Human uncoupling proteins and obesity.

MINI-REVIEW

Human Uncoupling Proteins and Obesity

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Abstract

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Uncoupling protein (UCP) 2 and UCP3 are newly discovered proteins that can uncouple ATP production from mitochondrial respiration, thereby dissipating energy as heat and affecting energy metabolism efficiency. In contrast to UCP1, which is only present in brown adipose tissue, UCP2 has a wide tissue distribution, whereas UCP3 is expressed predominantly in skeletal muscle. Some evidence of a role for UCPs in modulating metabolic rate was provided by linkage and association studies. Furthermore, UCP3 gene expression was found to correlate negatively with body mass index and positively with sleeping metabolic rate in Pima Indians. Treatment with thyroid hormone increases expression of the UCP2 and UCP3 genes. Other regulators of UCP2 and UCP3 gene expression are β_3-adrenergic agonists and glucocorticoids. Surprisingly, fasting has a stimulatory effect on UCP2 and UCP3 mRNA levels, possibly explained by the effects of free fatty acid on UCP2 and UCP3 gene expression.

Key words: uncoupling proteins, energy expenditure, obesity, humans

Introduction

Many processes in living cells consume energy. These processes include muscle contraction, protein turnover, Na^+,K^+-pump, Ca^{2+} pump, and substrate cycles. In these processes, the energy is provided by adenosine triphosphate (ATP). This molecule can release energy by donating 1 or 2 phosphate groups, leaving adenosine diphosphate (ADP) or adenosine monophosphate, respectively. At any time, the amount of ATP in the human body is sufficient to supply the body's energy needs for only a few seconds. Therefore, ATP has to be continuously resynthesized from ADP in a process called oxidative phosphorylation. During the oxidation of substrates (fat, carbohydrate, and protein), the cofactors NADH and a reduced form of flavin adenine dinucleotide are formed in the mitochondrial matrix. At the level of the inner mitochondrial membrane, NADH and a reduced form of flavin adenine dinucleotide are converted to NAD^+, flavin adenine dinucleotide, and H^+. According to the chemiosmotic hypothesis of Mitchell (1), the protons are then transported to the cytosolic side of the inner mitochondrial membrane by a series of reactions. This eventually generates a proton gradient across the membrane, which causes protons to flow back over the inner mitochondrial membrane. The energy generated is used by ATPase to transform ADP into ATP. Therefore, the processes of substrate oxidation are coupled to the formation of ATP.

The resting metabolic rate (RMR) represents the basal energy requirements of the body and constitutes 60% to 70% of total energy expenditure (2). Under resting conditions and without change in energy storage, all of the energy expenditure is lost as heat, because no external work is performed. The processes involved in energy expenditure can be divided into two categories: ATP consuming processes and non-ATP consuming processes (3).

ATP Consuming Processes

Many processes in the body require ATP as an energy-delivering substrate, including the Na^+,K^+-pump that is responsible for ~20% of the ATP consuming energy expenditure, protein turnover (12% to 25% of RMR), and the Ca^{2+} pump (4% to 6% of RMR). Muscle contraction and, more specifically, actin-myosin ATPase, is also a significant contributor to energy expenditure. Substrate cycling contributes up to 8% of RMR, composed mainly of cycling in glycolysis, triglyceride turnover, and the Cori cycle. Other ATP consuming processes include gluconeogenesis (5% to 8% of RMR), urea synthesis (2%), enzyme phosphorylation, and RNA/DNA turnover (3) (Figure 1). All of these processes are continuously operating because they are counterbalanced by opposing reactions: protein degradation vs. protein synthesis, Na^+ channels vs. Na^+ pumping, muscle relaxation vs. muscle contraction, etc. Therefore, a continuous supply

