Annu. Rev. Nutr. 1995. 15:263–91

THERMOGENESIS AND THYROID FUNCTION

Hedley C. Freake
Department of Nutritional Sciences, University of Connecticut, Storrs, Connecticut 06269-4017

Jack H. Oppenheimer
Department of Medicine, University of Minnesota, Minneapolis, Minnesota 55455

KEY WORDS: triiodothyronine, receptors, metabolism, substrate cycles, mitochondria

CONTENTS

ABSTRACT

The past 10 years have seen tremendous progress in the definition of the nuclear mechanism of action of thyroid hormones. Although the way in which these nuclear mechanisms underlie the 3,5,3′-triiodo-L-thyronine (T_3)–dependent stimulation of metabolic rate remains to be clarified, evidence favoring non-nuclear pathways is limited. Clearly, T_3 stimulates both the production and

0199-9885/95/0715-0263$05.00

consumption of energy within cells. It also exerts a number of parallel effects that result in increased oxygen consumption, e.g. on mitochondrial structure and composition; on the metabolism of lipids, carbohydrates, and proteins, and on cardiac function. Additionally, T_3 may increase the proton permeability of the inner mitochondrial membrane, which implies that it may decrease the efficiency of energy production. These metabolic effects of T_3 appear to be restricted to homeothermic animals, representing a coordinated response to the challenge of maintaining body temperature.

INTRODUCTION

The stimulatory effects of thyroid hormones on metabolic rate in humans were first described by Magnus-Levy (97) 100 years ago. The unique effect of these hormones on basal metabolism led to the use of basal metabolic rate (BMR) as an index of thyroid function. This method was the standard for many years, prior to the availability of more specific assays (149). This clinical utility not withstanding, little progress was made during the first 60 years of this century in determining how thyroid hormones functioned to produce these effects. However, as investigators began to understand mitochondrial structure and function, they suggested that thyroidal stimulation of metabolic rate resulted from the uncoupling of oxidative phosphorylation. Indeed, such effects were demonstrated in isolated mitochondria (90, 99). However, as Tata et al (149, 150) pointed out, these experiments used extraordinarily high amounts of hormone, and at any physiological concentration, respiration remained tightly coupled. Furthermore, in an analysis of the sequential biochemical changes induced by thyroid hormone, Tata & Widnell (151) observed that alterations in nuclear RNA synthesis preceded biochemical changes found in mitochondria. These findings raised the possibility that nuclear events mediate thyroid hormone–stimulated mitochondrial metabolism. As is discussed in greater detail below, subsequent studies have supported this hypothesis and have indicated that thyroid hormones exert multiple independent effects via specific receptors located in the nuclei of target tissues.

Although the biologic and clinical importance of thyroid hormone–stimulated thermogenesis has been generally recognized, the quantitative contributions of specific energy-consuming processes to total oxygen consumption in the hypo- and hyperthyroid states remain unclear. In this review, we describe the component elements in the mammalian thyroid hormone system, review current thinking with regard to the mechanism of action of thyroid hormones, and examine the specific components that contribute to thyroid hormone–stimulated thermogenesis. Lastly, we speculate on the significance of this process in the development of homeothermic vertebrates.

GENERAL CHARACTERISTICS OF THE THYROID HORMONE SYSTEM

Production and Metabolism

The only physiological function of iodide is as a precursor for thyroid hormones. Thus, any change in iodide status may affect thyroid hormone function. Following absorption in the gut, iodide is efficiently concentrated in the thyroid gland (57). The iodide is oxidatively coupled to tyrosine residues in the large protein thyroglobulin, forming mono- and diiodotyrosines. These in turn condense to produce the iodothyronines, L-thyroxine (T_4), and 3,5,3′-triiodo-L-thyronine (T_3) (57). Although both products are secreted by the thyroid gland, the tetra-iodo form predominates. The production and secretion of hormones by the thyroid gland is under the control of the pituitary glycoprotein thyroid-stimulating hormone (TSH), which in turn is negatively regulated by T_3, giving an efficient homeostatic mechanism for regulating thyroid hormone status (91).

T_3 is the most biologically active form of thyroid hormones (119). It may be produced directly from the thyroid gland, or peripherally from T_4 by the action of the deiodinase enzymes (94). There are two principal, quite different forms of this enzyme. Type I is found in liver and kidney, and type II is located in brain and brown adipose tissue (94). More recently, a type III thyroxine 5–deiodinase has been defined. Type III deiodinase inactivates T_4 and T_3 by removal of the iodine in position 5 of the inner ring (94).

The type I enzyme is nutritionally significant in that it is one of the very few known selenoproteins (9), and thus its activity is dependent on selenium status (6). There is an additional link between selenium and thyroid hormone status; the thyroid gland is dependent on the antioxidant function of another selenium enzyme, glutathione peroxidase, to neutralize the oxidative environment required for the production of thyroid hormones (24).

The deiodinase enzymes also convert T_3 to diiodothyronines (T_2). Diiodothryonines are considered inactive metabolites in the degradative pathway that have little, if any, action on calorigenesis in the whole animal (149) and low affinity for the nuclear T_3 receptor (85). However, some investigators have found T_2 to be quite effective in various in vitro systems (69, 89).

Mechanism of Action

The prescient observations of Tata and colleagues in the mid-1960s suggested that thyroid hormone might produce its manifold effects by stimulating nuclear mechanisms (151). However, this hypothesis did not receive immediate acceptance, and most authorities of the time adopted the view that thyroid hormone action was initiated at multiple cellular sites.

One of the major problems with this hypothesis for a nuclear mechanism

was that the site of interaction between thyroid hormone and nucleus had not been defined. In 1972, however, studies demonstrated limited-capacity, high-affinity sites for T_3 in the nuclei of rat liver and kidney following the injection of tracer [^{125}-I]-labeled T_3 together with increasing doses of unlabeled T_3 (116). Soon thereafter, similar nuclear-binding sites for T_3 sites were demonstrated in other rat tissues (120) and in lines of pituitary tumor cells (131). T_4-binding sites were not as easily demonstrated using these techniques because the receptor's affinity for T_4 is only one tenth that for T_3. The physiological implications of these differences were clarified by Braverman et al (16), who demonstrated that in humans, peripheral tissues convert T_4 to T_3. This finding was confirmed in total body analyses of rats following the injection of tracer T_4 (136). Additional studies showed a strong correlation between occupancy of nuclear receptors by thyroid hormone analogues and the biological response exerted by such analogues (85). Moreover, the correlation between occupancy of nuclear receptors and the level of specific mRNAs further strengthened the concept of a nuclear initiation (115).

In recent years, the operation of the nuclear signaling pathway has been defined in much more precise terms. A sine qua non for this progress was the identification in 1986 of the cellular counterparts of the v-erbA oncogene as the nuclear T_3 receptors (TRs) (132, 157). However, this discovery also complicated the picture of the nuclear thyroid hormone receptor because two separate genes, α and β, encode biologically active TRs in both rats (105) and humans (7, 157). These genes in turn encode multiple products that are expressed and regulated in a tissue-specific manner (92). These products include proteins that do not bind T_3 and that apparently can act as thyroid hormone antagonists (92).

Progress in defining the function of these receptors has been accelerated by the discovery that they are part of a large family of nuclear receptor molecules that includes those that mediate the action of steroid hormones, retinoic acid, and vitamin D (32). Although there are differences between the modes of action of the members of this large family of signaling molecules, they all function within the nucleus by binding to their receptors and changing the transcription rate of their target genes (5, 32). The receptors have a modular structure, with one region that binds the specific ligand and a second region, which is highly conserved across all members of the family, that binds to DNA (32). This DNA-binding region contains invariant cysteine residues, which chelate zinc. This mineral in turn stabilizes the protein structure, permitting DNA binding (8). A receptor identifies its target genes by the presence of specific response elements within the regulatory portions of the DNA that are usually found in the regions 5′ to the start site of transcription (5, 32). For the TR, the response element consists of a hexanucleotide in the form AGGTCA that is usually, and perhaps always, found in pairs or greater numbers, with

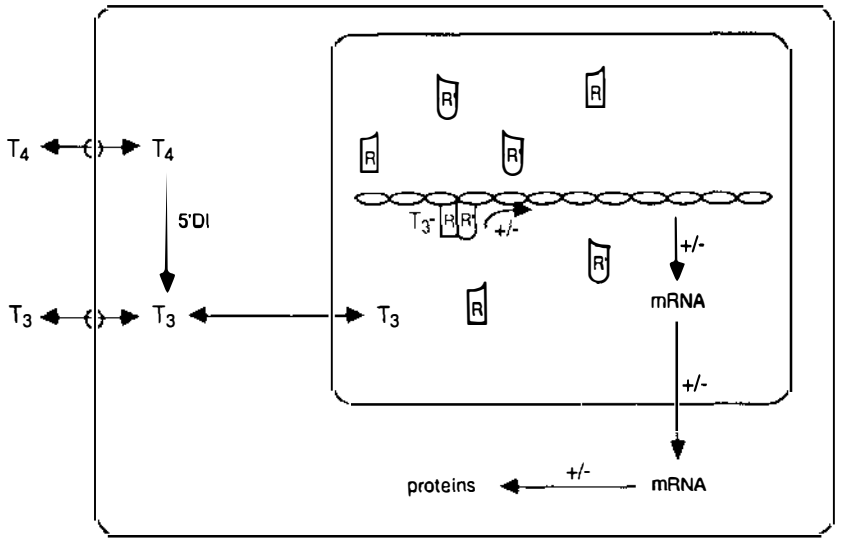

Figure 1 Nuclear action of thyroid hormone. Both T_4 and T_3 are delivered to the cell and taken up, probably by a carrier-mediated process. Cells that contain the deiodinase enzyme (5'DI) will convert T_4 to T_3; others will be dependent on a plasma source of T_3. Within the nucleus, T_3 stabilizes a complex consisting of a heterodimer between a T_3 receptor (R) and a 9-*cis* retinoic acid receptor (R'), and a thyroid hormone response element of a target gene. This process results in an altered rate of transcription, which in turn leads to altered mRNA and protein levels, and modified cellular function.

fixed separations between the sites (17). The precise spatial arrangement of these sites is critical because very similar sequences are used as response elements by other members of the nuclear receptor family (153).

The nuclear actions of T_3 are illustrated in Figure 1. In vitro experiments that examined the binding of homogeneous preparations of TRs to their response elements found that additional nuclear proteins were often required in order to demonstrate such interactions (20, 104). The identity of these proteins may vary from tissue to tissue (and from gene to gene), but in many cases, retinoid X receptor (RXR) has been identified as the active agent (93, 161). This protein is a member of the nuclear receptor superfamily; its ligand is 9-*cis* retinoic acid (58), an isomer of the normal all-*trans* form. Heterodimers between TRs and RXRs bind more tightly to TR response elements than do homodimers and also appear to elicit a more robust transcriptional activation of model target genes (93, 161). The receptors for all-*trans* retinoic acid and 1,25 dihydroxyvitamin D also heterodimerize with RXR (93, 161).

