



## *Molecular Biology of Thermoregulation*

### Invited Review: Uncoupling proteins and thermoregulation

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**Argyropoulos, George, and Mary-Ellen Harper.** Invited Review: Uncoupling proteins and thermoregulation. *J Appl Physiol* 92: 2187–2198, 2002; 10.1152/jappphysiol.00994.2001.—Energy balance in animals is a metabolic state that exists when total body energy expenditure equals dietary energy intake. Energy expenditure, or thermogenesis, can be subcategorized into groups of obligatory and facultative metabolic processes. Brown adipose tissue (BAT), through the activity of uncoupling protein 1 (UCP1), is responsible for nonshivering thermogenesis, a major component of facultative thermogenesis in newborn humans and in small mammals. UCP1, found in the mitochondrial inner membrane in BAT, uncouples energy substrate oxidation from mitochondrial ATP production and hence results in the loss of potential energy as heat. Mice that do not express UCP1 (UCP1 knockouts) are markedly cold sensitive. The recent identification of four new homologs to UCP1 expressed in BAT, muscle, white adipose tissue, brain, and other tissues has been met by tremendous scientific interest. The hypothesis that the novel UCPs may regulate thermogenesis and/or fatty acid metabolism guides investigations worldwide. Despite several hundred publications on the new UCPs, there are a number of significant controversies, and only a limited understanding of their physiological and biochemical properties has emerged. The discovery of UCP orthologs in fish, birds, insects, and even plants suggests the widespread importance of their metabolic functions. Answers to fundamental questions regarding the metabolic functions of the new UCPs are thus pending and more research is needed to elucidate their physiological functions. In this review, we discuss recent findings from mammalian studies in an effort to identify potential patterns of function for the UCPs.

brown adipose tissue; white adipose tissue; basal metabolic rate; reactive oxygen species

AT THE LEVEL OF the whole body, energy expenditure represents the sum total of the energy costs associated with obligatory and facultative metabolic processes. Energy expenditure can be measured directly as heat production (thermogenesis) but is more commonly assessed indirectly as oxygen consumption. The former

necessitates the use of a calorimeter and the latter an indirect calorimeter. During indirect calorimetry, carbon dioxide production is often measured simultaneously, allowing the calculation of respiratory quotients, which in turn allows the estimation of proportions of energy expenditure supported by dietary carbohydrate and fat.

Energy expenditure can be subdivided broadly into two categories of thermogenesis: obligatory and facultative. Obligatory thermogenic processes are essential for the life of all cells of the body and include those that support normal and consistent body temperatures (i.e.,

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endothermy) (72). The largest component of obligatory thermogenesis is provided by the basal metabolic rate. Basal metabolic rate is measured in the resting and postabsorptive state and in a thermoneutral environment. Also considered an obligatory thermogenic process is the portion of diet-induced thermogenesis that results from the digestion, absorption, and metabolism of dietary nutrients. The most important of the endocrine factors governing obligatory thermogenesis are the thyroid hormones (131).

Although obligatory thermogenesis occurs continuously in all organs of the body, facultative thermogenesis can be rapidly switched on or off and occurs mainly in two tissues: skeletal muscle and brown adipose tissue (BAT). Energy expenditure attributable to exercise occurs mainly in skeletal muscle, with the addition of some concomitant energy substrate cycling in the liver. It is in skeletal muscle and BAT that heat is produced when endothermic organisms are in a cold environment. Shivering thermogenesis takes place in muscle, and nonshivering thermogenesis occurs in BAT. Although the acute activation of shivering and nonshivering thermogenic reactions does not require any change in the expression of thermogenic genes, chronic cold exposure invokes the expression of many genes that are important in thermoregulatory processes.

BAT thermogenesis is an important component of facultative thermogenesis in many mammals, and its activity is regulated principally by norepinephrine and the sympathetic nervous system (72). In humans, BAT thermogenesis is important at birth and in infancy. With age, however, BAT atrophies, and during adulthood the amount of active tissue is normally small. In contrast, the contribution of BAT thermogenesis to overall energy expenditure can be very significant in rodents and many small mammals throughout their lives. This is evidenced by the approximate doubling of total body energy expenditure after an acute dose of a  $\beta_3$ -adrenergic agonist in mice or rats (63, 138).

When total body energy expenditure is equal to the metabolizable energy from the diet (the energy substrates actually absorbed), a state of energy balance exists. Simply put, energy expenditure equals energy intake. The regulation of energy balance is extremely complex and very poorly understood. The fact that most of us remain in a state of energy balance for most of our lives is quite remarkable, considering the day-to-day intra- and interindividual variation in energy demands. The outcome of an extended period of negative energy balance (when expenditure exceeds intake) is undernutrition, which is characterized by loss of bodily energy stores, primarily adipose triglyceride. Similarly, the outcome of an extended period of positive energy balance is overnutrition, which is characterized by accretion of fat mass and obesity (89). Overnutrition is an increasingly common occurrence in affluent countries and in urban centers of many developing countries (54). The aim of this review is to examine possible roles of the uncoupling proteins (UCPs) in energy expenditure and thermoregulation.