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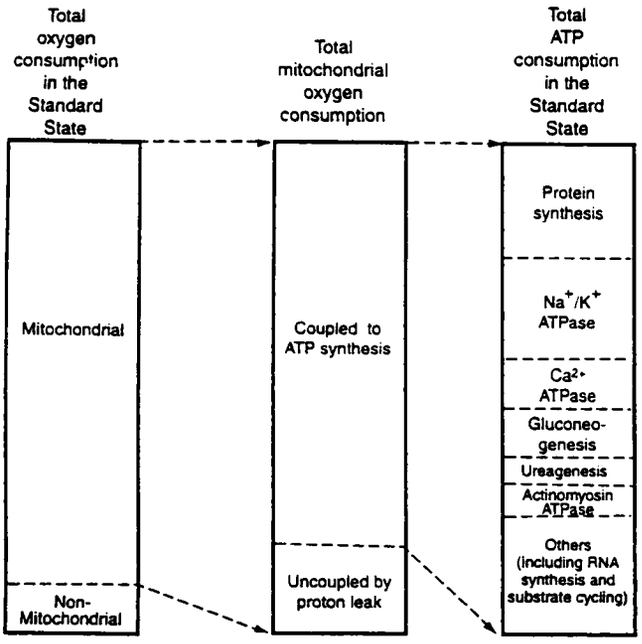

Figure 1: Estimated contribution of processes to energy utilization in standard state. First column shows contribution of mitochondrial (80%) and nonmitochondrial (10%) oxygen consumption to total respiration rate in standard state. Second column shows proportion of mitochondrial respiration used to drive ATP synthesis (80%) and proton leak (20%) in standard state. Data shown in first and second columns were calculated using data for proton leak and nonmitochondrial oxygen consumption from liver, heart, and skeletal muscle only and ignoring possible contribution of these processes in other tissues (see section II*B*). Third column represents contribution of ATP-consuming processes to total ATP consumption in standard state calculated as outlined in section II*E*. (From David F.S. Rolfe and Guy C. Brown. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiological Reviews, Vol 77 (3), July 1997.)

of ATP is required. It has been estimated that ATP consuming processes account for ~70% to 80% of RMR (3).

Non-ATP Consuming Processes

Not all energy expenditure in the human body is coupled to ATP use. There are at least two processes that contribute to energy expenditure and thus heat production without the involvement of ATP:

1. *Nonmitochondrial oxygen consumption:* There are a number of processes in mammals that use oxygen outside the mitochondria (e.g., peroxisomal fatty acid oxidation). The total contribution of nonmitochondrial oxygen consumption has been estimated at ~10% of RMR (3).
2. *Mitochondrial proton leak:* As previously described, the formation of ATP from ADP requires a proton gradient across the inner mitochondrial membrane. The flow of protons from the cytosolic side of the membrane into the mitochondrial matrix provides the energy to transform ADP to ATP. However, when inner mitochondrial membrane proton conductance is increased, insufficient proton gradient is generated and oxygen consumption is uncoupled from ATP production. In humans, such proton leaks are present in many tissues, partly uncoupling oxygen consumption from ATP synthesis and thus directly dissipating energy as heat. It has been calculated that the overall contribution of proton leaks to RMR is ~20% (3).

The Role of Brown Adipose Tissue in Energy Metabolism

Mammals have two types of adipose tissue: white adipose tissue that consists of lipid storing adipocytes; and brown adipose tissue that is composed of multilocular lipid storing adipocytes, and also contains abundant mitochondria. The functions of the two types of adipose tissue are different. The primary function of white adipose tissue is energy storage, and obesity is characterized by an increase in the amount of white adipose tissue. In contrast, brown adipose tissue has a very active metabolism resulting in thermogenesis. It has been shown that brown adipose tissue can account for up to 40% of the two-fold increase in RMR of rats infused with norepinephrine or exposed to cold (4,5). Food intake can also increase the thermogenic activity of brown adipose tissue, thereby contributing to diet-induced thermogenesis (6). Mitochondria of brown adipose tissue are exceptionally permeable to protons, and this could lead to leakage (7). Possible pathways for the mitochondrial proton leak are membrane proteins, the phospholipid bilayer, and protein/phospholipid interfaces (8). In 1978, it was demonstrated that a 32 kDa mitochondrial membrane protein, called thermogenin or uncoupling protein (UCP1), was responsible for the mitochondrial proton leak, and therefore the thermogenic activity of brown adipose tissue (9) (Figure 2). Uncoupling proteins act as H^+ or (OH^-) ions translocators in a carrier-like fashion, and can also function as anion (such as Cl^-) transporters. Both H^+ and anion transport are inhibited by purine nucleotides (7). Furthermore, fatty acids are known to activate UCP-mediated uncoupling, by a mechanism that is presently not completely understood (10).

