Thermogenic Mechanisms and Their Hormonal Regulation

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Silva, J. Enrique. Thermogenic Mechanisms and Their Hormonal Regulation. Physiol Rev 86: 435–464, 2006; doi:10.1152/physrev.00009.2005.—Increased heat generation from biological processes is inherent to homeothermy. Homeothermic species produce more heat from sustaining a more active metabolism as well as from reducing fuel efficiency. This article reviews the mechanisms used by homeothermic species to generate more heat and their regulation largely by thyroid hormone (TH) and the sympathetic nervous system (SNS). Thermogenic mechanisms antecede homeothermy, but in homeothermic species they are activated and regulated. Some of these mechanisms increase ATP utilization (same amount of heat per ATP), whereas others increase the heat resulting from aerobic ATP synthesis (more heat per ATP). Among the former, ATP utilization in the maintenance of ionic gradient through membranes seems quantitatively more important, particularly in birds. Regulated reduction of the proton-motive force to produce heat, originally believed specific to brown adipose tissue, is indeed an ancient thermogenic mechanism. A regulated proton leak has been described in the mitochondria of several tissues, but its precise mechanism remains undefined. This leak is more active in homeothermic species and is regulated by TH, explaining a significant fraction of its thermogenic effect. Homeothermic species generate additional heat, in a facultative manner, when obligatory thermogenesis and heat-saving mechanisms become limiting. Facultative thermogenesis is activated by the SNS but is modulated by TH. The type II iodothyronine deiodinase plays a critical role in modulating the amount of the active TH, T₃, in BAT, thereby modulating the responses to SNS. Other hormones affect thermogenesis in an indirect or permissive manner, providing fuel and modulating thermogenesis depending on food availability, but they do not seem to have a primary role in temperature homeostasis. Thermogenesis has a very high energy cost. Cold adaptation and food availability may have been conflicting selection pressures accounting for the variability of thermogenesis in humans.

I. THERMOGENESIS AND BODY TEMPERATURE HOMEOSTASIS

Core body temperature ($T_C$) is among the best-guarded constants in homeothermic species. This has resulted from the evolution of mechanisms to regulate the exchange of heat with the environment and mechanisms to increase the basal generation of heat by the body as well as to produce additional heat in response to colder environments in which basal heat production and heat-saving mechanisms are not sufficient to maintain $T_C$. The generation of heat or thermogenesis is thus essential to homeothermy, but it has an energy cost that is not usually appreciated. Thermogenic mechanisms are probably older than homeothermy, but in homeothermic species some are permanently activated, yet regulated both by
neural and hormonal signals, the intensity of which is commensurate with the gap in temperature between the core and the environment and the overall thermal conductance of the body. This review focuses on thermogenesis and its hormonal and sympathetic regulation. In this regard, thyroid hormone (TH) plays a pivotal role readily evident in experimental or clinical conditions associated with impairment or excess thyroidal secretion.

A. Evolutionary Aspects: Homeothermy and the Need for Additional Heat Production

Studies in poikilothermic species show that biological processes have in general an optimal temperature, particularly regarding locomotive activity and the utilization of energy (5). The advent of homeothermy, that is, of mechanisms to keep body temperature constant, independently from a broad range of environmental temperatures represented a leap forward in evolution as it allowed species to expand their niche. While mechanisms to save or dissipate heat are important in adjusting to sudden changes in temperature, if the body could not produce significant amounts of heat, the range of ambient temperatures for effective regulation would be severely limited. Biological machines are not any different from any other in that they require energy, in the form of fuels, to perform some form of work, which in the case of the biological machines is the functions inherent to life. In biological machines, the energy contained in substrate fuels is captured in an utilisable form, as purine nucleotides, largely ATP, which can be thus considered the energy currency of the cells, an immediate source of energy to sustain biological functions (Fig. 1A). Now, whenever there is energy transformation or transfer, some energy is lost as heat, and the energy transformations in the biological machine are no exception. Life, therefore, naturally produces heat, but this heat is insufficient to maintain $T_C$ if the environment is significantly colder, as evident in poikilothermic species, the body temperature of which parallels that of the environment. To maintain $T_C$ in cold habitats with just the minimal amount of heat derived from vital functions would require both vast thermal insulation and minimizing the body surface area for heat loss by irradiation and evaporation. Insulation would have to be massive, particularly in smaller species, and would limit substantially activity and displacements, as it would the adoption of sphere-shaped postures to minimize the heat-losing surface. Instead, mechanisms to increase heat production have evolved, as it is readily evident when comparing poikilothermic and homeothermic species metabolic rate. Thus, whether one looks at the whole animal or separate organs, mice have much faster metabolism than lizards of similar size and studied at the same ambient temperature (78, 119) (Fig. 1B). The fact that smaller animals, with a higher surface area-to-volume ratio, hence more prone to lose heat, have higher metabolic rates than larger species (102) is, in and of itself, evidence that species “learned” how to upregulate metabolism to produce sufficient heat to keep up with the demands.

B. Regulation of Body Temperature: Obligatory and Facultative Thermogenesis

The heat normally resulting from sustaining vital functions is called obligatory thermogenesis, and as men-

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**Fig. 1.** Schematic representation of energy transformations in biology and differences between poikilothermic and homeothermic species. A: energy transfer from substrates to ATP and from ATP to support vital functions such as transmembrane gradients, synthetic processes, growth, and physical activity. Heat is generated mainly in the synthesis of ATP and less so in the use of this in some activities. Heat generation can be increased by accelerating the use of ATP or by reducing the efficiency with which a cell captures energy of substrates in ATP. See text for details. B: comparative rates of oxygen consumption by a poikilothermic species (lizard) and a homeothermic one (mouse) of equal weight and studied at the same temperature. Data have been normalized to the corresponding value of the mammal. [Data from Else and Hulbert (78) and Hulbert and Else (119).] C: thermodynamic efficiency of skeletal muscle of poikilothermic and homeothermic species. [Data from Woledge (254).]
tioned above, homeothermic species have evolved an obligatory thermogenesis (ObT) higher than that in poikilothermic species (78).

Operationally, one can define a thermoneutral zone, a range of ambient temperatures where ObT is sufficient, without the participation of any other temperature homeostasis mechanism, to maintain Tc. In this range of ambient temperatures, the body is in thermal equilibrium with the environment (102). The thermoneutral zone is narrow, yet it is possible to define a lower and a higher limit to the range, but for the purpose of this discussion, I prefer to use the term thermoneutrality temperature (TN), defined as the lowest ambient temperature at which ObT suffices to maintain body temperature without the participation of other thermoregulatory mechanisms. Thus TN is identical to the lower limit of the thermoneutral zone. TN depends on the magnitude of ObT relative to the thermal conductance of the animal. As mentioned, this latter is strongly influenced by the surface area-to-volume ratio. Smaller species not only have higher metabolic rates relative to their mass than larger animals, but by virtue of their so much larger surface area-to-volume ratio, their thermal conductance is proportionally higher, and their TN cannot be as low as that of larger species. Thus TN is 30 and 28°C in mice and rats, respectively, compared with 23°C in humans (102).

When ambient temperature goes below TN, the immediate response is to activate heat-saving mechanisms, which include vasoconstriction, piloerection, and the adoption of a curled posture to reduce the surface of heat irradiation. Animals also reduce their mobility. Such mechanisms, as mentioned, are very limited, and additional thermogenesis is promptly activated. This additional heat, produced on demand, is called facultative or adaptive thermogenesis. Shivering is the earliest and most primitive response to increase heat production and is called shivering facultative thermogenesis. This form of thermogenesis consumes large amounts of energy, is not effective during severe cold (e.g., 4–6°C for rodents) or prolonged cold exposure, and interferes with normal activity. Understandably, homeothermic species have evolved a more efficient and long-lasting form of nonshivering facultative thermogenesis that uses pure metabolic mechanisms to generate heat. For simplicity, we will call this latter facultative thermogenesis (FcT) and the former just shivering. The site and mechanisms of FcT differ in the two classes of homeothermic animals, birds and mammals. In birds, the major site of FcT seems to be the skeletal muscle (72), whereas in mammals the major site of FcT is brown adipose tissue (BAT) (51). As discussed later, BAT can heat the body effectively and with a lower energy cost probably by virtue of its particular anatomical locations, rather than by the nature of the biochemical mechanism used to generate heat.

C. Thermogenic Mechanisms

As mentioned above, as machines, cells are not different from any other in that there is an obligatory loss of energy as heat during energy capture and utilization to support work or endothermic reactions. Therefore, one form of generating more heat is to increase energy utilization, in biology, basically to produce more ATP. Another form of increasing more heat is by reducing the thermodynamic efficiency of the energy utilization, that is, by sacrificing the work output for the sake of generating more heat. The comparison of the homeothermic with the poikilothermic machine suggests that both types of mechanism are used by homeotherms to increase heat production. As already mentioned, mammals have significantly faster metabolic rates than reptiles, all variables such as shape and ambient temperature being controlled for (78, 119), but also the thermodynamic efficiency of the homeothermic machine is lower. This is illustrated in Figure 1C, with data from Woledge (254), that shows that the ratio of mechanical work per change of enthalpy (W/ΔH) is significantly lower in mouse and rat muscle than in muscle from frog or tortoise.

I. Thermogenic mechanisms that increase ATP utilization

Being an endothermic reaction, ATP synthesis can only be increased by accelerating its utilization. ATP utilization is certainly a function of metabolic rate, that in turn is a function of ambient temperature, the so-called Q10 effect, but this explains only a minor fraction of the high metabolic rate of homeothermic species because the difference remains wide when metabolic rates are compared at the same ambient temperature (78). This leads to the conclusion that warm-blooded species spend ATP in a futile manner. Metabolic cycles resulting from coupling lipolysis and lipogenesis, glycolysis, and gluconeogenesis, etc., account for a significant fraction of ATP consumption. Such cycles are quantitatively important in liver and in the cycling substrates between this latter and muscle or adipose tissue. However, such cycles are activated as a consequence of increased metabolism rather than as a means to increase metabolic rate, and they do not seem to be used to a significant extent as thermogenic mechanisms. Even if activated by TH, metabolic cycles account for a comparatively low fraction of the thermogenic effect of this hormone (211). There seems to be significant differences between poikilothermic and homeothermic species in the reutilization of substrate, e.g., muscle lactate used as gluconeogenesis substrate in liver (43, 159, 178), but to the best of my knowledge no comparative quantitative studies are available in the literature.

On the other hand, cells spend an important fraction of their energy in maintaining certain ion gradients, and
there is evidence that such mechanisms may be used for the purpose of increasing ATP consumption in a futile manner. Thus Hulbert and Else (119) find that the metabolic rate is more sensitive to ouabain, an inhibitor of Na\(^+\)-K\(^+\)-ATPase, in tissues of mammals than in reptile counterparts. Not only the energy spent maintaining this gradient is higher in homeothermic than in poikilothermic species when expressed in absolute terms, but also when expressed as a fraction of total energy expenditure. On the other hand, when these ion gradients across the membrane are reduced either by leakage or by utilization of them to support transmembrane transport, the cell is forced to spend more ATP to keep the gradient. It has been postulated that this is one mechanism whereby TH increases thermogenesis, namely, by increasing the permeability of the cell membrane to Na\(^+\) and K\(^+\), allowing the passive entry of the former and exit of the latter, thus forcing the Na\(^+\)-K\(^+\)-ATPase to spend more energy in maintaining the gradients (108–110).

Another such mechanism to increase thermogenesis is the Ca\(^{2+}\) exchange between cytosol and sarcoplasmic reticulum. Cytosolic Ca\(^{2+}\) is an important intracellular second messenger and a signal to activate a number of processes, muscle contraction among others, but the cessation of the signal and the maintenance of the responsiveness to the signal requires the rapid reuptake of the cytosolic Ca\(^{2+}\) back into the endoplasmic reticulum, the energy for which is provided by the coupling of ATP hydrolysis by a Ca\(^{2+}\)-dependent ATPase located in the membrane of the organelle. Ca\(^{2+}\) cycling between cytosol and sarcoplasmic reticulum in a modified muscle segment around the eye of deepwater fish provides heat to maintain the function of the eye and adjacent brain at temperatures as high as 14°C over the water temperature (24, 26). This is a good example of a mechanism used by nature to produce heat that antecedes homeothermy. Interestingly, the ryanodine receptor (RyR) channel expressed in this organ is similar to that in slow-twitch muscle (161), which may be more important for thermogenesis than fast-twitch muscle, as discussed below. The “leakage” of Ca\(^{2+}\) from the reticulum will force the activity of the enzyme, with ensuing energy expenditure. A dramatic illustration of the potential of this mechanism to produce heat in mammals is malignant hyperthermia, wherein in genetically predisposed individuals or animals, certain environmental factors such as some anesthetics can make the sarcoplasmic reticulum frankly leaky (see Refs. 158, 166 for reviews), with an ensuing life-threatening hyperthermia. Furthermore, it is possible that some leakage occurs in the normal resting muscle contributing to ObT. We estimated based on observations made in isolated sarcoplasmic vesicles that the Ca\(^{2+}\) recycling across the sarcoplasmic membrane could account for 30–70% (depending on the calcium pool size in the muscle) of the resting muscle energy expenditure (153). As mentioned, muscle is a major site of FcT in birds (72), and it has been found that SERCA1 and RyR channels increase in muscle of ducklings during cold adaptation (74). Such observations altogether add support to the hypothesis that sarcoplasmic reticulum Ca\(^{2+}\) leak, coupled to rapid recapture by the sarco/endoplasmic reticulum Ca\(^{2+}\)-dependent ATPase (SERCA), could subserve a thermogenic role (reviewed in Refs. 25, 59).