#### FROM "THERMOGENIN" TO THE IDENTIFICATION OF NOVEL UCPs

*Uncoupling in BAT.* In most tissues most of the time, the oxidation of fuel substrates results in mitochondrial ATP production through oxidative phosphorylation (118). Oxidative phosphorylation is driven by the electrochemical gradient across the mitochondrial inner membrane. As protons move down the electrochemical gradient and enter the mitochondrial matrix through ATP synthase, the gradient is dissipated. In BAT, the presence of uncoupling protein 1 (UCP1) allows a unique alternative route of proton entry (99). Through UCP1, protons bypass the ATP synthase route of entry and the energy of the electrochemical gradient is dissipated as heat. As described above, the heat production in BAT is essential for effective thermoregulation in many small mammals and during human infancy, and its role in energy balance and obesity has been described (70). During uncoupling thermogenesis in BAT, the rate of heat production depends on, and is limited by, the rate of substrate oxidation (e.g.,  $C_{16}H_{32}O_2 + 23O_2 \rightarrow 16CO_2 + 16H_2O + \text{heat}$ ).

Mutational analysis has provided compelling evidence for a role of UCP1 in proton transport in conjunction with free fatty acids (FFAs) and pH-dependent nucleotide binding (70, 78). Purine nucleotides bind to the cytosolic portion of the protein, whereas fatty acids are thought to act as secondary messengers to facilitate proton conductance (100). Thus fatty acids act not only as energy substrates but also as activators of uncoupling. UCP1 was recently shown to recruit coenzyme Q (ubiquinone) as a cofactor for fatty acid-dependent proton transport (46, 47). The mechanism of FFA activation of UCP1 is not well understood; however, more is known about purine nucleotide inhibition of UCP1 activity. Studies of the heterologous expression of UCP1 in yeast have shown that the mutagenesis of the distal region of the UCP1 gene containing a nucleotide recognition element resulted in increased FFA activation of uncoupling (16). Furthermore, deletion of amino acids 261–269 resulted in permeation of molecules up to 1,000 Da, thus converting UCP1 into an unspecific pore (62). Finally, mutations in the three matrix loops resulted in the formation of a hydrophobic pocket (61). BAT thermogenesis is thus a remarkably complex phenomenon whose mechanism at the level of the protein alone involves proton translocation, purine nucleotide binding, FFA activation, and pH sensitivity.

*Mitochondrial proton conductance in tissues other than BAT.* For just over a decade, it has been known that another sort of proton conductance occurs across the mitochondrial inner membrane in tissues other than BAT (3, 117). Accurate assessments of proton leak require simultaneous measurements of mitochondrial protonmotive force and oxygen consumption. In effect, it represents mitochondrial oxygen consumption when ATP synthesis (i.e., phosphorylation) is not occurring. Estimates of proton leak-dependent oxygen consumption in isolated rat liver cells and perfused rat skeletal muscle show that ~26% of resting energy expenditure is due to leak in liver cells (20, 23, 67, 101) and 52% in

resting skeletal muscle (20, 117, 118). At the level of the whole body, it has been estimated that mitochondrial proton leak could account for ~15–20% of standard metabolic rate (118). The mechanism of proton leak is not well understood, although it has been thought that the UCP1 homologs are central to its mechanism. Recent findings however, suggest that these mitochondrial proton leak processes may be mediated primarily through other mechanisms (19, 25, 95). Several physiological functions have been proposed for proton leak: 1) heat production, 2) regulation of the efficiency of oxidative phosphorylation, 3) reduced production of reactive oxygen species (ROS) by mitochondria, and 4) a means to maintain the NAD<sup>+</sup>-to-NADH ratio sufficiently high to support carbon fluxes in biosynthetic processes (118).

**Discovery of UCP1 homologs.** In recent years, four genes were discovered and classified as UCP1 homologs by virtue of their amino acid identity levels with the prototypical UCP and on the basis of initial findings from their heterologous expression in yeast: UCP2, UCP3<sub>L</sub>/UCP3<sub>S</sub> (where L signifies the long transcript and S the short transcript), UCP4, and UCP5/BMCP1 (2, 17, 53, 58, 90, 122). Inevitably, UCP was renamed “UCP1” to distinguish it from its homologs. The question, however, remains as to whether the UCP1 homologs have true thermogenic properties *in vivo*. Experiments in yeast have frequently demonstrated that the UCP homologs can decrease mitochondrial membrane potential ( $\Delta\Psi$ ), which is consistent with, but certainly not conclusive of, uncoupling (80). These approaches and findings have recently been criticized for the possible erroneous interpretation of experimental artifacts due to overexpression (137). Infrared imaging has been used to evaluate the thermogenic properties of UCP2 in cultured cells (107), but this method has not been extended or duplicated with the other UCPs. Although it is well accepted that fatty acids interact directly with UCP1, their role in the activities of the novel UCPs is poorly understood (74, 134). Fatty acids activate UCP1 but are thought to be less effective in the activation of UCP2 and UCP3 (114). The latter may reflect true differences in the activation of uncoupling by the homologs, or it may indicate that the homologs have other physiological functions. Proton transport by UCP1 is dependent on a histidine pair that is absent in the homologs, suggesting a complete (UCP2) or partial (UCP3) inability in proton transport (9). The recent discovery of UCP2 homologs in the carp and the zebra fish (136), both of which are ectothermic organisms (11), has further perplexed scientists.