Brown adipose tissue thermogenesis is thought to play a role in the development of obesity in animals. For example, leptin-deficient *ob/ob* mice, which are morbidly obese, have defective brown adipose tissue thermogenesis and are cold-sensitive (11). In 1993, a transgenic mouse was created with targeted ablation of brown adipose tissue. These mice lacked UCP1 and became obese at a young age, before developing hyperphagia (12). However, UCP1 knock-out mice were cold-sensitive, but did not become obese (13). This indicates that the brown adipose tissue may

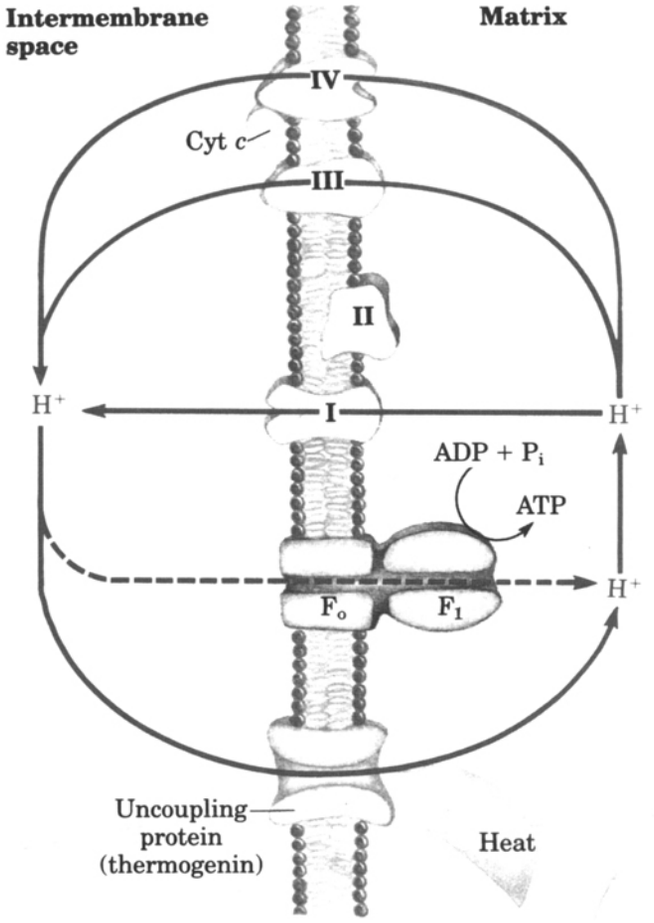

Figure 2: The UCP (thermogenin) of brown fat mitochondria, by providing an alternative route for protons to re-enter the mitochondrial matrix, causes the energy conserved by proton pumping to be dissipated as heat. (From Oxidative phosphorylation and photophosphorylation. In: Lehninger AL, Nelson DL, Cox MM, eds. *Principles of Biochemistry,* 2nd ed. chap. 18. New York: Worth Publishers; 1993.)

contain other signals that are involved in the regulation of body weight. Together, these results indicate the importance of UCP1 in the thermogenic activity of brown adipose tissue; however, the role of UCP1 in body weight regulation is still unclear.

A Novel UCP: UCP2

In adult humans, brown adipose tissue is very scarce. In neonates, brown adipose tissue is present but, later in life, the expansion of white adipose tissue overgrows brown adipose tissue (14). Under prolonged periods of cold exposure, some brown adipose tissue may remain in adult humans (15), but in general, the amount is very limited. Therefore, the role of UCP1 in human energy expenditure has long been controversial. However, Fleury et al. (16) and Gimeno et al. (17) recently cloned a homolog of UCP1, called UCP2. The amino acid sequence of UCP2 is 59% identical to UCP1 and consists of 309 amino acids with a molecular weight of 33 kDa. Several protein motifs are conserved in the two UCPs, including three mitochondrial carrier protein motifs and the amino acids essential for ATP binding, suggesting that UCP2 also has a functional role as a mitochondrial uncoupler. To test this, the decrease in mitochondrial potential was measured in yeast after introducing UCP2 in a vector. The results showed that UCP2 influenced mitochondrial activity and could partially uncouple respiration from ATP synthesis (16). The tissue distribution of UCP2 gene expression was investigated by Northern blot analysis and was shown to be markedly different from UCP1. UCP2 mRNA was present in skeletal muscle, lung, heart, and kidney as well as in tissues of the immune system (16). Further evidence for the uncoupling capacity of UCP2 was provided by Nègre-Salvayre et al. (18), who showed that, in brown adipose tissue, the production of H_2O_2 (an index of mitochondrial respiration) was increased by guanosine diphosphate (GDP), probably by an inhibiting effect on UCP1 and/or UCP2. GDP had a similar effect on H_2O_2 production in nonparenchymal cells as in brown adipose tissue, but had no effect on H_2O_2 production in hepatocytes (18). In contrast to hepatocytes, nonparenchymal cells do contain UCP2; therefore, these results suggested that the effect of GDP was through an inhibiting effect on UCP2, giving further evidence for an uncoupling effect of UCP2.