It would appear then, largely from comparisons of Na\(^+\)-K\(^+\)-ATPase activity between poikilothermic and homeothermic species, as well as from comparative observations regarding muscle calcium cycling, that nature has increased these ionic exchanges, to a significant extent in a futile manner, for the sake of producing heat in homeothermic species. In general, these mechanisms generate heat largely by forcing cells to produce more ATP to support the maintenance of the gradients. In contrast to other thermogenic mechanisms discussed below, the thermodynamic efficiency of ADP phosphorylation seems to be preserved. In agreement with this idea, ADP phosphorylation in the heater organ of deepwater fish, even under stimulation by Ca\(^{2+}\), seems to remain well coupled (172). However, it is possible that the net efficiency of ATP utilization in pumping Ca\(^{2+}\) into the sarcoplasmic reticulum be also reduced to some extent to producing heat, which has been called “uncoupling” of the Ca\(^{2+}\) pump (67, 219), as will be discussed below.

2. Thermogenic mechanisms that reduce the efficiency of mitochondrial ATP synthesis

The energy resulting from the electron transfer through the respiratory chain in the mitochondria is transiently trapped in a proton gradient across the inner membrane of this organelle, the so-called proton-motive force, which is then utilized by ATP synthase to produce ATP by phosphorylation of ADP (144) (Fig. 2A). In the presence of an oxidizable substrate, but in absence of ADP, the gradient builds up and respiration is kept at a minimum (state 4 respiration) (Fig. 2B), illustrating the low permeability of the membrane to protons and the “braking effect” of the gradient on respiration. If ADP is added, the ATP synthase will phosphorylate it to ATP utilizing the energy trapped in the gradient (Fig. 2A). This will reduce the gradient to some extent and allow respiration to proceed as a function of the gradient’s reduction, i.e., the rate of ATP synthesis (state 3 respiration) (Fig. 2B). However, the coupling of oxidations to ATP synthesis is not perfect, and a significant part of the energy liberated from the substrate will normally be dissipated as heat. If a low-impedance route is provided for protons to move back into the mitochondrial matrix, which can be done with substances appropriately called uncouplers [p-trifluoro-methoxy carbonyl cyanide phenylhydrazone (FCCP) in Fig. 2B], protons will more readily move back into the
matrix, bypassing the ATP synthase and collapsing the gradient, causing respiration to be accelerated but uncoupled from ADP phosphorylation. Since the passage of protons across the membrane, in the direction of the gradient, is an exergonic reaction, the energy will be now dissipated as heat instead of being captured in ATP (Fig. 2B) (144).

Until recently, uncoupling of respiration from ATP synthesis was known to occur in a regulated manner only in a specialized tissue called BAT. Here, a protein abundantly present in the inner mitochondrial membrane, called uncoupling protein (UCP), provides an alternative, low-impedance route for the protons back into the mitochondrial matrix, collapsing the gradient, bypassing the ATP synthase, producing heat, and accelerating respiration (see Refs. 167, 169 for historical reviews and original references). Although the intimate mechanism whereby UCP reduces the proton-motive force is still being debated (93, 101, 134), it has been long known that the activity of UCP, i.e., its proton conductance, is blocked by nucleotides and strikingly augmented by fatty acids (168, 186). BAT is found only in mammals (see Ref. 51 for review), and UCP is found only in this tissue (49), which was confirmed when it was cloned (34, 35). It appeared that regulated uncoupling of phosphorylation to produce heat was a late evolutionary acquisition, present only in one of the two classes of homeothermic species, mammals. However, parallel independent studies demonstrated the presence of a significant leak of protons through the mitochondrial inner membrane in several tissues, not restricted to BAT (38), and subsequent work provided evidence that it was regulated and could serve a thermogenic function (reviewed in Ref. 195). This proton leak was, thus, found to be more active in homeothermic than poikilothermic species (39) and estimated to account for 20–30% of the basal metabolic rate of the rat (194) and as much as 50% of muscle resting oxygen consumption (193).

Attempts to demonstrate such leak through artificial membranes with the exact composition of mitochondria membrane failed, leaving it unexplained how it could occur in native mitochondria (44). The cloning of two new UCPs, called UCP2 and UCP3 (BAT UCP became then UCP1), of more ubiquitous tissue distribution, brought the expectation that they could explain the proton leak detected in mitochondrial of tissues other than BAT (88, 98, 243), and indeed, when expressed in yeast mitochondria, these novel proteins could mimic the action of UCP1. The cloning of new UCPs in mammals stimulated the search in other species. Observations in potato mitochondria had already revealed the presence of a 32,000 kDa protein that conferred this mitochondria properties reminiscent of BAT mitochondria (241), and thus a UCP homolog was cloned from potato (138, 240) and subsequently demonstrated in all higher plants investigated (126). Homologs have been also found in birds (83, 183, 242). The phylogenetic analysis of these proteins shows they belong to a large family of mitochondrial anion carriers and that UCP1, UCP2, UCP3, and the bird homologs are clustered close to plant UCPs, whereas the other two mammalian homologs UCP4 and BMPC1 are more distant (30). UCP1 and bird and plant UCPs share many properties, such as being inactivated by nucleotides, activated by fatty acids, and induced by cold. Such observations clearly demonstrate that uncoupling of mitochondria respiration from ATP synthesis is not a late but an ancient strategy used by nature to produce heat. Notwithstanding these observations, a major role for all the UCPs in mammalian FeT is still to be convincingly demonstrated. Transgenic mice lacking either UCP2 or UCP3 do not

**FIG. 2.** Mitochondrial respiration and proton-motive force. A: respiratory chain complexes are represented by the purple circles with their Roman numerals embedded in the inner mitochondrial membrane. As electrons travel down the respiratory chain, complexes I, III, and IV extrude protons into the intermembrane space creating a gradient with the mitochondrial matrix. The energy of this gradient is used by the ATP synthase complex to produce ATP. B: schematic representation of proton gradient, respiration (oxygen consumption), and ATP synthesis when a substrate (succinate), ADP, and an uncoupler, p-trifluoromethoxycarbonyl cyanide phenylhydrazone (FCCP), are added to intact mitochondria in vitro. For details, see Ref. 144.
exhibit a readily evident thermogenic deficiency (7, 99, 244). While mice overexpressing UCP3 at very high levels are hyperphagic, yet leaner than controls (58), the expression of UCP2 in yeast mitochondria in amounts similar to those normally found in tissues failed to produce a detectable proton leak, suggesting the uncoupling originally observed in forced expression experiments in yeast was an artifact derived from the high levels of expression (227). On the other hand, it is possible that some of these proteins have different roles, one of which could be to contribute to ObT, as suggested by some genetic studies in humans (32, 203, 247), and by some data from our laboratory that will be discussed below. These proteins may also, or instead, accomplish other functions. For example, the deletion of the UCP2 gene in mice is associated with resistance to *Toxoplasma gondii*, as a consequence of uncontrolled production of reactive oxygen species in macrophages (7). In addition to limiting the production of reactive oxygen species, a role that UCP3 and plant UCPs (224) may share with UCP2, UCP2 may modulate insulin secretion (55, 258) and, along with UCP3, may be important for fatty acid metabolism (reviewed in Ref. 196). In the author's opinion, the lack of striking phenotypes in transgenic models of gene deletion must be taken cautiously, because it has been repeatedly found that phenotypes could be obscured by compensations or redundant mechanisms, as illustrated later with specific examples. More research is obviously needed.

Looking for additional thermogenic mechanisms, we have recently initiated the investigation of the NADH glycerol-3-phosphate (G3P) shuttle. Reducing equivalents \((\text{H}^+, \text{e}^-)\) generated from substrates oxidized in the cytoplasm ultimately reduce NAD to NADH, \(\text{H}^+\). This cannot, however, enter the mitochondria to complete the reduction of \(\text{O}_2\) to \(\text{H}_2\text{O}\), and thus cannot directly transfer the energy to ATP. This is accomplished by shuttle molecules than can enter the mitochondria and are reduced by NADH (144). There are two major shuttle systems: the so-called malate-aspartate shuttle and the G3P shuttle (Fig. 3). In the former, the molecule reduced by NADH is oxaloacetate, which is reduced to malate by the cytoplasmic malate dehydrogenase. The malate enters the mitochondria and in the matrix is reoxidized to oxaloacetate by the mitochondrial malate dehydrogenase that then transfers the reducing equivalents to the complex I of the respiratory chain, generating three ATP molecules per atom of oxygen reduced. The resulting oxaloacetate is returned to the cytosol in the form of aspartate, generated by transamination from glutamate, and in the cytosol, aspartate regenerates oxaloacetate by the transamination of \(\alpha\)-ketoglutarate to glutamate.

In the G3P shuttle, the molecule being reduced is dihydroyxacetone phosphate (DHAP), which results from the cleavage of fructose-1,6-diphosphate into two 3-carbon molecules, DHAP itself and glyceraldehyde-3-phosphate. These can be interconverted by a triose phosphate isomerase, and DHAP can thus continue in the glycolytic pathway or serve as an acceptor of reducing equivalents transferred from NADH [and NADPH (84), in a reaction catalyzed by the cytoplasmic G3P dehydrogenase (EC 1.1.1.8; cGPD). The resulting G3P is reoxidized to DHAP by the mitochondrial glycerol-3-phosphate dehydrogenase (EC 1.1.99.5; mGPD). This enzyme is a flavin adenine dinucleotide (FAD)-linked dehydrogenase, located on the outer surface of the inner mitochondrial membrane, that directly transfers the electrons to the complex III of the respiratory chain, producing only two ATPs per molecule of \(\text{H}_2\text{O}\) formed (64, 133).

By catalyzing the interconversion between DHAP and G3P, the G3P shuttle constitutes a crossroad between lipid and carbohydrate metabolism as well as the main

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**Fig. 3. NADH shuttles.** Left: malate-aspartate NADH shuttle. Glu, glutamate; \(\alpha\)-KG, \(\alpha\)-ketoglutarate. Malate and \(\alpha\)-KG, on one hand, and aspartate and glutamate, on the other, utilize transporters to get in and out the mitochondria. Right: glycerol-3-phosphate (G3P) shuttle. cGPD and mGPD, cytoplasmic and mitochondrial G3P dehydrogenases, respectively; DHAP, dihydroxy-acetone-3-phosphate; UQ, ubi-quinone; III, complex III of the respiratory chain. External mitochondrial membrane is completely permeable to the substances involved and is therefore not depicted, and intermembrane space from this point of view is contiguous with cytoplasm. See text and Ref. 144 for more details.
entry of glycerol into gluconeogenesis. Such relationships are shown in Figure 4. Thus G3P can be converted to DHAP by mGPD and thus enter the gluconeogenesis pathway mainly in liver and kidney. The other fate of G3P, quantitatively important in lipogenic tissues (e.g., adipose tissues, liver), is to be acylated to form triglycerides and other lipids. On the other hand, G3P can also be generated by phosphorylation of glycerol resulting from lipolysis, i.e., triglyceride hydrolysis, in a reaction catalyzed by glycerol kinase (GK). Liver has a large content of GK, and because of its size, this organ is the main site of production of G3P from circulating, lipolysis-derived glycerol (120). It should be noted that other tissues also have significant amounts of GK that plays the role of locally recycling glycerol derived from lipolysis. Notable among these are muscle, brain, and BAT (121, 248), where the enzyme is stimulated by the sympathetic nervous system (86), but interestingly GK is not present in white adipose tissue (WAT).

The activity of these two shuttles and their relative importance are tissue dependent. In liver, the malate-aspartate shuttle is very active, whereas the G3P shuttle is the main one in skeletal muscle (144). Accordingly, a mouse genetically deficient in cGPD shows a marked increase in DHAP and a reduction of G3P in muscle but not in liver (149). We have found increased levels of G3P and lactate in muscle but not in liver of transgenic mGPD/H11002/H11002 mice (3, 71) and have made several observations that support the concept of the different role of the shuttle and mGPD in muscle and liver, which is illustrated in Figure 5 and discussed in the ensuing paragraphs.