The elevated expression of both UCP2 and UCP3 in ground squirrels during hibernation has been cited to advocate a role for these UCPs in mammalian nonshivering thermoregulation (18). However, one could argue that increased expression correlates also with increased fatty acid oxidation. Similarly, the discovery of a UCP homolog in hummingbirds was described to have a potential thermogenic role of UCP in vertebrates other than mammals (144), in addition to an

interesting correlation of its expression with increased fatty acid oxidation. Several research groups have quite elegantly shown that UCPs might play a role in the regulation of ROS (19, 41, 75, 145). In the plant kingdom, UCP homologs have been implicated in fruit ripening and induction by cold (38, 84, 115). Potential UCP homologs have also been reported for *Drosophila melanogaster* and *Caenorhabditis elegans*. Phylogenetic analyses in putative UCP signature domains in the genomes of *Drosophila*, *C. elegans*, arabidopsis, and mammals have identified UCP4 (the brain-specific UCP) as the ancestral UCP with an additional role in apoptosis (66). Therefore, the UCP homologs in mammals as well as in other species, ranging from the fruit fly, to fish, and to plants, could perhaps play important common roles in mitochondrial metabolism in addition to, or instead of, thermogenesis.

**Mitochondrial biogenesis of the UCPs.** The UCPs belong to a superfamily of proteins that includes the oxoglutarate carrier (154) and the ADP/ATP carrier proteins, each consisting of ~300 amino acids and all having similar secondary structures (148). Like most mitochondrial carrier proteins, UCPs are encoded by nuclear DNA and likely use mechanisms of protein import similar to those described for yeast proteins, i.e., the TIM and TOM systems (translocators of the inner and outer mitochondrial membranes, respectively) (79, 91). The pathway of UCP1 import seems to involve the smaller components of the mitochondrial intermembrane space for the direct insertion of the UCPs into the inner mitochondrial membrane where they are expected to dimerize (7, 86, 119, 151). Experiments on the biogenesis of UCP1 have shown that the central matrix loop drives import of the protein into the mitochondria (125). Little is known about the specific mechanisms employed for the import of the other UCPs. However, a mutant human UCP3 variant (Arg70Trp, in the second matrix-facing loop), which was identified in our laboratory and showed no ability to alter  $\Delta\Psi$  in yeast (21), failed to be imported into rat liver mitochondria in an *in organello* import assay (unpublished observation). Therefore, the matrix-facing loops in the UCPs could perhaps play a significant role in the recognition and insertion of the proteins into the inner mitochondrial membrane. Blue gel electrophoresis experiments could advance our understanding of UCP import and confirm the functional conformation of UCPs as dimers in the inner membrane.

## ANIMAL STUDIES

**Physiological and expression studies.** Animal models have provided an excellent paradigm to analyze the possible physiological functions of UCPs. At the transcriptional level, mouse UCP1 (mUCP1) is regulated by adrenoreceptors (116) in brown adipocytes, whereas the promoter of mUCP2 was regulated by cAMP-dependent protein kinase in transiently transfected 3T3-L1 adipocytes (153). On the basis of the adipocytes' hypothesized function in adaptive thermogenesis, researchers have often exposed animals to cold as a

method of examining UCP expression and possible function. Skeletal muscle rat UCP3 (rUCP3) increased up to threefold after a 6- to 24-h exposure to cold, whereas rUCP2 showed only a small increase, suggesting a role for only rUCP3 in thermoregulatory (shivering or other) mechanisms in muscle (132). In another study, however, skeletal muscle rUCP3 expression did not change with cold exposure but increased with 1 wk of fasting (14). Acute exercise resulted in significant increases of rUCP3, but not rUCP2, in white and red gastrocnemius muscles, whereas chronic exercise had no effect on either gene (39, 56). Endurance-trained rats, in contrast, had significantly decreased rUCP2 and rUCP3 expression in the tibialis anterior and soleus muscles and in the heart in the case of rUCP2; researchers (13) suggested a metabolic role for the UCPs in the rapid weight gain that sometimes occurs when exercise training ceases.

