Thyroid hormones are the major endocrine regulators of metabolic rate, and their hypermetabolic effects are widely recognized. The cellular mechanisms underlying these metabolic effects have been the subject of much research. Thyroid hormone status has a profound impact on mitochondria, the organelles responsible for the majority of cellular adenosine triphosphate (ATP) production. However, mechanisms are not well understood. We review the effects of thyroid hormones on mitochondrial energetics and principally oxidative phosphorylation. Genomic and nongenomic mechanisms have been studied. Through the former, thyroid hormones stimulate mitochondriogenesis and thereby augment cellular oxidative capacity. Thyroid hormones induce substantial modifications in mitochondrial inner membrane protein and lipid compositions. Results are consistent with the idea that thyroid hormones activate the uncoupling of oxidative phosphorylation through various mechanisms involving inner membrane proteins and lipids. Increased uncoupling appears to be responsible for some of the hypermetabolic effects of thyroid hormones. ATP synthesis and turnover reactions are also affected. There appear to be complex relationships between mitochondrial proton leak mechanisms, reactive oxygen species production, and thyroid status. As the majority of studies have focused on the effects of thyroid status on rat liver preparations, there is still a need to address fundamental questions regarding thyroid hormone effects in other tissues and species.

Introduction

Thyroid hormones—the major endocrine controllers of metabolic rate

The hypermetabolic effects of thyroid hormones are widely known. Indeed a hallmark feature of hyperthyroidism is an elevated metabolic rate, and this has been recognized for well over a hundred years (1). A predominant outcome of thyroidectomy is decreased basal metabolic rate, which can be upward of 40% (2). There is also a direct relationship between circulating levels of thyroid hormones and metabolic rate, or energy expenditure. In fact the same allometric exponent in all mammals apparently describes the relationship between whole-body oxygen consumption and thyroid hormone turnover (3).

Thyroid hormones are thus widely regarded as the major endocrine controllers of energy expenditure. After thyroid-stimulating hormone (TSH) levels, resting energy expenditure is the most responsive parameter to circulating levels of thyroxine (T4) in patients receiving chronic T4 treatments (4). Before the development of thyroid hormone assays, the resting metabolic rate of an individual was the means through which thyroid status was assessed. The implications of thyroid hormone status for a healthy body weight have been widely studied. In one particularly interesting study, Astrup et al. (5) found that formerly obese (or “post-obese”) women had lower plasma triiodothyronine (T3) levels and basal metabolic rates than matched control women who had never been obese. Further, in the formerly obese women, the low free T3 levels in the plasma statistically accounted for the lower metabolic rates. Most obese individuals have normal thyroid function. However, subclinical hypothyroidism is common in adults, and in those with abnormally high TSH levels, decreased resting energy expenditure rates have been documented (6). Moreover, subclinical hypothyroidism may increase the risk for metabolic syndrome (7).
While it is clearly understood that energy expenditure is modulated by thyroid hormone status, much less well understood are the actual processes responsible for thyroid hormone effects on energy metabolism. Within cells of the body, mitochondria are responsible for the major energy transduction processes (e.g., oxidative phosphorylation). Mitochondria account for virtually all of a cell’s oxygen consumption. While it is well accepted that thyroid hormones have profound effects on mitochondrial energy expenditure, the mechanisms need further elucidation. This review focuses on the effects of thyroid hormones on mitochondrial energetics.

Mitochondrial energetics

Due to their importance in energy transduction processes, mitochondria have been traditionally referred to as the “powerhouses” of cells. It is in these cellular organelles that roughly 90% of a cell’s adenosine triphosphate (ATP) is produced through the oxidative phosphorylation system. Beyond energy transduction, mitochondria play essential roles in cell signaling (e.g., through alterations in the dynamics of calcium and of reactive oxygen species [ROS]) and in cell death through apoptotic and necrotic routes. The pathway of retrograde signaling from mitochondria to the nucleus is now known to affect many cellular and organismal events in health and in disease (8,9).

Scope of thyroid hormone effects

The scope of thyroid hormone effects upon metabolism is wide, even within the literature specifically describing thyroid hormone effects on mitochondria. However, within this literature there is the caveat that many of the studies have focused exclusively on the effects of T3. The biological effects of other thyroid hormone metabolites, such as T4, rT3, and 3,5-T2, are unfortunately less well studied. The unique effects of various thyroid hormone metabolites are described in Moreno et al. (10), another chapter of this monograph.

In addition, many studies have employed supraphysiologic doses of thyroid hormones. It is important to keep in mind that circulating concentrations of free hormone are in the pM range in a wide range of vertebrate species (11). The high doses of T3 that have frequently been used in in vivo and in vitro studies have resulted in micromolar concentrations in plasma and incubation media.