The entrance of reducing equivalents into the respiratory chain via mGPD is a rapid pathway for aerobic ATP generation. Accordingly, the expression of mGPD is particularly high in the tissues that require rapid generation of ATP such as the flight muscle of insects, sperm cells, and pancreatic β-cells. In rodents, mGPD is highly expressed in BAT (173). In the mouse, skeletal muscle ranks second, next to BAT in mGPD abundance, followed by brain, kidney, and liver (136), which we have just confirmed (3). This hierarchy is similar in the rat (142). The preferential use of G3P shuttle in muscle allows the rapid aerobic generation of ATP from NADH produced by glycolysis, reducing the accumulation of lactate. In the liver, and probably other gluconeogenic tissues, G3P is more of a glucose precursor, whereas in lipogenic tissues, such as adipose tissues and liver itself, G3P is the backbone for TG synthesis. Although a rapid way to generate ATP aerobically, ATP synthesis via this pathway is less efficient because only 2ATPs are generated per atom of oxygen fully reduced to H₂O. This results from the reducing equivalents entering the respiratory chain in complex III, with the energy of the missing third ATP being dissipated as heat (64). Thus the predominant use of the G3P shuttle could subserve a thermogenic function.

The function of the G3P shuttle in different tissues depends not only on the abundance of its enzymes, particularly the rate-limiting one, mGPD, but also on the makeup of other enzymes in the tissue, which in turn reflects the role the tissue plays in whole body metabolism. This will obviously depend both on how active this shuttle is and on how active the alternative malate-aspartate shuttle is. As mentioned, mGPD in rodents is most active in BAT and muscle (100, 136, 142, 173), whereas liver for comparison has lower levels of activity and a very active malate-aspartate shuttle. Thus respiration in liver,
but not in muscle, of normal mice is inhibited by amino-oxyacetate, an inhibitor of the malate-aspartate shuttle (Fig. 5A). One would also predict from this information that G3P would be mainly a fuel for muscle and BAT, whereas in liver G3P would be less of, or not at all, an oxidizable substrate. This is exactly what we have observed, as shown in Figure 5, A–C. The addition of G3P in sequence after succinate did not change or even decreased respiration in liver but increased it by a factor of four in muscle (tibialis anterior) (Fig. 5, A and B). Note that the absence of mGPD did not have an effect in liver respiration, whereas it completely prevented the G3P-stimulated respiration in muscle. When substrates were added on tissues respiring on Ringer glucose, G3P increased QO2 by sixfold in muscle and by threefold in BAT slices, to a level not different from that induced by succinate, whereas it did not significantly increase respiration of liver slices (Fig. 5D).

To investigate a role for mGPD in thermogenesis and intermediary metabolism, we have been studying a transgenic C57Bl/6J mouse with targeted disruption of the mGPD gene (81) (mGPD−/−). Results have been recently published (3, 71). The most salient characteristics of the phenotype are summarized in Table 1. Briefly, we have found that these animals do have a mild reduction in energy turnover, as judged by both food intake and indirect calorimetry (oxygen consumption, QO2) (71). They have lower blood glucose and higher triglycerides as well as G3P plasma levels. Consistent with the G3P shuttle being more active in muscle, G3P levels and the lactate-to-pyruvate ratio were elevated in muscle, but not in liver. Actually, in liver the lactate-to-pyruvate ratio was reduced probably due to large influx of lactate and the large capacity of the liver to oxidize it to pyruvate, dumping the reducing equivalents into the mitochondria via the malate-aspartate shuttle (3). Most interestingly, mGPD−/− mice had 15–20% higher levels of both T4 and T3, which was due to increased thyroidal secretion, as plasma binding or clearance was not affected by the deletion of the mGPD gene. Because these animals have reduced energy turnover (QO2 and food intake), this suggests that the elevated levels of thyroid hormones are part of a response to cold stress. Consistent with this idea, BAT had signs of being chronically stimulated when animals were reared at 22°C, but raising the ambient temperature to 32°C, slightly over thermoneutrality, resulted in regression of such signs, atrophy of the tissue, and reduction of the circulating levels of T4 and T3 to those of the wild-type genotype (WT) controls. We also found increased UCP3 expression in skeletal muscle in mGPD−/− males but not in the female counterpart. Altogether, these results suggested an attempt to compensate a thermogenic deficiency (71).

These results are consistent with the idea that the G3P shuttle contributes to ObT and that TH utilizes this metabolic pathway (among others) to increase ObT. Further evidence that the G3P shuttle represents a spend-thrift metabolic pathway is supported by observations we made when mGPD−/− mice were challenged with high fat or caloric restriction, in the form of either a short fasting or more prolonged but partial caloric restriction. Given a high-fat diet (3), mGPD−/− mice increased weight more rapidly than WT controls, even though the caloric intake was not different from that of WT. When fasted or caloric restricted, mGPD−/− mice lost less weight than the WT, as one would predict from eliminating a spend-thrift metabolic pathway.

FIG. 5. Effect of substrates on in vitro respiration by tissue slices (unpublished observations). A: effect of 1 mM succinate or 1 mM glycerol-3-phosphate (G3P) on liver from wild-type genotype (WT) and mGPD−/− mice. B: effect of same substrates on skeletal muscle (tibialis anterior) of WT and mGPD−/− mice. C: effect of amino-oxyacetate on glucose-supported respiration of liver and muscle (tibialis and vastus lateralis). D: stimulation of glucose-supported respiration by succinate or G3P in muscle, brown adipose tissue (BAT), or liver.
Furthermore, the attenuated weight loss in mGPD−/− was associated with a greater fall in $Q_O_2$ and $T_c$, consistent with the idea that thermogenesis was more reduced in these mice than in the WT controls in response to food restriction, ultimately indicating that the G3P shuttle contributes significantly to ObT.

Intriguingly, even though male and female mGPD−/− mice did not perceptibly differ in basal levels of glucose, TG, FFA, G3P levels, etc., markers of qualitative changes in intermediary metabolism, the thriftiness revealed by the high-fat diet or by energy restriction was evident only in females (3). The reasons for such gender differences have not been elucidated, but the data suggest that the increase in TH levels and UCP3 probably play a role. As mentioned, plasma $T_3$ was 15–20% higher in mGPD−/− males than in the WT males, whereas in mGPD−/− females plasma $T_3$ concentration was not higher than in their WT controls. Likewise, UCP3 was not as elevated in mGPD−/− females as it was in the male counterpart (3). In view of the responsiveness of UCP3 mRNA to $T_3$ (71), it is possible that the lack UCP3 elevation in mGPD−/− females was due to their failure to elevate $T_3$. On the other hand, while UCP3-deficient mice are not obese (99), in the context of absence of mGPD, UCP3 may play a role in the resistance to diet-induced obesity of mGPD−/− males because double-knockout males, i.e., UCP3−/− mGPD−/−, gain weight as mGPD−/− females when challenged with a high-fat diet (Stepanyan, Schifman, and Silva, unpublished observations).

While the work described above was in course, Brown et al. (45) published their observations on another mGPD-deficient model. They reported their mGPD-deficient mice have reduced viability and grew less than WT controls, as we find too. However, they did not detect a reduction in $Q_O_2$ or in food intake. Most notably, their female mGPD-deficient mice did not gain more weight than the WT controls when fed a high-fat diet since weaning, whereas mGPD-deficient males identically treated

### Table 1. Most relevant phenotypic characteristics of mice with targeted disruption of the mitochondrial glycerol-3-phosphate gene (mGPD−/−) at 3–6 mo of age

<table>
<thead>
<tr>
<th>Compared With Wild Type</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy balance</strong></td>
<td></td>
</tr>
<tr>
<td>Food intake</td>
<td>−10%</td>
</tr>
<tr>
<td>$Q_O_2$</td>
<td>−(5–10%)</td>
</tr>
<tr>
<td>Core temperature</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Weight</td>
<td>−(5–10%)</td>
</tr>
<tr>
<td>Blood concentrations</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>−14%</td>
</tr>
<tr>
<td>Serum FFA</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Serum triglycerides</td>
<td>+84%</td>
</tr>
<tr>
<td>Serum G3P</td>
<td>+45%</td>
</tr>
<tr>
<td>Serum $T_4$</td>
<td>+22%</td>
</tr>
<tr>
<td>Serum $T_3$</td>
<td>+18%</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>G3P</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Lactate</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>Increased</td>
</tr>
<tr>
<td>Lactate/pyruvate</td>
<td>Decreased</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
</tr>
<tr>
<td>G3P</td>
<td>Increased</td>
</tr>
<tr>
<td>Lactate</td>
<td>Increased</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>Decreased</td>
</tr>
<tr>
<td>Lactate/pyruvate</td>
<td>Increased</td>
</tr>
<tr>
<td>UCP3 mRNA</td>
<td>Increased</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td></td>
</tr>
<tr>
<td>UCP1</td>
<td>Increased</td>
</tr>
<tr>
<td>Responses to challenges</td>
<td></td>
</tr>
<tr>
<td>High-fat diet</td>
<td>Obesity</td>
</tr>
<tr>
<td>Fasting</td>
<td>Less weight loss and hypothermia</td>
</tr>
<tr>
<td>Starvation</td>
<td>Less weight loss, reduced $Q_O_2$, and hypothermia</td>
</tr>
</tbody>
</table>

$Q_O_2$, oxygen consumption; FFA, free fatty acids; G3P, glycerol-3-phosphate; UCP, uncoupling protein. [Data from Alfadda et al. (3) and DosSantos et al. (71).]

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gained less weight than WT. In addition, they did not
demonstrate a thermogenic deficiency in mGPD-deficient
mice of either gender, but this was investigated less thor-
oughly than we did. While there are many differences in
the experimental approach in both studies, it is likely that
the major reason for the different findings derives from
some unidentified difference(s) in the genetic back-
ground. While the nominal genetic background of both
genotypes was the C57Bl, the original mGPD transgene
carrying the disruption was of different origin, and the
C57Bl background of our mice is one that has been for
innumerable generations in Japan. The differences be-
tween the two models illustrate the caveats of using trans-
genic models of gene deletion, as the consequences of it
may be obscured by compensatory mechanisms and ge-
etic background. Notwithstanding, rather than finding
these differences disturbing, their investigation could be
revealing of unsuspected factors contributing to the phe-
notype.

II. HORMONAL REGULATION
OF THERMOGENESIS

A. Thyroid Hormone Acquires a New Role
With the Advent of Homeothermy

The thyroid gland is present in all vertebrates, where
it is essential for a coordinated development and to con-
trol specific functions. The effects of TH on amphibian
metamorphosis have been known for nearly a century
(105; for a review, see Ref. 92), and the devastating ef-
fects of congenital hypothyroidism on mammalian brain de-
tevelopment are well known as well (see Refs. 14, 75, 143 for
most recent reviews and references). However, only in
homeothermic species TH increases metabolic rate and
thermogenesis (107, 249; reviewed in Ref. 207). The im-
portant role TH plays in temperature homeostasis is
backed by the well-known cold or heat intolerance of
animals and humans with hypothyroidism or hyperthy-
roidism, as well as by the hypothermia and hyperthermia
associated, respectively, with extreme forms of hypo- and
hyperthyroidism (see Ref. 207 for review). TH stimulates
ObT, and it is essential for FcT.

As already mentioned, homeothermic species gener-
ate more heat than poikilothermic species, and this is due
to both the more active metabolism and the lower ther-
modynamic efficiency of the homeothermic machine (78,
254). These two differences are largely dependent on TH.
Indeed, the absence of TH makes homeothermic animals
(humans included) regress into a quasi-poikilothermic
state. Not only TH increases the number of energy trans-
formations, as evidenced by 30–50% reduced basal meta-
bolic rate, but it also reduces the thermodynamic effi-
ciency of the homeothermic machine for the sake of
producing more heat. For example, for any amount of
mechanical work, euthyroid rat muscles generate more
heat than the hypothyroid counterpart (145) (Fig. 6, fur-
ther discussed below). Likewise, the energy cost of pro-
ducing a given amount of glycogen from lactate is higher
in euthyroid than hypothyroid hepatocytes (15). More-
ever, studies in hepatocytes from rats with manipulated
thyroid status show that the difference in oxygen con-
sumption between the hypo- and the euthyroid state is
much greater than the difference in ATP turnover (an
estimate of the amount of effective work) between the
two states (114).

We have demonstrated in humans that resting energy
expenditure (REE), a close measure of ObT, is remark-
ably sensitive to TH around the euthyroid state (2), un-
derscoring the physiological relevance of this TH action.

FIG. 6. Heat production by muscle as a function of tension de-
veloped in a short tetanus in muscle from euthyroid and hypothyroid mice.
A: data from extensor digitorum longus (EDL), a fast-twitch muscle. B:
data from soleus, a slow-twitch muscle. In both cases, heat increased
linearly with mechanical work (tension development). For EDL, slopes
were not affected by thyroid status, but the euthyroid curve was signif-
icantly higher, with a y-intercept significantly higher. For soleus, both
slopes and y-intercepts were significantly higher in the euthyroid state.
See text for details and discussion. [Lines represent the regression lines
derived from experimental data by Leijendekker et al (145).]
Thus, in athyreotic patients maintained euthyroid on exogenous thyroxine, minimal changes in their daily dose, which did not move serum free T4 concentration out of the normal range, were associated with readily detectable changes in REE. Except for thyroid stimulating hormone (TSH), none of the indexes of TH action used clinically were significantly affected by the dose changes, yet there was a remarkable negative correlation between TSH and REE ($r = -0.82; P < 0.001$). The T4 dose changes in these patients moved TSH between 0.05 and 10 mU/l (normal: 0.4–4.5 mU/l), which was associated with a 15% excursion of REE (2). Moreover, we found that spontaneous fluctuations in free T4 concentration in lean normal males are also associated with significant changes in REE (29).

