is now clear that the body's ability to regulate its own temperature is crucial to the maintenance of life. In fact, when the body temperature strays too far from the norm, the consequences may be lethal.

The production of heat is partly a consequence of the exothermic nature of many of the metabolic pathways involved in energy metabolism, such as those involved in ATP synthesis and respiratory-chain activity as well as those involved in ATP consumption. The thermogenic system of the body is both fascinating and complex, and several different mechanisms are required for the homeostatic control of body temperature. Thermogenic mechanisms are routinely classified as either obligatory or facultative. Obligatory thermogenesis is represented by the energy released as heat as a result of the activities of cells and organs within the body, and the bulk of this heat is provided by the basal metabolic rate (BMR). The BMR represents the minimal amount of energy that the body has to expend in order to maintain vital processes, and thus heat production. Facultative thermogenesis, on the other hand, represents the additional heat produced in response to a change in environmental temperature or diet. In particular, non-shivering thermogenesis (NST) is a crucial element in thermal physiology as it: (1) provides the heat needed for thermal homeostasis; (2) almost completely replaces shivering as the major source of metabolic heat production during cold acclimation; (3) can act as an energy buffer during periods of overeating, thereby reducing metabolic efficiency and participating in the regulation of weight gain. In the facultative thermogenesis it may also be included the 'voluntary thermogenesis' that is associated with the physical activity.

Thyroid hormone play an important role in both obligatory and facultative thermogenesis. During obligatory thermogenesis in particular, the role of TH seems basically to consist in (1) the stimulation of several biological processes involved in energy transduction within tissues, and (2) the stimulation of some processes that may have been positively selected during evolution to generate heat in warm-blooded species. Mechanisms such as the maintenance of transmembrane ion gradients through the action of $Na^{+/}K^{+}$-ATPase, Ca^{2+}-ATPase and Ca^{2+} cycling in muscle have been reviewed in several recent articles (Silva 1995, 2001, 2003, Lanni *et al.* 2001) and are not further described here.

Concerning the importance of TH in facultative thermogenesis, there is much evidence that T3 plays a role in this phenomenon, with BAT in rodents attracting the principal interest of investigators (Seydoux *et al.* 1982, Bianco & Silva 1987), as described below. The existence of certain TH-associated clinical manifestations such as hypo- and hyperthyroidism and thyrotoxicosis in which the body's heat production is altered, support an essential role for TH in both obligatory and facultative thermogenesis also in humans (Silva 2003).

Calorigenic effects of thyroid hormones: old and new perspectives

Even though the notion that TH are able to modify the BMR has a long history (Dubois 1936), the mechanisms by which they achieve this end are still poorly understood. Although most of the early studies regarding the effects of TH focused on T3, a growing number of researchers became excited by the possibility that iodothyronines other than T3 might exhibit biological activities. Among these iodothyronines, 3,5-T2 appears to have biological relevance, in particular in the context

of energy metabolism (Horst *et al.* 1989, Kventy 1992, O'Reilly & Murphy 1992a, Lanni *et al.* 1993, Leary *et al.* 1996, Hulbert 2000, Goglia 2005). In fact, both T3 and 3,5-T2 have significant effects on metabolic rate, even though they do not share the same mechanism of action (Lanni *et al.* 1996, Moreno *et al.* 1997, 2002, Goglia *et al.* 1999, Goglia 2005).

It is evident that the control of energy metabolism requires the participation of a multitude of biochemical and molecular mechanisms within a number of cellular compartments. Indeed, both nuclear-mediated and extranuclear-mediated actions may underlie the influence exerted by TH over energy metabolism. It is well established that the calorigenic effects induced by TH are largely mediated by the binding of T3 to TR (which belong to the superfamily of nuclear receptors), thereby acting on TH response elements (TRE) and resulting in transcriptional activation/repression of the expression of genes coding for proteins with effects on both cytoplasmic and mitochondrial functions. Various aspects of the molecular mechanisms involved in this pathway have recently been discussed in a number of articles (Hulbert 2000, Zhang & Lazar 2000, Flamant & Samarut 2003), and interested readers are referred to them. Actually, the nuclear-mediated signalling pathway is universally accepted, while the extranuclear-mediated one is still a matter of debate.

Historically, Tata and co-workers provided, in the early 1960s, the first line of evidence for a nuclear-mediated effect of T3 on energy metabolism (Tata *et al.* 1962, 1963a, Tata 1963b). Unfortunately, both the number and the identity of the T3-controlled genes remain unknown to this day, as do their relative contributions. By virtue of their central role in the energy-transduction pathway, mitochondria are natural candidates as the target for the calorigenic effects of TH (Goglia *et al.* 1999). In fact, T3 not only increases the number of these organelles but also their activity in several metabolically very active tissues, such as skeletal muscle, heart, kidney and liver (Soboll 1993, Goglia *et al.* 1999). In these tissues, it regulates mitochondrial biogenesis which, in turn, influences the proliferation, differentiation, maturation and death of cells (Scheffler 2000, Duchen 2004).

During the last three decades, on the basis of results purporting to show either the mitochondrion or the nucleus as the location of the major signalling pathway, several mechanism have been proposed to explain the calorigenic effects of TH. However, none has received universal acceptance, possibly because of the perceived problems arising from the wide variety of experimental methods and conditions used in the various investigations (Lanni *et al.* 1996, Goglia *et al.* 2002). It is possible to distinguish early and late effects of T3 on thermogenesis (also called short-term and long-term effects), the first being evident within minutes or a few hours, whereas the second occurs over several hours or days (Soboll 1993, Davis & Davis 1996, Goglia *et al.* 1999). These short- and long-term effects have often been taken to reflect the extranuclear- and nuclear-mediated actions of T3, respectively. However, simply looking at the latency of a response to T3 may not necessarily allow us to discriminate nuclear-mediated effects from extranuclear-mediated ones and ultimately it seems evident that TH can influence mitochondrial activity both indirectly and directly. The indirect pathway relies on the influence that T3 exerts on nuclear-encoded components of the respiratory machinery and of mitochondrial specific transcription factors such as mtTFA (Garstka *et al.* 1994, Pillar & Seitz 1997, Goglia *et al.* 1999, Choi *et al.* 2001), thus indirectly increasing the expression of mitochondrial encoded genes. The direct pathway, on the other hand, can be mediated both by a direct interaction of TH with the mitochondrial energy apparatus and by a direct effect of TH on mitochondrial genome transcription. The existence of a direct interaction of TH with the mitochondrial respiratory chain is supported by evidence indicating the presence of specific binding sites for T3 and for other iodothyronines (such as 3,5-T2) (Goglia *et al.* 1981, 1994a, 1999, Moreno *et al.* 1997, Arnold *et al.* 1998). Mitochondria contain, in particular, a truncated TRα isoform of 43 kDa called p43 (Wrutniak *et al.* 1995), and this acts as a ligand-dependent mitochondrial transcription factor, binding specifically to mt-DNA response elements (mt-T3REs) and able to heterodimerize with mitochondrial peroxisome proliferator-activated receptor (mt-PPAR) and mt-retinoid X receptor (mt-RXR) (Casas *et al.* 2003). The presence within the mitochondrion of a 28 kDa truncated isoform of TRα (p28) also support a direct influence of T3 on this organelle (Wrutniak-Cabello *et al.* 2001). Mitochondria, moreover, contain several nuclear receptor variants, including a glucocorticoid-receptor isoform, a truncated PPARγ2 (mt-PPAR), and a truncated RXRα (mt-RXR), as well as certain TR-related proteins (Wrutniak *et al.* 1998, Casas *et al.* 1999, 2000, Scheller *et al.* 2000) that have the potential to act as mitochondrial transcription factors.