Importantly, the administration of FFAs to rats via intralipid plus heparin infusions caused significant increases in rUCP3, suggesting that FFAs may be an important mediator of the increase of rUCP3 in muscle during fasting (150). Streptozotocin administration increased levels of circulating FFAs and induced a 9.4-fold increase of rUCP3 but not rUCP2 in heart mRNA (68). In studies that used multiple muscle and adipocyte cell lines, peroxisome proliferator-activated receptors- $\gamma$  (PPAR- $\gamma$ ) agonists, however, induced UCP2 expression (26, 135).

*UCPs as regulators of the fuel substrate mix.* The issue regarding whether UCP2 or UCP3 are mediators of thermogenesis or regulators of lipids as fuel substrates was recently raised. When the antilipolytic agent nicotinic acid was given to fed and fasted rats, there was a threefold increase in serum FFA and significantly increased rUCP2 and rUCP3 expression in muscle (120). The greatest increases occurred in the fast-twitch glycolytic gastrocnemius and tibialis anterior muscles rather than in the slow-twitch oxidative soleus muscle (120). These findings indicate a muscle-type dependency in the regulation of UCP2 and UCP3 expression and suggest a role in the regulation of fatty acid vs. glucose oxidation in muscle (120). In another experiment (121), the refeeding of isocaloric amounts of a low-fat diet in rats resulted in lower energy expenditure and lower mRNA levels of rUCP2 and rUCP3 mRNA in muscle, compared with ad libitum-fed control rats. This downregulation of rUCP2 and rUCP3 was abrogated by the refeeding of high-fat diets; in addition, regression analysis suggested that insulin resistance could explain the variability of rUCP2 and rUCP3 in muscle, emphasizing the possibility that UCPs may play a role in the regulation of lipids as fuel substrates (121). Furthermore, feeding a 45% high-fat diet for 8 wk resulted in a twofold increase of mUCP3, but not mUCP2, expression in skeletal muscle of obesity-prone but not obesity-resistant mouse strains (59). The increases of rUCP3 (and to a lesser extent rUCP2) in fasted rats were confirmed by a separate study (25); however, proton conductance was unchanged, suggesting that rUCP2 and rUCP3 might not be responsible

for proton leak in skeletal muscle mitochondria. In *ob/ob* mice, mUCP2 mRNA levels in liver or white adipose tissue (WAT) were higher than in wild-type animals, but fasting did not increase mUCP2 expression (92). This multifaceted array of experiments in rodents advocates the possible important involvement of UCP2 and UCP3 in fuel substrate utilization (44).

*Effects of leptin.* Leptin administration in rats resulted in a twofold increase of rUCP1 in BAT, a 62% increase of rUCP3 in BAT, and a twofold increase of rUCP2 in epididymal WAT (123, 124). Leptin also increased mUCP1 expression via the  $\beta$ -adrenoreceptors (36), whereas it reduced adipose tissue via an mUCP1-dependent mechanism in BAT (35). Further evidence for a role by leptin in the regulation of UCP expression has come from adenoviral-mediated leptin expression in *ob/ob* mice that resulted in mUCP3 and mUCP2 increases of expression in the muscle and pancreatic islets, respectively (88). In other studies (48, 123), leptin administration induced rUCP2 and rUCP3 in a nonsympathetic innervation pathway, whereas  $\beta_3$ -adrenergic agonists also stimulated rUCP3. Leptin did not induce mUCP2 expression in either mouse epididymal WAT or mouse retroperitoneal WAT, but it increased mUCP1 in retroperitoneal WAT and mUCP2 in epididymal WAT and BAT, although this occurred in mUCP1 knockout mice only (35). mUCP2, on the other hand, did not differ between lean and *ob/ob* mice in both epididymal WAT and retroperitoneal WAT (37).

*Transgenics and knockouts.* The deletion and/or overexpression of genes in animals are commonly used to elucidate physiological functions of genes in whole organisms. The interaction of such genetic interventions with altered environmental conditions (e.g., diet) or with effects of pharmaceuticals often assists in the identification of metabolic phenotypes. The knockout and transgenic animal models for UCP function generated thus far are summarized in Table 1. mUCP1 knockout mice (mUCP1<sup>-/-</sup>) were nonobese and nonhyperphagic and did not become obese when fed high-fat diets (49). However, the animals were markedly cold sensitive (49), confirming the important role that mUCP1 plays in cold-induced thermogenesis. Another phenotype of the mUCP1<sup>-/-</sup> mice was the higher leak-dependent oxygen consumption rate in muscle (95), an observation that may in part explain how these mice remain in energy balance in the absence of UCP1 thermogenesis. However, the increased proton leak in muscle of mUCP1<sup>-/-</sup> mice was not accompanied by any increase in the expression of UCP2 or UCP3. Additional studies have shown that only UCP1 can mediate adaptive nonshivering thermogenesis and that during prolonged thermogenic demand there was no evidence for any UCP1-independent adaptive nonshivering thermogenesis in muscle or any other organ (57). Finally, the ectopic expression of UCP1 (mUCP1<sup>-/-</sup>) in skeletal muscle results in resistance to obesity, lower levels of glucose, insulin, and cholesterol, and increased metabolic rates both at rest and with exercise (87).