In addition, TH is essential for FcT, which is inherent to homeothermy. In mammals, particularly in small ones including the human newborn, BAT is the main site of FcT. Interacting with sympathetic nervous system, TH is essential for the full thermogenic response of BAT (reviewed in Refs. 207, 212). Hypothyroid rodents develop profound hyperthermia if placed in the cold, and this is promptly corrected, within 24–48 h of giving small doses of T4 that normalize the thyroid status of BAT but not other tissues (21). In both hypothyroid rats and mice, norepinephrine fails to increase BAT temperature, and this defect is again rapidly corrected by the administration of small doses of T4 or T3 (187, 188). TH is essential to sustain the norepinephrine signaling cascade, acting at the receptor as well as the postreceptor level (see Refs. 52, 197, 198, 230 and references therein), but T3 and norepinephrine also interact synergistically at a more distal level, regulating the expression of several genes (20), most notably the expression of the UCP1 gene (reviewed in Ref. 218). Moreover, both UCP1 expression and BAT temperature elevation in response to norepinephrine correlate nicely with TH receptor occupancy by T3 (36).

B. Thyroid Hormone and Obligatory Thermogenesis

1. How does TH affect the thermodynamic efficiency of the homeothermic machine?

It has been known for more than a century that TH increases basal metabolic rate (150), the closest expression of resting OBT, yet still we do not know the precise underlying mechanisms. As reviewed above (Fig. 1), there are basically two major ways to generate more metabolic heat, namely, to increase ATP turnover, by stimulating its utilization, and to reduce the thermodynamic efficiency of ATP synthesis, that is, capturing less energy of the substrate in the form of ATP and dissipating more of it as heat (207). There is ample evidence that TH may stimulate both types of thermogenic mechanisms in homeothermic species.

2. TH stimulation of ATP consumption as a thermogenic mechanism

TH can increase ATP utilization, to a significant extent in a futile manner, for the sake of producing heat. Thus TH increases lipolysis and lipogenesis, proteolysis and protein synthesis, glucose oxidation, and gluconeogenesis. However, the energy cost of stimulating these metabolic cycles is small, probably explaining together not more than 10% of the TH-dependent stimulation of energy expenditure (see, for example, Ref. 91). Perhaps more important is the increase in ATP consumption to maintain ion gradients, namely, the Na+ and K+ gradient across the cell membrane and the Ca2+ gradient between the sarcoplasmic reticulum and cytosol. Directly or indirectly, TH can stimulate the entrance of Na+ in the cells or the extrusion of K+, forcing Na+–K+–ATPase activity to restitute the gradients of these two ions across the cell membrane, with the attendant ATP consumption (110, 125), whereas TH also stimulates the expression of Na+–K+–ATPase (124). There was initially great enthusiasm over this mechanism (76), but today it is felt that it only explains a comparatively minor fraction of TH thermogenesis (59, 90, 205). Even though estimates derived from measurements of ouabain-sensitive QO2 suggest that −20% of basal metabolic rate (BMR) could be explained by the Na+–K+–ATPase in humans (59), about two-thirds are accounted for by brain and kidney, where the effect of TH on ouabain-sensitive QO2 is small or nil. Revisiting early studies examining the effect of ouabain on QO2 in rats suggests that the contribution of Na+–K+–ATPase to TH-dependent energy expenditure is very small in the euthyroid state, i.e., in the transition from hypothyroidism to euthyroidism, whereas it could be more important in thyrotoxidosis (77).

Another postulated mechanism of increased ATP consumption is the stimulation of Ca2+ transfer from cytosol to sarcoplasmic reticulum (59). This is thought to be largely due to increased activity of the sarcoplasmic/ endoplasmic reticulum Ca2+-dependent ATPase (SERCA). TH indeed stimulates the expression of SERCA1 in skeletal muscle (reviewed in Ref. 219). This sole effect could explain the increase in the sarcoplasmic calcium pool, the increased release of calcium during contraction, and ultimately the additional energy expenditure to return the calcium back into the sarcoplasmic reticulum. TH administration to hypothyroid rats increases the SERCA activity and the amount of sarcoplasmic reticulum, which is associated with a substantial increase in the amount of energy spent in Ca2+ pumping (221). Slow-twitch muscle is more responsive to T3 than fast muscle, largely owing to the predominant stimulatory effect of TH on SERCA1 gene expression, the baseline expression of which is low in this type of muscle (219, 220, 239). While TH can stimulate the expression of SERCA2 in individual muscle
fibers, the effect is modest and diluted by a reduction in the number of SERCA2-expressing fibers so that the global effect on SERCA2 can even be negative (163, 239).

It has been debated whether there is flux of calcium across the sarcoplasmic membrane in the resting muscle, i.e., a leak of Ca\textsuperscript{2+}. The most recent evidence suggests that the resting cytosolic calcium is in a dynamic equilibrium with the sarcoplasmic calcium (153 and references therein). Independent calculations of the ATP's need to maintain the resting cytosolic Ca\textsuperscript{2+} concentration in muscle indicate that it may amount to 30–70% of resting muscle ATP demands in the rat (153). This is interesting, since TH also increases the number and activity of ryanodine receptors, involved in the release of Ca\textsuperscript{2+} from sarcoplasmic reticulum into the cytosol, both in heart and skeletal muscle (60, 127), suggesting that TH could also stimulate sarcoplasmic Ca\textsuperscript{2+} efflux into the cytosol, both in the resting state and during contraction, enhancing the demands of ATP to return it into the sarcoplasmic reticulum.

Overall, skeletal muscle Q\textsubscript{O2} increases ~30% in the transition of hypothyroidism to euthyroidism (11), and SERCA activity in such transition increases 2.5- to 3-fold and 1.5-fold in slow- and fast-twitch muscle, respectively (219, 220). The stimulation of calcium pumping by TH thus accounts for a large fraction of the physiological TH-dependent stimulation of resting muscle energy expenditure. Because skeletal muscle normally contributes ~20–30% of REE or BMR (260 and references therein), Ca\textsuperscript{2+} transport in muscle could explain as much as 10% of the effect of TH on resting thermogenesis. Because the flux of Ca\textsuperscript{2+} is increased during muscle activity, the associated heat production is also increased. This has been examined by direct measurement of heat production as a function of tension developed in either twitch or tetanus in both a fast-twitch muscle (extensor digitorum longus, EDL) and a slow-twitch muscle (soleus) (145). Results relevant to this discussion are depicted in Figure 6. In both muscles, either twitch or tetanus activity produced heat linearly as a function of the developed tension, but for any level of tension, there was significantly more heat produced in the euthyroid muscle than in the hypothyroid counterpart, that is, the energy cost of mechanical work is greater in the euthyroid than in the hypothyroid state, the difference being accounted for by the extra heat produced in the euthyroid state. The slopes of the curves, i.e., the increase in heat production with tension, was not affected by the thyroid status in EDL, but it was significantly greater in the euthyroid soleus than in the corresponding hypothyroid muscle, suggesting that in a slow-twitch muscle TH stimulates heat production by an additional mechanism. This may be the predominant stimulatory effect on SERCA1 expression, which is more accentuated in slow- than in fast-twitch glycolytic muscle (219) and the greater capacity for reducing the Ca\textsuperscript{2+}/ATP ratio of this SERCA isoform (68). Interestingly, when the lines are extrapolated to tension zero, the intercepts, which the authors call tension-independent heat production (145), are significantly greater, by >50%, in the euthyroid than in the hypothyroid muscles, providing independent support to the idea that resting muscle heat production is also greater in euthyroidism.

Finally, it is important to recall that slow-twitch muscles are relatively more abundant in larger mammals, such as humans, who in addition have less brown fat, so that these mechanisms are quantitatively more important in larger species. The presence of type II iodothyronine 5'-deiodinase in human but not in rodent skeletal muscle (201) may also be an indication that muscle TH-dependent thermogenesis is more important in humans. As mentioned earlier, resting energy expenditure is very sensitive in athyreotic humans to changes in the dose of thyroxine. The small dose changes in these studies did not significantly affect serum concentrations of the active thyroid hormone T\textsubscript{3}, suggesting that local T\textsubscript{4}-to-T\textsubscript{3} conversion in the site of thermogenesis could be more important than circulating levels of T\textsubscript{3} (2).

3. TH reduction of ATP synthesis efficiency as a thermogenic mechanism

The concept that TH could uncouple oxidative phosphorylation in the mitochondria is not new, but it was ignored for many years (118) until Brand and colleagues (111) postulated, based on observations made in isolated mitochondria, that TH increased the leak of protons through the mitochondrial inner membrane, thus reducing the proton-motive force and stimulating oxidations to maintain the rate of ATP synthesis. These authors showed later in liver cells that in the transition from hypo- to euthyroidism there was an increase in respiration, without a significant increase in ATP turnover, indicating that the TH-induced energy expenditure in this transition was probably dissipated as heat via the stimulated proton leak. Indeed, about half the TH-induced increment in Q\textsubscript{O2} could be accounted for by the stimulation of the proton leak, whereas the other half was thought to be nonmitochondrial. In the transition from the euthyroid to the hyperthyroid state, there was a more marked increase in Q\textsubscript{O2}, and now half was accounted for by ATP synthesis whereas the other half was explained by the proton leak, with no significant change in the nonmitochondrial component (114) (Fig. 7). Such findings suggest that physiological levels of TH primarily stimulate heat production, rather than ATP consumption. Such interpretation is in agreement with the early observation that Na\textsuperscript{+}-K\textsuperscript{-} ATPase activity in liver membranes is clearly increased in the thyrotoxic state but is not significantly reduced in the hypothyroid state relative to the euthyroid level (77).

It was initially hypothesized that TH increased the proton leak by changing the phospholipid composition of the mitochondrial membrane, but such hypothesis did not
find support (44). The cloning of UCP2 and UCP3 (88, 98, 243) raised the expectation that they could mediate not only a regulated dissipation of the proton gradient across the inner mitochondrial membrane, as discussed earlier, but also the portion of the thermogenic effect of TH not accounted for by mechanisms increasing ATP utilization. Of the two novel UCPs, it was promptly noted that UCP3, expressed in muscle, heart, and BAT, responds clearly to TH and adrenergic stimulation (98), while UCP2 does not respond to sympathetic stimulation (88) and its response to TH in vivo is quite variable and inconsistent (see, for example, Ref. 98). Such findings made UCP3 a more likely mediator of TH thermogenesis. However, such expectations have not been fulfilled. For one thing, hepatic mitochondria, in which the proton leak had been demonstrated and characterized, do not express either UCP2 or UCP3. Besides, even though when expressed at high concentrations in mitochondria UCP2 and UCP3 share with UCP1 the property of reducing the proton-motive force, at the concentrations normally found in cells they do not cause a detectable drop in the proton gradient (recently reviewed in Ref. 196). Changes in either UCP2 or UCP3 associated with manipulations of the thyroid status are not associated with changes in the proton gradient in human muscle (10). Lastly, neither ucp2 nor ucp3 knockout mice show any thermogenic defect, and UCP3-deficient mice increase QO2 and body temperature in response to T3 injections as the WT genotype (99, 196). Altogether, these observations make it unlikely, but do not exclude, that these proteins mediate the thermogenic effect of TH, and further studies are necessary to define their role in TH thermogenesis.

As mentioned earlier, the NADH-G3P shuttle constitutes another mechanism of producing ATP with lower efficiency, particularly in skeletal muscle, where there is abundant extramitochondrial generation of NADH and where the alternate NADH-malate-aspartate shuttle is of little or no significance (144). It has been known for a long time that TH stimulates the activity of several mitochondrial enzymes, one of which is the mGPD, the rate-limiting enzyme of the shuttle (142 and references therein). Most interestingly, there is a good correlation between the stimulation of QO2 by TH in rat tissues (11) and the stimulation of mGPD activity (142) or its mRNA (97). Moreover, TH does not stimulate mGPD in those tissues where it does not stimulate QO2, nor does it stimulate the enzyme in species where it does not increase QO2 (249). For all these reasons, we undertook the study of transgenic mice lacking mGPD, as described earlier. Results have been published (3, 71), are partially summarized on Table 1, and have been discussed above. Relevant to this part of the discussion, male mGPD−/− mice living at room temperature (21°C), which represents a modest but significant cold stress for mice, have elevated levels of T4 and T3 and increased UCP3 mRNA and protein, and their BAT shows signs of being more stimulated than in controls, yet they have modestly reduced QO2, altogether suggesting a reduction in ObT despite the elevated levels of TH (71). These phenotypic differences were reduced or abrogated when animals were acclimated at 32°C, slightly over the nominal Tm for mice (30°C), and the reduction of QO2 of mGPD−/− mice relative to WT was doubled, altogether indicating such phenotype represented a compensatory response to an ObT deficiency.