Thirdly, while some effects of thyroid hormones on mitochondrial energetics have been explained as direct/nongenomic effects upon membrane function, most are thought to be mediated through genomic mechanisms, that is, through altered expression of nuclear and mitochondrial genes. Characteristics of the genomic mechanisms are covered in other sections of this monograph. Nongenomic effects of thyroid hormones are more controversial and less extensively discussed in the literature, but have been studied for over 50 years [e.g., (12)]. However, studies of direct/nongenomic effects in human erythrocytes have clearly demonstrated increased Ca++ ATPase activity (13). Some of the nongenomic effects of thyroid hormones have implicated the physical association of thyroid hormones, which are amphipathic hormone molecules, with membranes (13), while others implicate the interaction of thyroid hormones with a cell surface G protein–sensitive receptor (14,15). Recent work demonstrates that integrin αvβ3 binds T4 specifically and that the integrin–thyroid hormone complex is needed for mitogen-activated protein kinase (MAPK) activation by physiological concentrations of T4 (16). High affinity receptors for thyroid hormone have been detected in liver mitochondria (17,18), with both the full-length and truncated forms of TRα1 detected in mitochondria from multiple tissues, including heart, skeletal muscle, brain, and spleen (19). Both the truncated and full-length forms were found to directly bind mitochondrial DNA, supporting the possibility that intramitochondrial T3 can promote transcription of mitochondrial DNA (19,20). Nongenomic effects have been reviewed by others (14,15,21,22).

Oxidative Phosphorylation

Oxidative phosphorylation—costs and benefits of imperfection

Oxidative phosphorylation is the mitochondrial system through which the oxidation of energy substrates in a cell is coupled to the activity of ATP synthase in the mitochondrial inner membrane. The ATP produced drives energy-demanding reactions not only in the mitochondrion but also in the cytoplasm after the ATP is exported by adenosine diphosphate (ADP)/ATP translocase. The activity of ATP synthase is powered by the electrochemical gradient existing across the mitochondrial inner membrane. The gradient, or “protonmotive force” (PMF), is produced by the three proton pumping complexes of the electron transport chain. Altogether, the oxidation of fuel substrates drives the activity of the mitochondrial electron transport chain, which in turn produces PMF, which then drives ATP synthase activity, and the ATP is used to support cellular work. These fundamental processes are depicted in Figure 1.

Mitochondrial fuel conversion efficiency is not perfect. In other words, the coupling of the oxidation of fuel substrates, such as fatty acids, to the synthesis of ATP is variable. To some extent, substrate oxidation is uncoupled from ATP synthase activity most of the time. As will be outlined in more detail below, many intra- and inter-species (e.g., mammalian vs. reptilian) studies have demonstrated intriguingly that the coupling efficiency of oxidative phosphorylation is correlated with many of the factors known to affect basal metabolic rate (23,24). Thyroid hormone status is one such factor.

Mitochondrial uncoupling directly affects the proportion of the energy in a fuel substrate that is captured as ATP and that which is simply “lost” as heat. While ATP-driven work within cells is also thermogenic (i.e., produces heat), the only outcome of uncoupled oxidative phosphorylation, in energetic terms, is heat. Clearly, the latter impacts energy balance and thermoregulation.

One form of mitochondrial uncoupling that plays a very important role in thermoregulation occurs in brown adipose tissue (BAT). BAT thermogenesis is important in thermoregulatory processes at birth in humans, in hibernation processes (e.g., arousal) in various animals, and in thermoregulatory processes overall in small mammals (25–27). Uncoupled oxidative phosphorylation in BAT is well controlled by the sympathetic neural system and by thyroid hormones, such that it can be rapidly activated and inactivated (26,27). The cellular mechanism is ultimately dependent on the unique presence of uncoupling protein-1 (UCP1) in this tissue. When
activated, the 32-kDa mitochondrial inner membrane protein allows protons to flow, or "leak," back into the mitochondrial matrix, thereby bypassing ATP synthase and dissipating PMF. As PMF drops, electron transport chain activity is increased in an "attempt" to restore a functional PMF. Thus, energy substrate oxidation is increased, but ATP is not produced; instead, the energy is released as heat. The importance of UCP1 in thermoregulatory processes is evidenced by the observed cold intolerance in the UCP1 knockout mouse (28).

As will be expanded upon below, thyroid hormones not only increase the capacity for UCP1-mediated uncoupling in BAT, but also activate other, as yet poorly understood uncoupling mechanisms in mitochondria of many other tissues. The important roles of the interactions between thyroid hormones and the sympathetic nervous system are reviewed by Silva and Bianco (29) in another chapter of this monograph.

**Thyroid hormone induction of mitochondriogenesis**

While the increased mitochondrial content of a cell or tissue does not in itself result in increased energy expenditure, it does increase the maximal "thermogenic capacity" of that cell or tissue. The hypermetabolic effects of thyroid hormones are partially due to an increased demand for ATP and, at least in some tissues, partially due to increased uncoupling. Thus, there is a need for increased thermogenic capacity through mitochondriogenesis (i.e., synthesis of new mitochondria). Since thyroid hormones activate ATP-consuming processes, such as the activities of the Na⁺-K⁺ ATPase and Ca⁺⁺ ATPase, there is an increased demand for ATP and thus for coupled oxidative phosphorylation. As will be reviewed in detail below, thyroid hormones also activate uncoupled oxidative phosphorylation through UCP1-independent mechanisms in tissues other than BAT. It is thus not surprising then that thyroid hormones are potent activators of mitochondriogenesis.