Three separate genes encode RXRs and multiple products of these genes (98). The additional multiplicity of TRs (92) suggests a high degree of regulational complexity. For example, a given response element might favor spe-

cific forms of each receptor. This preference might be influenced in a tissue-specific manner by the presence of other proteins that interact either directly with the receptors or with neighboring portions of the target gene DNA. The specifics of such interactions remain to be worked out, especially in vivo, but these receptor systems are clearly sufficiently complex to account for the physiology of thyroid hormone action observed in intact animals.

Proposed Nonnuclear Mechanisms

Remarkable progress has been made toward defining a nuclear pathway for thyroid hormone action. Investigations of pathways originating in other portions of the cell, however, have not been as productive. Reports of extranuclear pathways are frequently based on the putative demonstration of specific binding sites in subcellular fractions. In general, analysis of functional responses to thyroid hormones and their analogues, especially those occurring under in vivo conditions, does not support a physiological role for such sites. Additional studies are clearly needed to define the role of the proposed extra nuclear mechanisms. However, we briefly cite some of the suggestions that have been put forth in this area of research.

MITOCHONDRIAL MECHANISMS The effects of thyroid hormone on oxidative processes have focused considerable attention on the mitochondrion as a potential direct target for hormone action (144). Sterling & Milch (146) reported the existence of specific mitochondrial binding sites for thyroid hormone in 1975. However, another report was unable to confirm their existence (47), and the characterizations of such sites by two other groups differ sharply from each other as well as from the initial report (43, 55). The physiologically relevant effects of thyroid hormone on mitochondrial respiration are very likely mediated by the regulation of gene expression at the nuclear level (see below).

CELL MEMBRANE–INITIATED MECHANISMS Binding sites for thyroid hormone also have been described in the cell membrane (65, 86, 126). The overall process whereby thyroid hormones, or in fact any other hormone with a nuclear site of action, are taken up by the cell and delivered to the nucleus is rather obscure. For thyroid hormones, cellular uptake is stereospecific (117); saturable in isolated cell systems (86, 126), although apparently not in vivo (117); and sensitive to metabolic inhibitors (65). Whether such sites are simply involved in the transport of iodothyronines or whether they mediate some action of thyroid hormone remains unclear. For example, some investigators have reported that plasma membrane T_3-binding sites on thymocytes are saturable and can facilitate the transport of 2-deoxyglucose from the medium into the cell (139). However, the fractional rate of accumulation of labeled 2-deoxyglucose failed to decrease at concentrations of T_3 as high as 10^{-5} M (138).

Rapid membrane-based actions of thyroid hormone on calcium transport

also have been reported (72, 137). However, direct linkages between occupation of binding in the membrane and changes in cellular calcium have not been demonstrated. Indeed, these effects may result from receptor-independent interactions of hormone with membrane lipids and proteins (144). To envisage how these rapid and transient effects result in a delayed and long-lasting phenomenon such as stimulation of metabolic rate is difficult. Furthermore, the analogue specificity of some of these membrane actions is not the same as that of the calorigenic effects of thyroid hormones (72, 144, 149). Thus, although membrane-based effects of thyroid hormone may yet prove to be important, no clear relationship between these phenomena and the regulation of metabolic rate is apparent.

MECHANISMS INITIATED IN THE CYTOPLASM Thyroid hormones also appear to bind to a number of proteins located in the cytoplasm of cells (73). These cytoplasmic proteins may simply help to move the hydrophobic T_3 and T_4 molecules to the nucleus, or they may play a more active role in T_3 transport. Oppenheimer & Schwartz have suggested that the concentration of free T_3 in the nucleus is manyfold higher than that in the cytoplasm, suggesting that an active transport system may carry T_3 from the cytosol to the nucleus (117). Hashizume et al (56) reported a possible mechanism for establishing such a gradient, having found that the properties of one cytosolic T_3-binding protein are dependent on the NADP/NADPH ratio of the cell. NADP favors the delivery of T_3 to the nucleus, whereas NADPH causes the protein to hold T_3 in the cytosol. Along the same lines, Ashizawa & Cheng (1) have shown that cellular glucose, via its metabolite fructose 1,6-bisphosphate, inversely regulates the T_3-binding activity of another cytosolic protein, a monomer of pyruvate kinase. The transcriptional activity of T_3 and its transport to the nucleus are favored by glucose. These findings indicate an interesting mechanism whereby the nuclear signaling pathway might be modulated by both the substrate supply and the redox state of the cell.

COMPONENTS OF METABOLIC RATE

Thyroid hormones are unique in their ability to regulate the basal portion of metabolic rate. Danforth & Burger (25) divided basal heat production in homeotherms into two components (Figure 2), essential heat and obligatory heat. Essential heat is generated as a byproduct of all the normal metabolic processes. Under most circumstances, this heat is insufficient to maintain the required body temperature. It is therefore supplemented with obligatory heat, which can be regulated and which is dependent on thyroid hormones. Some environmental conditions cause greater heat losses and require supplementation of this basal heat production with adaptive heat. This supplementation is under control of the central nervous system and comprises both shivering and nonshivering thermo-

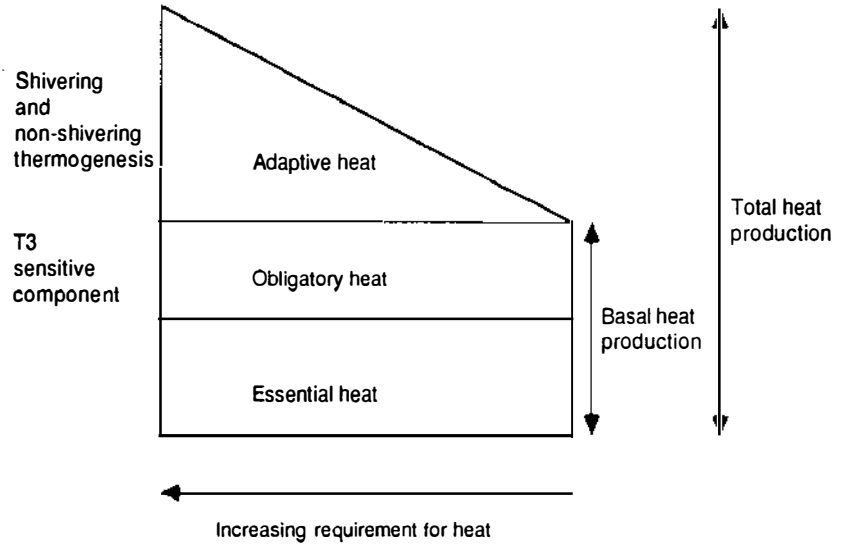

Figure 2 The components of metabolic rate at rest. The total amount of heat required to maintain body temperature depends on the environmental conditions. When obligate and essential heat are insufficient, adaptive heat is activated. Any increase in the amount of obligatory heat that is generated tends to decrease the requirement for adaptive heat. See text for definition of terms.

genesis. The latter process has been localized to brown adipose tissue (BAT) (38). This tissue contains a unique mitochondrial protein, uncoupling protein (UCP), which uncouples electron transport from the phosphorylation of ADP (110). Nonshivering thermogenesis and BAT are intimately connected with thyroid hormones, but they are not part of what is usually considered thyroid hormone thermogenesis. In hypothyroid animals, cold exposure does not increase UCP function (124) even though T_3 is necessary for the transcriptional induction of the UCP gene (10). However, BAT may be deactivated in the hyperthyroid state. Thus UCP induction by cold exposure is reduced in the hyperthyroid animal, and the tissue accumulates fat, presumably because its fatty acid oxidation is slower (148, 152). In the hyperthyroid state, when obligatory heat production is enhanced, the requirements for adaptive heat are reduced, and thus BAT is less active. Therefore, hyperthyroidism may actually reduce rather than stimulate thermogenesis in BAT.

PHYSIOLOGY OF THE CALORIGENIC EFFECTS OF THYROID HORMONES

Before considering the mechanisms that underlie thyroid hormone–induced thermogenesis, we delineate this process as clearly as possible. The literature

is not new, and although we have no particular reason to question it, fresh data may be desirable for some aspects of thyroid hormone–induced thermogenesis.

Magnitude

It is generally agreed that hypothyroidism results in an ~30% reduction in basal oxygen consumption in humans and rats; hyperthyroidism is associated with an ~50% increase in oxygen consumption (3, 118, 133, 142, 150). Overall, therefore, thyroid hormone approximately doubles the BMR. Although this result is obviously of great physiological importance, a twofold change in many biochemical assays is not particularly large. Such a change may not achieve statistical significance, and its interpretation may depend on the investigator. For example, thyroid hormone stimulation of expression of the β-subunit of F1-ATPase was deemed physiologically important by one group (76) but not by another (96).

Tissue Distribution

The exact location of the tissues responsible for the increase in BMR remains to be determined. Barker & Klitgaard (3) treated hypothyroid rats with a large dose of T_4 and measured oxygen consumption both in the whole animal and in numerous tissue preparations at various times after treatment. All tissues examined, with the exception of brain, spleen, and testis, exhibited increased oxygen consumption. Interestingly, the percentage increase in oxygen consumption of the whole animal exceeded that of any individual tissue, with the exception of heart (3). This finding suggests that either a crucial site of thyroid hormone–induced thermogenesis was omitted from the study or that the oxygen consumption measured in vitro did not accurately reflect the activity in vivo. The latter problem may be characteristic of many studies that attempt to assess regulation of oxygen consumption. This basic cellular parameter, which results from the integrated operation of the tissue, seems to be dependent on such whole-body functions as substrate delivery and removal by the circulation, as well as on nerve supply. An in vitro preparation will not only remove these factors but may also disrupt the physical organization of the tissue in crucial ways.

Rothwell & Stock (130) attempted to overcome these limitations by using radiolabeled microspheres to track blood flow in control and hyperthyroid rats. This technique had been used previously with great success to demonstrate the importance of BAT as the site of nonshivering thermogenesis (38). The greatest effect of the thyroid hormone measured was to stimulate blood flow to white adipose tissue (130). Hepatic arterial flow was also increased, as was supply to the gut, which ended up in the liver via the portal circulation. A lack of stimulation of blood flow to muscle was noted. Surprisingly, this study failed to demonstrate any increase in cardiac output, which, at least in humans,

is considered a standard consequence of hyperthyroidism (83). In addition, to determine oxygen consumption of a tissue in vivo, measurements of blood flow must be analyzed in conjunction with determinations of oxygen extraction by that tissue. Because these investigators did not perform such an analysis, we cannot exclude the possibility that muscle oxygen consumption, for example, was increased without significant change in blood flow. This result was observed in livers of hyperthyroid patients (107). Thyrotoxicosis resulted in little change in hepatic blood flow but caused a marked increase in oxygen extraction.