The lower QO2 in the presence of elevated TH levels is an indication that mGPD is somehow necessary for the stimulation of ObT by T3, but is obviously not essential, nor is it the only mechanism because hypothryroid mice have a QO2 30–40% lower than euthyroid controls (71). In agreement with this view, when hypothyroid mGPD−/− mice were treated with a moderately high dose of T3 (5 times the estimated production rate, 20 ng · g−1 · day−1) for 3 wk, they increased QO2 with the same temporal profile and to the same level as the WT controls (71), so higher levels of T3 in the absence of mGPD can totally overcome the lack of mGPD. Interestingly, this was associated with a greater increase of UCP3 mRNA in mGPD−/− than in WT mice (71). As mentioned earlier, the elevation of UCP3 mRNA in mGPD−/− mice is both ambient temperature dependent and TH dependent, since this mRNA was reduced by acclimating both mGPD−/− and WT mice to 32°C, but was still higher in mGPD−/− mice, whereas levels decreased further, to an equally low level, when mice acclimated at 32°C were rendered hypothyroid (71). Furthermore, as indicated earlier, mGPD−/− males, but not females, have an elevation of plasma T3, and only the males show increased muscle expression of UCP3 (3), whereas mGPD−/− females but not males were sensitive to diet-induced obesity and exhibited a thrifty phenotype.
Finally, double-knockout male mice, UCP3−/− mGPD−/− become sensitive to diet-induced obesity, as mGPD−/− females. Thus the elevation of UCP3 in muscle of mGPD−/− males appears to be a relevant compensatory response to the lack of mGPD and is TH dependent. The participation of several mechanism may well explain why the injection of high doses of T₃ to transgenic UCP3−/− mice induces comparable response to those of WT controls (99). In these studies there was no measurement of circulating TH levels nor was attention paid to alternative mechanisms, e.g., mGPD, that could compensate for the lack of UCP3.

An important corollary from the results summarized in the preceding paragraphs is that more care should be exerted in interpreting the phenotypes of transgenic animals with targeted gene deletions, particularly when the phenotype does not show an expected deficiency. As we showed, when made moderately thyrotoxic, mGPD−/− mice increase QO₂ as the WT controls (71). Had we not done the other experiments described above, the conclusion would have been that mGPD is not necessary for the thermogenic effect of TH, in the same way that such experiments led the investigators to conclude that UCP3 was not necessary for TH thermogenesis (99). Neither mGPD nor UCP3 is per se essential for thyrotoxicosis-induced increase in metabolic rate, but they may both contribute to the physiological stimulation of ObT by TH.

In summary, TH has been known to stimulate basal metabolic rate, the closest expression of ObT at rest, for over a century. Everything staying equal, increased basal metabolic rate alone is coupled to proportionally more heat production. A good fraction of the acceleration of metabolism by TH may be futile, that is, TH increases ATP consumption for the sake of heat production. Such mechanisms work basically by increasing ATP utilization, forcing cells to increase oxidations to maintain ATP levels for other cell functions. Although imprecise, the estimation is that these mechanisms increasing the utilization of ATP, whether futile or not, do not account for more than 50% of the thermogenic effect of TH. A large fraction of TH thermogenesis may result from the hormone reducing the efficiency of ATP synthesis, i.e., increasing the fraction of energy lost as heat in its synthesis, forcing the cells to burn more fuel to maintain the required production of ATP to sustain vital functions. Thus studies in mitochondria isolated from animals with manipulated thyroid status, notably from liver and skeletal muscle, indicate that TH reduces the proton-motive force by facilitating the leak of protons through the inner membrane. The recently cloned UCP homologs raised the expectation that these proteins could mediate the TH-mediated increase in proton leak, but the evidence has not so far supported this expectation, although such evidence does not exclude that these proteins, particularly UCP3, be necessary to support the thermogenic effect of TH. Finally, we have explored the possibility that mGPD could participate or be necessary for the stimulation of ObT by TH and obtained evidence that this enzyme is at least necessary because ObT is ~17% lower in mGPD−/− mice when the contribution of BAT and compensatory mechanisms are minimized. However, as it occurred with UCP3, the induction of mild thyrotoxicosis caused the same stimulation of QO₂ as in WT genotype controls. We must accept the concept that TH uses several mechanisms to stimulate ObT.

C. Thyroid Hormone and Facultative Thermogenesis

The discovery of type II iodothyronine 5′-deiodinase (D2) in BAT and its stimulation by the SNS (146, 216) allowed the demonstration of how important BAT FcT is in cold adaptation and how important TH is for its activation. Indeed, as discussed further below, numerous studies demonstrated previously that TH was somehow necessary for normal BAT responses to cold challenges (see Ref. 117 for a review of the time), but the activation of D2 by the SNS allowed the demonstration of a more complex and synergistic interaction between this latter and TH. Prompted by the potential of BAT to generate T₃ both locally and for circulation (215), and the estimated important contribution of D2 to the levels of T₃ in BAT even at room temperature (19), we performed experiments in hypothyroid rats treated acutely with T₄ or T₃. Two days of T₄ treatment in doses equal to the daily production rate sufficed to restore cold tolerance and were associated with normalization of the UCP levels in BAT (21), while during this limited regimen of T₄ there was no change of the thyroid status of the liver and plasma T₃ levels remained subnormal, that is, these animals could tolerate severe cold (4°C) for 48 h without normalizing ObT, only normalizing BAT, as judged by the levels of UCP1. The inhibition of D2 with iopanoic acid prevented the normalization of both cold tolerance and BAT UCP1 levels by T₄, even though we gave T₃ to avoid the reduction of plasma T₃ that could result from the inhibition of T₄-to-T₃ conversion elsewhere (20, 21). In contrast, to accomplish the same result with T₃ injections required longer treatment and doses that increased plasma T₃ and liver enzymes, used as markers of general thyroid status, to thyrotoxic levels (21). These observations have recently received decisive support from transgenic mice with disruption of the D2 gene (dio 2), which show cold intolerance, in spite of normal plasma T₃ concentration (65).

1. TH-adrenergic interactions

The SNS and the adrenal medulla are considered part of a system, centrally controlled at the level of the hypo-
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thalamus and brain stem, called sympathoadrenal system. Norepinephrine, the main SNS neurotransmitter, is synthesized and stored in peripheral sympathetic nerve endings and released in response to coordinated nerve impulses targeted to specific tissues or organs. The adrenal medulla is an endocrine gland that produces predominantly epinephrine, which is secreted in response to impulses carried in the splanchnic nerves and which globally influences processes throughout the body, including many also stimulated by norepinephrine. The activity of the SNS and adrenal medulla is regulated centrally, in a coordinated manner, suited to changes in the environment. Thus both SNS and adrenal medulla are activated together in response to cold and strenuous exertion, whereas in hypoglycemia the adrenal medulla is stimulated but the activity of the SNS is suppressed.

TH plays an important role in the responses of the sympathoadrenal system to the environment (reviewed in Refs. 209, 210). The sympathoadrenal system triggers rapid adjustments, whereas TH enhances the capacity of the cells to respond to most actions of catecholamines and maintains a metabolic rate appropriate for the availability and mobilization of substrates essential to ensure vigorous adrenergic responses. On the other hand, catecholamines increase T₄-to-T₃ conversion in selected tissues, notably BAT, and may increase the retention of the TH receptor in the nucleus or the recognition of DNA sequences through PKA-mediated phosphorylation of the T₃ receptor (170, 231, 237), the significance of which is yet to be defined. In general, a synergistic interaction is needed in circumstances when the delivery of substrate or release of energy is required, such as during cold adaptation or overeating. In opposite situations, for example, starvation, both systems are turned down independently, at separate levels: sympathetic outflow decreases (257), and thyroidal secretion as well as T₄-to-T₃ conversion are reduced. In general, sympathetic output to the tissues is reduced in hyperthyroidism and increased in hypothyroidism (139, 156, 236). In patients with thyrotoxicosis, plasma and urinary levels of norepinephrine have been reported as either normal or diminished (12, 62), whereas in hypothyroid patients urinary norepinephrine excretion and plasma norepinephrine levels are elevated (57, 62), reflecting an augmented production rate (61, 179).

The coordinated responses between the sympathoadrenal system and TH are particularly important in temperature homeostasis. As outlined above, BAT D2 plays a key role in the response to cold. Cold exposure triggers vigorous SNS stimulation of BAT, which, in addition to activating the thermogenic response of the tissue, activates D2, with the resulting increase in BAT T₃ amplifying SNS effects such as lipolysis and stimulation of the UCP1 gene. In the hyperthyroid states, ObT is increased and there is less need for BAT FcT. Appropriately, SNS activity is reduced and as a result D2 is less stimulated. In addition, D2 is rapidly inactivated by T₄ (213, 217), which makes the enzyme more susceptible to ubiquitination and degradation by proteasomes (226; reviewed in Ref. 17). Also, as it will be discussed later, TH reduces the expression of β₃-AR in BAT. In the opposite situation, the hypothyroid state, there is an attempt to compensate the reduction of ObT with increased BAT thermogenesis, and this tissue shows indeed signs of increased stimulation (162), and there is an increase in norepinephrine turnover, a sign of increased SNS activity (256). The resulting increase in D2 activity, further enhanced by the reduced levels of T₄, is critical to maintain the levels of T₃ in BAT with plasma T₄ concentrations as low as 25–30% of the euthyroid ones (53, 213). However, with the deepening of the hypothyroidism, the D2 response will be insufficient, there will be lack of T₃ in BAT, and the tissue responses to norepinephrine will be ultimately reduced. These relationships are schematically illustrated in Figure 8A.

Thermogenesis obviously increases energy needs. Essential to support thermogenesis is the provision of extra energy. It makes teleological sense, therefore, that TH also stimulates food intake and lipogenesis (207, 212 and references therein). The former provides additional energy and the latter, a mechanism to store it in a high caloric density form, fat. Hepatic de novo synthesis of fatty acids and of triglycerides is increased (91), and these are rapidly mobilized to WAT and muscle, where TH stimulates lipoprotein lipase (175, 200). In addition, TH enhances the lipolytic responses to catecholamines in WAT (116, 191, 246). Increased thermogenesis also demands additional oxygen supply to tissues and TH clearly stimulates cardiac function, increasing cardiac performance and output as well as the capacity of blood to transport oxygen (129), acting directly at the end organs, as well as potentiating the actions of catecholamines on the heart and peripheral circulation. TH is as essential for the full responsiveness of the cardiovascular system to catecholamines as it is for BAT to express all its thermogenic potential in response to SNS stimulation. Likewise, the stimulation of lipolysis by norepinephrine in white adipose tissue needs TH (209), as it does the epinephrine or glucagon stimulation of phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme in gluconeogenesis (113). Where in the signaling pathways and how is TH necessary to ensure optimal responsiveness to the sympathoadrenal system is tissue dependent (reviewed in Refs. 209, 210). Here, the focus will be on how TH affects the thermogenic function of BAT.

2. TH and BAT thermogenesis

That TH is necessary for a full BAT thermogenic response in cold adaptation has been long known (see Ref. 117 for early references). Most of the studies focused
on the diminished or lost responsiveness of BAT to sympathetic stimulation in the hypothyroid state. Mory et al. (162) noted that BAT of hyperthyroid rats showed morphological changes seen in cold adaptation, yet UCP1 was not stimulated nor did it respond to cold challenge. Seydoux et al. (206) reported impaired responses of BAT to sympathetic nerve stimulation or catecholamines that they attributed to reduced number of β-adrenergic receptors (β-AR), but noted that the responses were reduced to a much greater extent than the number of β-AR, suggesting an additional defect(s) downstream. Sundin et al. (230) showed reduced respiratory and lipolytic responses of brown adipocytes to isoproterenol or forskolin, and again pointed to a more distal effect as neither cAMP analogs nor forskolin stimulated normally respiration in hypothyroid brown adipocytes. Altogether these studies, among others, indicate that TH is essential to maintain the norepinephrine signaling in BAT, but they also clearly pointed to additional steps where TH is needed for a normal response as well as to complex interactions between this hormone and SNS. Thus, while TH was necessary for the response of UCP1 to cold, as assessed by GDP binding, treatment of rats with doses of T4 greater than the daily production rate, inducing a significant degree of thyrotoxicosis, was associated with a reduction of GDP binding or a diminished stimulation of GDP binding by cold (229, 233), leading to the concept that TH played a permissive role on BAT function (162, 233).