In eukaryotic cells, mitochondriogenesis is the result of complex interactions between the nuclear and mitochondrial genomes (30–33). Key events in thyroid-hormone-induced mitochondriogenesis include the increased expression of the mitochondrial transcription factors, nuclear respiratory factor-1 (NRF-1), and peroxisome-proliferator-activated receptor gamma coactivator-1α (PGC-1α) (34–36). Thyroid hormone effects on the expression of oxidative phosphorylation genes can occur through the interaction of the thyroid hormone receptor complex with hormone response elements in nuclear and mitochondrial genes. They can also occur indirectly through the induction of the nuclear-encoded mitochondrial transcription factors (36). In addition, transcriptional and posttranscriptional mechanisms are important for the effects of T3, for example, in the synthesis of cytochrome c oxidase subunits (37).

**The "futile" effects of thyroid hormones on oxidative phosphorylation**

The hypothesis that thyroid hormones (initially T4, and then T3) exerted their hypermetabolic effects by "uncoupling an oxidative phosphorylation" was put forth by Lardy and colleagues over 50 years ago (12,38). Indeed, they...
demonstrated that enzyme preparations from livers of hyperthyroid rats were less efficient in coupling phosphorylation with oxidation than were preparations from normal rats (12). Of note, these and other observations came a decade or so prior to Mitchell’s formulation of the chemiosmotic theory of oxidative phosphorylation. Thereafter, a number of studies focused on the acute effects of thyroid hormones on mitochondria from thyroidectomized rats [e.g., (39,40)]. Other studies examined the effects of thyroid status more generally on futile cycling pathways [e.g., (41)]. Table 1 provides a summary of characteristics of some of these early studies as well as some recent studies.

The acute effects of thyroid hormones on oxidative phosphorylation have been controversial to the extent that they have not always been detectable upon the addition of hormones to tissue preparations and isolated mitochondria. However, acute effects of thyroid hormones have been demonstrated in some laboratories through the addition of micromolar amounts of T3 to tissue preparations from thyroidectomized rats. For example, Corrigall et al. (42) demonstrated that the incubation of liver homogenate for 15 minutes with 1 μM T3 before isolating mitochondria restored the efficiency of oxidative phosphorylation toward normal. However, the direct addition of the hormone to the mitochondria was without effect. Acute effects were also investigated in the intact perfused liver of rats. Seitz et al. found that within 2 hours of the addition of T3 to the perfusate, the mitochondrial ATP/ADP ratio in hypothyroid rat liver was significantly decreased (43). They also demonstrated that the ATP/ADP ratio in livers from T4-treated hyperthyroid rats was significantly decreased in mitochondria and increased in the cytosol. Altogether they concluded that thyroid hormones increase mitochondrial respiration and ATP regeneration, which is associated with accelerated adenine nucleotide translocator (ANT) activity; i.e. increased translocation of ADP and ATP.

Acute effects have also been demonstrated in preparations of isolated mitochondia when incubated under defined conditions. The addition of 10 μM T3 to hypothyroid rat liver mitochondria doubles ANT activity at low ADP concentrations (44). Nicotinamide inhibits this, simultaneous to its inhibition of the ADP-ribosylation of a mitochondrial inner membrane protein having a similar molecular weight. The authors put forth the idea that the covalent modification of ANT to form its externally facing C-conformation increases its leakiness to cations (e.g., Ca++, H+, and K+) and increasing its capacity for ADP import. A related observation is that 30 nM Ca++ apparently stabilized the less leaky conformation of ANT through a mechanism that was distinct from ADP-ribosylation. With the more recently recognized importance of ANT in uncoupled thermogenesis through a proton leak (45), this idea should be further examined. The acute effects of 3,5-T2 and other thyroid metabolites are well described by others in this monograph. The following section focuses on the effects of in vivo thyroid status on proton leak.

Mitochondrial proton leak—basal and inducible

As described above, while mitochondrial oxidative phosphorylation is responsible for the synthesis of about 90% of a cell’s ATP, it is well known that this fuel conversion process is variable in its efficiency. Again, the oxidation of fuel substrates (e.g., glucose and fatty acids) is uncoupled from ATP synthase activity to a certain extent most of the time. The uncoupling caused by UCP1 activation in brown adipocytes was described above. In cells that do not express UCP1, uncoupling occurs through an as yet poorly understood process, basal mitochondrial proton leak (24).

Basal mitochondrial proton leak is thought to be physiologically important. In isolated hepatocytes it accounts for 20–30% of oxygen consumption (46). In perfused resting skeletal muscle in rats it accounts for approximately 50% of oxygen consumption (47). However, in vivo NMR determinations of mitochondrial energetics have supported the idea that only a small fraction of resting oxygen consumption in mouse skeletal muscle is due to uncoupling (48). Altogether, Rolfe and Brand have estimated that the contribution of basal mitochondrial proton leak to resting energy expenditure at the whole-body level could be in the range of 20–25% (47,49).