UCP in Skeletal Muscle: UCP3

Inspired by the discovery of UCP2, by the fact that skeletal muscle determines 40% of whole-body adrenaline-induced thermogenesis (19) and that chronic treatment of mice with β_3-agonists induced expression of UCP1 in skeletal muscle (20), Boss et al. (21) searched for UCP homologs in skeletal muscle. They found three products similar to the rat muscle UCP1 product, with amino acid lengths of 309, 312, and 275. The 309 amino acid product turned out to be the recently discovered UCP2 protein. The other two products were identical for the first 275 amino acids, suggesting that they were isoforms of the same protein. They had 57% and 73% amino acid identity to UCP1 and UCP2, respectively, and were called UCP3 long and short forms (UCP3L and UCP3S). When comparing UCP1, UCP2, and UCP3, it appeared that many of the nonidentical residues were conservative substitutions and in regions that also showed substantial variation in UCP1 between species (21). This indicated that all three UCPs belonged to the same gene family. Both UCP2 and UCP3L contain six transmembrane domains and have a potential purine nucleotide binding region (aa 279–301). Binding of GDP to this nucleotide binding region in UCP1 has been shown to result in a change in conformation and inhibition of H^+ and Cl^- permeability (22). UCP3S only has five transmembrane do-

mains and lacks the purine nucleotide binding region. Northern blotting revealed that the UCP3 gene was expressed predominantly in skeletal muscle and brown adipose tissue, and at low levels in heart muscle, and that UCP3 gene expression in skeletal muscle was four-fold higher than that of the UCP2 gene (23). Furthermore, the ratio of UCP3L to UCP3S gene expression was 1 (21). The genomic structure of the UCP3 gene was first described by Solanes et al. (24), who reported that the UCP3 gene consists of 7 exons, of which exon 1 is untranslated (Figure 3). Furthermore, intron 6 contains a cleavage and polyadenylation site that terminates the message elongation ~50% of the time. If this occurs, message elongation terminates in exon 6, where a stop codon (codon 275) is located and UCP3S is produced. If message elongation is not ended by the polyadenylation signal in intron 6, message elongation continues to a polyadenylation signal in exon 7, producing UCP3L. The last exon (exon 7) encodes a 37 amino acid C-terminus. In UCP1, this segment has been shown to be important in purine nucleotide-mediated inhibition of UCP1 activity (25). It is therefore suggested that UCP3S may have altered uncoupling activity.

In summary, both UCP2 and UCP3 have uncoupling activity and are expressed in tissues that have an important role in energy expenditure. Therefore, it can be assumed that those UCPs might have a major influence on energy balance.

Role of UCP2 and UCP3 in Obesity and Energy Expenditure

In animals, a direct comparison of UCP2 gene expression in obesity-resistant (A/J) mice and obesity-prone (B6) mice showed higher UCP2 mRNA levels in the obesity-resistant mice (16). mRNA levels of UCP2 were increased in white adipose tissue by a high-fat diet in the A/J strain, but not in the B6 strain mice (16). This result suggested that UCP2 plays a role in preventing obesity in A/J mice fed a high-fat diet, possibly by increasing energy expenditure. Surprising results were provided by Enerbäck et al. (13), who showed that mice lacking UCP1 (UCP1 knock-out) did not become obese when fed a high-fat diet. Therefore, additional mechanism(s) (other than UCP1) for maintaining body weight must be present. UCP2 was upregulated five-fold in brown adipose tissue of these mice, possibly compensating for the absence of UCP1. These results suggest that UCP2 may be involved in the regulation of body weight.