The finding of D2 and its activation by norepinephrine alone, as judged by the denervation of BAT in hypothyroid rats, is quite modest, 2- to 3-fold, whereas the stimulation is 18- to 20-fold in the presence of TH. Likewise, the stimulation of UCP1 by T3 on denervated BAT was also quite modest, barely significant (18). A complex TH response element (TRE) was identified upstream in the rat UCP1 gene (182), included in a critical enhancer well conserved in both the rat and mouse genes (54, 137). Such TRE was actually composed of two TREs in tandem located immediately below a cAMP response element (CRE) in that upstream enhancer region of the UCP1 gene (182). The synergism between cAMP and T3 was demonstrated to occur at the gene level (181), with TH and its TRE being necessary for the recruitment of an additional CRE downstream, in the proximal 5'-flanking region of the gene (reviewed in Ref. 218). Such multiple-level synergism makes it even more intriguing that the induction of a hyperthyroid state was associated with a reduction of the response of the tissue to adrenergic stimulation (229). The answer is probably D2 and its responsiveness to adrenergic stimulation and T4. Thus the synergism between T3 and the SNS makes BAT responses very sensitive to the removal of either. D2 is highly responsive to endogenous or exogenous norepinephrine stimulation (214–216), but it is also powerfully inhibited by T4, reverse T3, or other substrates that facilitate its rapid degradation by proteasomes (226), not only in BAT but also in other tissues (208, 213, 217). Thus the reduction of D2 activation by high levels of T4 will substantially reduce the response of UCP1 to adrenergic stimulation, which is backed by experiments wherein the enzyme is blocked with iopanoic acid, a competitive inhibitor (20, 21), or its adrenergic activation prevented with prazosin, an α1-AR antagonist (53). In addition to
inhibiting D2, hyperthyroxinemia is associated with reduced central sympathetic stimulation not only of BAT but also of heart and other organs (135) so that, even though there may be some T₃ entering BAT from plasma, there will not be enough cAMP to induce a full response. Thus, in hyperthyroidism, particularly if induced by T₃, there is a reduction in the central stimulation of BAT as well as an inhibition of D2 activity, explaining the observations mentioned above (220, 233).

The reduced responsiveness of hypothyroid BAT or brown adipocytes from hypothyroid rats directed the attention to the effects of TH on norepinephrine signal transduction (206, 230). While β-AR number was found reduced (206), this latter and subsequent studies showed the responses were reduced to a much greater extent than the receptor number (206). It was also observed that the respiratory responses of isolated brown adipocytes to forskolin, which directly stimulates adenyl cyclase, as well as to free fatty acids were reduced (230). Altogether, these findings indicated the need for TH at various levels, not only in the norepinephrine signaling pathway, for a full BAT thermogenic response. Of note, the authors of this latter study (230) did not find a reduction in the cAMP response to forskolin in hypothryoid brown adipocytes, but subsequent studies have consistently reported a reduction in the cAMP response to both β-AR agonists and forskolin in BAT cells from hypothyroid animals (180, 197). [Such discrepancy was probably due to the short time of exposure (15 min) to isoproterenol or forskolin in the study by Sundin et al. (230).]

Besides the poor response of cAMP to forskolin, additional evidence indicates that a reduction in β-AR number is but a small factor in the limited responsiveness of BAT to SNS stimulation. Rubio et al. (197) found that cAMP responses were reduced by more than 60%, but the normalization of the number of β₁-AR and β₂-AR receptors after T₃ treatment preceded the normalization of cAMP generation for nearly 24 h. In another paper, these authors reported that β₂-AR are increased in BAT of hypothyroid rats (whereas they are reduced in WAT) (198) and that norepinephrine generated a larger fraction of the cAMP via this receptor in the hypothryoid than in the euthyroid state (198). Interestingly, β₂-AR number was rapidly reduced to the euthyroid levels by treatment with T₃, whereas the cAMP response improved during this time. Lastly, it is possible that the reduction in β₁,₂-AR was the result of the desensitization owing to the sustained increase in SNS stimulation of BAT (202, 236), rather than being the direct result from the lack of TH, because the difference in BAT β₁,₂-AR number between hypothyroid and euthyroid rats became statistically insignificant within 2 days of acclimation to thermoneutrality (30°C) (197).

The mechanisms leading to decreased responsiveness or sensitivity to catecholamines of WAT in hypothyroidism have received substantially more attention than those affecting BAT. In WAT, hypothyroidism causes the following: reduction in the number of β-adrenergic receptors and increased presence of α-adrenergic receptors (22); enhanced inhibitory responses to adenosine (48, 128, 255), probably related to increases in G₂α or G₂β protein subunits (147, 157, 176, 184); and augmented phosphodiesterase activity (103). The α₂-AR, which causes inhibition of the cAMP production through coupling to G₁ proteins, is little affected by thyroid dysfunction (66, 189). We explored each of these possibilities in BAT. We promptly excluded increased phosphodiesterase activity as a mechanism because inhibition of the enzyme with IBMX increased the accumulation of cAMP by the same factor in hypothyroid and euthyroid brown adipocytes, without reducing the gap between the two thyroid states (197). Likewise, we found that increased sensitivity to adenosine or increased G₁α or G₁β subunits were of limited relevance, if at all significant, in the defective cAMP response to β₂-AR activation or forskolin stimulation (52). While intact brown adipocytes from hypothyroid rats consistently show reduced cAMP response to β₂-AR or forskolin, these responses were not reduced but increased in isolated membranes from hypothyroid brown fat when assayed for adenyl cyclase in the standard conditions (52). Further studies showed that adenyl cyclase was exquisitely sensitive to inhibition by Ca²⁺ and affected by GTP or ATP in a biphasic manner, with stimulation in the micromolar range and pronounced inhibition at concentrations >0.3–0.4 mM (52), a profile suggesting that the predominant isofrom of adenyl cyclase in hypothyroid brown adipocytes is AC-VI (112). It was proposed that the reduced capacity of hypothyroid brown adipocytes to generate cAMP was due to the intracellular conditions prevailing in these cells, namely, high Ca²⁺ and nucleotides levels, not favorable for the activity AC-VI (52). However, subsequent studies based on adenyl cyclase isofrom mRNA measurements found that the predominant form of adenyl cyclase mRNA in BAT is AC-III (104) and that this mRNA is increased in hypothyroidism (56). In addition to adenyl cyclase activity not being characterized in this latter study, mRNA was extracted from whole tissue, which contains a large proportion of stromal and vascular cells of BAT (164). Because AC-III mRNA levels are responsive to sympathetic stimulation (104), it is possible that the increased abundance of this mRNA in total BAT RNA resulted from the increased sympathetic tone of the hypothyroid state on cells other than brown adipocytes, as vascular cells are also abundantly innervated (164).

Additional evidence of the need of TH for BAT normal responsiveness norepinephrine comes from studying mice with deletion of the TH receptor α (T₃Rα). These mice are cold intolerant and have severely impaired BAT thermogenic response to norepinephrine. In WT mice,
BAT temperature increases linearly with time during a norepinephrine infusion, which is associated with a parallel increase in $T_c$. In contrast, BAT temperature virtually failed to increase in TRα-0/0 mice, suggesting that this receptor is crucial for the thermogenic response to norepinephrine (155). Apparently, there is a very specific step in the response that strictly requires TRα1 for TH stimulation because other responses to SNS stimulation, such as D2, UCP1, and PGC1α1 mRNAs were normal. Consistent with these findings, previous studies giving the TRβ1,2-AR are reduced while β1-AR which is reduced, despite that the generation of cAMP is reduced in isolated brown adipocytes, even to stimulation by forskolin. Such reduced adenylyl cyclase response does not seem to be due to a reduction in total enzyme activity but to the isofrom predominantly expressed in the hypothyroid state, probably AC-VI, and to the prevailing intracellular conditions that are not favorable to its activity. However, there are other defects further downstream that may derive from the lack of TH at the some critical gene levels, most notably UCP1. Norepinephrine-stimulated lipolysis is blunted in hypothyroidism, but the respiratory responses of brown adipocytes to added fatty acids are also blunted (230), again supporting the idea of multiple level defects, in this case, a blunted lipolytic response and a reduced respiratory response probably reflecting the low levels of UCP1. Moreover, an action of T3 specifically mediated by TRα seems to be critical for the actual heat production by BAT in response to norepinephrine (155).

In summary, TH appears necessary for a full BAT thermogenic response. This statement derives largely from the observed defects in hypothyroidism. Such observations show that β1,2-AR are reduced while β1-AR are increased, despite that the generation of cAMP is reduced in isolated brown adipocytes, even to stimulation by forskolin. Such reduced adenylyl cyclase response does not seem to be due to a reduction in total enzyme activity but to the isofrom predominantly expressed in the hypothyroid state, probably AC-VI, and to the prevailing intracellular conditions that are not favorable to its activity. However, there are other defects further downstream that may derive from the lack of TH at the some critical gene levels, most notably UCP1. Norepinephrine-stimulated lipolysis is blunted in hypothyroidism, but the respiratory responses of brown adipocytes to added fatty acids are also blunted (230), again supporting the idea of multiple level defects, in this case, a blunted lipolytic response and a reduced respiratory response probably reflecting the low levels of UCP1. Moreover, an action of T3 specifically mediated by TRα seems to be critical for the actual heat production by BAT in response to norepinephrine (155). In addition, the enhancement of the α1-AR pathway in hypothyroidism (171) may elevate intracellular $Ca^{2+}$ levels that, jointly with increased levels of nucleotides, may inhibit the activity of AC-VI, which may be compounded by this isofrom being relatively more abundant in the hypothyroid state. However well-founded some of these hypotheses may be, they need to be tested rigorously. Lastly, it is important to keep in mind that some of the defects in BAT thermogenesis in hypothyroidism are not necessarily an indication of loci directly affected by TH, as some of the defects may result from hypothyroidism elsewhere, such as the downregulation of β1,2-AR which is caused to a significant extent by desensitization due to centrally mediated sympathetic stimulation of the tissue.

Altogether, the studies reviewed here and others suggest complex interactions between TH and the SNS on BAT thermogenesis. Such interactions take place at various levels, including the central regulation of the SNS output to BAT and seem to endow temperature homeostasis with mechanisms to adjust FcT responses to ambient temperature as well as to the level of ObT. When ObT is reduced, such as in hypothyroidism, SNS stimulation of BAT is increased and the activation of D2, in this case amplified by the reduced levels of $T_4$, results in increased fractional conversion of $T_4$ to $T_3$ in the tissue, maintaining the tissue levels of $T_3$ needed to sustain the overall thermogenic response to norepinephrine signaling. In the rat, studies replacing $T_4$ in a graded manner in the hypothyroid state indicate that the enhanced D2 activity allows the maintenance of thermogenic responses with reductions of circulating $T_4$ down to 30% of the euthyroid levels (53). Direct measurements of tissue $T_3$ hypothyroid rats treated with graded infusions of $T_4$ from implanted minipumps show that BAT and brain can effectively maintain the levels of $T_3$ within a broad range of plasma $T_4$ concentrations (80). We have recently provided another example of BAT FcT adjustment to reduced ObT in a transgenic model lacking mGPD (mGPD $-/-$), as mentioned before in this article, as such mice show evidence of increased BAT stimulation (71). When ObT increases, as in hyperthyroid states, there is concerted reduction of SNS stimulation of BAT and of D2 activity, which is further reduced by the higher plasma $T_3$ concentration so that the tissue is less stimulated and the concentration of the active form of TH, $T_3$, is maintained low, disabling the synergism between norepinephrine and $T_3$. These relationships are schematically depicted in Figure 8A.

3. Is there another site of nonshivering facultative thermogenesis in mammals?

The evidence that supports BAT as the only site of FcT comes from observations in rodents (51). This concept has been reinforced by observations made in UCP1 knockout mice. These mice have cold intolerance (79), which came as a surprise to no one. However, the gradual exposure of these animals to cold results in improved cold tolerance. While in WT controls the initial shivering was rapidly replaced by BAT thermogenic activity during cold exposure, these authors found persistent muscle electrical activity during cold adaptation in the UCP1-deficient mice, for which they attributed the cold tolerance to a form of “chronic shivering” (96). They argued that the lack of respiratory response to norepinephrine in the UCP-deficient mice was an indication of the lack of BAT FcT or of any other form of nonshivering FcT in the UCP-deficient mice (96), and they concluded that UCP1 “remains the only protein the activity of which can be recruited for the purpose of facultative thermogenesis” (165). While there is good evidence that skeletal muscle is a site of FcT (nonshivering) in birds (72, 74), there is no agreement whether muscle, or for that matter other tissues, may be a site of nonshivering FcT in mammals. Certainly BAT is not prominent in large mammals, at least...
during adulthood, and it has been proposed on solid grounds that muscle can take that function (see Ref. 219 for a recent review and additional references).

Even in small rodents, where BAT plays such an evident role in cold adaptation, there are findings that could be interpreted as evidence of an alternate form of nonshivering FcT. Thus, while UCP1 knockout mice were, as expected, cold intolerant, it was surprising that these mice were not obese (79). Moreover, when challenged with a high-fat diet, the UCP1 ablated mice gained less weight than WT controls. Interestingly, this obesity resistance was ambient temperature dependent because it was evident when they were reared at room temperature, but they gained weight faster than the WT, rapidly reaching their level of obesity, promptly after transferring them to an ambient closer to TN (148). The authors reasonably interpreted these findings as the expression of an alternate form of FcT that was more energy demanding than BAT thermogenesis and thus protected the UCP1 knockout mice from gaining weight when adapted to room temperature (148). Acclimating these mice to a higher temperature eliminated the need of this putative form of FcT and thus made them prone to gain weight on a high-fat diet. We have made a similar observation in mice.