In the increasingly complex literature on mitochondrial proton leak, it is essential to distinguish between “basal” and “inhibitor-sensitive inducible” proton conductance. It is the former, basal proton leak/conductance, that is proposed to affect whole-body energy expenditure. This is the form of proton leak that varies between tissue types (50), between animals with different body mass (as well as mass-specific metabolic rates) (51–53), between endotherms and some ectotherms (54–56), and between different thyroid hormone states (57–59).

An influential study into the sites of thyroid hormone action in oxidative phosphorylation was that of Verhoeven et al. (60), in which metabolic control analysis was employed. The latter is a quantitative and integrative approach to identify the distribution of metabolic control within a pathway, or system of pathways. Liver mitochondria were isolated from hypothyroid rats or from rats 24 hours after treatment with a single dose of T3. T3 treatment resulted in increased control over oxidative phosphorylation flux by the AN, the dicarboxylate carrier, and cytochrome c oxidase, and decreased control by bc1 complex. As well, after T3 treatment, ANT operated at a higher (ATP/ADP)$_{baso}$ supporting previous findings on the stimulation of ANT by thyroid hormones.

Hafner and Brand (61) first demonstrated that basal proton leak was lower in mitochondria from hypothyroid rats and was higher in mitochondria from hyperthyroid rats, compared to euthyroid controls. That such effects were not restricted to isolated mitochondia was revealed by analyses of the kinetics of proton leak reactions in intact hepatocytes of hypothyroid and euthyroid rats (57). Basal proton leak was lower in hypothyroid than in euthyroid hepatocytes.

Shortly thereafter, metabolic control analysis was again applied to the oxidative phosphorylation system, including proton leak, as well as ATP turnover reactions in hepatocytes from hypo- and hyperthyroid rats (58,59). The aim overall was to identify the quantitatively most important sites of thyroid hormone action in the oxidative phosphorylation system. Hypothyroid hepatocytes had significantly lower resting rates of oxygen consumption than euthyroid hepatocytes (58). Approximately half (52%) of this decrease was due to reduced basal proton leak, and the other half was...
### Table 1. Summary of Characteristics of Some Relevant Studies Examining the Role of Thyroid Status on Mitochondrial Energetics

<table>
<thead>
<tr>
<th>Animal model (species, strain, gender, age/wt)</th>
<th>Treatment</th>
<th>Biological preparation studied</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat, SD, male, 150–175 g</td>
<td>Hypo: 0.1% PTU in diet for 3 weeks</td>
<td>Plasma membrane, colonocytes</td>
<td>(87)</td>
</tr>
<tr>
<td>Rat, CFY, male 200–250 g</td>
<td>Hypo: thyroidectomized-parathyroidectomized, Hyper: 20–30 μg T3/180–230 g BWt for 24 h</td>
<td>Liver mitochondria</td>
<td>(86)</td>
</tr>
<tr>
<td>Rat, Wistar, male, 12 weeks</td>
<td>Hypo: PTU 0.05% in drinking water for 4–5 weeks, Hyper: T3 150 μg/kg daily i.p. for 10 days</td>
<td>Heart mitochondria</td>
<td>(94)</td>
</tr>
<tr>
<td>Rat, Wistar, male, n/a</td>
<td>Hypo: T3 200 μg/kg daily i.p. for 7 days</td>
<td>Heart mitochondria</td>
<td>(75)</td>
</tr>
<tr>
<td>Rat, Wistar, male, 200 g</td>
<td>Hypo: T3 200 μg/kg daily s.c. for 25 days</td>
<td>Soleus and plantaris muscle mitochondria</td>
<td>(74)</td>
</tr>
<tr>
<td>Rat, Wistar, male, 220–230 g</td>
<td>Hypo: PTU 1 mg/100 g BWt daily i.p. plus iopanoic acid 6 mg/100 g BWt weekly i.p. for 3 weeks, Hyper: 15 μg T3/100 g BWt daily for 7 days</td>
<td>Skeletal muscle mitochondria</td>
<td>(72)</td>
</tr>
<tr>
<td>Rat, Wistar, male 200–250 g</td>
<td>Hypo: 0.05% (w/v) PTU in drinking water, Hyper: 0.8 mg T4/100 g BWt i.p.; one treatment given to euthyroid rats, Hyper: 40 μg T3/100 g BWt i.p.; one treatment given to hypothyroid rats</td>
<td>Liver mitochondria</td>
<td>(60)</td>
</tr>
<tr>
<td>Rat, Wistar, male</td>
<td>Hypo: 0.05% (w/v) PTU in drinking water, for 6–8 weeks, started at 4–5 weeks of age, Hyper: 15 μg T3/100 g BWt i.p. daily for 10 days, started at 8–9 weeks of age</td>
<td>Hepatocytes</td>
<td>(58)</td>
</tr>
<tr>
<td>Rat, Wistar, male</td>
<td>Hyper: 15 μg T3/100 g BWt i.p. daily for 10 days, started at 8–9 weeks of age</td>
<td>Hepatocytes</td>
<td>(59)</td>
</tr>
<tr>
<td>Rat, Wistar, male, 100–160 g</td>
<td>Hypo: thyroidectomized-parathyroidectomized (with 0.2% calcium lactate in drinking water); studies begun 6–10 weeks later</td>
<td>Hepatocytes</td>
<td>(57)</td>
</tr>
<tr>
<td>Rat, Wistar, male, 100–160 g</td>
<td>Hypo: thyroidectomized-parathyroidectomized (with 0.2% calcium lactate in drinking water); studies begun 8 weeks later</td>
<td>Liver mitochondria</td>
<td>(95)</td>
</tr>
<tr>
<td>Rat, Wistar, male, 100–160 g</td>
<td>Hypo: thyroidectomized-parathyroidectomized (with 0.2% calcium lactate in drinking water); studies begun 8 weeks later, Hyper: 0.05% (w/v) PTU in drinking water, for 8 weeks, Hyper: 8 mg T4 i.p. one treatment, Hyper: 15 μg T3/100 g BWt i.p. daily for 10 days</td>
<td>Liver mitochondria</td>
<td>(80)</td>
</tr>
</tbody>
</table>