Support for the significance of muscle in the process of thyroid thermogenesis also comes from the earlier work of Tata et al (150), who found that the changes in oxygen consumption in the whole animal correlated better in time with effects on mitochondria from muscle than with those on mitochondria from liver. In addition, Capo & Sillau (21) found that thyroid hormone increased the capillarity of rat muscle in a manner dependent on muscle type.

In summary, although a large body of evidence from in vitro studies suggests that most, but not all, tissues of the body respond to hyperthyroidism by increasing oxygen consumption, the situation in vivo remains unclear, particularly in terms of the quantitative significance of different tissues.

Time Course

Another hallmark of the stimulation of metabolic rate by thyroid hormone is a distinct lag time following administration of hormone before any response is seen. Indeed, until more recent studies showed very fast transcriptional effects of T_3 and very rapid membrane-based phenomena (see above), all actions of thyroid hormone were thought to occur only after a delay of several hours. Although this delayed increase in whole-body oxygen consumption appears to be widely accepted, the exact length of the delay is unclear. In Barker & Klitgaard's study (3), whole-body oxygen consumption was increased one day following hormone administration, the first time point at which consumption was measured. Oppenheimer et al (118) subsequently confirmed this finding. Tata and coworkers (150) suggested a lag time of 20–30 h, and their data include some early time points at which no change is apparent. Myant & Whitney detected an increase in oxygen consumption 18 h after giving T_3 to hypothyroid rats (106). Thus, although many hours pass before any effects of thyroid hormones on metabolic rate are apparent, the exact duration of this lag time is not clear. In any event, it is much longer than that required to see thyroid hormone–dependent changes in oxygen consumption in mitochondria in vitro (60) or even in perfused liver (101). These results lead us to question to what extent these phenomena are associated with the calorigenic response in vivo.

Analogue Specificity

The analogue specificity of thyroid hormone stimulation of oxygen consumption is another question worth reexamining. Tata (149) reviewed a large body of data from contemporary studies in 1963. He reported that T_3 was more active than T_4, that the L forms were more active than the D forms, and that the loss of an iodide from the inner ring to give 3,3′,5′-triiodothyronine (reverse T_3) or any of the diodothyronine analogues resulted in an almost total loss of activity. Some calorigenic potency could be regained by substitution of methyl groups for the iodide. The question of analogue specificity was revisited recently following the discovery of multiple forms of the nuclear receptor. The response of a given tissue to thyroid hormone analogues is determined by the affinity of the receptor for the analogue and the steady-state concentration of the analogue. The affinity of thyroid hormone receptors for a given analogue is generally independent of the isoform of the receptor or the tissue of origin. Therefore, it would be most unexpected to find two analogues that exhibit different relative potencies in two tissues or differences in potency between an in vivo response system and a relevant in vitro response system. Reports that T_2 exhibits a greater metabolic effect than T_3 in some in vitro systems (69, 89) contradict the finding that T_2 has at best 1/30 the activity of T_3 when stimulating metabolic rate (149) and is a poor competitor for the nuclear receptor (85). A report that a synthetic T_3 analogue exhibited a preferentially affected liver metabolism while sparing thyroid hormone action on the heart (154) attracted much attention because it indicated a potential role for T_3 in the treatment of lipid disorders, but this finding has not yet been confirmed.

MECHANISMS BY WHICH T_3 REGULATES METABOLIC RATE

In the past, investigators frequently assumed that the calorigenic effects of thyroid hormone reflected a simple effect on either energy production (e.g. uncoupling of oxidative phosphorylation) or energy expenditure [e.g. stimulation of Na^+,K^+-ATPase (74, 75)]. It now appears likely that T_3 affects both arms of this axis, probably independently. For instance, although higher thyroid hormone levels are associated with increased amounts of respiratory chain components, those components are found in a more reduced state within the cell (66). Thus, although T_3 increases the oxidative capacity of the system, it also disproportionately increases the supply of reducing equivalents. Furthermore, a predominant effect of T_3 on either energy production or energy consumption might be expected to disturb the phosphorylation potential of the cell, but, as reviewed by Sestoft (141), no such changes are evident. More recently, Seitz et al (140) found that hyperthyroidism was associated with

increased cytosolic and decreased mitochondrial ATP/ADP ratios, changes they attributed to an effect of thyroid status on mitochondrial adenine nucleotide transport (see below). However, Kalderon et al (81) confirmed that T_3 treatment reduced the mitochondrial phosphate potential but showed that the cytosolic ratio was unaffected. They suggested that the reduced mitochondrial potential was caused by a decrease in ATP production. At any rate, T_3 clearly regulates both the production and use of energy within cells.

T_3 and Energy Production

Because thyroid hormones regulate oxygen consumption, once the role of mitochondria in this process was established, they appeared to be likely targets for hormone action. Direct action of T_3 on mitochondria has been suggested based on three pieces of evidence. First, high-affinity binding sites were found in the mitochondrial membrane (43, 55, 146). However, as discussed above, evidence linking these sites to the biological function of the hormone is still lacking. Second, some effects of T_3 on mitochondria are so rapid that it is difficult to envisage a nuclear pathway operating in that span of time (18, 101). Third, thyroid hormone may have direct effects on isolated mitochondria (121, 147). However, one cannot assume that the simple replication in vitro of an effect of thyroid hormone observed in vivo means that the mechanisms involved are identical. Although adding thyroid hormone to isolated mitochondria in vitro simulates the increased amino acid incorporation observed in vivo, different mechanisms appear to initiate these processes (45).

Regardless of whether T_3 has direct nonnuclear actions on mitochondria, at least some of the calorigenic effects of the hormone are manifest by changes in these organelles. The thyroid state–dependent changes in respiration, observable in the whole animal, are also evident in both intact hepatocytes and isolated mitochondria prepared from those animals (12, 62). These in vitro effects persist with a variety of substrates and in the absence (state 4) or presence (state 3) of ADP. A variety of experimental approaches have been used to analyze the mechanism(s) underlying T_3-dependent alterations in mitochondrial function.

EFFECTS ON THE AMOUNTS AND ACTIVITIES OF MITOCHONDRIAL PROTEINS

Given the clearly established nuclear pathway for thyroid hormone action, it is tempting to suppose that its effects on mitochondrial respiration are due to increased expression of genes encoding proteins required for this process. Thyroid hormone has a general stimulatory effect on mitochondrial biogenesis (49, 108), particularly on the surface area of the inner membrane (79). Candidate proteins include the components of the respiratory chain; the F1-ATPase; and the adenine nucleotide translocase, which is required for the transport of ADP into the mitochondria. Effects of hypothyroidism and the consequent

administration of T_3 have been observed both for nuclear-encoded proteins, such as cytochrome c (66, 134) and cytochrome c_1 (96) and for mitochondrially encoded proteins such as subunits II and III of cytochrome c oxidase (96, 156, 159). Indeed, the synthesis of all mitochondrially encoded mRNAs is stimulated by T_3. However, this process may occur via a nuclear mechanism, since the mitochondrial genome does not encode any proteins capable of regulating its own transcription (96, 156, 159). The data are not in agreement as to whether T_3 regulates the synthesis of some mitochondrial proteins, e.g. the β subunit of F1-ATPase (76, 96) and some of the nuclear-encoded subunits of cytochrome oxidase (96, 156, 159). As suggested above, this discrepancy may reflect the technical difficulties associated with measuring small changes in the levels of specific mRNAs, particularly against the backdrop of generalized effects of thyroid state on production of both messenger and ribosomal RNA.

Although changes in the levels of mitochondrial proteins may cause some of the effects of thyroid hormone on mitochondrial respiration, they are not sufficient to explain the full effects. Increased flow through the respiratory chain can be accomplished either by increasing amounts of the components of the respiratory chain or by increasing their reduction state. Thyroid hormone does both, but the effects on reduction state occur earlier than those on amounts (66). Indeed, the increased expression of the genes encoding mitochondrial proteins may not be a direct effect of T_3 but rather a secondary response. The direct stimulus could be a general signal resulting from an increased load on mitochondrial function or a *trans*-acting factor specifically induced by T_3. Some sequence information is available about the promoter regions of the genes in question, and potential thyroid hormone response elements have been suggested (76, 159). However, there is no proof that these putative response elements are biologically active. Thus the question of whether the expression of these mitochondrial proteins is directly or secondarily regulated by T_3 remains open.

PROTON LEAK The extent to which the amounts of the components of the respiratory chain limit respiration also has been questioned (12). Metabolic control analysis is a technique that determines the proportional control that individual steps in a complex pathway exert over flux through that pathway (33). Groen et al (48) first applied this approach to oxidative phosphorylation, and in recent years Brand and coworkers have refined and adapted it to examine how thyroid state influences the control exerted over mitochondrial respiration by individual steps (13). These latter authors initially examined hepatic mitochondria isolated from rats of different thyroid states. In state 4 mitochondria, they found that the most significant regulator of oxygen consumption was the passive leak of protons across the mitochondrial membrane (51, 53). Relative to euthyroid controls, this leak was reduced in hypothyroid

mitochondria and increased in mitochondria prepared from hyperthyroid rats. In state 4, mitochondrial respiration is limited by the absence of ADP, but when ADP is not limiting (state 3), the role of proton leak in mitochondrial function is insignificant (48, 53). In state 3 mitochondria, hypothyroidism reportedly limited oxygen consumption by reducing the activities of F1-ATPase and the adenine nucleotide translocator, whereas hyperthyroidism stimulated oxygen consumption through effects on the respiratory chain (53). Interpretation of these experiments in isolated mitochondria is complicated because in vivo respiratory control is between states 3 and 4. In addition, thyroid state also may affect reactions at other sites in the cell that consume ATP or affect the redox potential (and thereby influence mitochondrial function). However, results of studies in hepatocytes isolated from rats of different thyroid states were similar (54). Thus proton leak may be an important contributor to changes in oxygen consumption caused by both hypo- and hyperthyroid states, accounting for ~50% of the differences between the two. Interestingly, a reduction in nonmitochondrial oxygen consumption also was important in hypothyroid cells. This reduction did not contribute to the change in oxygen consumption that occurs during the euthyroid-to-hyperthyroid transition. The latter change was dependent on increased ATP turnover and increased proton leak (54).