Leaving aside for the moment the extent to which short- or long-term mechanisms and nuclear or extranuclear pathways underlie the observed effects of TH, we find that the candidates put forward to explain their calorigenic effects include a number of intracellular processes. The problem that arises is that the number of processes influenced by T3 is considerable, and it is then a question as to whether the calorigenic effects of TH can better be explained by (1) all of them contributing, each in a small way and in an additive manner, or (2) by a modulation of single fundamental process. Another

important question that needs to be considered is whether TH enhance cellular respiration by a mechanism capable of increasing the respiratory capacity of the cell in a quantitative manner (e.g. by inducing a change in the number of mitochondria, in the surface area of the cristae, and/or in the concentration or activity of enzymes), or whether instead they influence respiration in a qualitative manner (e.g. by inducing a change in the efficiency of energy transduction), or indeed both. No definitive answer can be given to this question at the moment.

As stated before and in general terms, several mechanisms have been proposed as contributors of the effect of TH on energy metabolism, including: (1) the already cited maintenance of transmembrane ionic gradients, such as the Na^+ and K^+ gradients across the cell membrane and Ca^{2+} gradients within the cell; (2) substrate cycles, such as the lipogenesis–lipolysis cycle; (3) the glycerol-3-phosphate/NADH shuttle; and (4) direct and/or indirect actions at the level of the mitochondrial energy-transduction apparatus (Fig. 1). The quantitative contribution made by the first three categories to the calorigenic effect of T3, seems to be small (Soboll 1993, 1995, Silva 1995, Lanni *et al.* 2001), and as mentioned before, will be dealt with only briefly in this article, while the effects of TH on the mitochondrial energy transduction apparatus will be our major concern. Moreover, it should be realized that the effects of TH on some cellular pathways, such as substrate cycles (lipogenesis–lipolysis cycle) and the mitochondrial energy-transduction apparatus, may be due to mechanisms that are not completely independent of each other, but instead integrated.

For a deeper consideration of the role that mechanism number (1) has in TH-mediated thermogenic effects, the reader is referred to Soboll (1993, 1995), Silva (1995, 2003), Lanni *et al.* (2001). As to substrate cycling (mechanism number 2), it is well known that T3 influences the intermediary metabolism of carbohydrates, lipids and proteins. The influence of T3 can be apparent as an activation of so-called 'futile cycles' by means of an acceleration of the turnover of substrates

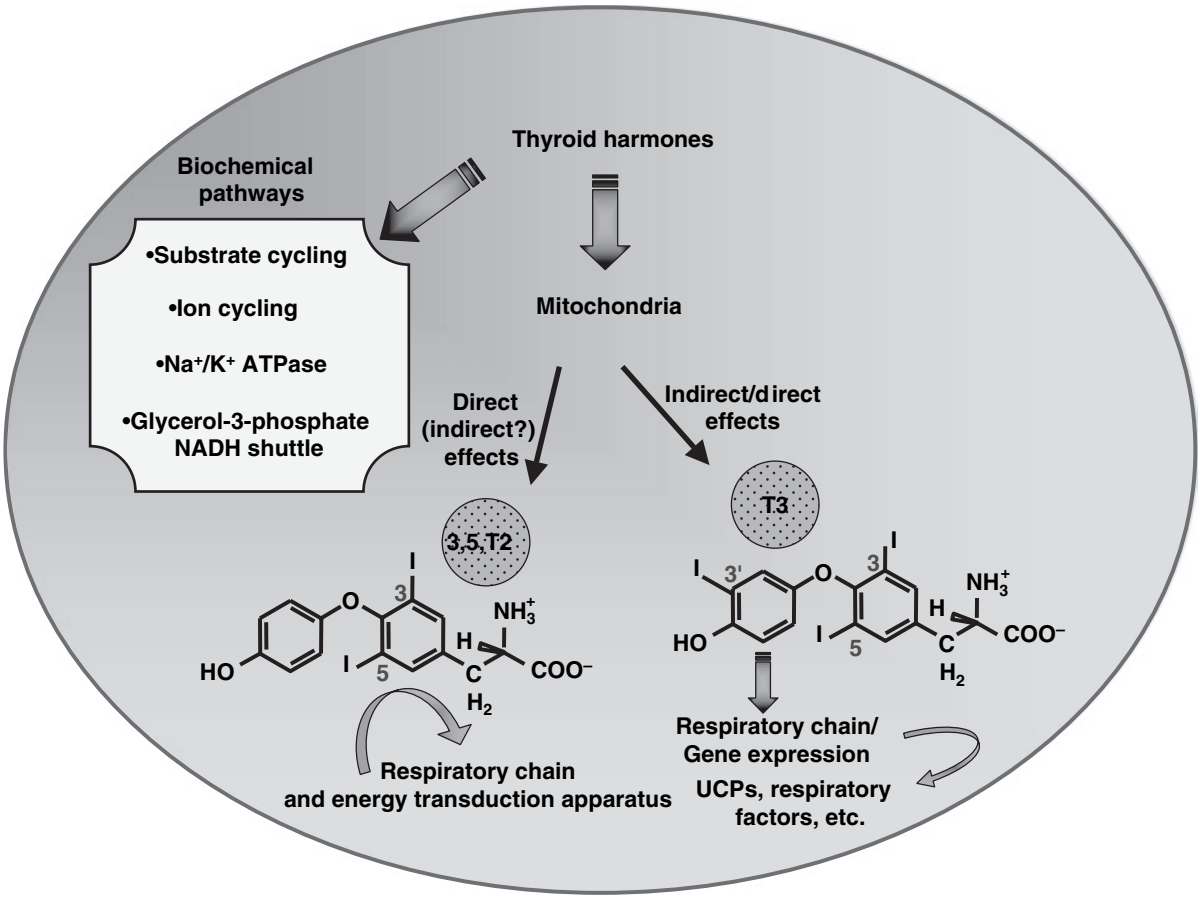

Figure 1 Schematic representation of the mechanisms underlying the control of energy metabolism by thyroid hormones. The figure shows, in general terms, the cellular processes that have been put forward as candidates to explain the effects of thyroid hormones on energy expenditure (for more details, see text). Question mark indicates the need of further confirmation or uncertainty.

without a change in their steady-state level. The greatest attention has been focused on the role of the lipogenesis–lipolysis cycle in the effect of T3 on the energy expenditure (Freake & Oppenheimer 1995). However, it has been reported that the contribution made by this substrate cycle to the thermogenic effect of T3 is small (3–10%) (Silva 1995). Concerning the role of the glycerol-3-phosphate NADH shuttle (mechanism number 3), it is known that cytosolic NADH is oxidized within mitochondria via two shuttles: the malate–aspartate shuttle and the glycerol-3-phosphate shuttle. Mitochondria participate in the glycerol-3-phosphate shuttle by means of an FAD-linked α-glycerophosphate dehydrogenase (α-GDP) that is stimulated by T3, as shown almost 40 years ago (Lee & Lardy 1965). This stimulation could involve transcriptional regulation of the corresponding gene by T3; however, the actual contribution made by this shuttle to the thermogenic effect of T3 remains poorly understood. It has been reported that the hypothyroid–hyperthyroid transition is associated with a switch from the malate–aspartate to the glycerol-3-phosphate shuttle, leading to a reduction of about 5% in the efficiency of mitochondrial ATP production (Kneer & Lardy 2000). Support for a role for α-GPD in TH-induced thermogenesis has recently been provided by a study on α-GPD-deficient mice. These mice have, despite increased plasma T4 and T3 levels, a clear reduction in obligatory thermogenesis, and this is compensated both by an increased facultative thermogenesis in BAT and by TH-dependent mechanisms utilizing other proteins, possibly UCP3 (DosSantos *et al.* 2003). In conclusion, all these mechanisms may contribute to 'thyroid thermogenesis' but their quantitative contribution still remains undefined. The problem that arises may be: are there fundamental processes that deeply influence the energy transduction which could better permit to explain the stimulatory effect of TH on metabolic rate with a concomitant decrease in metabolic efficiency?