(continued)
due to reduced extramitochondrial oxygen consumption. The decreased nonmitochondrial oxygen consumption in the hypothyroid hepatocytes was most likely due to decreases in peroxisomal and/or microsomal oxidative reactions. It had earlier been demonstrated that hypothyroidism results in a 75–85% decrease in microsomal P450 reductase protein and activity (62). Hyperthyroid hepatocytes had significantly greater oxygen consumption than euthyroid hepatocytes. In this case, approximately half was due to increased basal proton leak, and half was due to increased ATP turnover in the cells (58). The results support earlier findings demonstrating that thyroid hormones increase the activity of Na\(^+\)-K\(^+\) ATPase, Ca\(^{2+}\) ATPase, and gluconeogenesis (63). A novel nontranscriptional mechanism for regulation of Na\(^+\)-K\(^+\) ATPase by thyroid hormone was identified by Lei et al., who showed T3-induced translocation of Na\(^+\)-K\(^+\) ATPase to plasma membrane, rather than its increased gene transcription in rat alveolar epithelial cells (64,65). Metabolic control analysis of hepatocyte oxidative phosphorylation revealed that substrate oxidation reactions, and ATP synthesis and turnover reactions exert most of the control over resting state respiration; hypothyroidism and hyperthyroidism did not

Table 1. (continued)

<table>
<thead>
<tr>
<th>Animal model (species, strain, gender, age/wt)</th>
<th>Treatment</th>
<th>Biological preparation studied</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat, Wistar, male, 100–160 g</td>
<td>Hypo: thyroidectomized-parathyroidectomized (with 33 μM calcium lactate in drinking water); studies begun 6 weeks later. Hypo: 0.05% (w/v) PTU in drinking water, for 6 weeks</td>
<td>Liver mitochondria</td>
<td>(61)</td>
</tr>
<tr>
<td>Mice, Swiss Black, 129/SvC, male, 10 weeks</td>
<td>Hyper: 1 mg T3/kg i.p.; one treatment, cells collected 24 h later.</td>
<td>Hepatocytes</td>
<td>(96)</td>
</tr>
<tr>
<td>Rat, Wistar, male, 180–200 g</td>
<td>Hypo: 250 μCi Na(^{125})I i.p.; experiments conducted 3–4 weeks later. Hyper: 50 μg T4/100 g BWt/day for 3–4 weeks. Acute T3: 1 mM T3 added to perfusate in hypothyroid livers.</td>
<td>Isolated, perfused liver</td>
<td>(43)</td>
</tr>
<tr>
<td>Rat, Sprague Dawley, male, 130–150 g</td>
<td>Hypo: thyroidectomized-parathyroidectomized with calcium lactate in diet, or 0.01% (w/v) PTU in drinking water for 6 weeks. Acute in vivo T3 treatment: 1.0 nmol/100 g BWt injected into tongue vein 15 min before tissue collection.</td>
<td>Liver mitochondria and mitoplasts</td>
<td>(44)</td>
</tr>
<tr>
<td>Rat</td>
<td>Hyper: 0.25% desiccated thyroid gland in diet for over 2 weeks.</td>
<td>Kidney homogenate</td>
<td>(12)</td>
</tr>
<tr>
<td>Rat, Wistar, male, 8–12 weeks</td>
<td>Hypo: thyroidectomized-parathyroidectomized.</td>
<td>Liver mitochondria</td>
<td>(40)</td>
</tr>
<tr>
<td>Rat, Sprague Dawley, male, 250–275 g</td>
<td>Hypo: thyroidectomized-parathyroidectomized, studied 6–8 weeks later. Hyper: injection of 50 μg T3/100 g BWt, daily for 3 days.</td>
<td>Perfused isolated hindquarter</td>
<td>(41)</td>
</tr>
<tr>
<td>Mouse, C57Bl6, 1 month</td>
<td>Hypo: thyroidectomized-parathyroidectomized and 2% w/v calcium lactate in drinking water for 5–8 weeks. Hyper: 2.5 μg T3 i.p. daily for 6 days.</td>
<td>Liver and hind limb muscle mitochondria</td>
<td>(71)</td>
</tr>
<tr>
<td>Rat, SD, male, 325–350 g</td>
<td>Hyper: 200 μg T3/day for 14 days (osmotic mini-pump).</td>
<td>Soleus and plantaris muscles, liver, and heart (apex of left ventricle)</td>
<td>(73)</td>
</tr>
</tbody>
</table>