Brand and coworkers examined chronic alterations in thyroid state and reported that these changes were slow to develop and not related to any rapid effects of T_3 on mitochondrial function (51, 53). Horrum et al looked at the effects of T_3 treatment on respiration and proton permeability of the inner mitochondrial membrane and found both to be increased after 9–12 h (68). However, whereas the increase in oxygen consumption (measured in isolated mitochondria) was inhibited by actinomycin D, the effects on proton permeability were not altered by this inhibitor of transcription. These authors' data, which require confirmation, not only suggest a nontranscriptional route for T_3 regulation of the proton permeability of the inner mitochondrial membrane but also call into question the role of this process in stimulation of oxygen consumption. Brand et al (15) also have shown that T_3-dependent changes in the proton leak across the inner mitochondrial membrane can be attributed to (*a*) an increase in the surface area of the membrane and (*b*) an enhanced permeability of the membrane for protons. The latter factor may be associated with changes in the mitochondrial phospholipids, particularly the degree of unsaturation of the fatty acids.

MEMBRANE LIPID COMPOSITION Changes in the lipid composition of the mitochondrial membrane appear to be a well-established feature of altered thyroid states (62). Hypothyroidism is associated with an increase in the degree of saturation of fatty acids in membrane phospholipids, notably decreased 20:4

and increased 18:2 (22). These changes also are seen in other cellular membranes and may result from general effects of T_3, presumably mediated at a nuclear level, on the metabolism of fatty acids. For example, T_3 affects the synthesis, desaturation, elongation, and oxidation of these compounds (62). A higher saturation index implies a more tightly structured membrane, which will contain less water and therefore have reduced proton permeability (62). Changes in membrane lipids can affect not only the permeability of the membrane, but also the activities of membrane proteins. Cardiolipin is a mitochondrial phospholipid whose synthesis is regulated by thyroid hormones (70). In rat heart mitochondria, levels of cardiolipin are decreased by hypothyroidism (122) and increased by hyperthyroidism (123), although this may not be the case in liver (63). Proteins whose activity depends on membrane lipid composition in general and on cardiolipin content specifically include cytochrome c oxidase (122, 123) and the adenine nucleotide translocase (62).

ADENINE NUCLEOTIDE TRANSLOCASE The process of ADP transport into mitochondria is dependent on thyroid hormone (2, 61) and is clearly important for the control of respiration. However, the T_3-induced stimulation of ADP uptake is achieved without an alteration in the number of translocators. On northern blot analyses, T_3 has no effect on expression of the translocase gene (64). Thus the changes in translocase activity appear to result from alterations in its lipid environment (62). It is interesting to speculate to what extent other mitochondrial changes are caused by T_3 effects on membrane composition. Indeed, Soboll (144) has suggested that some of the short-term effects of hormone treatment that are seen in various systems may mimic the long-term effects because thyroid hormone directly interacts with membrane lipids. Uptake of thyroid hormones into the membrane may cause changes in membrane fluidity similar to those associated with changes in fatty acid composition and thereby affect membrane protein function. Whether these effects ever occur in vivo remains to be determined.

DOES THYROID HORMONE UNCOUPLE RESPIRATION? For the past 30 years, reviews on thyroid hormone and thermogenesis have stated that T_3 does not uncouple oxidative phosphorylation. This conclusion is based largely on the work of Tata et al (150). However, these findings appear to contradict the idea, put forward by Brand, that T_3 stimulates the proton leak (53). If respiration is normally tightly coupled to ATP production, how can T_3 loosen this linkage by stimulating the passive return of protons without affecting the P/O ratio? First, P/O ratios are almost always measured under state 3 conditions, i.e. in the presence of saturating levels of ADP. This measurement is usually performed in isolated mitochondria (59), and sometimes in vivo (36), and gives a ratio of ~2.5. This measurement eliminates any influence of the proton leak because

proton leak plays a very minor role in state 3 respiration (48, 53), and measured P/O ratios are therefore close to the ideal. As mentioned above, under most circumstances, the respiratory state of mitochondria is usually somewhere between states 3 and 4. In state 3, the P/O ratio will be the ideal, i.e. 2.5 (59), whereas in state 4 it will be close to 0 because there is no ADP to phosphorylate. Thus in reality, the P/O ratio will likely be considerably less than 2.5, with the precise value depending on the state of activation of the tissue.

Brand et al (14) confirmed this finding by measuring P/O ratios in hepatic mitochondria at various respiration rates intermediate between states 3 and 4. They also calculated P/O ratios in hypothyroid and euthyroid hepatocytes respiring at less-than-maximal rates and observed only minor differences (14). If proton leak accounts for a significant proportion of oxygen consumption, then this leak could be increased by thyroid hormone without substantially changing the P/O ratio, provided the processes involved in producing ATP were also stimulated in parallel, which is exactly what Harper & Brand suggested (54). Interestingly, in skeletal muscle and (to a lesser extent) heart, respiration rates are relatively low under basal conditions, the conditions in which the influence of thyroid state on metabolic rate is usually measured. Under these circumstances, respiration should be shifted in the direction of state 4. This shift should increase the significance of proton leakage as a contributor to respiration.

Brand and colleagues have put forward interesting and provocative ideas about the control of respiration in general and by thyroid hormone in particular (14, 53). However, these ideas require confirmation using other experimental approaches before they are widely accepted. For example, Gregory & Berry (46) measured rates of gluconeogenesis and ureogenesis in hepatocytes taken from rats of different thyroid states. Assuming a P/O ratio of 3, they calculated the cost of the observed rates of synthesis and concluded that a T_3-dependent stimulation of noncoupled respiration occurred. This conclusion could still hold true if a P/O ratio of 2.5 were used. Thus, the debate as to whether thyroid state affects the efficiency of energy production has been reopened.

T_3 and Energy Usage

Even if T_3 decreases the efficiency of oxidative phosphorylation, it also stimulates increased production of ATP. This ATP must be consumed at a faster rate, or respiration will be limited owing to a reduced supply of ADP. The question then is as follows: Which of the ATP-consuming processes within the cell are stimulated by T_3, and to what extent do they contribute to the enhanced respiration?

HEART WORK A familiar clinical consequence of hyperthyroidism is increased heart rate, which results in an enhanced ability to supply oxygen to tissues.

Cardiac output is approximately doubled in hyperthyroidism, and cardiac size and stroke volume are also increased (83). Direct effects of T_3 on the heart have been demonstrated. For example, T_3 stimulates the transcriptional rate of the α-myosin heavy chain (α-MHC) gene, with a concomitant inhibition of the β form (78). The promoter of the α-MHC gene has been well investigated and thyroid hormone response elements delineated (37, 77). Other cardiac genes also are affected directly by T_3, including stimulation of myosin ATPase and sarcoplasmic reticulum Ca^{2+}-ATPase (30, 128). However, the influence of thyroid state on the heart appears to go beyond these direct effects. Klein and colleagues transplanted a second heart into a rat such that it was exposed to the circulation but under no hemodynamic load (84). The hypertrophy evident in the functioning heart upon thyroid hormone treatment was not seen in the transplanted heart, suggesting that at least some of the hormonal effects resulted from the increased work load. However, T_3 still affected the expression of specific genes in the unloaded heart (113). This observation demonstrates that the overall influences of thyroid state on the heart result from a combination of direct transcriptional effects and indirect stimulation of performance brought about by the increased load. This well-documented regulation of cardiac physiology provides an interesting parallel to the aforementioned mitochondrial effects of T_3, which also appear to result from the aggregate of the direct effects on some of the relevant genes and the indirect effects caused by the enhanced demand for ATP.

The increased heart work, which can be attributed to both direct and indirect effects of T_3, will require ATP and enhanced oxygen consumption. Sestoft (141) assumed that cardiac oxygen consumption would be increased proportionately to cardiac work and therefore suggested that as much as 30–40% of the thyroid hormone–dependent oxygen consumption could be attributed to the increased energy needs of the heart. Although this figure may be partially inflated because T_3 also decreases systemic vascular resistance (83), the heart is likely a major contributor to the increased respiration in hyperthyroidism.

ION PUMPING Maintaining ion gradients across cellular membranes is an energetically costly process, particularly for sodium and potassium. However, there are wide variations in the proportions of cellular energy consumption attributable to the cell membrane Na^+,K^+-ATPase (23). There are major differences in the energetic significance of this process in different tissues. Clausen et al (23) ascribe less than 5% of total oxygen consumption in liver and heart of euthyroid animals and slightly more than 5% of total consumption in skeletal muscle of euthyroid animals to the Na^+,K^+-ATPase. Almost 50% of total oxygen consumption in brain and an even greater percentage of consumption in kidney, where the enzyme functions to reabsorb sodium, are attributed to the Na^+, K^+-ATPase (23). In 1970, Ismail-Beigi & Edelman (74) showed

that the activity of this transporter was regulated by thyroid hormone and that it could account for as much as 90% of the T_3-stimulated oxygen consumption in some tissues. They treated rats with thyroid hormones in vivo, removed tissues, and determined the extent to which the T_3-dependent increment in oxygen consumption in vitro could be blocked by ouabain, a specific inhibitor of the Na^+,K^+-ATPase (74, 75). T_3 was subsequently shown to increase expression of the genes encoding Na^+,K^+-ATPase (41), although whether this increase is a direct transcriptional effect of T_3 or secondary to an increased leak of sodium and potassium across the membrane (50) is unclear.

The quantitative significance of cation transport for thyroid thermogenesis has been questioned. The use of tissue slices and homogenates in the original work (74, 75) might result in artificially high estimates of Na^+,K^+-ATPase activity because the damaged cell membranes would be exposed to extremely high concentrations of sodium, resulting in maximal rates of pump operation (23, 141). Furthermore, long-term incubation of preparations with ouabain may also lead to artificially high estimates of pump energetics because changes in intracellular sodium and potassium would depress oxygen consumption (23, 111). Measurements using more intact preparations suggest much lower contributions, i.e. in the range of 5–10%, of this process to thyroid thermogenesis (23, 141), although some studies with hepatocytes have indicated values approaching 30% (111). At any rate, the energy costs of the Na^+-K^+-ATPase clearly do not constitute the major determinant of thyroid hormone thermogenesis but rather form one part of a much broader set of actions.

SUBSTRATE CYCLES Another well-known characteristic of thyroid hormone is its effect on the metabolism of all macronutrients. Hyperthyroidism is associated with stimulation of both anabolic and catabolic processes. The enhanced flow through these substrate cycles appears energetically wasteful and clearly contributes to the increased heat production caused by thyroid hormone (109).