Table 1. *Animal models for the study of functional properties of the UCPs*

Type of Model	Phenotype	Reference
mUCP1 <sup>-/-</sup> KO	Nonobese but cold sensitive	49
mUCP1 <sup>↑</sup> TG	98% Higher O <sub>2</sub> consumption	87
mUCP1 <sup>↑↑</sup> TG	246% Higher O <sub>2</sub> consumption, lower glucose, higher metabolic rate	87
UCP1-DTA TG	Reduction of BAT, obese, leptin resistant	82
mUCP2 <sup>-/-</sup> KO	Nonobese, higher ROS	6
<i>ob/ob</i> mUCP2 <sup>-/-</sup> KO	Higher ATP, higher glucose-stimulated insulin	155
mUCP3 <sup>-/-</sup> KO	Nonobese, not sensitive to cold	60
mUCP3 <sup>-/-</sup>	Nonobese, not sensitive to cold, higher ROS	145
mUCP1 <sup>-/-</sup> mUCP3 <sup>-/-</sup> double KO	Similar to mUCP1 <sup>-/-</sup>	60
hUCP3 <sup>↑↑↑</sup> TG	Hyperphagic, lean, higher glucose clearance rate	32
MAC16 mUCP1 <sup>↑</sup> mUCP2-3 <sup>↑</sup>	Cancer cachexia, hypophagia, hypothermia	10
A-ZIP/G-1 TG	No WAT, lipotrophic diabetes	112

UCP, uncoupling protein (m = mouse, h = human); BAT, brown adipose tissue; ROS, reactive oxygen species; WAT, white adipose tissue; KO, knockout; TG, transgenic; ↑, low expression of transgene; ↑↑, high expression of transgene; ↑↑↑, very high expression of transgene.

mUCP2 knockout mice were nonobese, similar to mUCP1<sup>-/-</sup> mice, and had normal responses to cold exposure and a high-fat diet (6). These data strongly support the notion that mUCP2 does not have the same thermogenic properties as mUCP1. However, mUCP2<sup>-/-</sup> mice were quite resistant to *Tocoplasma gondii* infection and had higher levels of ROS, supporting a role for mUCP2 in ROS regulation (6). Hepatocytes from *ob/ob* mice had increased mUCP2, relative to lean mice, and mitochondria from *ob/ob* mice had an increased rate of proton leak and reduced ATP stores, which made them vulnerable to necrosis after hepatic ischemia (30). These findings were confirmed in *ob/ob* mUCP2<sup>-/-</sup> mice that had higher ATP levels and increased glucose-stimulated insulin secretion, suggesting that mUCP2 may play a role in β-cell glucose sensing and might be a link between obesity and Type 2 diabetes (155).

mUCP3<sup>-/-</sup> mice were created by two separate groups (60, 145), and the phenotypes were shown to be consistent in that neither line was obese. Furthermore, there were no discernable differences between the double mUCP1<sup>-/-</sup>/mUCP3<sup>-/-</sup> and the single mUCP1<sup>-/-</sup> mice, suggesting that mUCP3 might have no significant thermoregulatory function (60). However, one of the two lines of mUCP3<sup>-/-</sup> mice had more coupled mitochondria and increased ROS production (145). At the other end of the spectrum, transgenic mice overexpressing the human UCP3 ortholog (hUCP3<sup>↑↑↑</sup> mice) were hyperphagic, were lean, had significantly reduced adipose tissue mass, and had an increased clearance rate for glucose (32). These data suggest that overexpression of hUCP3 in mice could play a significant role in resistance to obesity and the development of diabetes. It must be pointed out that very high levels of UCP3 overexpression (about 66-fold) were achieved in this transgenic model.

Another interesting mouse model for studying the role of UCPs and fatty acid metabolism is the transgenic A-ZIP/F-1 mouse, which has a phenotype similar to the human severe lipotrophic diabetes (29, 55, 112). A-ZIP/F-1 mice lack WAT (113) and could possibly make an effective tool to study the expression of mUCPs in BAT and in muscle.