In humans, Millet et al. (26) showed a positive correlation between UCP2 mRNA levels in adipose tissue and body mas index (BMI). However, no difference in the expression of UCP2 and UCP3 in skeletal muscle was found between subjects with obesity and lean subjects, suggesting no major role for UCPs in obesity (26). In contrast, we recently showed a negative correlation between skeletal

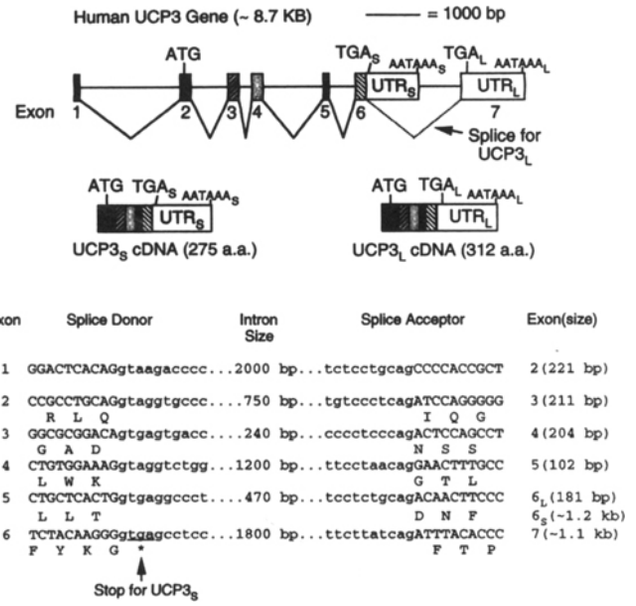

Figure 3: Human *UCP3* gene structure. Human *UCP3* gene with start codon (ATG), stop codons (TGA_S for $UCP3_S$ and TGA_L for $UCP3_L$), and cleavage and poly(A) adenylation signals ($AATAAA_S$ for $UCP3_S$ and $AATAAA_L$ for $UCP3_L$) are shown herein. Exons are coded from 1 through 7. 3′-Untranslated regions for $UCP3_S$ and $UCP3_L$ are shown as UTR_S and UTR_L, respectively. The GenBank™ accession numbers for each exon and flanking intronic sequences are consecutive from exon 1 to exon 7: AF012196, AF012197, AF012198, AF012199, AF012200, AF012201, and AF012202. Schematic cDNAs are shown below the gene structure. On the bottom is the exact location of the splice donors and splice acceptors (uppercase letters refer to exon sequence; lowercase letters refer to intron sequence). Amino acids adjacent to the splice sites are shown below the nucleotide sequence. (From Gemma Solanes, Antonio Vidal-Puig, Danica Grujic, Jeffrey S. Flier, and Bradford B. Lowell. The human uncoupling protein-3 gene: genomic structure, chromosomal localization, and genetic basis for short and long form transcripts. *J. Biol. Chem.* 1997;272:25433–25436.)

muscle UCP3 expression and BMI, and a positive correlation between UCP3 mRNA levels and RMR in Pima Indians (27) (Figure 4). Assuming that mRNA levels reflect UCP3 protein concentrations and activity, these data indicate that reduced skeletal muscle UCP3 may result in a reduced RMR. Because a low relative RMR is a predisposing factor for weight gain (28), it was expected that individuals with low UCP3 gene expression would have increased BMI, which is in accordance with the negative correlation between BMI and UCP3 gene expression in our study (27).

Both the UCP2 and UCP3 genes have been mapped to human chromosome 11q13. Solanes et al. (24) demonstrated that P1 clones containing human genomic inserts contained both the UCP2 and UCP3 genes. This indicates that the UCP2 and UCP3 genes were located within 75-150