Surprisingly, they do not show signs of being cold-stressed when reared at room temperature (155), when BAT normally makes a substantial contribution to overall thermogenesis (51). Furthermore, despite having a non-functional BAT, the metabolic rate (QO2) of TRα-0/0 mice was higher at room temperature than that of WT controls (155). Such difference in QO2 disappeared when TRα-0/0 mice were acclimated to TN (30°C), indicating that it is cold dependent, hence by definition the expression of a form of FcT. It is important to consider in the analysis that the two models, UCP1-deficient and TRα-0/0 mice, congenitally lack BAT function. In this regard, the situation is similar to that in piglets, which normally do not have BAT (232). In this species, early cold exposure results in the accelerated establishment of a muscle phenotype leading to enhanced thermogenesis (16).

In the light of the observation of persistent muscle electrical activity in cold-challenged UCP1-deficient mice, it cannot be excluded that the development of cold tolerance in those mouse models is ultimately due to “chronic shivering.” However, this was demonstrated at a very low ambient temperature (96), when an alternative form of nonshivering FcT may not have been sufficient, and persistent shivering was necessary. Such chronic shivering at room temperature remains to be demonstrated. On the other hand, shivering, and for the matter muscle activity, may be associated with variable amounts of heat production. In the TRα-0/0 mice reared at room temperature, QO2 was 40–45% greater than the appropriate WT controls at night, when animals are more active, whereas it was only 20% higher during the day, a significantly smaller difference (155). There are mechanisms whereby heat production associated with muscle activity can be increased.

When the gradient of Ca2+ between the sarcoplasmic reticulum vesicles and cytosol increases, the optimal Ca2+ transport-to-ATP hydrolysis coupling ratio decreases from 2 to values close to 1, due to “slippage” of the pump (123) with the ensuing increase of ATP demands and heat production. Moreover, SERCA can mediate Ca2+ efflux following the osmotic gradient with the resulting heat liberation (this is an exothermic process) and increased ATP expenditure to pump calcium back into the sarcoplasmic reticulum (69). In addition, the heat released in the ATP hydrolysis by the SERCA can vary over a rather wider range in skeletal muscle (67). As discussed earlier, TH can increase both resting and tension-associated heat production (reviewed in Ref. 219) (Fig. 6), and TH stimulates the expression of SERCA1 content predominantly in slow-twitch muscle. Since the slow-twitch, resistance-endurance motor units are more frequently active than fast-twitch muscle, the substitution for SERCA1 in these motor units, by virtue of the higher catalytic activity of this isoform, will double the rate of Ca2+ turnover, resulting in more ATP turnover, steeper gradients, and more Ca2+ efflux (219). In this context, it is notable that slow muscle is substantially more abundant in large mammals (6, 177) than small rodents. Conceivably, the loss of the function of BAT with age and growth, or for that matter the absence of it in some species, probably stimulates the development of muscle thermogenesis under the control of TH.

In summary, even though BAT is unquestionably the major site of FcT in small rodents and bigger mammals during the neonatal period, muscle and perhaps other tissues may participate in nonshivering FcT in larger mammals or even in small ones, when BAT function is lost, as it occurs in UCP1-ablated or TRα-deficient mice. Such may also be a role of muscle in mammals not having BAT, such as pigs, or during adulthood when BAT thermogenesis becomes less prominent, for example, in humans. Even in small rodent there is evidence suggesting that muscle may be an alternate site of FcT, although other evidence suggests this is merely a form of chronic shivering. There are mechanisms that allow muscle to produce more heat at rest and during activity, and TH stimulates such mechanisms. Therefore, whether the muscle electrical activity seen in the discussed mouse models of BAT dysfunction is chronic shivering or increased muscle tone, the amount of heat associated with any form of muscle activity may be increased in response to sustained cold stimulation. While the separation of FcT between shivering and nonshivering may be useful, it may
become tenuous when it comes to muscle. Lastly, whether shivering or nonshivering, the alternate facultative thermogenesis is more costly and less effective than BAT thermogenesis, as illustrated by the increased \( Q_{O2} \) of TRα-0/0 mice and the obesity resistance of UCP-ablated mice when reared at room temperature vis-à-vis their inability to keep \( T_C \) when more severely cold-challenged.

### III. OTHER HORMONE ROLES IN THERMOGENESIS

As discussed in previous sections, TH plays a pivotal role in temperature homeostasis by increasing ObT and supporting FcT. Such a role appears to be a late evolutionary acquisition because there is no evidence that TH stimulates thermogenesis in poikilothermic species, which, however, plays other important functions, shared with all vertebrates. No other hormone seems to have such a direct action on thermogenic mechanisms and temperature homeostasis as TH does. Malfunctioning of other endocrine organs or altered sensitivity of target cells to their hormones, e.g., to insulin, is often associated with disruptions on energy balance and thermogenesis, but these seem to result from a variety of indirect mechanisms. Most commonly, other hormones play a permissive role, mainly by affecting substrate availability and partition. Thus epinephrine and glucagon will stimulate glycogenolysis, gluconeogenesis, and lipolysis, thus increasing circulating levels of glucose and fatty acids. Insulin is essential for fuel storage in the form of glycogen, in liver and muscle, and fat, in the adipose tissue, but also for blood glucose availability in skeletal muscle. Of all hormones, TH excluded, insulin likely plays an essential role in the so-called thermal effect of food or “specific dynamic action” of the old German literature (199). Glucocorticoids play an essential permissive role in gluconeogenesis and mobilizing labile proteins and other glucoseogenic precursors but have effects at various levels geared to reduce energy expenditure and thermogenesis in situations of stress. Leptin, a comparatively new hormone, stimulates energy expenditure via SNS, acting at the central level, as probably does insulin as well, while it also enhances the secretion of TRH, upregulating thyroid function. Ghrelin, also a recently discovered hormone, induces satiety but may reduce energy expenditure.

#### A. Insulin, Glucagon, and Epinephrine

1. **Insulin and thermogenesis**

   Insulin may affect thermogenesis, but it does not appear to have a primary regulatory role. It is largely permissive, as one can infer from observation made in patients with diabetes or insulin resistance, or in experimental models of these. Patients with insulin resistance progressing into type 2 diabetes have increased basal metabolic rate, but because of the resistance to insulin action at the muscle level, the increase in metabolic rate normally associated with food, the thermal effect of food (TEF) is reduced in proportion to the insulin resistance at the muscle level (250). When insulin is given in increasing amounts, in hyperinsulinemic-euglycemic clamps, there is a rapid increase in glucose storage and in glucose oxidation, both exhibiting saturation kinetics, but the latter reaches a plateau at insulin levels <20% of those required to saturate storage (see Ref. 85 for review and references). The partition between oxidation and storage is probably variable depending, among other factors, on the muscle sensitivity to insulin relative to other tissues. This has the support of observations made on transgenic mice with tissue-selective deletion of the insulin receptor (IR). Mice with skeletal muscle deletion of IR oxidize less glucose and store more as fat (47, 130), in contrast to mice with deletion of the protein-tyrosine phosphatase-1B, in which there is an increase in insulin sensitivity in muscle. These mice are less susceptible to diet-induced obesity and have increased metabolic rate without change in UCPs expression, while hyperinsulinemic-euglycemic clamp studies show an elevated capacity to oxidize glucose (132). Despite PTP-1B being expressed in many tissues, WAT among them, insulin-stimulated glucose uptake was not increased in this latter (132), suggesting that muscle responsiveness to insulin is a major determinant in the partition of energy. Furthermore, normal muscle has the capacity to increase glucose consumption if more is available, as it occurs in the selective deletion IR in adipose tissue, which is associated with resistance to obesity and more glycolysis and glucose oxidation (27). A corollary of these studies is that even in small rodents skeletal muscle is important for a form of thermogenesis, the TEF.

   In these latter species BAT also seems to participate in TEF, with insulin being an essential signal. For one thing, the selective deletion of IR in BAT is associated with loss of adipogenic or lipogenic transcription factors (CEBP, PPARγ), as well as with loss of lipogenic enzymes such as fatty acid synthase and malic enzyme and with fat depletion, but most notably, these animals show age-dependent related glucose intolerance (106). Cold exposure is associated with increased glucose uptake by all tissues, but most markedly by BAT (110-fold) (238), and on this insulin also plays a permissive role regulating the expression and activity of glucose transporters (151), as well as BAT D2 activity. A single injection of insulin stimulated 8- to 10-fold D2 activity, and this effect was enhanced by prefeeding carbohydrates or in streptozotocin-induced diabetes (214). Interestingly, a single meal increased BAT \( Q_{O2} \) and weight over appropriate controls (94) and doubled D2 activity and GDP binding, an expres-
sion of active UCP1 (95), while diabetes or fasting re-
duced the stimulation of D2 by cold or exogenous norepi-
nephrine (214), underscoring the importance of insulin
for BAT responses to food, but to cold as well.

Another manner for insulin to contribute to thermo-
genesis and energy expenditure is by increasing sympa-
thetic activity, probably acting at a hypothalamic level
(see Refs. 85, 204 for reviews). Hyperinsulinemic-euglyce-
cemic clamps are associated with increased sympathetic
activity in a number of tissues (85), notably in skeletal
muscle, where the sympathetic nerves cause vasodilation
(4, 245). Such an effect facilitates the muscle glucose
uptake and may be an important factor in TEF. Whether
the increased sympathetic activity to the muscle per se
increases glucose oxidation is not clear (245). Indepen-
dent limiting factors are obviously glucose uptake and
oxidation, which are normally stimulated by insulin but
does not occur in states of insulin resistance, such as
metabolic syndrome and type 2 diabetes (250). The insu-
lin-induced sympathetic activation is believed to result
from direct action of the hormone in the hypothalamus,
where it can pass the blood-brain barrier and reduce
neuropeptide Y (NPY) levels from the arcuate nucleus,
contributing to TEF and satiety, provided concomitant
blood glucose levels are normal or higher (204). In states
of food deprivation, the associated low levels of insulin
may be a factor in reduced sympathetic stimulation of
muscle and reduced thermogenesis as well as increased
hunger (204). On the other hand, studies in a transgenic
model of neuronal insulin receptor deletion suggest even
broader effects for insulin in brain. Expectedly, these
mice have increased food intake and are more susceptible
to diet-induced obesity, but they also show central de-
pression of reproductive function (46).

2. Glucagon and epinephrine

Even though the injection of glucagon or epinephrine
is associated with an increase in oxygen consumption, it
is not clear that they play a key role regulating thermo-
genesis. As mentioned before, they play a permissive role,
by mobilizing stored fat and carbohydrate and stimulating
gluconeogenesis. Epinephrine causes vasodilation in skel-
etal muscle, augments glucose and oxygen consumption
(222), and has been proposed that it could participate in
thermogenesis in humans (223), specifically in FcT in-
duced by carbohydrates (8). However, the threshold con-
centration for the thermogenic effect is rather high, for
which this effect is probably of significance in states of
stress, when epinephrine levels rise well over the thresh-
hold (63). Despite the earlier belief that glucagon increased
oxygen consumption in muscle (73), this has not been
directly demonstrated (154). Both glucagon and epineph-
rine increase liver oxygen consumption, but this seems to
be a reflection of the stimulation of gluconeogenesis in
this organ (13, 87). Both epinephrine and glucagon can
also stimulate BAT, but it is not likely that such an effect
is of major importance in temperature and energy ho-
meostasis. Epinephrine will only exceptionally reach con-
centrations high enough at BAT β-AR to stimulate the
tissue, as does the locally, sympathetically released nor-
epinephrine. However, in patients with pseudochromocy-
toma, in whom there is a sustained elevation of circulat-
ing catecholamines, predominantly epinephrine, BAT
shows signs of histological and biochemical signs of stim-
ulation, e.g., an increase in UCP1, which may reach levels
comparable to those in cold-exposed rodents (140, 190).
In the case of glucagon, this does not significantly in-
crease GDP binding acutely, but it does after repeated
high doses (23), and although it elicited a thermogenic
response, this could be blocked with propranolol and
occurs equally in rats and hamsters, which do not have
glucagon receptors in BAT (70). Acutely, and again in high
doses, glucagon also increases BAT D2, but this may as
well be an indirect effect (214).

B. Glucocorticoids

Glucocorticoids also have complex effects on ther-
mogenesis and temperature homeostasis. It is not a pri-
mary function of these hormones to increase thermogen-
esis, but rather to coordinate thermogenic responses to
substrate or food availability. For example, in a stress
situation where food is reduced, e.g., illness, glucocorti-
coids will have an inhibitory effect on BAT, but in cold
stress, the inhibition of BAT thermogenesis would be
counterproductive. Here, substrate mobilization is equally
needed, but not the inhibitory effect on facultative ther-
mogenesis. The stress reaction has many components.
Initial loci are the corticotropin releasing hormone
(CRH)-producing neurons, which activate the adrenal and
thyroid axis, and the locus ceruleus-norepinephrine,
which will activate the branches of the sympathetic ner-
vous system (reviewed in Ref. 235). CRH and products of
the POMC gene will inhibit appetite and activate path-
ways to increase thermogenesis (235, 253), but the net
effect of these signals will depend on others, such as NPY
and agouti-related protein (AGRP), which in turn depend
on peripheral signals, notably leptin and probably insulin
as well. In the absence of such input, there is a higher
output of NPY and AGRP from the arcuate nucleus that
will antagonize the thermogenic effect (see Ref. 89 for
review). It is interesting that POMC expression stimula-
tion in the hypothalamus will also stimulate TRH secre-
tion, upregulating thyroid function and thermogenesis, an
effect that will obviously be reduced or abrogated in the
presence of signals of limited energy in the periphery,
largely reduced leptin and insulin (141). So, the stimula-
tion of the hypothalamic-pituitary-adrenal (HPA) axis is
primarily associated with reduced appetite and increased thermogenesis, but the magnitude of this stimulation is strongly influenced by peripheral signals of abundance.