The literature describing the physiological induction of the UCP homologs includes many paradoxical findings, the most prominent of which is the significant increases in mUCP2 and mUCP3 gene expression that occur during fasting and severe food restriction. Both of the latter conditions are situations in which efficient energy metabolism is well recognized and not in which energy wastage would be desirable. Significant increases in mUCP3 expression were observed in muscle of fasted rats, but there were no changes in mitochondrial proton leak (25). In a similar study (8), fasting caused a fourfold increase in mUCP3 and a twofold increase in mUCP2 in muscle of wild-type mice, whereas proton leak was unchanged (8). Importantly, the results also showed significant differences in respiratory quotients between mUCP3<sup>-/-</sup> mice and wild-type mice, suggesting impaired fatty acid oxidation in the absence of UCP3. This, in conjunction with the tight correlation between the expression of UCP3 and metabolic states in which fatty acid oxidation is high, supports the idea that UCP3 plays a physiological role in fatty acid metabolism (120, 121).

A mechanism to explain how UCP3 could enhance fatty acid oxidation has been recently proposed (70) where UCP3 facilitates rapid rates of fatty acid oxidation by acting as a mitochondrial fatty acid efflux protein. UCP3 may act in concert with mitochondrial thioesterase(s) (MTE) to remove FFA (produced by MTE) from the matrix and thereby liberate CoA. The relative demand for CoA during fatty acid oxidation is high; during the complete oxidation of palmitate for example, the relative molar requirement for CoA is 15-fold that of palmitate. Thus the UCP3 fatty acid export cycle would liberate CoASH to allow continuous high rates of fatty acid oxidation and other CoASH-requiring processes. It also removes from the mitochondrion potentially damaging molecules of fatty acid anions. Recent studies provide some support for this hypothesis. In the mouse that overexpresses human UCP3 (hUCP3) in muscle, MTE-1 and lipoprotein lipase expression was increased (96). In addition, the UCP3<sup>-/-</sup> mouse and wild-type control mice were unable to metabolize fatty acids (8). Changes in mUCP3

expression in *db/db* mice and *db/+* mice treated with selective PPAR agonist were closely correlated with MTE-1 expression (33). Although the idea that UCP3 functions to export fatty acid anions from the mitochondrial matrix remains as yet only a hypothesis, it merits further investigation.

In summary, the data from animal studies have not as yet clearly identified the physiological roles of mUCP2 and mUCP3. There is no doubt, however, that UCP1 is crucial for thermogenesis, and it has been suggested that it is the only true UCP (98). Results generated from hUCP3  $\uparrow\uparrow\uparrow$  mice suggest roles for this gene in lowering circulating levels of glucose and cholesterol and in resistance to obesity. This possibility is supported by findings from mUCP1  $\uparrow\uparrow$  mice, thus providing some phenotypic similarities, i.e., a potential involvement in fuel substrate partitioning and obesity. Physiological studies that showed an induction of mUCP2 and mUCP3 by fasting further implicate these two homologs in fatty acid oxidation. It can be concluded from the animal studies that mUCP1 is thermogenic, whereas mUCP3 (and possibly mUCP2) seems to play significant roles in fatty acid oxidation, glucose clearance, and ROS production. It is far too early to speculate on the functional properties of UCP4 and UCP5, given the scarcity of experimental data for these two homologs.

#### HUMAN STUDIES

*Studies of UCP mRNA expression in muscle.* UCP expression has been examined quite extensively in humans. In response to peak exercise and after 1–2 h of recovery, the expression of hUCP3 was increased up to sevenfold (108), whereas two 2-h bouts of treadmill running attenuated the 24-h fasting-induced transcriptional activation of hUCP3 (69). In trained subjects, however, hUCP3 was significantly reduced (127) but was upregulated by a high-fat diet (126). Fasting increased hUCP2 and hUCP3 in both lean and obese individuals, whereas insulin failed to modify the overexpression of these genes (93). In obesity, hUCP2 was overexpressed in skeletal muscle by 1.5-fold compared with lean states and its content was increased by 15% when increased muscular contractile activity of knee extensor muscles was reduced by several weeks of low-frequency electrical stimulation (132).

*Studies of UCP mRNA expression in adipose.* A positive correlation has been reported between hUCP2 mRNA concentrations in adipose tissue, hUCP3 expression in the muscle, and components of metabolic rate (85). hUCP1 mRNA concentrations were higher in the intraperitoneal than in the extraperitoneal tissue in both obese and lean individuals, but morbidly obese individuals had significantly lower hUCP1 mRNA in the intraperitoneal tissue only (102). Expression of hUCP2 was increased in omental adipose tissue relative to subcutaneous adipose tissue, which may relate to the functional attributes of this subpopulation of adipocytes (42). hUCP2 mRNA in adipose tissue was inversely related to adiposity and independently linked

to local plasma leptin levels but was not acutely regulated by food intake, insulin, or fatty acids (109). Increased plasma nonesterified fatty acids induced hUCP3, but not hUCP2 expression, whereas triglyceride infusion during a hyperinsulinemic clamp prevented induction of hUCP3 mRNA (76).