Thyroid hormone, mitochondrial efficiency and uncoupling proteins: a new approach to an old question?

Mitochondria are the site of oxidative phosphorylation, the step-by-step process recognized as the principal mechanism responsible for ATP synthesis in aerobic cells. Mitochondria synthesize ATP via the activity of the respiratory chain complex, which is able to generate a proton-gradient across the inner membrane coupled to oxidative phosphorylation. The electrochemical proton gradient, $\Delta\mu_{H^+}$, produced by the H^+-pumping activity of the chain is in fact used by ATP synthase to transduce the energy of the gradient itself into the form of ATP molecules. However, the system is not completely efficient, there being several mechanisms capable of uncoupling phosphorylation from respiration. One established uncoupling mechanism is the leakage of protons back across the mitochondrial inner membrane, the so-called 'proton leak'. In this process protons, bypassing ATP synthase, directly transduce the energy of the gradient into heat. Another mechanism underlying mitochondrial inefficiency is the so-called 'redox slip' (Fig. 2). This involves a decreased proton-pumping activity at a given electron-transport rate (i.e. decreased H^+/e^- stoichiometry), and is mediated by certain components of the respiratory chain such as cytochrome oxidase (complex IV). In fact, this process can involve the allosterically regulated subunit IVa of cytochrome oxidase (Kadenbach *et al.* 1998). Among the other mechanisms that can uncouple mitochondrial respiration is an increased activity of the glycerol-phosphate shuttle; this competes with the more efficient aspartate–malate shuttle for the transfer of cytosolic NADH to the mitochondrial matrix for the purpose of respiration (Kozak *et al.* 1991).

All of the above mechanism have been proposed at one time or another as the molecular basis of the metabolic inefficiency induced by TH in mitochondria. Uncertainty still exists as to the possible role of redox slip as an uncoupling mechanism induced by TH (Hafner *et al.* 1988, Hafner & Brand 1991, Brand *et al.* 1992, Schmehl *et al.* 1995). For instance, Schmehl *et al.* (1995) suggested the occurrence of a slip mainly at the level of the cytochrome oxidase complex. The kinetics of this complex depend on the lipid-membrane environment, surface charge, pH and phosphorylation state (Kadenbach *et al.* 2000) as well as on its tight association with cardiolipin (Robinson 1993). T3 is able to change the phospholipid composition of the inner membrane and to stimulate cardiolipin-synthase activity, leading to an increased amount of cardiolipin, which in turn stimulates several carriers and enzymes within the mithochondrion (Paradies *et al.* 1991). Luvisetto *et al.* (1992) reported that in isolated mitochondria, hyperthyroidism increases both proton-leak and redox-slip kinetics. These results, however, cannot be considered conclusive as the experiments were carried out at 25 °C, and the temperature is a fundamental parameter determining the relative contribution made by proton leak and redox slip to the basal respiration rate. In fact, low temperature (such as 25 °C) reduces the contribution made by proton leak while increasing that made by redox slip. It is still not clear whether T3 only affects redox slip at unphysiologically low temperatures (Luvisetto 1997). There is some evidence that 3,5-T2 is also able to affect the cytochrome oxidase complex. For instance, we reported that 3,5-T2 stimulates the activity of isolated cytochrome oxidase from bovine heart mitochondria, while T3 induces little or no stimulation (Goglia *et al.* 1994b).

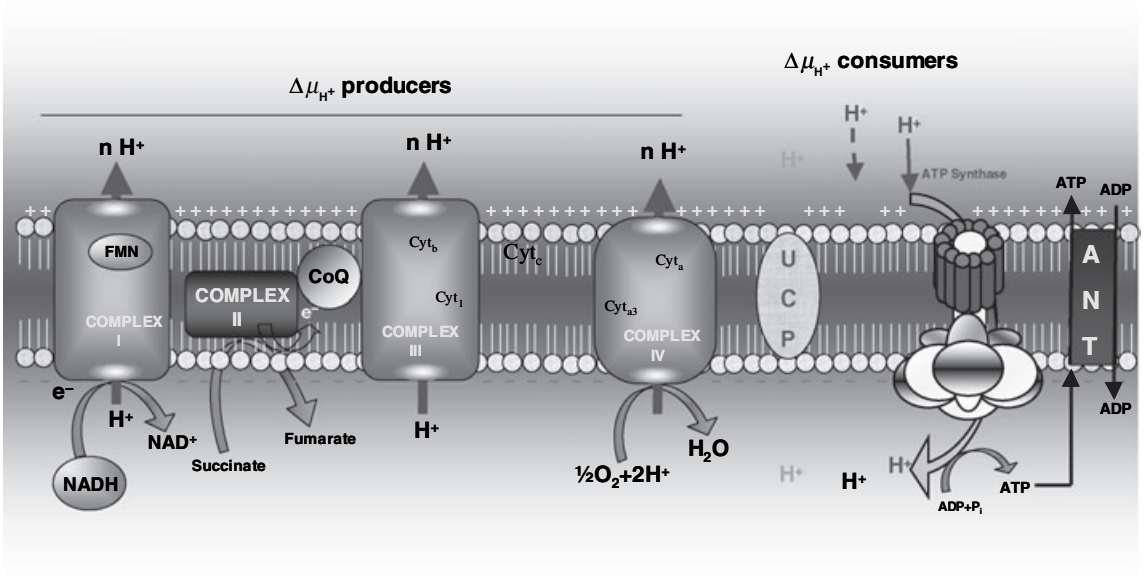

Figure 2 Schematic representation of oxidative phosphorylation. Oxidative phosphorylation is the process by which ATP is formed as a result of the transfer of electrons from NADH (or $FADH_2$) to O_2 by a series of electron carriers. The flow of electrons from NADH (or $FADH_2$) to O_2 through protein complexes located in the mitochondrial inner membrane leads to the pumping of protons out of the mitochondrial matrix ($\Delta\mu_{H^+}$ producers). The resulting uneven distribution of protons generates a pH gradients and a transmembrane electrochemical potential, creating a proton-motive force. ATP is synthesized when proton flow back to the mitochondrial matrix through the enzyme ATP synthase. On the other hand, when H^+ enters the mitochondrial matrix – either directly across the lipid bilayer or by protein-mediated process [UCPs or ANT (mechanism not represented)] – respiration is not coupled to ATP synthesis. These last two events represent what is called 'mitochondrial proton leak' (and together with ATP synthesis represent the $\Delta\mu_{H^+}$ consumers). Incomplete coupling of respiration can also be due to the so-called 'redox slip' (not shown) that occurs when fewer protons are pumped per oxygen consumed or electrons transported, causing a reduced $\Delta\mu_{H^+}$ (electrochemical proton potential) production.

In fact, binding of the 3,5-T2 to cytochrome oxidase leads to conformational changes, as evidenced by the modified visible spectrum of the oxidized enzyme (Goglia *et al.* 1994b). These data suggest that the 'short-term' effects of TH on mitochondrial respiration may be at least partly due to the allosteric interaction of 3,5-T2 with the cytochrome oxidase complex. Concerning cytochrome oxidase activity, it has been hypothesized that *in vivo* it is in a steady state between the phosphorylated and the dephosphorylated forms (Bender & Kadenbach 2000, Kadenbach *et al.* 2000), with the dephosphorylated form no longer controlled by the intramitochondrial ATP/ADP ratio, but instead by the $\Delta\mu_{H^+}$. At high ATP/ADP ratios, ATP inhibits cytochrome oxidase. The activity of the enzyme is then reversibly switched on by cAMP-dependent phosphorylation (which is called 'a second mechanism of respiratory control') (Kadenbach 2003).