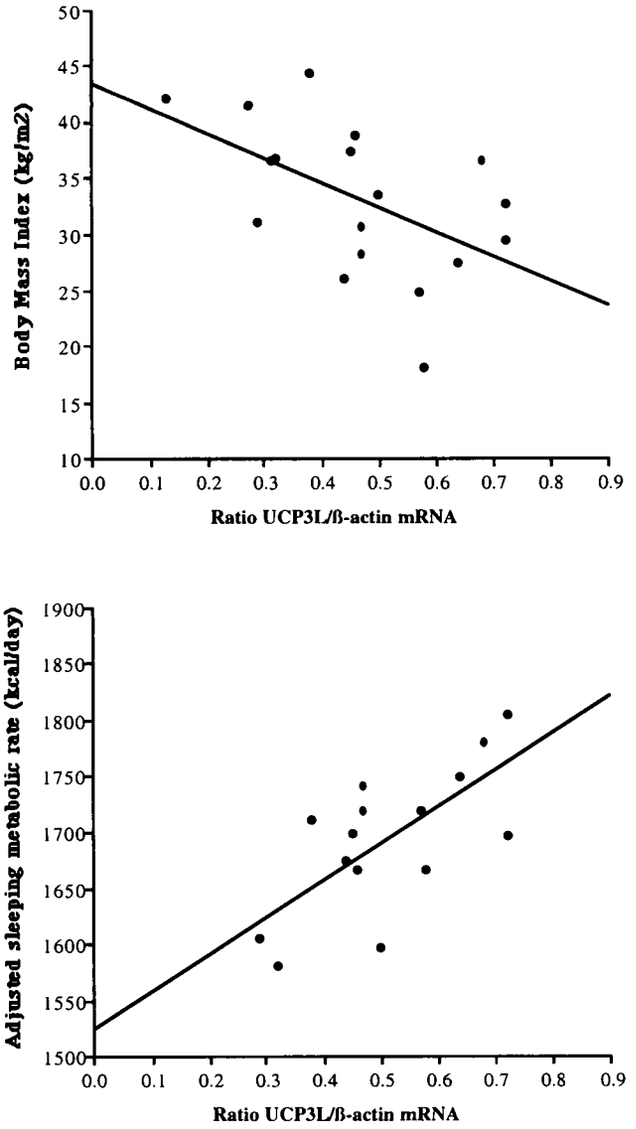

Figure 4: From Patrick Schrauwen, James Xia, Clifton Bogardus, Richard Pratley and Eric Ravussin. Skeletal muscle UCP3 expression is a determinant of energy expenditure in Pima Indians. Submitted 1998.

kb of each other. Surwit et al. (29) showed that the mouse UCP3 gene is 5′ to the UCP2 gene and that the two genes are only 8 kb apart. Chromosome 11q13 is in the proximity (~15 cM) of a locus (11q21–q22), which was found to be linked to percent body fat in Pima Indians (30). Bouchard et al. (31) typed three markers in the vicinity of 11q13 in 640 individuals from 155 pedigrees from the Québec Family Study. A linkage between marker D11S911 and RMR was found, suggesting a role for UCP2 and/or UCP3 in energy metabolism (31). In contrast, Elbein et a. (32) did not find linkage between markers in the 11q13 region and BMI in 42 North European families with type 2 diabetic siblings.

Urhammer et al. (33) were the first to report a common polymorphism in the UCP2 gene. This polymorphism was a single nucleotide substitution, which resulted in an alanine to a valine amino acid substitution at position 55. The Ala55Val variant was located in a nonfunctional domain and the substitution of valine for alanine is a conservative amino acid substitution, probably not resulting in major changes in the structure of the protein. In Danish Caucasians, this polymorphism was not associated with BMI, fat mass, or waist-to-hip ratio (33). We identified a second polymorphism in UCP2, a 45 base pair insertion/deletion (I/D) variant located 158 base pairs after the stop codon in exon 8. This polymorphism was found to be associated with sleeping metabolic rate in a small group ($n=76$) of Pima Indians. There was also some evidence of association between this polymorphism and BMI in Pima Indians over 45 years of age ($p=0.04$, $n=105$) (34). The insertion/deletion variant was highly polymorphic in this population, with a genotype frequency of 16%, 49%, and 35% for I/I, I/D, and D/D, respectively. Although the position of this polymorphism suggests possible involvement in mRNA stability, no differences in UCP2 mRNA levels in skeletal muscle were found between the genotypes.

In summary, the previously described data suggest a role for these new uncoupling proteins in energy balance. Impairment of the expression of these proteins, or expression of less active forms, could lead to reduced energy expenditure and therefore contribute to the development of obesity. Therefore, UCPs may represent a new possible target for the treatment of obesity. However, a lot more data is required regarding the regulation of these genes in normal human physiology.