*Expression in relation to obesity and Type 2 diabetes.* mRNA levels of hUCP3, but not hUCP2, were significantly reduced in skeletal muscle of Type 2 diabetes patients, compared with control subjects, and there was a positive correlation between hUCP3 expression and the whole body insulin-mediated glucose utilization rate, suggesting that hUCP3 regulation could be altered by insulin resistance (83). Increased content of hUCP2 in skeletal muscle of obese subjects was positively correlated with percent body fat, and it coincided with reduced postabsorptive rates of lipid oxidation in muscle (132). It has been suggested that fatty acids may induce hUCP2 expression and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) can provoke a twofold decrease in hUCP2 mRNA levels, whereas hUCP2 in cultured human adipocytes is increased by activators of PPAR- $\gamma$  (146). PPAR- $\gamma$  agonists can also induce hUCP1 in isolated human adipocytes (43). In the Pima Indians, body mass index (BMI) was negatively correlated with hUCP3, but not hUCP2, expression levels, and the metabolic rate during sleep was positively correlated with the long isoform of hUCP3, suggesting a role for hUCP3 in energy expenditure and metabolic efficiency in this population (128, 129). hUCP2 is an important negative regulator of  $\beta$ -cell insulin secretion; it reduces  $\Delta\Psi$  and increases the activity of ATP-sensitive potassium channels, possibly contributing to the loss of glucose responsiveness in obesity-related Type 2 diabetes (28). Moreover, hUCP3 stimulated glucose transport and GLUT4 translocation to the cells in cardiac and skeletal muscle by increasing phosphotyrosine-associated phosphoinositide 3-kinase activity (73).

*Genetic variants in the human UCPs.* Several genetic variants have been identified in the three UCPs: hUCP1, hUCP2, and hUCP3 (149). A synopsis of the human genetic variants in the UCPs, reported to date, is provided in Table 2. Single nucleotide polymorphisms (SNPs) have been identified in the structure and promoter regions of hUCP1 (81, 110). A SNP in the 5'-UTR, A > T, and a structural SNP resulting in an amino acid substitution (Met229Leu) were in linkage disequilibrium and could be associated with Type 2 diabetes (97). Other 5'-UTR and structural missense SNPs have also been identified in hUCP1 (Arg40Trp, Ala64Thr, Val137Met, and Lys257Asn) but were not associated significantly with obesity- or diabetes-related phenotypes (142). However, a SNP in the distal hUCP1 promoter, -3826G >A (27), was significantly associated with intraperitoneal hUCP1 mRNA (51) and could play a role in the pathogenesis of obesity and arteriosclerosis (34, 143, 156).

The gene structure and the promoter of hUCP2 have also been determined (139, 152). A structural, missense SNP was identified, Ala55Val, but was not associated with BMI and percent body fat (4, 141). In

Table 2. Genetic variants in human UCPs

	Genetic Variant	Type of SNP	Reference
hUCP1	-112A > T	5'-UTR	97
	Met229Leu	Missense	97
	-3826A > G	Promoter	27, 156
	X > Y	5'-UTR	142
	W > Z	5'-UTR	142
	Arg40Trp	Missense	142
	Ala64Thr	Missense	142
	Val137Met	Missense	142
	Lys257Asn	Missense	142
	hUCP2	Ala55Val	Missense
-2723T > A		Promoter	52
-1957G > A		Promoter	52
-866G > A		Promoter	52
-371G > C		Promoter	52
13nt-del/ins		Promoter	52
hUCP3		-439InsA	Promoter
	-155C > T	Promoter	103
	-55C > T	Promoter	130
	+5G > A	5'-UTR	103
	Val9Met	Missense	104
	Arg70Trp	Missense	22
	Ala83Ala	Silent	104
	Tyr99Tyr	Silent	104
	Val102Ile	Missense	5
	Arg143*	Stop codon	5
	Tyr210Tyr	Silent	104
	Arg308Trp	Missense	104
	Intron4C > T	Intronic	104
	IVS6 + 1G > A	Exon 6 splice	5
	hUCP2-hUCP3 locus	D11S911	Linkage for RMR ( $P = 0.000002$ )

SNP, single nucleotide polymorphism; del/ins, deletion/insertion.

another study, the same SNP was not associated with obesity and the metabolic syndrome (77). However, the same polymorphism was in linkage disequilibrium with other SNPs in the region and was associated with maximal O<sub>2</sub> uptake in Caucasians (24) and with metabolic rate during sleep in young Pima Indians (147). An insertion polymorphism of 45 nucleotides (nt) located 150 nt downstream of the stop codon in the 3'-UTR was not associated with BMI or resting metabolic rate (140). SNPs in the promoter of hUCP2 have also been identified (-2723T >A, -1957G >A, -866G >A, -371G > C, 13-nt deletion/insertion), and the -866G >A SNP was associated with a high risk for obesity (52).