The proton leak across the inner mitochondrial membrane accounts for a significant part of an animal's resting metabolic rate (RMR), and it represents a potential mechanism for energy dissipation or heat production. The proton leak, however, can have a role not only in heat production, but also: (1) in the regulation of the efficiency of oxidative phosphorylation, (2) in reducing the production of reactive oxygen species (ROS) by mitochondria, and (3) in maintaining the NAD^+-to-NADH ratio at a sufficiently high level to support carbon fluxes in biosynthetic processes (Rolfe & Brown 1997). It has been estimated that in isolated rat liver cells and perfused rat skeletal muscle, the proton leak-dependent oxygen consumption may represent ~26 and 52% of resting energy expenditure, respectively (Brown *et al.* 1990, Nobes *et al.* 1990, Harper & Brand 1993, Brand *et al.* 1994, Rolfe & Brand 1996, Rolfe & Brown 1997). Several processes have been put forward to explain the mitochondrial proton leak (Cadenas *et al.* 1999, Monemdjou *et al.* 1999, Brand 2000), but its underlying mechanism remains poorly understood. Actually, two types of proton leak may be present: basal and inducible. The former is present in the mitochondria within every tissue, and while the mechanism regulating it is unclear, it may be related to the lipidic environment of the membrane (Rolfe *et al.* 1994). The inducible type, on the other hand, is tightly regulated and occurs through specific proteins, among which uncoupling proteins (UCPs) are currently attracting particular interest

(Brand *et al.* 1999, 2004). UCPs are homologous proteins constituting a subfamily of mitochondrial inner membrane anion carriers (Ježek & Ježek 2003) that are evolutionarily related and possibly derived from an ancestral protein that acted as a proton/anion carrier.

UCP1, cloned in 1985 and simply called UCP until 1997, is located on human chromosome 4, and is exclusively expressed in BAT (Ricquier & Bouillaud 2000). It is recognized as the site of NST in that tissue. Located in the inner mitochondrial membrane, UCP1, by uncoupling BAT mitochondria, generates a physiologically important, hormonally regulated heat production in response either to cold or to an inadequate diet (Cannon & Nedergaard 2004). Since 1997, several genes have been discovered that encode proteins related to UCP1. In mammals, four such genes have been described: UCP2, UCP3, UCP4 and BMCP1 (brain mitochondrial carrier protein 1, also termed UCP5). However, a recent report highlighted that from a phylogenetic point of view a distinct family consist of UCP1, UCP2 and UCP3, while UCP4 and UCP5 actually result misnamed and are not more close to UCP1/2/3 than they are to certain other mitochondrial carriers (Sokolova & Sokolov 2005). On the other hand, taking a non-phylogenetic view, UCPs could be defined as proteins that when ectopically expressed depolarize mitochondria but in such case also other mitochondrial carriers should be included in the group [i.e. oxoglutarate carrier (Yu *et al.* 2001)]. UCP2 mRNA is present in a large number of tissues, and is at high levels in white adipose tissue (WAT), spleen and pancreatic β cells. Despite the ubiquitous presence of UCP2 mRNA, UCP2 protein has been detected in only a few tissues (Pecqueur *et al.* 2001). UCP3 mRNA is strongly expressed in skeletal muscle and to a lesser extent in heart, BAT and WAT, while UCP4 mRNA is present in brain, and the mRNA for BMCP1/UCP5 is present in brain and liver (Boss *et al.* 2000, Ricquier & Bouillaud 2000). With the exception of the UCP2 in Kupffer cells and UCP5, no typical proteins able to induce a depolarization (when ectopically expressed) have been detected in liver parenchymal cells; however, Tan and co-workers recently reported the cloning and identification of a novel cDNA fragment, called HCC-downregulated mitochondrial carrier protein (HDMCP). This encodes a protein of 308 amino acids that has all the hallmark features of mitochondrial carrier proteins and that when transiently overexpressed in transfected cancer cells leads to the dissipation of mitochondrial membrane potential (Tan *et al.* 2004). However, its function is yet to be thoroughly investigated. The homology of both UCP2 and UCP3 with UCP1 (55 and 57%, respectively) has led to the assumption that they also have a specific proton/anion carrier function. However, both the physiological functions and the putatively transported substrate/s of UCP2 and UCP3 remain uncertain. A number of experimental models – proteoliposomes (Jaburek *et al.* 1999, Echtay *et al.* 2001), yeast heterologous expression systems (Zhang *et al.* 1999, Harper *et al.* 2002) and transgenic mice (Clapham *et al.* 2000, Gong *et al.* 2000) – have been successful in demonstrating that both UCP2 and UCP3 can uncouple mitochondrial oxidative phosphorylation, and it is now quite clear that heterologous or transgenic expression of these proteins leads to an increase in the proton conductance of the inner membrane. However, it is less obvious whether the uncoupling observed in the above-mentioned conditions is due directly to the activity of the proteins or whether it is the consequence of a more general perturbation of mitochondrial function (Harper *et al.* 2002). Increases in the levels of the mRNAs for UCP2 and UCP3 have been demonstrated not only in situations in which energy expenditure is significantly increased [fever (Fleury *et al.* 1997), hyperthyroidism (Gong *et al.* 1997, Lanni *et al.* 1997, 1999), high levels of leptin (Cusin *et al.* 1998), cold exposure (Simonyan *et al.* 2001)] but also in starvation, in which energy expenditure would be expected to be depressed (Weigle *et al.* 1998). Moreover, an increase in their expressions is not always associated with increased mitochondrial uncoupling (Cadenas *et al.* 1999, Hesselink *et al.* 2003). In addition, surprisingly, other UCP1 homologues have been described also in poikilothermic organisms [i.e. in fish (Argyropoulos & Harper 2002) and plants (Vercesi *et al.* 1995, Laloi *et al.* 1997)], and indirect indications have been obtained for the existence of UCPs in an amoeba (Sluse & Jarmuszkiewicz 2002) and a parasitic yeast (Jarmuszkiewicz *et al.* 2000), further suggesting that thermogenesis/uncoupling is hardly the primary function of the UCP1 homologues, but rather an effect secondary to their main function. Indeed, the postulated roles for the newly discovered UCPs include their involvement in: modulating the production of ROS and preventing their dangerous effects, lipid handling, preventing lipotoxicity, modulation of energy expenditure and the functioning of pancreatic islet cells (Dulloo & Samec 2000, Himms-Hagen & Harper 2001, Schrauwen & Hesselink 2002b, Goglia & Skulachev 2003, Lanni *et al.* 2003, Rousset *et al.* 2004). However, because of their putative uncoupling properties, UCP2 and UCP3 are thought to be good candidates for roles as molecular determinants in the modulation of energy metabolism by T3. In fact, administration of T3 to rodents upregulates the expressions of both UCP2 and UCP3 in heart and skeletal muscle (Gong *et al.* 1997, Lanni *et al.* 1997, 1999). The hypothyroidism–hyperthyroidism transition, moreover, leads to an increase in UCP3 mRNA expression in skeletal muscle and also to increased

mitochondrial uncoupling activity in both rats and humans (Lanni *et al.* 1999, Jucker *et al.* 2000, Lebon *et al.* 2001). Indeed, we showed that in hypothyroid rats given a single injection of T3, it was possible to identify a strict correlation, in terms of time course, between the induced increase in UCP3 protein level in muscle mitochondria, the decrease in mitochondrial respiratory efficiency, and the increase in the RMR of the whole animal (de Lange *et al.* 2001). This result provided *in vivo* evidence that UCP3 has the potential to be a molecular determinant in the influence of T3 over RMR even though it seems reasonable to us that only a part of the T3-induced thermogenesis may be attributed to UCP3 activation and T3 thermogenesis consequent to the activation of UCP3 could be a secondary effect. However, surprising and astonishing results (Sprague *et al.* 2004) showing that: (1) methamphetamine (METH)-induced hyperthermia was not evident in thyroparathyroidectomized animals and that T4 treatment restored the hyperthermic response; (2) UCP3 knockout mice develop a markedly blunted hyperthermic response to METH compared with wild-type animals, indicate that TH and UCP3 have a major role in METH-induced hyperthermia, obliging to reconsider the thermogenic role of UCP3 also in relation to T3 action.