PTU: 6-n-propyl-2-thiouracil; BWt: body weight; i.p.: intraperitoneal; n/a: not available; s.c.: subcutaneously.
change the overall distribution of control (58,59). (Hypothyroidism and hyperthyroidism did, however, change the flux through various blocks of reactions, as described above.) Resting state cells were defined as cells isolated from fed rats, and incubated in a minimal medium without the addition of substrates for anabolic reactions (58). When the hypothroidy, euthyroid, and hyperthyroid cells were treated with oligomycin to induce state 4 (nonphosphorylating) respiration, metabolic control analysis indicated that respiration is predominantly controlled by the proton leak (58,59). Again, results were consistent with the idea that while thyroid status affects flux through branches of the oxidative phosphorylation system, the overall distribution of control is not substantially affected.

The mechanism(s) underlying basal proton leak and the thyroid-hormone-induced changes in leak are still poorly understood. Importantly, recent studies have demonstrated that half to two-thirds of the basal proton leak of mitochondria is catalyzed by ANT (45). While this uncoupling was shown to be independent of the ATP/ADP exchange function of ANT and of the known fatty-acid-induced leak function of ANT, further research is needed to re-examine the role of thyroid hormone effects on ANT-mediated basal proton leak.

Inhibitor-sensitive inducible proton leak has been the subject of intense research since the identification 10 years ago of the novel uncoupling proteins UCP2, UCP3, UCP4, and UCP5 (BMCP1). UCP2 mRNA is expressed almost ubiquitously, but it is only in cell types that express very high levels of UCP2 mRNA that there appears to be any UCP2 protein (66); skeletal muscle is not one of these tissues. Important to keep in mind when reviewing the literature on the effects of thyroid hormones on liver oxidative phosphorylation is that the liver parenchymal cell does not express UCP2 (or UCP3). In the liver, it is the Kupffer cells, the “resident macrophages,” that express UCP2. Compared to UCP2, UCP3 has a much more restricted pattern of tissue expression, with protein expression in skeletal muscle, BAT, and heart (67, 68). Although it was initially thought that UCP2 and UCP3 might cause the increased leak associated with hyperthyroidism (particularly because their promoters contain thyroid response elements), it is now generally agreed, as described below, that the novel UCPs do not contribute to basal proton leak (69,70).

The potential role of thyroid-hormone-induced expression of UCP2 and UCP3 on leak-dependent oxygen consumption has been examined by several groups. Jekabsons et al. (71) found that T3 treatment of thyroidectomized mice stimulated whole-body energy expenditure and UCP2 and UCP3 mRNA expression in gastrocnemius muscle, and UCP2 expression in liver. However, there was no increase in leak-dependent oxygen consumption in liver and muscle mitochondria. Reasons for the discrepancy between the latter finding and previous findings showing a direct relationship between leak and thyroid hormone status could relate to species differences (previous work was conducted in rats, not mice) and to the T3 concentration that was sixfold lower than that used in previous studies (61,72). Moreover, in a study of the effects of T3 on UCP expression and ATP production in tissues of the rat, it was found that T3 increases mitochondrial ATP production in oxidative muscle despite the coincident increased expression of UCP2 and UCP3 (73). While these findings are consistent with earlier findings supporting increased ANT activity and ATP turnover in hyperthyroidism, unfortunately, leak-dependent (uncoupled) respiration was not assessed. In a more recent study of the effects of thyroid hormones in oxidative (soleus) and glycolytic (planaris) muscles in rats treated with T3, Bahl et al. (74) demonstrated that T3 has a major effect on gene transcription, mitochondrial content, and leak-dependent (ADP-independent) respiration in slow oxidative muscle, but a lesser effect in glycolytic muscle. Findings are consistent overall with T3-induced increases in both coupled (ATP-dependent) and uncoupled (leak-dependent) energy expenditure mechanisms. It was postulated that the higher expression of thyroid hormone receptors and PGC-1z in oxidative muscle than in glycolytic muscle was at least in part responsible for the differences between the muscle types.