Lipid Metabolism Thyroid hormone stimulates fatty acid synthesis, particularly in liver but also in several other tissues of the rat (11, 26, 29, 39). This stimulation is the result of the coordinate induction of the expression of genes encoding the enzymes of fatty acid synthesis, as well as those genes responsible for generating the necessary reducing equivalents (29, 44, 119, 129).

The first increase in hepatic lipogenesis is not seen until 12–16 h after hormone injection; maximal levels are reached after ~4 days (39). This time course is quite similar to that of oxygen consumption following T_3 treatment (3, 150). The ATP costs of thyroid-dependent lipogenesis can be calculated using equations generated by Flatt (35) and then converted to an oxygen requirement. When these values were divided by the changes in total oxygen consumption seen in similar animals, a contribution of 5–10% for the process

of fatty acid synthesis was calculated (39). From a subsequent study that determined energy expenditure using measurements of caloric balance, investigators concluded that the energy costs of additional lipogenesis accounted for only 3–4% of the increment in energy output in the transition from euthyroidism to hyperthyroidism (118). Thus fatty acid synthesis per se does not appear to contribute significantly to the calorigenic actions of thyroid hormone.

Several additional pathways of fatty acid metabolism are responsive to thyroid hormone (62). Anabolically, thyroid hormone stimulates fatty acid chain elongation and desaturation (62, 88), as well as esterification into phospholipids and triglycerides (52, 62, 129, 160). This latter role is somewhat controversial; in perfused liver there is an inverse relationship between thyroid state and triglyceride export (82, 87). However, in perfused liver one does not have the enhanced substrate supply, including free fatty acids and glycerol, associated with the hyperthyroid state (52). Because fatty acid oxidation is also stimulated by hyperthyroidism (see below), competition between this process and esterification may limit the activity of both pathways if substrate supply is not enhanced.

Lipolysis is also positively correlated with thyroid state. T_3 enhances lipolysis by increasing the sensitivity of the process to catecholamines (27, 28, 34). These hormones interact with membrane receptors to enhance intracellular levels of cyclic AMP, which results in the activation of hormone-sensitive lipase and thereby lipolysis. T_3 may augment this signal either by enhancing amounts of proteins in the membrane signaling pathway (127) or by reducing the activity of phosphodiesterase (155). In humans, hyperthyroidism is associated with enhanced levels and increased turnover of plasma free fatty acids, thus accounting for the finding that fatty acids constitute the principal fuel in this state (31, 52). In the rat, lipolysis is required to fuel T_3-induced thermogenesis, at least early after hormone treatment before an increase in food intake occurs (118). In addition to promoting lipolysis, T_3 also stimulates the oxidation of these fatty acids by increasing the activity of carnitine palmitoyl transferase, the enzyme responsible for transporting fatty acids into the mitochondria (62, 145).

Various authors have noted that the production of fatty acids by lipolysis exceeds their rate of oxidation in humans (31, 52, 141). The balance is presumably reesterified in a futile cycle; Sestoft has estimated the energy costs of this process to be as much as 15% of the hyperthyroid increment in oxygen consumption in humans (141). He assumed that this process occurred primarily within adipose tissue because the data discussed previously indicated a negative effect of hyperthyroidism on triglyceride production in the perfused liver. However, others have suggested a key role for the liver in this process (52), and an adipose tissue/liver cycle appears more likely.

Carbohydrate Metabolism Sestoft (141) has argued that carbohydrate substrate cycles, specifically gluconeogenesis, are insignificant in thyroid hormone thermogenesis. This conclusion is based largely on the lack of effect of hyperthyroidism on the activity of fructose bis-phosphatase in rat and human liver (103, 112) and on perfusion experiments in which the increased gluconeogenesis observed in hyperthyroid vs euthyroid preparations was not seen when free fatty acids were included in the perfusate (4). However, several studies, summarized and reviewed by Muller & Seitz (102), have subsequently shown increased endogenous production and utilization of glucose in hyperthyroid rats and humans. For example, tracer studies in rats have shown that both the Cori cycle (glucose/lactate/glucose) and the glucose/glucose-6-phosphate substrate cycle are increased in hyperthyroidism and decreased in hypothyroidism (71, 114). Similarly, both radio- and stable isotope studies in humans have revealed significant thyroid hormone–dependent glucose substrate cycling (100, 142). The quantitative contribution of these processes to thyroid thermogenesis has been estimated at 10% for the rat (71). In humans, different investigators have measured some but not all of the glucose substrate cycles and have given estimates of up to 2% of BMR for various cycles (100, 142).

In the case of lipid metabolism, extensive evidence, reviewed above, indicates that the effects of thyroid hormone on lipogenesis are dependent on receptor-mediated alterations in gene expression. For carbohydrate metabolism, fewer data are available. However, Loose et al have shown that T_3 stimulates the expression of the phosphoenolpyruvate carboxykinase gene (95), and Giralt et al have demonstrated the presence of a thyroid hormone response element in the promoter of this gene (42). As discussed above, very rapid effects of thyroid hormone on glucose uptake have been reported (138). In addition, T_3 increased gluconeogenesis from alanine in the isolated perfused rat liver in a matter of minutes (101). Whether these findings are associated with the long-term changes in oxygen consumption and the substrate utilization seen in vivo is not yet apparent. Interestingly, Shulman et al found that thyroid hormone failed to increase glucose substrate cycling after one week of treatment in hypothyroid individuals (142), suggesting that these phenomena represent long-term, perhaps secondary, effects of the hormone.

Protein Metabolism In addition to increasing the transcriptional rate of a specific set of mRNAs in target tissues, thyroid hormone has a more generalized stimulatory effect on total RNA synthesis (143, 151). Thyroid hormone also increases RNA levels in muscle, which results in higher levels of protein synthesis (19). This finding has been well documented in both cardiac and skeletal muscle (19, 83). However, T_3 also stimulates protein degradation such that clinical thyrotoxicosis may be associated with a myopathy (125). This accelerated rate of tissue renewal is likely to be energetically expensive (25),

although the calculation of the ATP costs of these processes is rather complex. Recalculation of values given by Flatt (35) suggests an energy cost of 1 kcal per gram of protein synthesized. In humans, this would translate to an energy cost of 75 kcal, given a 25% stimulation of normal protein synthetic rates of 300 g/day, which would probably represent 5–10% of the thyroid-dependent portion of metabolic rate.

THYROID HORMONE, THERMOGENESIS, AND EVOLUTION

Thyroid hormones and the receptors that mediate their actions are present in all vertebrate species studied to date (158). The ability of thyroid hormones to stimulate thermogenesis nonetheless is restricted to the homeothermic species, i.e. birds and mammals, which evolved more than 200 million years ago. The function of thyroid hormones in species that evolved earlier is only partially understood. However, thyroid hormones likely play an important role in developmental processes, as illustrated most dramatically by the changes accompanying amphibian metamorphosis (40). The developmental actions of thyroid hormone are retained by homeotherms along with the more recently acquired functions of stimulating thermogenesis (135).

The ability of thyroid hormone to stimulate lipogenesis and lipolysis probably also developed at the time that the homeotherms evolved. Thus, malic enzyme activity is present in fish liver but is not affected by thyroid hormone administration (158). Many of the thyroid hormone effects on mitochondrial function may be dependent on changes in membrane lipid composition (15, 62). We can reasonably suppose that the mechanisms allowing these membrane effects to occur are similarly undeveloped in poikilotherms, which explains why metabolic rate is unaffected by thyroid hormone in these species.

The concomitant evolution of thermogenesis, lipogenesis, and lipolysis may be of adaptive value to homeotherms (118). As pointed out above, the percentage contribution of lipogenesis to total energy utilization of the rat is relatively small. However, total thyroid hormone–supported thermogenesis in the euthyroid rat is increased ~50% over values registered in hypothyroid animals (3, 150). This increase in the normal set point for energy metabolism in the euthyroid animal therefore requires a corresponding increase in caloric intake and an ability to store caloric reserves in the form of fat depots for rapid release on sudden demand. Estimates of lipid turnover in rats in the transition between the euthyroid and hyperthyroid states suggest that thyroid hormone administration results in a two- to threefold increase in fractional lipid turnover (118). A similar increase may well occur in the transition between the hypothyroid and euthyroid states. For fat depots to be maintained at physiologically required levels, lipogenesis must increase proportionately. This require-

ment may explain the concurrent increase in thermogenesis, lipolysis, and lipogenesis in the transition between the hypothyroid and euthyroid states and potentially account for the use of the thyroid hormone system to achieve these changes in the evolution of homeothermic species.

Of interest in this regard is that the thyroid hormone–induced increase in lipogenesis appears to be activated by T_3 before any increase in oxygen consumption is observed. The mRNA for the S14 protein, which is believed to be intimately involved in lipogenesis, rises within 20 min of injection of T_3 (80), whereas increases in oxygen consumption are delayed ~24 h. These considerations suggest that the increase in lipogenesis is activated directly by T_3 rather than secondarily as a metabolic consequence of augmented thermogenesis.

CONCLUSIONS

In the past two decades, multiple converging lines of evidence have provided convincing data that thyroid hormones initiate their actions by interacting with specific nuclear receptors and that such T_3-receptor complexes in turn interact with *trans*-acting factors at designated response elements of target genes. Whether nonnuclear pathways are also involved in this process remains to be determined. Despite gratifying progress at the molecular level, many of the central questions pertaining to the thermogenic actions of the thyroid hormones remain unanswered. The effects of thyroid hormone on cellular systems relevant to thermogenesis are summarized in Figure 3. We are still uncertain as to the relative contributions made by specific organs or metabolic processes to the total increase in oxygen consumption. The possibility that respiration

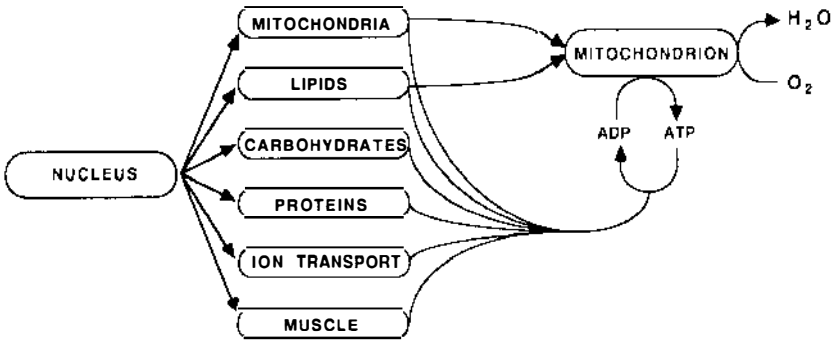

Figure 3 Mechanisms underlying the stimulation of oxygen consumption by thyroid hormone. T_3, acting via its nuclear receptors, regulates the levels of proteins required for the synthesis and breakdown of macronutrients, for ion transport, and for muscle contraction. Both the synthesis and operation of these proteins consume ATP and therefore stimulate respiration. Thyroid state also affects mitochondrial function by changing the membrane lipid composition and by altering the level of mitochondrial proteins encoded by both the mitochondrial and nuclear genomes.

may not be tightly coupled has had a profound effect on these calculations, and reaching a new consensus on the influence of T_3 on the efficiency of oxidative phosphorylation should be a priority. Although the actions illustrated in Figure 3 have been drawn in parallel, which thyroid hormone effects are mediated directly by T_3 and its receptors at the nuclear level and which represent the indirect effects of as yet unidentified primary targets remain unclear. However, the diverse thyroid hormone effects observed likely result from evolutionary pressures to meet the specific needs of the organisms.