Regulation of UCP2 and UCP3 Gene Expression

Cold Exposure

In brown adipose tissue, UCP1 is responsible for the increase in energy expenditure during cold exposure (4,5). The increase in UCP1 leads to more energy dissipated as heat for the regulation of body temperature. Therefore, cold exposure is one of the candidates for upregulation of UCP2 and UCP3 expression. In one study, UCP2 in mice was not upregulated after 10 days of cold exposure (4°C) in brown adipose tissue, white adipose tissue, skeletal muscle, or liver (16). In contrast, Boss et al. (35) found that both UCP1 and UCP2 mRNA in brown adipose tissue were upregulated by 48 hours of cold exposure (6°C) in rats. As previously described, UCP1 knock-out mice had a five-fold increase in brown adipose tissue UCP2, but were not able to maintain body temperature when exposed to cold (5°C) (13). This indicates that, even if UCP2 expression is increased in brown adipose tissue by cold exposure, this increase is not sufficient for the maintenance of body temperature. In two studies, UCP3 mRNA expression was found to be upregu-

lated by 10 days of cold exposure (4°C or 6°C) in brown adipose tissue of rats, but not in skeletal muscle (21,36). Overall, these results indicate a role for the UCPs in the adaptive response to cold exposure in rodent brown adipose tissue, but not skeletal muscle. The role of these UCPs in human response to cold exposure is unclear.

Thyroid Hormone

An explanation for the different effects of cold exposure on UCP2 and UCP3 in brown adipose tissue and skeletal muscle was provided by Larkin et al. (36). In brown adipose tissue, cold exposure leads to an increase in triiodothyronine (T_3), by the conversion of thyroxine to T_3. This conversion is catalyzed by thyroxine 5′ deiodinase type II, an enzyme induced 17-fold by cold exposure in brown adipose tissue, but not present in skeletal muscle. T_3 is secreted by the thyroid gland and has widespread stimulatory effects on metabolism, such as increasing the rate of oxygen consumption, protein synthesis, glycogenolysis, and lipolysis. T_3 had a stimulatory effect on the expression of UCP2 and UCP3 (36). Masaki et al. (37) gave rats daily infusions of T_3 for 7 days. This procedure made the rats hyperthyroid and they lost weight, compared with a control group given daily infusions of saline. UCP2 mRNA expression increased in brown adipose tissue, white adipose tissue, and skeletal muscle, and UCP1 increased in brown adipose tissue of the T_3-treated rats. A stimulatory effect of T_3 injection for 1 week on UCP2 mRNA in heart and skeletal muscle was also found by Lanni et al. (38). The effect of thyroid hormone on UCP3 mRNA expression was examined by Larkin et al. (36), who showed a five-fold increase in UCP3 mRNA expression after 5 days of T_3 hormone treatment. In hypothyroid rats, skeletal muscle UCP3 mRNA levels were decreased 3-fold, and treatment of these rats with a single dose of T_3 increased the UCP3 mRNA levels 6-fold (39). Overall, these results provide clear evidence of a role for thyroid hormone on both UCP2 and UCP3 gene expression in rodents.

Fasting and High-Fat Feeding

Fasting is known to cause a decrease in RMR. In rodents, UCP1 expression was decreased in brown adipose tissue with fasting (40). However, after a 48-hour fast, UCP2 gene expression was not changed in brown adipose tissue of rats, but was increased in skeletal muscle (35). Fasting (48 hours) decreased UCP3 mRNA in brown adipose tissue of rats, but increased UCP3 expression six-fold in skeletal muscle (39,41). In humans, Millet et al. (26) showed that a 5-day hypocaloric diet (1045 kJ/day) resulted in a two- to three-fold increase in UCP2 mRNA in white adipose tissue and skeletal muscle, and two- to three-fold increase in UCP3 mRNA in skeletal muscle. These results are surprising, because fasting decreases metabolic rate, and an increase in UCP2 and UCP3 in skeletal muscle and white adipose tissue would be expected to increase metabolic rate. One possible explanation is that the increase in lipolysis and free fatty acid (FFA) during fasting may upregulate UCP2 and UCP3 gene expression. FFAs are ligands for peroxisome proliferator-activated receptors, which were shown to stimulate UCP2 expression (42,43), although results are still somewhat inconclusive. Therefore, Weigle et al. (44) examined whether the effects of fasting on UCP3 mRNA expression could be explained by the effects of FFAs. They administered infusions of an emulsion of triglycerides to rats, resulting in FFA concentrations similar to those induced by fasting, and showed an increase in UCP3 mRNA in skeletal muscle similar to that induced by fasting. This suggests an important role for FFAs in the regulation of UCP3 expression. Recently, Samec et al. (45) also showed an increase in UCP2 and UCP3 mRNA in skeletal muscle in response to fasting and a subsequent decrease in response to refeeding, following the pattern of FFA levels. In addition, in obesity-resistant A/J mice, UCP2 mRNA levels in white adipose tissue were increased after consumption of a high-fat diet for 7 days, 18 days, and 25 days. Matsuda et al. (46) also found increased UCP3 levels in skeletal muscle and increased UCP2 levels in white adipose tissue of rats after 4 weeks of high-fat diet. In contrast, Surwit et al. (29) observed no change in UCP2 or UCP3 mRNA levels in skeletal muscle after 2 weeks of a high-fat diet in obesity-prone and obesity-resistant strains of mice. Overall, these results suggest a role for diet and FFA in the regulation of UCP2 and UCP3 expression.