The resulting effect of the HPA axis is an increase in adrenal hormones, most importantly glucocorticoids, cortisol in humans, and corticosterone in rodents. By feeding back negatively at the hypothalamic levels, glucocorticoids will reverse the central effects of activating the HPA axis described above. This is a very important effect particularly in the absence of the positive signals of insulin and, especially, leptin. This is supported by the repeated and consistent observation that adrenalectomy is associated with improvement of obesity and cold tolerance of models of genetic obesity lacking leptin or leptin signal, such as the ob/ob mouse and the fa/fa (Zucker) rat (see Refs. 40, 42 for reviews). In addition, glucocorticoids have multiple peripheral effects that will ultimately affect thermogenesis and thermogenic responses. In rodents, these hormones clearly inhibit the function of BAT by inhibiting UCP1 expression (160, 225). Glucocorticoids also inhibit the expression of the β3-AR in BAT but not in other tissues such as gastrointestinal tract and WAT (82, 122). However, glucocorticoids may not always inhibit, but may stimulate, β3-AR in vivo, as it occurs in models of genetic obesity (174). Furthermore, the effect may be initially inhibitory and then stimulatory in lean C57Bl/6J mice (82), later found also in normal CD1 mice (9). The inhibitory defect of glucocorticoids on BAT β3-AR is direct and can be demonstrated in isolated brown adipocytes or derived cell lines (9), but even in such cell lines it is possible to demonstrate a dual, opposing effect (9).

Glucocorticoids induce the CAAT enhancer binding protein beta (C/EBPβ) that stimulates the transcription of β3-AR gene (9), and this transcription factor is also stimulated by the sympathetic nervous system (50). Based on these observations, we proposed a model in which glucocorticoids will directly inhibit β3-AR expression by acting on a putative negative CRE, upstream in the gene, but at the same time they would induce C/EBPβ, the magnitude of which will depend on the sympathetic stimulation, and if high enough will offset the inhibition (9). As mentioned earlier, β3-AR is also differentially regulated by TH in BAT and WAT (198), suggesting that this receptor is key to BAT responsiveness to adrenergic stimulation and is hence involved in the physiological regulation of BAT thermogenesis. It is possible that β3-AR also participate in signals emanating from the gastrointestinal tract as well as in mediating central effects on food intake (40).

C. Leptin

The role of leptin in regulating energy balance is dramatically illustrated by the phenotype of the so-called ob/ob mouse, characterized by hyperphagia and cold intolerance (reviewed in Ref. 234). With the cloning of leptin (259) we learned that this hormone produced by white adipocytes is a major signal controlling the energy balance. Its production is a function of the replenishment of the fat stores, but fasting reduces its levels rapidly, faster than WAT is depleted of fat (115). Leptin inhibits food intake and increases thermogenesis via activation of the sympathetic nervous system, and its reduction is associated with hyperphagia and reduced thermogenesis, as dramatically illustrated by the ob/ob mouse that has an inactivating mutation in the gene. On the other hand, the large volume of research work triggered by the cloning of leptin has revealed that it not only is a regulator of appetite and thermogenesis, but also plays an important role in concerting other responses to the nutritional state, notably reproductive and thyroid functions (1, 115, 141). Although leptin receptors and actions in peripheral tissues have been described, it appears that its thermogenic effect is largely central (152).

IV. THERMOGENESIS AS A SOURCE OF VARIABILITY IN ENERGY EXPENDITURE

It is well known that basal metabolic rate (BMR) in humans is closely correlated to lean body mass, yet the variability around the regression line, for any lean body mass, is ~600 kcal, which represents a huge individual variability considering that people of 70 kg of lean body mass have BMRs between 1,600 and 2,200 kcal/day (28, 251). BMR also correlates with body temperature (192), and a large fraction of BMR variability is due to muscle thermogenesis (260). Furthermore, such variability is familial, largely genetically based (28, 228; reviewed in Ref. 31), and possibly resulted from varying pressures on our remote ancestors wherein the need for heat production was in competition with food availability. As mentioned at the beginning of this review, homeothermic species have evolved mechanisms to constantly produce more heat, facilitating the maintenance of Tc in environments much colder than this temperature. Thus the rate of oxidations in homeothermic species is more than fivefold higher than in poikilothermic species, even when measured in similar-sized species and at the same ambient temperature, and whether measured in the whole animal or isolated tissues (78) (Fig. 1). Not only the metabolism is more active, but also the thermodynamic efficiency of the homeothermic machine is lower when the energy cost of certain functions, such as muscle contraction, is considered (254) (Fig. 1). So, there is significant energy cost attached to homeothermy.

It follows that food availability may have been a limiting variable for the amount of heat to be produced for the sake of maintaining Tc during evolution, so in the end, the need to produce heat and food availability were se-
lection pressures of variable magnitude that resulted in biological “machines” of variable fuel efficiency. These views are supported by observations made on overfeeding or starving humans. The responses are genetically determined (33, 252), and in general there is correlation between the increase in energy expenditure with overfeeding and its reduction in fasting, defining a range from spend-thrift to thrifty phenotypes (252). Many animal models, notably transgenic with deletion or overexpression of a variety of genes exhibit spend-thrift or thrifty phenotypes, indicating that many genes may have been the target of selection. Now, reduced BMR is associated with an increased risk of obesity (28, 185), as a reduction in thermogenesis in response to caloric restriction is a limiting factor in weight loss in obese subjects (41). So, the understanding of the mechanisms regulating thermogenesis is bound to be enlightening not only in the field of temperature homeostasis, but it may be also of benefit to the understanding and treatment of obesity.

V. SUMMARY AND CONCLUSIONS

The advent of homeothermy represented an important leap in evolution, as it expanded the niche of species providing not only a constant internal temperature but also by setting it at the temperature most conducive to biochemical and physiological processes inherent to life. Central to homeothermy is an augmented heat generation out of biological processes to facilitate the maintenance of $T_C$ in habitats that are usually colder than $T_C$. Not only is basal heat production greater in homeothermic than in poikilothermic species, but the former have the capacity to increase heat production on demand, in a facultative manner, hence facultative thermogenesis. Interestingly, the mechanisms used by homeothermic species to generate heat are not new, and they antedate homeothermy, as evidenced by their presence in lower species, even plants. Good examples of these are the heater organ of the eye and brain of certain deep-water fish (24) and the uncoupling proteins, until not long ago believed to be unique to mammals, but now known to be already present in plants (30). So, it would appear that nature resorted to preexisting mechanisms to produce heat in concerted and coordinated manner in the homeothermic species. What is evolutionarily new is then the control and regulation of this function.

For the purpose of clarity, we have divided thermogenic mechanisms into those that increase ATP demands and those that increase heat generation during mitochondrial production of ATP. Mechanisms increasing the demands of ATP generate heat by virtue of forcing the cells to produce more ATP, with its inherent energy loss as heat. Quantitatively most important among these mechanisms appear to be those that accelerate the movement of ions against gradients, most notably, sodium and potassium across the cell membrane and calcium across the sarcoplasmic reticulum membranes. In both cases, mechanisms reducing these gradients seem to be key in forcing the cell to spend more ATP to maintain it, but as discussed for cytotoxic-sarcoplasmic Ca$^{2+}$ exchange, the coupling of ion exchange to ATP consumption may be also reduced, forcing more ATP consumption to maintain the flux of ions needed to keep the gradient up. It is possible that mechanisms increasing ATP turnover, clearly more active in homeothermic than poikilothermic species, are more important for the basal heat production, that is, are a major component of ObT.

Estimates of the contribution of mechanisms increasing ATP demands suggest that they are insufficient to explain ObT in homeothermic species. The existence of a regulated proton leak in the inner mitochondrial membrane, more active in homeothermic species and regulated by TH, has been the main support to the idea that regulated reductions in ATP synthesis efficiency could be an important thermogenic mechanism (37). The finding of uncoupling proteins outside of BAT reinforced the concept that there could be a regulated proton leak or uncoupling mechanism elsewhere. A central thermogenic role for the novel UCPs, though, has so far not been supported by studies in transgenic mice with deletion or overexpression of these proteins. It is possible that non-BAT UCPs play different roles, one of which could be to support other thermogenic mechanisms, for example, to reduce the formation of superoxides during increased ATP synthesis. On the other hand, evidence from polymorphisms in human as well as observations in mGPD deficient mice, are consistent with UCP3 participating in ObT without being essential (196). Another mechanism that could produce ATP with lower efficiency is the NADH-G3P shuttle, particularly in muscle. G3P is an important fuel for muscle to rapidly produce ATP but with a lower efficiency. The transgenic deletion of the rate-limiting enzyme in the shuttle, mGPD, results in a reduction in ObT that is partially compensated by increased FcT in BAT and possibly by an increase in UCP3 mediated by increased levels of $T_P$.

Thermogenic mechanisms are regulated in homeothermic species. TH is the major regulator, a new function that this hormone acquired with the advent of homeothermy. TH not only directly controls critical steps of thermogenic mechanisms, but also contributes to increase fuel availability by increasing appetite and stimulating lipogenesis in liver and to increase the delivery of oxygen and fuel to tissues by stimulating cardiovascular function, lipolysis, and gluconeogenesis. In stimulating thermogenesis, TH works synergistically with the SNS, practically at all levels. TH is essential to maximize the responsiveness to catecholamines acting at the adrenergic receptor level as well as at several postreceptor steps.
in the catecholamine signaling pathways, particularly those initiated in the β-ARs. The synergistic interaction is most important and evident on BAT FcT, where the SNS is sine qua non to activate BAT thermogenesis, but where the responsiveness of the tissue is dependent on the presence of high tissue concentrations of the most active form of TH, T3, for which the SNS activates D2. This enzyme acts as a gatekeeper for thermogenesis, since the excess of TH inhibits it, directly and indirectly by reducing SNS stimulation, protecting BAT from the elevated ambient levels of TH in hyperthyroidism and hence reducing the responses to residual SNS stimulation.

Other hormones’ participation in thermogenesis is less direct. Insulin is essential to provide fuel for thermogenesis. However, this effect is strongly influenced by the relative responsiveness of adipose tissue and muscle to its action, as is revealed by studies in transgenic mice lacking or overexpressing the insulin receptor in one of these tissues (131). Prevention of entry of glucose to muscle results in reduced glucose oxidation, while increased glucose availability, in the presence of insulin, increases glucose oxidation and thermogenesis as revealed by the transgenic deletion of the protein tyrosine phosphatase B1 (132) or the insulin receptor gene selectively in adipose tissue (27). Insulin and leptin are major signals for the central nervous system to change food intake and energy expenditure via various mechanisms, most importantly SNS and TH. Being signals of abundance, leptin and insulin reduce food intake and increase thermogenesis by using these mechanisms, whereas their suppression by starvation and diseases is associated with reduced sympathetic activity and thyroid function, hence, reduced energy expenditure and thermogenesis, and, depending on other signals, hunger and increased food intake.

An unresolved issue is whether there is FcT in sites other than BAT in mammals. Skeletal muscle seems an undisputed site of FcT in birds. In transgenic models with BAT deficiency, there is evidence of increased energy expenditure in cool environments but not a thermoneutrality, i.e., by definition a form of FcT, which is more energy demanding than BAT thermogenesis. While studies in one of these models suggest that this alternate form of FcT could simply be chronic shivering, independent evidence suggests that muscle activity, including shivering, may be associated with more or less heat production in a regulated manner, by TH and possibly by other signals. Such conception makes the line between FcT and BAT tenuous in the absence of BAT, a situation that becomes quiescent with adulthood. Thus, in these species a substantial fraction of ObT is normally generated in muscle, but this tissue may be more or less thermogenic in response to the environment.

Finally, understanding of thermogenesis and temperature homeostasis is not only important for better comprehending these processes but also for getting insight into the variability in energy expenditure in humans and into the risk for developing obesity and type II diabetes, conditions wherein the capacity to burn fuel becomes limiting. Regardless of the psychosocial and economical factors behind the excess eating that is plaguing our society, multiple studies show that the capacity to dispose of the excess calories varies markedly in humans, on genetic bases (33). Not only thermogenesis may become limiting, but also this is promptly reduced in response to a shortage in the influx of energy, which is important to survive famine and disease but severely limits the effectiveness of the mainstay of obesity treatment, namely, caloric restriction. We are well protected against food restriction, but seemingly not as well against food excess.

ACKNOWLEDGMENTS
I acknowledge the dedicated and enthusiastic work of my technicians, students, and postdoctoral fellows.

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GRANTS
The work from my laboratory presented in this review has been supported over the years by the Howard Hughes Medical Institutes and the National Institutes of Health (while in the United States) and by the Medical Research Council and Canadian Institutes of Health Research.

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