Boehm et al. (75) found in heart mitochondria of hyperthyroid rats that UCP2 and UCP3 protein expressions were increased in association with increased palmitate-activated proton leak. However, it is not known whether the increased leak was caused by the uncoupling proteins per se (e.g., fatty acid activation of ANT is a possibility). That mice lacking UCP3 (i.e., UCP3 knockout mice) exhibit a normal response to thyroid hormones further supports the conclusion that this UCP does not play a significant role overall in thyroid-induced energy expenditure (76).

Thus, while it remains possible that the novel uncoupling proteins may be involved in inhibitor-sensitive inducible proton conductance, perhaps for the purpose of protection from ROS (77–79), it is quite clear that thyroid-hormone-induced increases in the expression of UCP2 and UCP3 do not cause the increased basal proton leak caused by hyperthyroidism.

Mitochondrial Inner Membrane Lipid Composition

Given that thyroid hormones do not seem to be mediating their effects on energy expenditure through the UCPs, other mechanisms must be explored. Beyond the questions that remain regarding the role of the ANT in thyroid-hormone-induced changes in basal proton leak, there are also important questions regarding the impact of thyroid hormone modifications in mitochondrial inner membrane lipid composition.

Basal proton leak has long been correlated with the fatty acyl composition of the membrane phospholipids (52,53,55, 56,80). The fatty acid having the strongest positive association with basal proton leak is docosanoate (C22:6 n-3); the fatty acids having a strong negative association with leak are oleate (18:1 n-9) and linoleate (18:2 n-6) (52,53). However, as basal proton conductance does not change when the fatty acid composition of liposomes is changed (81,82), it is possible that fatty acyl–protein interactions play a significant role in thyroid effects.

Indeed, while there is a relationship between mitochondrial proton leak and mass-specific metabolic rate (51,83), there is no relationship between either free T4 or free T3 levels and mass-specific metabolic rate (11). For example, mice have a mass-specific metabolic rate that is 20 times that of cattle and horses, but circulating levels of free thyroid hormones are alike. On the other hand, as discussed in detail by Hulbert
(11), there is a strong correlation between tissue T4 concentration and the phospholipid composition of the tissue. The effects of thyroid hormones in a diverse range of animal species, and experimental approaches, demonstrate with little exception that almost every membrane system shows decreases in linoleic acid (18:2 n-6) content with hyperthyroidism, and increases with hypothyroidism (11). The opposite effects are observed for arachidonic acid (i.e., increases with hyperthyroidism and decreases with hypothyroidism). Consistent with these observations are the increased delta-6 desaturase activity with thyroid hormone treatment and decreased activity in hypothyroidism (84,85). As changes in mitochondrial fatty acyl composition and delta-6 desaturase have been observed within 1–2.5 hours after T3 injection (84), it has been argued that direct effects of thyroid hormones (not mediated through nuclear receptors) can be significant.

Physical characteristics of membranes and their fatty acid (FA) composition are also affected by thyroid hormone status, and such changes have been proposed as mechanisms through which oxidative phosphorylation could also be altered by thyroid hormones (11,84). Regardless of mechanism (nuclear receptor or direct interaction of thyroid hormones with the mitochondrial membranes), it is clear that thyroid status has an impact on membrane fluidity and lipid composition. Alterations specifically in mitochondrial membrane characteristics parallel those in other cellular membranes, such as the plasma membrane. Mitochondrial membranes as well as mitoplasts (mitochondria lacking the mitochondrial outer membrane, but retaining the inner membrane) isolated from livers of hypothyroid and hyperthyroid rats have decreased and increased membrane rigidity, compared to the euthyroid mitoplasts (86). Findings were corroborated in studies of liver mitochondria from thyroidectomized rats (87). In vitro studies in liposomes have demonstrated that T3 and T4 “rigidify” liposome bilayers in the liquid-crystalline phase (88). Moreover, the latter studies demonstrated that T3 can have the opposite effect (i.e., increase fluidity) when the membrane is in the gel state. In this regard, thyroid hormone effects are similar to effects of cholesterol on the biophysical characteristics of mitochondrial membranes.

Mitochondrial Reactive Oxygen Species

Mitochondria produce ROS as a byproduct of energy metabolism; approximately 0.2–2.0% of the oxygen consumed by cells is converted to superoxide and other, ROS (89,90). Since thyroid hormones impact upon mitochondrial energetics, it is not surprising that they also impact upon ROS.