Actually, we feel that it is necessary to distinguish between two mechanisms: direct thermogenesis and indirect thermogenesis. In the case of T3, the former mechanism would require metabolic modulation by this hormone of cellular processes directly involved in heat production (i.e. BAT/UCP1 activation). The latter, on the other hand, would involve cellular processes (i.e. UCP3 expression and activity) that lead indirectly to heat production despite having a different primary function. The quantitative and/or qualitative relationship between the two systems is not understood, and it would be interesting to investigate it thoroughly. Barbe *et al.* (2001) showed that T3 upregulates both UCP2 and UCP3 mRNA expressions in human skeletal muscle and adipose tissue. They reported that the increase in the free T3 level in the plasma, following T3 injection, was strictly related to both the increase in RMR and the decrease in respiratory quotient, pointing once again towards a role for UCP3 in mediating the effect of T3 on RMR. However, in UCP3-knockout mice a rise in BMR is observed after T3 administration, a finding that seems not to be in agreement with the above notion (Gong *et al.* 2000). However, the discrepancy may be apparent rather than real. Possible reasons for this may be: (1) the dose used in the study of Gong *et al.* is very high (100 μg/100 g body weight) and non-specific thermogenic mechanisms may be activated; (2) other mechanisms such as those involving ANT may be overstimulated at this dose; and (3) in UCP3 knockout mice, the deiodinase enzymes are active and some of the injected T3 may be converted into 3,5-T2, known to be able to stimulate RMR and mitochondrial respiration (Goglia 2005). In addition, the authors report an increase in RMR of normal mice after T3 treatment about 24% higher when compared with that of knockout ones (89% increase in wild type mice vs. 72% increase in knockout animals) that even if not significantly different still may represent an effect due to the presence of UCP3.

To date, T3 seems unique in its ability to induce both UCP2 and UCP3 expressions and mitochondrial uncoupling. This could be a consequence of its capacity synergistically to stimulate the complex network of biochemical pathways underlying the activation of the newly discovered UCP, such as those related to fatty acid utilization, mitochondrial Coenzyme-Q (CoQ) levels, and ROS production (Lanni *et al.* 2003). Recently, we provided evidence that T3 is able to stimulate, at one and the same time, UCP3 expression and activity, suggesting that *in vivo*, T3 activates the integrated biochemical pathways leading to the establishment of the conditions needed for UCP3 activity: mitochondrial ROS formation and adequate levels of fatty acids and CoQ (Moreno *et al.* 2003). Recently, we also showed that muscle mitochondria from hyperthyroid rats, apart from having a higher free fatty acid content than those from euthyroid animals, also have a higher sensitivity to fatty acids in terms of the levels of respiration rate and membrane potential, parameters whose levels correlated well with the higher level of UCP3 protein in the former than in the latter mitochondria (Lombardi *et al.* 2002). T3 seems directly to affect the UCP3 gene. Indeed, very recently Solanes *et al.* (2005) published evidence suggesting that the human UCP3 gene is directly regulated by T3 via TR binding to its promoter, which seems to behave as a multihormonal responsive element, sensitive to T3, PPAR and retinoic acids. In addition, the authors showed that human UCP3 gene is more sensitive to thyroid-dependent stimulation than the mouse gene with both the human and moue UCP3 gene promoter requiring a similar TRE in the proximal promoter region, but the mouse TRE appearing to be weaker than the human TRE (Solanes *et al.* 2005). These results stress the possible existence of a species-specific response of UCP3 gene to T3 and underscore the need for caution in comparing/evaluating data related to T3 effects on metabolism between different species; this may be true also for the previously reported data.

Thyroid hormones and nutritional status

Among the conditions that elicit adaptive thermogenesis, diet is a major player. Starvation decreases RMR by as much as 40% (Blaxter 1989), while feeding increases

energy expenditure with an acute increase in RMR of 25–40% in humans and rodents, the so-called thermic effect of food (Shibata & Bukowiecki 1987).

Thanks to studies performed over the past four decades, it is now well established that there is an endocrinological system for the homeostatic regulation of body weight. The observation that a change in nutritional status leads to an alteration in the thyroid axis made the TH – together with other hormones (such as leptin, glucocorticoids, insulin), the newly discovered stomach-derived hormone (i.e. ghrelin), neuromediators and other factors such as glucose levels – candidates for roles as modulators of the metabolic adaptations to fasting and altered nutrition. However, the interrelationship between TH and the substrates and other hormones implicated in these adaptations remains poorly understood to this day. Factors related to the level and composition of the energy intake (including whether an organism is in energy balance) are important signals directing the hormonal adaptation to any change in the animal's nutritional status (Danforth & Burger 1989).

Within the last decade, our understanding of the neuroendocrine control of the thyroid axis – in particular of the mechanism regulating TRH–TSH synthesis and release – has made considerable progress. In addition, as a result both of new findings related to the UCPs and of the elucidation of the role played by the peripheral metabolism of TH (via deiodinase pathways), we may expect a rapid advance in our understanding of the mechanisms recruited at the cellular level during modifications in an animal's nutritional status.

It has been reported that starvation is associated with reduced serum levels not only of TH (Harris *et al.* 1978, Rondeel *et al.* 1992), but also of TSH and TRH, in both human and rodents (in contrast to the situation seen in primary hypothyroidism, in which the reduced feedback action of TH results in increases in serum TSH and TRH) (Danforth & Burger 1989, Blake *et al.* 1991). This suggests that starvation, a situation involving caloric-intake restriction, is associated with a depression of the level of TH that serves functionally to reduce energy expenditure. In this condition, any mechanism that permits TSH secretion to remain low, despite the decrease in serum T3, will allow the above adaptation to starvation to persist. In fact, several mechanisms may underlie the above change in the thyroid–hormone axis. The identity of the responsible mechanism may be different depending on the duration and type of food restriction, and they may involve the entire system of the thyroid axis. In rats, acute starvation leads to decreases in both the hypothalamic proTRH mRNA level and in TRH release (Blake *et al.* 1991, Rondeel *et al.* 1992). Diano *et al.* (1998a) felt that the difference in the effects on TRH production and release between primary hypothyroidism (increased) and fasting (decreased) might be due to a difference in the hypothalamic expression of D2 between fasted animals and animals that were hypothyroid due to a failure of the gland. They showed that short-term fasting leads to an increased negative feedback action of the thyroid on the hypothalamus due to locally formed T3, which in turn would suppress TRH through a thyroid–hormone–receptor binding site, termed Site 4, in the proximal promoter of TRH (Hollenberg *et al.* 1995, Satoh *et al.* 1996). This site appears to be both critical for the binding of thyroid–receptor isoforms and necessary for the negative regulation of TRH by T3.