Literature Cited

1. Ashizawa K, Cheng S-Y. 1992. Regulation of thyroid hormone receptor–mediated transcription by a cytosol protein. *Proc. Natl. Acad. Sci. USA* 89:9277–81
2. Babior BM, Creagan S, Ingbar S, Kipnes RS. 1973. Stimulation of adenine diphosphate uptake by thyroid hormone. *Proc. Natl. Acad. Sci. USA* 70:98–102
3. Barker SB, Klitgaard HM. 1952. Metabolism of tissues excised from thyroxine-injected rats. *Am. J. Physiol.* 170: 81–86
4. Bartels PD, Sestoft L. 1980. Thyroid hormone induced changes in the regulatory interrelationship between gluconeogenesis and ketogenesis in perfused rat liver. *Biochim. Biophys. Acta* 633:56–67
5. Beato M. 1989. Gene regulation by steroid hormones. *Cell* 56:335–44
6. Beckett GJ, MacDougall DA, Nicol F, Arthur JR. 1989. Inhibition of type I and type II iodothyronine deiodinase activity in rat liver kidney and brain produced by selenium deficiency. *Biochim. J.* 259:887–92
7. Benbrook D, Pfahl M. 1987. A novel thyroid hormone receptor encoded by a cDNA clone from a human testis library. *Science* 238:788–91
8. Berg J. 1990. Zinc fingers and other metal-binding domains. *J. Biol. Chem.* 265:6513–16
9. Berry MJ, Banu L, Larsen PR. 1991. Type I iodothyronine deiodinase is a selenocysteine containing enzyme. *Nature* 349:438–40
10. Bianco AC, Sheng X, Silva JE. 1988. Triiodothyronine amplifies norepinephrine stimulation of uncoupling protein gene transcription by a mechanism not requiring protein synthesis. *J. Biol. Chem.* 263:18168–75
11. Blennemann B, Moon YK, Freake HC. 1992. Tissue-specific regulation of fatty acid synthesis by thyroid hormone. *Endocrinology* 130:637–43
12. Brand MB, Murphy MP. 1987. Control of electron flux through the respiratory chain in mitochondria and cells. *Biol. Rev.* 62:141–93
13. Brand MD, Chien L-F, Rolfe DFS. 1993. Control of oxidative phosphorylation in liver mitochondria and hepatocytes. *Biochem. Soc. Trans.* 21: 757–62
14. Brand MD, Harper M-E, Taylor HC. 1993. Control of the effective P/O ratio of oxidative phosphorylation in liver mitochondria and hepatocytes. *Biochim. J.* 291:739-48
15. Brand MD, Steverding D, Kadenbach B, Stevenson PM, Hafner RP. 1992. The mechanism of the increase in mitochondrial proton permeability induced by thyroid hormones. *Eur. J. Biochem.* 206: 775–81
16. Braverman LE, Ingbar SH, Sterling K. 1970. Conversion of thyroxine to triiodothyronine in athyreotic human subjects. *J. Clin. Invest.* 49:855–64
17. Brent GA, Moore DD, Larsen PR. 1991. Thyroid hormone regulation of gene expression. *Annu. Rev. Physiol.* 53:17–35
18. Bronk JR. 1966. Thyroid hormone: effects on electron transport. *Science* 153: 638–39
19. Brown JG, Bates PC, Holliday MA, Millward DJ. 1981. Thyroid hormones

and muscle protein turnover. *Biochim. J.* 194:771–82
20. Burnside J, Darling D, Chin W. 1990. A nuclear factor that enhances binding of thyroid hormone receptors to thyroid hormone response elements. *J. Biol. Chem.* 265:2500–4
21. Capo LA, Sillau AH. 1983. The effect of hyperthyroidism on capillarity and oxidative capacity in rat soleus and gastrocnemius muscles. *J. Physiol.* 342:1–14
22. Chen YDI, Hoch FL. 1976. Mitochondrial inner membrane in hypothyroidism. *Arch. Biochem. Biophys.* 172: 741–44
23. Clausen T, Van Hardeveld C, Everts ME. 1991. Significance of cation transport in control of energy metabolism and thermogenesis. *Physiol. Rev.* 71: 733–74
24. Corvilain B, Van Sande J, Laurent E, Dumont JE. 1991. The H_2O_2 generating system modulates protein iodination and the activity of the pentose phosphate pathway in the dog thyroid. *Endocrinology* 128:779–85
25. Danforth E, Burger A. 1984. The role of thyroid hormones in the control of energy expenditure. *Clin. Endocrinol. Metab.* 13:581–95
26. Dayton S, Dayton J, Drimmer F, Kendall FE. 1960. Rates of acetate turnover and lipid synthesis in normal, hypothyroid and hyperthyroid rats. *Am. J. Physiol.* 199:71–76
27. Debons AF, Schwartz IL. 1961. Dependence of the lipolytic action of epinephrine in vitro upon thyroid hormone. *J. Lipid Res.* 2:86–89
28. Deykin D, Vaughn M. 1963. Release of free fatty acids by adipose tissue from rats treated with triiodothyronine or propylthiouracil. *J. Lipid Res.* 4:200–3
29. Diamant S, Gorin E, Shafrir E. 1972. Enzyme activities related to fatty acid synthesis in liver and adipose tissue of rats treated with triiodothyronine. *Eur. J. Biochem.* 26:553–99
30. Dillman WH. 1984. Hormonal influences on cardiac myosin ATPase activity and myosin isoenzyme distribution. *Mol. Cell Endocrinol.* 34:169–81
31. Eaton RP, Steinberg D, Thompson RH. 1965. Relationship between free fatty acid turnover and total body oxygen consumption in the euthyroid and hyperthyroid states. *J. Clin. Invest.* 44: 247–60
32. Evans RM. 1988. The steroid and thyroid hormone receptor superfamily. *Science* 240:889–95
33. Fells DA. 1992. Metabolic control analysis: a survey of its theoretical and experimental development. *Biochim. J.* 286:313–30
34. Fisher JN, Ball EG. 1967. Studies on the metabolism of adipose tissue: the effect of thyroid status upon oxygen consumption and lipolysis. *Biochemistry* 6:637–67
35. Flatt JP. 1978. The biochemistry of energy expenditure. In *Recent Advances in Obesity Research*, ed. G Bray, pp. 211–28. London: Newman
36. Flatt JP, Pahud P, Ravussin E, Jequier E. 1984. An estimate of the P:O ratio in man. *Trends Biol. Sci.* 9:466–68
37. Flink IL, Morkin E. 1990. Interaction of thyroid hormone receptors with strong and weak *cis*-acting elements in the human α-myosin heavy chain gene promoter. *J. Biol. Chem.* 265:11233–37
38. Foster DO, Frydman ML. 1979. Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-adapted rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Can. J. Physiol. Pharmacol.* 57:257–70
39. Freake HC, Schwartz HL, Oppenheimer JH. 1989. The regulation of lipogenesis by thyroid hormone and its contribution to thermogenesis. *Endocrinology* 125: 2868–74
40. Galton VA. 1983. Thyroid hormone action in amphibian metamorphosis. In *Molecular Basis of Thyroid Hormone Action*, ed. JH Oppenheimer, HH Samuels, pp. 293–324. New York: Academic
41. Gick GG, Ismail-Beigi F, Edelman IS. 1988. Thyroidal regulation of the rat renal and hepatic Na,K-ATPase gene expression. *J. Biol. Chem.* 263:16610–18
42. Giralt M, Park EA, Gurney AL, Liu J, Hakimi P, Hanson RW. 1991. Identification of a thyroid hormone response element in the phosphoenolpyruvate carboxykinase (GTP) gene. *J. Biol. Chem.* 266:21991–96
43. Goglia F, Torresani J, Bugli P, Barletta A, Liverini G. 1981. In vitro binding of triiodothyronine to rat liver mitochondria. *Pflügers Arch.* 390:120–24
44. Goodridge AG, Back DW, Wilson SB, Goldman MJ. 1986. Regulation of genes for enzymes involved in fatty acid synthesis. *Ann. NY Acad. Sci.* 478:46–62
45. Gordon A, Surks MI, Oppenheimer JH. 1973. Thyroxine stimulation of amino acid incorporation into mitochondrial protein: differences between in vivo and