In many respects the effects of hypo- and hyperthyroidism upon ROS production and damage appear to be tissue specific. Overall, however, hyperthyroidism is associated with increased and hypothyroidism is associated with decreased ROS production and damage. The mechanisms include not only altered production of ROS by the electron transport chain but also some changes in the protective mechanisms (e.g., superoxide dismutase and glutathione peroxidase activities). In human polymorphonuclear leukocytes, within minutes of the addition of T4, T3, or T2, superoxide production was increased; this occurred in a dose-dependent manner and, interestingly, it was Ca++ dependent (91). In a fairly comprehensive study of the effects of hyper- and hypothyroidism in rats, Asayama et al. (92) demonstrated that hyperthyroidism was associated with increased lipid peroxides in oxidative muscle (soleus) and heart, which was accompanied by increased mitochondrial superoxide dismutase. These changes were not observed in glycolytic muscle (extensor digitorum longus) or liver. The activity of another protective enzyme, glutathione peroxidase, was found to decrease in all tissues studied (oxidative and glycolytic muscles, heart, and liver), and a similar decrease in catalase activity in many of the tissues was also found. On the contrary, hypothyroidism resulted in decreases in several markers of oxidative stress, including a marginal decrease in lipid peroxides. Mitochondrial superoxide dismutase activity decreased. The authors suggested that their findings support the idea that hyperthyroidism results in increased ROS damage, particularly in the highly oxidative tissues (heart and soleus muscle), due to augmented oxidative metabolism and decreased glutathione protection.

Mitochondrial ROS production is affected by the flux of electrons through the respiratory chain and by PMF. It is highest under state 4 (nonphosphorylating respiration, equivalent also to basal proton leak respiration) conditions, when PMF is higher than it is during high rates of ATP synthase (e.g., during state 3 respiration). An important factor is the occupancy of the outer Q-site of complex III with the semiquinone anion (93), which occurs when ATP demand is low and PMF is high (i.e., at or near state 4 respiration). Unfortunately, only a few studies to date have examined thyroid effects in conjunction with mitochondrial bioenergetic characteristics (e.g., rates of state 3 and state 4 respiration and PMF values). However, Lopez-Torres et al. (94) took such an approach and studied also the sites of ROS production along the mitochondrial electron transport chain that were affected by thyroid hormone status. T3-treated rats and 6-n-propyl-2-thiouracil (PTU)–treated Wistar rats were compared to euthyroid Wistar rats. Hypothyroidism reduced cardiac mitochondrial hydrogen peroxide production when mitochondria were studied under state 4 and state 3 conditions. Decreased DNA damage was also noted in cardiac genomic DNA. Most of the decrease in the production of ROS occurred at Complex III of the respiratory chain, but there were decreases also at Complex I of the chain. Hyperthyroidism resulted in significantly increased state 4 respiration of the cardiac mitochondria. Interestingly, however, hyperthyroidism did not result in increased ROS production, and the authors postulate that this was due to the increased state 4 respiration. Had PMF been simultaneously assessed (and found to be decreased), empirical data for the latter would have been strong.

Priorities for research in this area would include investigations into the effects of thyroid hormones on ROS production and damage in mice lacking some of the proteins proposed to have roles in either basal proton leak or inhibit the inhibitor-sensitive inducible proton leak (e.g., UCP knockout mice and ANT knockout mice). The prevailing thoughts regarding the function of the novel UCPs hold that the UCP's act in a negative feedback loop to mitigate ROS production and damage. Moreover, thyroid hormones stimulate UCP expression (at least UCPs 1–3), but also increased ROS production and damage. The question that needs to be addressed is, are the UCPs playing any role in mitigating ROS production or damage?
Conclusion

Altogether it is clear that thyroid hormones have profound effects on energy metabolism. At the cellular level, many of these effects stem from changes in ATP demand (e.g., ion transport, including Ca$^{2+}$ ATPase and Na$^{+}$-K$^{+}$ ATPase), which necessitates changes in ATP synthesis through oxidative phosphorylation in mitochondria. Corresponding alterations in mitochondrial biogenesis also occur.

Additional effects include alterations in the efficiency of oxidative phosphorylation processes. In brown adipocytes, thyroid hormones stimulate uncoupling through UCPI, which is necessary for effective thermoregulation in some mammals. In other cell types thyroid hormones stimulate uncoupling, though the mechanisms are as yet poorly understood. Most of the literature on the effects of thyroid hormones on non-UCP1 uncoupling is based on studies conducted in liver, where the expression of novel uncoupling proteins is restricted to a very small proportion of cells (Kupffer cells). Thus, the uncoupling that is stimulated by thyroid hormones in liver parenchymal cells is not due to uncoupling proteins. Some of the basal uncoupling stimulated by thyroid hormones may be caused by alterations in the lipid composition of the mitochondrial inner membrane. Evidence also suggests that thyroid hormones may affect basal uncoupling through ANT, which has long been linked to the hypermetabolic effects of thyroid hormones. In some tissues thyroid hormones may induce mild uncoupling through the novel uncoupling proteins (e.g., UCP2 and UCP3), perhaps in a negative feedback loop to minimize ROS production. However, this is, at present, speculative, and it is not yet known if these proteins can function physiologically as true uncoupling proteins. The implications for thyroid hormones on the efficiency of energy expenditure and the handling of ROS are important, as hyperthyroidism and hypothyroidism are associated with increases and decreases in ROS damage. Thus, there is still much scope for research into the energetic effects of thyroid hormones. As the mechanisms underlying thyroid hormone activation of basal and inducible types of mitochondrial proton leak are not well understood, this is one area that requires specific attention.

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