The mechanisms by which a differential regulation of D2 occurs during fasting are unknown. Some recent studies seem to support the notion that non-thyroidal signals might be involved in the fasting-induced changes seen in the thyroid axis. During starvation, the serum leptin level decreases. This fall have an inhibitory effect on hypothalamic D2 expression and activity, playing an important role in the changes in the thyroid axis observed during starvation as recently reported by Coppola *et al.* (2005). Interestingly, Ahima *et al.* (1996) showed that the suppression of the thyroid axis seen during starvation can be reversed by leptin administration. This finding was extended by Legradi *et al.* (1997), who demonstrated that the effects attributable to leptin were due to an upregulation of TRH-gene expression. Conceivably, leptin might have a direct action on TRH neurones, which contain the long form of the leptin receptor, or an indirect action (Nillni *et al.* 2000) on other neurones, such as neuropeptide Y (NPY), agouti-related protein (AgRP), or pro-opiomelanocortin (POMC) neurones, all of which project onto the paraventricular TRH cells (Diano *et al.* 1998b). The existence of a direct pathway is supported by data from Harris *et al.* (2001), who showed that leptin regulates the promoter of the TRH gene. On the other hand, the indirect pathway is supported by data from Legradi *et al.* (1998) who showed that ablation of the arcuate nucleus blocks the effect of leptin on TRH expression. Kim *et al.* (2000) furnished data supporting the indirect model, and they suggested that the melanocortin pathway may mediate the nutritional response to leptin shown by TRH neurones. Although these data may have provided new insights into the mechanisms operating within the CNS during starvation, the actual mechanisms may differ depending on the type and magnitude of the reduction in food intake (for a schematic representation, see Fig. 3). Indeed, van Haasteren *et al.* (1996) showed that a reduction in food intake to one-third of normal, for a period of 3 weeks, has significant effects, at various levels, on the hypothalamic–pituitary–thyroid axis. However, in

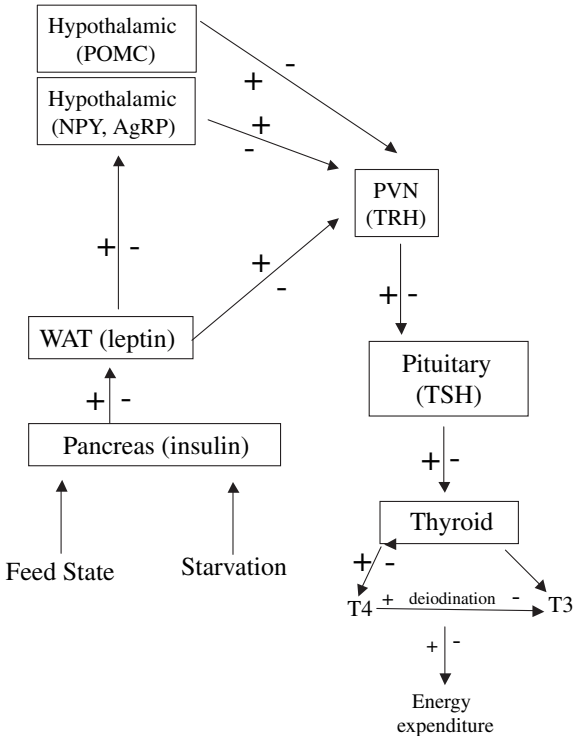

Figure 3 Schematic representation of some possible relationships between TH and nutritional status. Reported is the interrelation between insulin, leptin, hypothalamic neurones and the hypothalamic–pituitary–thyroid axis during starvation and feed state as discussed in the text. The information (stimulation or inhibition, represented here as plus or minus, respectively) relative to the 'feed state' are always reported on the left of the arrows, while those relative to the 'starvation' are reported on the right. Also showed are both the direct and the indirect pathways leading to leptin modulation of TRH release from PVN (for major details, see text).

contrast to the situation seen in acute starvation, the suppression of serum TSH does not involve decreases in the expressions of the genes for hypothalamic proTRH or pituitary TSH-β.

Although most of the above-cited studies were performed in rodents, it may be that similar effects occur in humans. However, starvation is less immediately threatening for humans than for rodents, because humans have greater energy stores compared with their metabolic rate. In fact, suppression of the thyroid axis during food restriction in humans occurs more slowly and is of smaller magnitude than that seen in rodents. This suggests that more studies should be carried out on the signalling pathways involved in the regulation of the hypothalamic–pituitary–thyroid axis in humans. Fasting, caloric restriction and altered dietary composition all exert influences over the peripheral metabolism of TH, too. Unlike T3, rT3 shows a marked increase during fasting and caloric restriction (Danforth & Burger 1989). During caloric overfeeding, on the other hand, the serum T3 concentration increases while the rT3 concentration decreases. In contrast, caloric restriction or overfeeding only slightly alters the serum T4 concentration. During fasting, the decrease in the T3 level is attributable to a decreased rate of extrathyroidal production, while the increased rT3 level is attributable to a decreased metabolic clearance rate. The explanation for these changes might seem likely to be a decrease in the activity of D1, which catalyses both the 5′-monodeiodination of T4 to T3 and that of rT3 to 3,3′-diiodothyronine (Einsestein *et al.* 1978, Suda *et al.* 1978). However, in the serum of D1-deficient C3H mice, T3 remains normal and T4 is increased (Berry *et al.* 1993, Maia *et al.* 1995) suggesting that some other mechanism must be considered. Two possibilities are: (1) that caloric deprivation inhibits the uptake of T4 and T3 into the tissues or (2) that the cytosolic cofactors required for deiodination undergo a decrease during caloric restriction. In fact, the former mechanism seems to be active in humans (Van der Heiden *et al.* 1986) while the latter is active in rodents (Balsam & Ingbar 1979). Upon refeeding, serum TH levels return to baseline. However, the composition of the food is a relevant factor determining the effectiveness of the return to baseline; for example, refeeding with glucose is much more effective than refeeding with an equicaloric amount of protein or fat (Wartofsky & Burman 1982). One possible explanation for this is that carbohydrate may be required for the induction of deiodinase activities (Gavin & Moeller 1983).

The results obtained so far and discussed above emphasize the complexity of the pathways underlying the role played by TH in the modulation of energy balance. It is evident that much more information is needed if we want to have a clear picture of the endocrinological system involved in the homeostatic regulation of body weight.

Thyroid hormones and cold

Thyroid thermogenesis in BAT

Homeotherms have evolved several strategies for survival during prolonged cold exposure, including metabolic adaptations serving to increase heat production, as well as the adoption of such strategies as torpor and hibernation. In rodents, cold exposure evokes a variety of adaptive mechanisms, which together ensure that heat production will balance the increased heat loss. In fact, during both acute and chronic cold exposure in rodents, energy expenditure at rest increases two- to fourfold in response to the drop in environmental temperature (Hart *et al.* 1956, Davis *et al.* 1960). Part of the acute response is due to shivering, but during adaptation the shivering disappears and the mechanisms

of adaptive thermogenesis (non-shivering) become prominent. In humans too, energy expenditure is sensitive to the environmental temperature, even if the effect on metabolic rate is smaller due to the fact that humans are able to adjust the amount of clothing they wear.

Thyroid hormones play a major role during the acute phase of cold acclimation, as clarified by numerous studies over the past 20 years. However, the influence of TH on thermogenesis during long-term cold exposure has attracted little attention. Actually, it has been suggested that TH may not be necessary for the maintenance of normothermia in the cold-acclimated state because when the brain detects cold, it activates other pathways leading to energy dissipation (principally the sympathetic nervous system, which heavily innervates effector targets such as BAT and skeletal muscle) (Lowell & Spiegelman 2000).

In newborns and small mammals (e.g. rodents), cold-induced NST is primarily the role of BAT, which produces additional heat whenever the organism is in need of it (cold acclimation, postnatally, febrile states and during arousal from hibernation) (Cannon & Nedergaard 2004, Rodriguez & Palou 2004, Sell *et al.* 2004). The rate of thermogenesis in BAT is controlled via a pathway originating in the hypothalamus, and it is also stimulated by the sympathetic nervous system, but has an absolute requirement for TH (Silva & Rabelo 1997). Whichever mechanism is involved, activation of this tissue leads to large amounts of lipids and glucose being combusted.