in vitro effects. *Acta Endocrinol.* 72: 684–96
46. Gregory RB, Berry MN. 1992. Stimulation by thyroid hormone of coupled respiration and of respiration apparently not coupled to the synthesis of ATP in rat heptocytes. *J. Biol. Chem.* 267:8903–8
47. Greif RL, Sloane D. 1978. Mitochondrial binding sites for thyroid hormone. *Endocrinology* 103:1899–902
48. Groen AK, Wanders RJA, Westerhoff HV, van der Meer R, Tager JM. 1982. Quantification of the contribution of various steps to the control of mitochondrial respiration. *J. Biol. Chem.* 257: 2754–57
49. Gustaffson R, Tata JR, Linbberg O, Ernster L. 1965. The relationship between the structure and activity of rat skeletal muscle mitochondria after thyroidectomy and thyroid hormone treatment. *J. Cell. Biol.* 26:555–78
50. Haber RS, Loeb JN. 1982. Effect of 3,5,3′-triiodothyronine treatment on potassium efflux from isolated rat diaphragm: role of increased permeability in the thermogenic response. *Endocrinology* 111:1217–23
51. Hafner RP, Nobes CD, McGown AD, Brand MD. 1988. Altered relationship between protonmotive force and respiration rate in non-phosphorylating liver mitochondria isolated from rats of different thyroid hormone status. *Eur. J. Biochem.* 178:511–18
52. Hagenfeldt L, Wennlund A, Felig P, Wahren J. 1981. Turnover and splanchnic metabolism of free fatty acids in the hyperthyroid state. *J. Clin. Invest.* 67: 1672–77
53. Harper ME, Ballantyne JS, Leach M, Brand MD. 1993. Effects of thyroid hormone on oxidative phosphorylation. *Biochem. Soc. Trans.* 21:785–92
54. Harper ME, Brand MD. 1993. The quantitative contributions of mitochondrial proton leak and ATP turnover reactions to the changed respiration rates of hepatocytes from rats of different thyroid status. *J. Biol. Chem.* 268:14850–60
55. Hashizume K, Ichikawa K. 1982. Localization of 3,5,3′-L-triiodothyronine receptor in rat mitochondria membranes. *Biochem. Biophys. Res. Commun.* 106: 920–26
56. Hashizume K, Miyamoto T, Ichikawa K, Yamauchi K, Sakurai A, et al. 1989. Evidence for the presence of two active forms of cytosolic 3,5,3′-triiodo-L-thyronine (T3)-binding protein (CTBP) in rat kidney. *J. Biol. Chem.* 264:4864–71
57. Hetzel BS, Maberly GF. 1986. Iodine. In *Trace Elements in Human and Animal Nutrition*, ed. W Mertz, pp. 139–208. New York: Academic
58. Heyman RA, Mangelsdorf DJ, Dyck JA, Stein RB, Eichele G, et al. 1992. 9-*cis* retinoic acid is a high affinity ligand for the retinoid X receptor. *Cell* 68:397–406
59. Hinkle PC, Kumar MA, Resetar A, Harris DL. 1991. Mechanistic stoichiometry of mitochondrial oxidative phosphorylation. *Biochemistry* 30:3576–82
60. Hoch FL. 1968. Thyroid hormone action on mitochondria. *Arch. Biochem. Biophys.* 124:238–47
61. Hoch FL. 1977. Adenine nucleotide translocation in liver mitochondria of hypothyroid rats. *Arch. Biochem. Biophys.* 178:535–45
62. Hoch FL. 1988. Lipids and thyroid hormones. *Prog. Lipid Res.* 27:199–270
63. Hoch FL, Subramanian C, Dhopeshwarkar GA, Mead HF. 1981. Thyroid control over biomembranes: VI. Lipids in liver mitochondria and microsomes of hypothyroid rats. *Lipids* 16:328–35
64. Hoppner W, Rasmussen UB, Abuerreish G, Wohlrab H, Seitz HJ. 1988. Thyroid hormone effect on gene expression of the adenine nucleotide translocase in different tissues. *Mol. Endocrinol.* 2: 1127–31
65. Horiuchi R, Cheng S-Y, Willingham M, Pastan I. 1982. Inhibition of the nuclear entry of 3,3′,5-triiodo-L-thyronine by monodansylcadaverine in GH3 cells. *J. Biol. Chem.* 257:3139–44
66. Horrum MA, Tobin RB, Ecklund RE. 1985. Thyroxine-induced changes in rat liver mitochondrial cytochromes. *Mol. Cell Endocrinol.* 41:163–19
67. Deleted in proof
68. Horrum MA, Tobin RB, Ecklund RE. 1992. The early triiodothyronine-induced changes in state IV respiration is not regulated by the proton permeability of the inner mitochondrial membrane. *Biochem. Int.* 28:813–21
69. Horst C, Rokos H, Seitz HJ. 1989. Rapid stimulation of hepatic oxygen consumption by 3,5-di-iodo-L-thyronine. *Biochim. J.* 261:945–50
70. Hostetler KY. 1991. Effect of thyroxine on the activity of mitochondrial cardiolipin synthase in rat liver. *Biochim. Biophys. Acta* 1086:139–40
71. Huang M-T, Lardy HA. 1981. Effects of thyroid states on the Cori cycle and futile cycling of glucose metabolism in rats. *Arch. Biochem. Biophys.* 209:441–51
72. Hummerich H, Soboll S. 1989. Rapid stimulation of calcium uptake into rat

liver by L-tri-iodothyronine. *Biochim. J.* 258:363–67
73. Ichikawa K, Hashizume K. 1991. Cellular binding proteins for thyroid hormones. *Life Sci.* 49:1513–22
74. Ismail-Beigi F, Edelman IS. 1970. Mechanism of thyroid calorigenesis: role of active sodium transport. *Proc. Natl. Acad. Sci. USA* 67:1071–78
75. Ismail-Beigi F, Edelman IS. 1971. The mechanism of the calorigenic action of thyroid hormone. *J. Gen. Physiol.* 57: 710–22
76. Izquierdo JM, Cuezva JM. 1993. Thyroid hormones promote transcriptional activation of the nuclear gene coding for mitochondrial beta-F1-ATPase in rat liver. *FEBS Lett.* 323:109–12
77. Izumo S, Mahdavi V. 1988. Thyroid hormone receptor α isoforms generated by alternative splicing differentially activate myosin HC gene expression. *Nature* 334:539–42
78. Izumo S, Nadal-Ginard B, Mahdavi V. 1986. All members of the MHC multigene family respond to thyroid hormone in a highly tissue specific manner. *Nature* 231:597–60
79. Jacovic S, Swift HH, Gross NJ, Rabinowitz M. 1978. Biochemical and stereological analysis of rat liver mitochondria in different thyroid states. *J. Cell. Biol.* 77:887–901
80. Jump DB, Narayan P, Towle HC, Oppenheimer JH. 1984. Rapid effects of triiodothyronine on hepatic gene expression. *J. Biol. Chem.* 259:2789–97
81. Kalderon B, Hertz R, Bar-Tana J. 1992. Effect of thyroid hormone on redox and phosphate potentials in rat liver. *Endocrinology* 131:400–7
82. Keyes WG, Heimberg M. 1979. Influence of thyroid status on lipid metabolism in the perfused rat liver. *J. Clin. Invest.* 64:182–90
83. Klein I. 1990. Thyroid hormone and the cardiovascular system. *Am. J. Med.* 88: 631–37
84. Klein I, Hong C. 1986. Effects of thyroid hormone on cardiac size and myosin content of the heterotopically transplanted rat heart. *J. Clin. Invest.* 77: 1694–98
85. Koerner D, Schwartz HL, Surks MI, Oppenheimer JH, Jorgensen EC. 1975. Binding of selected iodothyronine analogues to receptor sites in isolated hepatic nuclei. *J. Biol. Chem.* 250:6417–23
86. Krenning EP, Docter R, Bernard B, Visser T, Hennemann G. 1981. Characteristics of active transport of thyroid hormones into rat hepatocytes. *Biochim. Biophys. Acta* 676:314–20
87. Laker ME, Mayes PA. 1978. Thyroid status and hepatic lipid metabolism. *Trans. Biochem. Soc.* 6:584–85
88. Landriscina C, Gnoni GV, Quagliariello E. 1976. Effect of thyroid hormones on microsomal fatty acid chain elongation synthesis in rat liver. *Eur. J. Biochem.* 71:135–43
89. Lanni A, Moreno M, Cioffi M, Goglia F. 1993. Effect of 3,3′-di-iodothyronine and 3,5-di-iodothyronine on rat liver mitochondria. *J. Endocrinol.* 131:59–64
90. Lardy HA, Feldcott G. 1951. Metabolic effects of thyroxine in vitro. *Ann. NY Acad. Sci.* 54:636–48
91. Larsen PR. 1982. Thyroid-pituitary interactions. Feedback regulation of thyrotropin secretion by thyroid hormones. *New Engl. J. Med.* 306:23–32
92. Lazar MA. 1993. Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocrinol. Rev.* 14:184–93
93. Leid M, Kastner P, Lyons R, Nakshatri H, Saunders M, et al. 1992. Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. *Cell* 68:377–95
94. Leonard JL, Visser TJ. 1986. Biochemistry of deiodination. In *Thyroid Hormone Metabolism*, ed. G Hennemann, pp. 189–229. New York: Dekker
95. Loose DS, Cameron DK, Short HP, Hanson RW. 1985. Thyroid hormone regulates transcription of the gene for cytosolic phosphoenol pyruvate carboxykinase (GTP) in rat liver. *Biochemistry* 24:4509–12
96. Luciakova K, Nelson BD. 1992. Transcript levels for nuclear encoded mammalian mitochondrial respiratory chain components are regulated by thyroid hormones in an uncoordinated fashion. *Eur. J. Biochem.* 207:247–51
97. Magnus-Levy A. 1895. Über den respiratorischen Gaswechsel unter dem Einfluss der Thyroidea sowie unter verschiedenen pathalogischen Zustanden. *Berl. Klin. Wochenschr.* 34:650
98. Mangelsdorf DJ, Borgmeyer U, Heyman RA, Zhou JY, Ong ES, et al. 1992. Characterization of three RXR genes that mediate the action of 9-*cis* retinoic acid. *Genes Dev.* 6:329–44
99. Martius C, Hess B. 1951. The mode of action of thyroxin. *Arch. Biochem. Biophys.* 33:486–87
100. McCulloch AJ, Nosadin R, Pernet A, Piniewska M, Cook DB, et al. 1983. Glucose turnover and indices of recycling in thyrotoxicosis and primary thyroid failure. *Clin. Sci.* 64:41–47
101. Muller MJ, Seitz HJ. 1980. Rapid and

direct stimulation of hepatic gluconeogenesis by L-triiodothyronine (T3) in the isolated-perfused rat liver. *Life Sci.* 27:827–35