Leptin

Leptin is absent in a strain of obese mice (*ob/ob*), and injection of these mice with leptin caused weight loss by decreasing food intake and increasing energy expenditure (47,48). UCP3 mRNA levels in skeletal muscle of *ob/ob* mice were not different to wild-type mice (39). Treatment of the *ob/ob* mice with leptin (2 μg/day) resulted in an increase in UCP3 mRNA levels in skeletal muscle and brown adipose tissue (39). However, no data on food intake were presented in this study, so the increase in UCP3 gene expression may have been the result of decreased food intake and weight loss. Zhou et al. (49) studied UCP2 mRNA levels in hyperleptinemic rats, compared with pair-fed controls, thereby controlling for the effect of food intake. UCP2 mRNA levels in pancreatic islets were increased 10-fold in hyperleptinemic rats, compared with pair-fed controls. These data suggest a role for leptin in the regulation of expression of UCP2 and UCP3. However, it should be remembered that both of these animal models are very unusual, compared with the physiology of human obesity. Evidence against a role for leptin on UCP expression was given by Surwit et al. (29). They found no effect of twice-daily leptin (20 μg) injections for 4 days on UCP2 expression in white adipose tissue of mice (50). Indirect evidence against

a role for leptin was provided by the stimulatory effects of fasting on UCP2 and UCP3 levels. Fasting decreases plasma leptin, yet UCP2 and UCP3 are upregulated (26). Overall, data indicate that leptin has no direct effect on the expression of the UCP2 and UCP3 genes under normal physiological conditions.

β_3-Agonists

The sympathetic nervous system is thought to play an important role in energy balance. A low sympathetic output has been associated with obesity in some animal models, and low sympathetic nervous system activity predicts weight gain in Pima Indians (51). β_3-Adrenergic stimulation of adipocytes results in increased lipolysis and thermogenesis, largely mediated by β_3-adrenoreceptors. UCP1 is induced by β_3-agonists, but treatment of mice for 10 days with a β_3-agonist (C316243) did not affect UCP2 mRNA levels in brown adipose tissue, white adipose tissue, muscle, or liver (16). In contrast, an increase in UCP2 mRNA levels in brown adipose tissue after treatment with β_3-agonist (Ro-168714) for 32 hours was reported in rats (35). Interestingly, treatment of rats with a β_3-agonist (CL214613) increased UCP3 mRNA levels in white adipose tissue, in which no UCP3 can normally be detected (39). It has been shown previously that, under strong β_3-adrenergic stimulation, white adipose tissue develops some characteristics of brown adipose tissue.

Glucocorticoids

During fasting, an increase in glucocorticoids is observed, and glucocorticoids are regulators of fuel metabolism and gene transcription. Gong et al. (39) showed an increase in UCP3 mRNA in muscle and a decrease in brown adipose tissue 18 hours after a single dose (3.7 μg/g) of dexamethasone in mice. However, food intake was not controlled in this study. In contrast, Weigle et al. (44) compared fasted rats, fed rats, and fed rats given twice-daily injections of hydrocortisone (50 mg/kg). They found no effect of glucocorticoids on UCP2 and UCP3 mRNA expression.

Conclusions and Future Directions

The discovery of the UCP2 and UCP3 genes could be a breakthrough in understanding the complex mechanisms regulating energy expenditure and has given new stimuli for research in this field. The results so far strongly suggest a role for the UCPs in energy balance and obesity. However, a lot of questions have yet to be answered to understand fully the importance of these novel genes. The first barrier to be overcome is generating antibodies for the proteins and developing assays to measure protein levels directly. Only then the question of whether mRNA levels reflect protein concentrations can be answered. Furthermore, it would be even better to measure the activity of the proteins in vivo. Alterations of the activity of the UCPs could be a new therapeutic target for obesity.

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