The amount of T3 in BAT is strongly influenced by D2. T3 contributes to BAT thermogenesis both by stimulating the noradrenaline signalling pathway and by directly inducing an expression of UCP1 (Ribeiro *et al.* 2001). Indeed, the UCP1 level varies with the T3 concentration in BAT. The idea that a synergism exists between the noradrenaline signalling pathway and T3 is based (1) on the presence of two functional TREs and a cAMP-response element in the UCP1 gene promoter, and (2) on the ability of T3 to regulate the adrenergic system itself (Ribeiro *et al.* 2001). The brown fat-selective expression of UCP1 also depends on the presence in its promoter region of binding sites for PPARγ (Tai *et al.* 1996). Actually, the T3 formed inside brown adipocytes has both autocrine and endocrine significance. An increase in the intracellular T3 concentration is functional to TR occupancy (Bianco & Silva 1988) that, in turn affects UCP1 expression. On the other hand, adipocyte-produced T3 may be functional to contribute to the systemic level of T3. T3 release by BAT was studied by measurements of arterio-venous differences and blood flow across the tissue in rats acutely or chronically exposed to cold (Fernandez *et al.* 1987). It was demonstrated that BAT is a source of systemic T3 and that the basal net BAT release of T3 is increased 10-fold by cold exposure (Fernandez *et al.* 1987). However, data from mice lacking D2 enzyme (de Jesus *et al.* 2001, Schneider *et al.* 2001) apparently argue against an endocrine significance of BAT in term of T3 release, at least in the mouse. In fact, despite the absence of D2 activity, in D2 knockout mice serum T3 levels are maintained close to wild-type ones. However, in PTU-treated rats, in which both hepatic and renal D1 activity are greatly reduced, extrathyroidal conversion of T4 to T3 is reduced only 60–70% (Frumess & Larsen 1975, Larsen & Frumess 1977). In addition, Nguyen *et al.* (1998) provided quantitative evidence that in the rat a significant fraction of the plasma T3 is derived from T4 conversion by D2. From this point of view, it may be that in D2 knockout mice the deficient production of T3 by BAT resulting from the absence of D2 activity is compensated for from other sources, among which, in particular, the thyroid and the liver, with the thyroid likely over stimulated, seen the high serum levels of TSH in these animals (Schneider *et al.* 2001). In addition, it can also be speculated that the maintenance of plasma T3 in D2 knockout mice could be explained by a reduction in the rate of clearance of T3 from plasma. Thus, the results obtained from D2 knockout mice showing no variation in T3 serum levels do not permit to exclude that BAT may represent an additional source of systemic T3.

In the rat, the adaptive response to cold exposure involves, at the level of BAT, cellular processes such as proliferation, differentiation, mitochondrial biogenesis and UCP1 expression, and produces a rise in BAT temperature that is associated with increased thyroid activity, an elevated serum level of T3, and an increased rate of T3 production (Hefco *et al.* 1975). Another marker of BAT activation is an upregulation of the expression of peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1). This factor is highly expressed not only in BAT, but also in skeletal muscle, heart and kidney (Puigserver & Spiegelman 2003). Cold exposure rapidly induces this effect in both BAT and skeletal muscle through a pathway involving the sympathetic nervous system, β-adrenergic receptors (βAR) and cAMP (Puigserver *et al.* 1998, Boss *et al.* 1999). It is known that PGC-1 binds to PPARγ and to other nuclear receptors, including retinoic acid and TR (Puigserver & Spiegelman 2003) (for a schematic representation, see also Fig. 4). In addition, the several-fold increase in mitochondrial protein content that occurs during cold acclimation recruits a large capacity for oxidative phosphorylation (Klingenspor 2003). Cold exposure also increases D2 expression (10–50-fold) and D2 activity (50-fold) in BAT, with an effect that is already evident after 30 min (Brizzi *et al.* 1998). The increase in D2 in the cold is achieved through

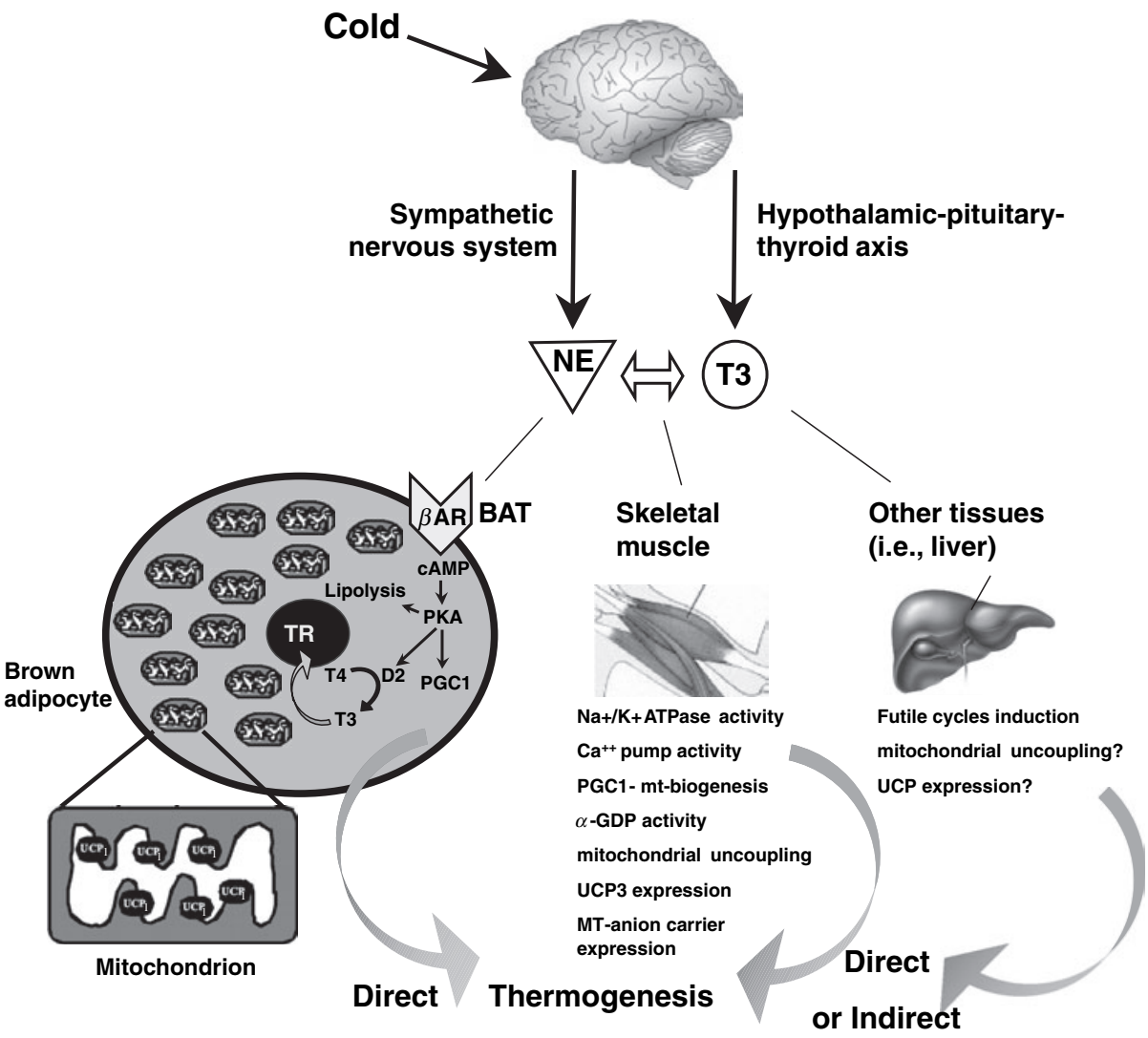

Figure 4 Schematic representation of some mechanisms underlying cold-induced adaptive thermogenesis. The double-headed arrow between NE and T3 simply indicates a possible interplay in some cases between the influence exerted by NE and that exerted by T3 (for details, see text). Question marks indicate the need of further confirmation or uncertainty.