102. Muller MJ, Seitz HJ. 1984. Thyroid hormone action on intermediary metabolism. Part I: respiration, thermogenesis and carbohydrate metabolism. *Berl. Klin. Wochenschr.* 62:11–18
103. Murad S, Freedland A. 1967. Effect of thyroxine administration on enzymes associated with glucose metabolism in the liver. *Proc. Soc. Exp. Biol. Med.* 124: 1176–78
104. Murray MB, Towle HC. 1989. Identification of nuclear factors that enhance binding of a thyroid hormone receptor to a thyroid hormone response element. *Mol. Endocrinol.* 3:1434–42
105. Murray MB, Zilz ND, McCreary NL, MacDonald MJ, Towle HC. 1988. Isolation and characterization of rat cDNA clones for two distinct thyroid hormone receptors. *J. Biol. Chem.* 263:12270–77
106. Myant NB, Whitney S. 1967. The time course of the effect of thyroid hormones upon basal oxygen consumption and plasma concentration of free fatty acid in rats. *J. Physiol.* 190:221–28
107. Myers JD, Brannon ES, Holland BC. 1950. A correlative study of the cardiac output and the hepatic circulation in hyperthyroidism. *J. Clin. Invest.* 29: 1069–77
108. Nelson BD. 1987. Biogenesis of mammalian mitochondria. *Curr. Top. Bioenerg.* 15:221–72
109. Newsholme EA, Crabtree B. 1976. Substrate cycles in metabolic regulation and heat production. *Biochem. Soc. Symp.* 41:61–109
110. Nicholls DE, Locke RM. 1984. Thermogenic mechanisms in brown fat. *Physiol. Rev.* 64:1–64
111. Nobes CD, Lakin-Thomas PL, Brand MD. 1989. The contribution of ATP turnover by the Na^+/K^+-ATPase to the rate of respiration of hepatocytes. Effects of thyroid status and fatty acids. *Biochim. Biophys. Acta* 976:241–45
112. Nolte J, Petter D, Bachmeier B, Kiefhaber B, Schneider H, Scriba PC. 1972. Enzyme response to thyrotoxicosis and hypothyroidism in human liver and muscle: comparative aspects. *Eur. J. Clin. Invest.* 2:141–49
113. Ojamaa K, Samarel AM, Kupfer JM, Hong C, Klein I. 1992. Thyroid hormone effects on cardiac gene expression independent of cardiac growth and protein synthesis. *Am. J. Physiol.* 263: E534–40
114. Okajima F, Ui M. 1979. Metabolism of glucose in hyper- and hypo-thyroid rats in vivo. *Biochim. J.* 182:565–75
115. Oppenheimer JH. 1979. Thyroid hormone action at the cellular level. *Science* 203:971–79
116. Oppenheimer JH, Koerner D, Schwartz HL, Surks MI. 1972. Specific nuclear triiodothyronine binding sites in rat liver and kidney. *J. Clin. Endocrinol. Metab.* 35:330–33
117. Oppenheimer JH, Schwartz HL. 1985. Stereospecific transport of triiodothyronine from plasma to cytosol and from cytosol to nucleus in rat liver, kidney, brain and heart. *J. Clin. Invest.* 75:147–54
118. Oppenheimer JH, Schwartz HL, Lane JT, Thompson MP. 1991. Functional relationship of thyroid hormone–induced lipogenesis, lipolysis and thermogenesis in the rat. *J. Clin. Invest.* 87: 125–32
119. Oppenheimer JH, Schwartz HL, Mariash CN, Kinlaw WB, Wong NCW, Freake HC. 1987. Advances in our understanding of thyroid hormone action at the cellular level. *Endocrinol. Rev.* 8:288–308
120. Oppenheimer JH, Schwartz HL, Surks MI. 1974. Tissue differences in the concentration of triiodothyronine nuclear binding sites in the rat: liver, kidney, pituitary, heart, brain, spleen, and testis. *Endocrinology* 95:897–903
121. Palacios-Romero R, Mowbray J. 1979. Evidence for the rapid direct control both in vivo and in vitro of the efficiency of oxidative phosphorylation in rats. *Biochim. J.* 184:527–38
122. Paradies G, Ruggiero FM, Dinoi P, Petrosillo G, Quagliariello E. 1993. Decreased cytochrome oxidase activity and changes in phospholipids in heart mitochondria from hypothyroid rats. *Arch. Biochem. Biophys.* 307:91–95
123. Paradies G, Ruggiero FM, Petrosillo G, Quagliariello E. 1994. Enhanced cytochrome oxidase activity and modification of lipids in heart mitochondria from hyperthyroid rats. *Biochim. Biophys. Acta* 125:165–70
124. Park IRA, Mount DB, Himms-Hagen J. 1989. Role of T3 in thermogenic and trophic responses of brown adipose tissue to cold. *Am. J. Physiol.* 257:E81–87
125. Ramsay ID. 1965. Electromyopathy in thyrotoxicosis. *Q. J. Med.* 34:255–67
126. Rao GS, Eckel J, Rao ML, Breuer H. 1976. Uptake of thyroid hormone by isolated rat liver cells. *Biochem. Biophys. Res. Commun.* 73:98–104
127. Rapiejko PJ, Watkins DC, Ros M, Malbon CC. 1989. Thyroid hormones regu-

late G-protein β-subunit mRNA expression in vivo. *J. Biol. Chem.* 264:16183–89

128. Rohrer D, Dillman WH. 1989. Thyroid hormone markedly increases the mRNA coding for sarcoplasmic reticulum Ca^{2+}-ATPase in the rat heart. *J. Biol. Chem.* 263:6941–44
129. Roncari DAK, Murthy VK. 1975. Effects of thyroid hormones on enzymes involved in fatty acid and glycerolipid synthesis. *J. Biol. Chem.* 250:4134–38
130. Rothwell NJ, Stock MJ. 1984. Tissue blood flow in control and cold-adapted hyperthyroid rats. *Can. J. Physiol. Pharmacol.* 62:928–33
131. Samuels HH, Tsai JS. 1973. Thyroid hormone action in cell culture: demonstration of nuclear receptors in intact cells and isolated nuclei. *Proc. Natl. Acad. Sci. USA* 70:3488–92
132. Sap J, Munoz A, Damm K, Goldberg Y, Ghsydael J, et al. 1986. The c-erb-A protein is a high affinity receptor for thyroid hormone. *Nature* 324:635–40
133. Saunders J, Hall SEH, Sonksen PH. 1980. Glucose and free fatty acid turnover in thyrotoxicosis and hypothyroidism, before and after treatment. *Clin. Endocrinol.* 13:33–44
134. Scarpulla RC, Kilar MC, Scarpulla KM. 1986. Coordinate induction of multiple cytochrome c mRNAs in response to thyroid hormone. *J. Biol. Chem.* 261: 4660–62
135. Schwartz HL. 1983. Effect of thyroid hormone on growth and development. In *Molecular Basis for Thyroid Hormone Action*, ed. JH Oppenheimer, HH Samuels, pp. 413–44. New York: Academic
136. Schwartz HL, Surks M I., Oppenheimer JH. 1971. Quantitation of extrathyroidal conversion of L-thyroxine to 3,5,3′-triiodo-L-thyronine in the rat. *J. Clin. Invest.* 50:1124–30
137. Segal J. 1990. In vivo effects of 3,5,3′-triiodothyronine on calcium uptake in several tissues in the rat: evidence for a physiological role for calcium as the first messenger for the prompt action of thyroid hormone at the level of the plasma membrane. *Endocrinology* 127: 17–24
138. Segal J, Ingbar SH. 1979. Stimulation by triiodothyronine of the in vitro uptake of sugars by rat thymocytes. *J. Clin. Invest.* 63:507–15
139. Segal J, Ingbar SH. 1982. Specific binding sites for triiodothyronine in the plasma membranes of rat thymocytes. Correlation with biochemical response. *J. Clin. Invest.* 70:919–26
140. Seitz HJ, Muller MJ, Soboll S. 1985. Rapid thyroid hormone effect on mitochondrial and cytosolic ATP/ADP ratios in the intact liver cell. *Biochim. J.* 227: 149–53
141. Sestoft L. 1980. Metabolic aspects of the calorigenic effect of thyroid hormone in mammals. *Clin. Endocrinol.* 13:489–506
142. Shulman GI, Ladenson PW, Wolfe MH, Ridgway EC, Wolfe RR. 1985. Substrate cycling between gluconeogensis and glycolysis in hypothyroid and hyperthyroid man. *J. Clin. Invest.* 76:757–64
143. Simat BM, Towle HC, Schwartz HL, Oppenheimer JH. 1980. Difference between thyroidectomized and hypophysectomized animals in their hepatic response to thyroid hormone. *Endocrinology* 107:1338–44
144. Soboll S. 1993. Thyroid hormone action on mitochondrial energy transfer. *Biochim. Biophys. Acta* 1144:1–16
145. Stakkestad JA, Bremer J. 1983. The outer carnitine palmitoyltransferase and regulation of fatty acid metabolism in rat liver in different thyroid states. *Biochim. Biophys. Acta* 750:244–52
146. Sterling K, Milch PO. 1975. Thyroid hormone binding by a component of the mitochondrial membrane. *Proc. Natl. Acad. Sci. USA* 72:3225–29
147. Sterling K, Milch PO, Brenner MA, Lazarus JH. 1977. Thyroid hormone action: the mitochondrial pathway. *Science* 197:996–99
148. Sundin U. 1981. GDP binding to rat brown fat mitochondria: effects of thyroxine at different ambient temperatures. *Am. J. Physiol.* 241:C134–39
149. Tata JR. 1963. Basal metabolic rate and thyroid hormones. *Adv. Metab. Dis.* 1: 153–89
150. Tata JR, Ernster L, Lindberg O, Arrhenius E, Pedersen S, Hedman R. 1963. The action of thyroid hormones at the cellular level. *Biochim. J.* 86:408–28
151. Tata JR, Widnell CC. 1966. Ribonucleic acid synthesis during the early action of thyroid hormones. *Biochim. J.* 98: 604–20
152. Triandafillou J, Gwilliam C, Himms-Hagen J. 1982. Role of thyroid hormone in cold-induced changes in rat brown adipose tissue mitochondria. *Can. J. Biochem.* 60:530–37
153. Umesono K, Murakami K, Thompson CC, Evans RM. 1991. Direct repeats as selective response elements for the thyroid hormone, retinoic acid and vitamin D_3 receptors. *Cell* 65:1255–66
154. Underwood AH, Emmett JC, Ellis D,

Flynn SB, Leeson PD, et al. 1986. A thyromimetic that decreases plasma cholesterol levels without increasing cardiac activity. *Nature* 324:425–29

155. Van Inwegen RG, Robinson GA, Thompson WJ, Armstrong KJ, Stouffer KE. 1975. Cyclic nucleotide phosphodiesterases and thyroid hormones. *J. Biol. Chem.* 250:2452–56
156. Van Itallie CM. 1990. Thyroid hormone and dexamethasone increase the levels of a messenger ribonucleic acid for a mitochondrially encoded subunit but not for a nuclear encoded subunit of cytochrome c oxidase. *Endocrinology* 127: 55–62
157. Weinberger C, Thompson CC, Ong ES, Lebo R, Gruol DJ, Evans RM. 1986. The c-erb-A gene encodes a thyroid hormone receptor. *Nature* 324:641–46
158. Weirich RT, Schwartz HL, Oppenheimer JH. 1987. An analysis of the interrelationship of nuclear and plasma triiodothyronine in the sea lamprey, lake trout, and rat: evolutionary considerations. *Endocrinology* 120:664–77
159. Wiesner RJ, Kurowski TT, Zak R. 1992. Regulation by thyroid hormone of nuclear and mitochondrial genes encoding subunits of cytochrome-c oxidase in rat liver and skeletal muscle. *Mol. Endocrinol.* 6:1458–67
160. Young DL, Lynen F. 1969. Enzymatic regulation of 3-*sn*-phosphatidylcholine and triacylglycerol synthesis in states of altered lipid metabolism. *J. Biol. Chem.* 244:377–83
161. Zhang X, Hoffman B, Tran P, Graupner G, Pfahl M. 1992. Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature* 355:441–46