The gene structure of hUCP3 was also determined (1, 12), and hUCP3 transcripts were transcribed by tissue-specific promoters involving different transcription start sites (50). hUCP3 encodes two isoforms termed hUCP3<sub>L</sub> (long transcript) and hUCP3<sub>S</sub> (short transcript). The two transcripts differ by the last coding exon that is missing in hUCP3<sub>S</sub>. A SNP in the promoter of the gene, -55C > T, was associated with elevated hUCP3 expression in male nondiabetic Pima Indians (130) but was not associated with BMI in a French Caucasian population (103) or a Danish Caucasian population (40); however, it was negatively correlated with BMI in a British Caucasian population (65). Six structural SNPs were identified in a French Caucasian population but were not associated with

obesity (104). Another two genetic variants have been reported, but they were not associated with obesity or Type 2 diabetes (103). A missense mutation, Arg70Trp, was identified in an adolescent (16 yr old) of Chinese descent with morbid obesity and Type 2 diabetes (22). However, the father of the child was nonobese but had Type 2 diabetes (21). A Chinese population has yet to be screened to examine for the frequency of this SNP. In the Gullah-speaking African-Americans, two SNPs and a rare mutation have been reported (5). The rare mutation that resulted in the insertion of a premature stop codon (R143X) was identified in a morbidly obese individual who was also heterozygous for an exon 6 splice donor SNP (5). This compound heterozygote represents the only known diabetic and morbidly obese individual having one allele encoding just half of hUCP3 and one allele encoding hUCP3<sub>S</sub>. The exon 6 splice donor polymorphism (IVS6+1G > A) was associated with elevated respiratory quotient in the Gullah African-Americans (5) but not so in the Maywood African-Americans (31). It should be pointed out, however, that the genetic structure between the Maywood and the Gullah African-Americans is markedly different. The Maywood individuals have 17% European admixture levels (106), whereas the Gullah individuals have only 3.5% European admixture (105), which could have a significant mediating effect on the impact of the IVS6+1G > A SNP on the respiratory quotient of the two populations. Functional analysis of hUCP3 mutants showed that the IVS6+1G > A SNP, which results in a truncated protein that is identical to hUCP3<sub>S</sub>, had equal ability to alter ΔΨ as hUCP3<sub>L</sub>, when tested in heterologous yeast systems (21, 64). Other mutations resulted in a reduced (Arg143\*) or completely absent (Arg70Trp) ability of the mutant proteins to alter ΔΨ in yeast (21). Significant linkage at the hUCP2 and hUCP3 locus has also been reported with resting metabolic rate ( $P = 0.000002$ ) and percent body fat ( $P = 0.04$ ) (15). Therefore, the UCPs could perhaps be involved in the development of obesity, but the genetic data are inconsistent and population dependent.

In summary, the human studies provide inconclusive evidence for an involvement of hUCP1, -2, and -3 in thermoregulation. However, the expression levels of hUCP2 and hUCP3 during and after exercise as well as after fasting parallel those reported for the animal studies, suggesting a consistent role for hUCP2 and hUCP3 in substrate fuel utilization in muscle. The genetic variants provide some evidence for a possible involvement of the three genes in glucose disposal, resting metabolic rate, and possibly Type 2 diabetes. However, differences in the overall genetic structures (113) and the impact of environmental factors (i.e., diet, exercise, climatic temperatures) may obscure the expression of detectable and consistent phenotypes across human populations.

CONCLUSIONS AND FUTURE DIRECTIONS

Although it was initially proposed that the recently identified UCPs caused thermogenic uncoupling simi-

lar to that described for UCP1 in BAT, it is becoming increasingly clear that the novel UCPs have somewhat different physiological functions. Findings from the wide range of studies in rodents and in humans are only consistent to the extent that fasting and some types of exercise result in the upregulation of UCP2 and UCP3 expression. The finding that mUCP1<sup>-/-</sup> mice are cold sensitive confirms the importance of UCP1 in thermoregulation. mUCP2<sup>-/-</sup> and mUCP3<sup>-/-</sup> mice have relatively normal phenotypes, with the common exception of increased ROS production and decreased proton conductance. Recent findings from mUCP3<sup>-/-</sup> mice, as assessed by indirect calorimetry, revealed impairments in fatty acid oxidation. mUCP1 and hUCP3 overexpressing mice are hyperphagic, obesity resistant, and more efficient in glucose disposal. Therefore, a possible role for the novel UCPs in fatty acid metabolism, glucose clearance, and ROS production is emerging. Studies in humans are unfortunately complicated by the fact that they are usually poorly controlled. It is virtually impossible to impose a uniform environment to study participants for extended periods of time, and, in the absence of multiple twins, it is even more difficult to achieve genetic homogeneity. The genetic variants in humans are also likely to play a role, but variations in diet, exercise, and other genetic factors might mask such effects. In summary, the UCP literature currently indicates that the physiological roles of the UCP1 homologs in mammals extend into fatty acid oxidation, energy substrate partitioning, glucose disposal rates, insulin secretion, ROS production, apoptosis, and aging. Much more research is needed.

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