synergistic α- and β-adrenergic effects (Raasmaja & Larsen 1989). Incidentally, mice lacking this enzyme display profound alterations in BAT functions: in particular, after cold exposure they activate shivering, which ensures survival but does not prevent them suffering mild hypothermia (de Jesus *et al.* 2001, Christoffolete *et al.* 2004). The increase in D2 activity seen in the cold is associated with an acceleration of the conversion of T4 to T3, leading as stated before, to the saturation of TR and to intracellular thyrotoxicosis specifically in BAT (Bianco & Silva 1988). This in turn increases the adrenergic responsiveness of the tissue (Sundin *et al.* 1984, Rubio *et al.* 1995a,b) in a feed-forward mechanism, allowing BAT to produce heat in a sustainable manner. Hypothyroid rats are unable to survive cold stress as their BAT fails to respond to cold exposure, and the consequent hypothermia, if not corrected by the administration of TH, proves fatal (Bianco & Silva 1987). The brown adipocytes of hypothyroid rats, apart from containing lower levels of UCP1 than those of euthyroid controls, respond to adrenergic stimuli by generating much less cAMP, due to changes in adrenergic-receptor density, G proteins and adenyl cyclase expression (Rubio *et al.* 1995b, Carvalho *et al.* 1996). Administration of physiologic doses of T4 (but not of T3) rapidly restores adrenergic responsiveness, UCP1 gene expression and BAT thermogenesis, by a mechanism that is blocked by IOP. Higher doses of T4 are associated with a blunted BAT response, an apparent paradox that can be explained by the finding (1) that sympathetic stimulation of BAT has an inverse relationship to the thyroid state (Silva 2001),

and (2) that D2 is activated by the sympathetic nervous system, but inhibited by its substrate T4 (Silva & Larsen 1986). All this would seem to explain the observation of a blunted BAT response to cold in hyperthyroidism.

During cold exposure, the activity of the lipogenic enzymes in BAT increases three- to fourfold. It has been reported that in isolated brown adipocytes, noradrenaline stimulates lipogenesis only in the presence of T4, and that pre-treatment with IOP blocks this induction, suggesting that D2 is required for the generation of T3 from T4, thus permitting the normal acute thermogenic function of BAT (Schneider *et al.* 2001). Exogenously administered T3 improves the cold tolerance of hypothyroid rats with a stimulating effect on oxidative capacity that, although greatest in BAT, is also evident in other metabolically active tissues (such as liver, heart and skeletal muscle). Underlying the effect of T3 in these tissues there is also a modulation of their trophism, a response that is fundamental to survival in the cold when high levels of energy expenditure are needed (Lanni *et al.* 1998). As 3,5-T2 is able to stimulate metabolism, as mentioned before, we compared the effects of T3 with those of 3,5-T2 (Lanni *et al.* 1998). We found that hypothyroid rats survived cold for 3–4 days, but when injected with 3,5-T2 their cold tolerance was improved, with an effect on both the specific and total oxidase activity of the analysed organs, and the animals survived, like those treated with T3, for at least the duration of the treatment. However, the action of 3,5-T2 does not affect the tissue trophism, and we, on the basis of previous studies (O'Reilly & Murphy 1992b, Goglia *et al.* 1994a,b), raised the possibility that the effects exerted by 3,5-T2 may be mediated by its direct interaction with mitochondria.

Ribeiro *et al.* (2001) have suggested that the effects of T3 on the noradrenaline signalling pathway in BAT are mediated by TH receptor $\alpha 1$ (TRα1) while, on the other hand, the TRβ isoform is not strictly required for the stimulation of UCP1. It has been reported that TRα1-knockout mice have a reduced body temperature, a phenotype never described for TRβ-knockout mice or in humans with TH-resistance syndrome due to mutations in TRβ genes (Flamant & Samarut 2003). Recently, it has been reported that mice devoid of all TR, despite having a slightly decreased body temperature and a greatly decreased BMR, can markedly increase their metabolic rate in the cold while being cold intolerant due to inadequate total heat production at low temperatures (Golozoubova *et al.* 2004). Moreover, in mice devoid of all TR, BAT is chronically stimulated by and desensitized to noradrenaline, indicating that such mice are chronically cold-stressed (Golozoubova *et al.* 2004).

Thyroid thermogenesis in other tissues

The blunted response of BAT seen after administration of a high dose of T4 suggests that tissues other than BAT can participate in adaptive thermogenesis, mainly skeletal muscle and liver (Fig. 4). In particular, the role of muscle remains unclear, although it could play an important role due to its large mass and high potential metabolic capacity. Indeed, experiments with cold-exposed UCP1-knockout mice, who continue to shiver even after several months in the cold (Golozoubova *et al.* 2001), have indicated that these animals are unable to recruit alternative sources of NST, a result that seems to argue against the participation of muscle in such thermogenesis. However, this does not mean that muscle is not a site of NST in its broadest sense. The liver has also been suggested as a possible site of adaptive NST (Gordon 1993, Jansky 1995), but its true involvement remains unclear (Depocas 1958, 1960) (Fig. 4).

The known UCP1 homologues could play a central role in the mechanism underlying the mitochondrial proton leak in tissues other than BAT, but this is still an open question. As already mentioned, T3 can affect the mitochondrial proton leak and the expression of UCPs (Lanni *et al.* 1999, 2003, de Lange *et al.* 2001). After the first report of the cloning of UCP1 homologues, it was believed that they might explain part of the thermogenic effects of T3 in tissues other than BAT (Ricquier & Bouillaud 2000, Lanni *et al.* 2003, Silva 2003). A role for UCP3 in the metabolic adaptation to cold exposure has been suggested in both rodents and humans (who, unlike rodents, possess very little brown fat in adult life) (Simonyan *et al.* 2001, Hagen & Vidal-Puig 2002, Schrauwen *et al.* 2002a, Wang *et al.* 2003). However, evidence is accumulating that thermogenesis and regulation of body weight may not be the main physiological functions of UCP2 and UCP3 and according to this, mice deficient in UCP2 or UCP3, do not display cold-intolerance and do not develop obesity (Hagen & Vidal-Puig 2002, Goglia & Skulachev 2003).

Recently, Zaninovich *et al.* (2003) studied the effects of long-term cold exposure on muscle and liver mitochondrial oxygen consumption in hypothyroid and normal rats. They demonstrated that TH, in the presence of noradrenaline, are major determinants of the thermogenic activity of both muscle and liver in cold-acclimated rats. Indeed, they showed that in both hypothyroid and normal rats, it was possible, upon cold exposure, to induce hypothermia *in vivo* and falls in muscle, liver, and BAT mitochondrial respiration *in vitro* only when β- and α_1-adrenergic receptors were blocked.

In recent years, another tissue seems to be assuming an increasing important role as a component of the complex network of mechanisms underlying energy homeostasis

and body weight control: namely the white adipose tissue (WAT). Particularly relevant are the studies indicating that WAT is a major site of endocrine secretion and release, and that it is involved in a number of functions besides simply fat storage (Trayhurn & Beattie 2001).

Conclusions and perspectives

The extensive range of studies that has accumulated in recent decades has progressively revealed more and more information about the key molecular components underlying the hormonal mechanisms implicated in energy balance, bodyweight control and facultative thermogenesis. However, the requirement for data has grown as the mechanisms underlying facultative thermogenesis became a target for the development of antiobesity therapies. Over the next few years, we are hopeful that the identification of the molecular determinants of such mechanisms will lead to the development of potential drugs capable of inducing an increase in energy dissipation. Among the still unresolved issues, there remain particularly relevant questions about the role of β-adrenergic agents, PGC-1, UCP1 homologues in tissues other than BAT, and the contribution of tissues other than BAT to adaptive thermogenesis, as well as questions about the role of T3 and other iodothyronines, such as 3,5-T2, in modulating energy expenditure. With regard to human physiology, issues that demand further investigation concern (1) the roles played by UCP3, Ca^{2+} homeostasis, and as-yet-unknown pathways in determining thermogenesis in muscle; (2) the possible roles played by other tissues, such as white adipose tissue, in energy balance; (3) how TH, nutritional status, and other hormonal and environmental factors interact with each other to determine energy expenditure and body weight; (4) the precise roles played by the different TR isoforms in the pathways underlying the observed effects of TH.

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