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The role of uncoupling proteins in the regulation of metabolism

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Abstract
Investigations of variations in metabolic efficiency and thermogenesis have a short and turbulent history. In small animals, non-shivering thermogenesis and diet-induced thermogenesis have a great impact on overall body weight, and the question is whether mechanisms to waste energy have evolved also in human energy metabolism. The candidate molecules for this adaptive thermogenesis are the uncoupling proteins. This is a newly discovered family of proteins, consisting of at least five proteins, namely UCP1, UCP2, UCP3, UCP4 and UCP5. Although a role for UCP1 in thermogenesis is unequivocal, the physiological function of the newer uncoupling proteins is as yet unclear. UCP1 is present in brown adipose tissue and has a well-documented role in cold-induced thermogenesis. The targeted disruption of the UCP1-gene rendered animals that were cold sensitive, but not obese. UCP2 mRNA has a ubiquitous distribution in tissue, namely, in skeletal muscle, white and brown adipose tissue, the gastro-intestinal tract, the lung and the spleen. By targeting the UCP2-gene there was no effect on whole body energy metabolism, but instead, a reduced ability to protect against free-radical oxygen species. UCP2 has also been shown to act as a negative regulator for insulin secretion. UCP3 is present in skeletal muscle. Targeted disruption of the UCP3-gene gave no effect on whole body energy metabolism, but showed the mitochondria in muscle to be more coupled. In conclusion, the uncoupling proteins may be important in various specific ways, as protectors of free radical oxygen species and as regulators of ATP-dependent processes.
Keywords ATP, energy, free oxygen radical, mitochondria, obesity, thermogenesis.

In order to maintain its structure and function, the cell must produce energy. The predominant part of the energy comes from the mitochondria, through the process called oxidative phosphorylation. In this process, the energy from our energy substrates is conserved by energy-rich electrons, which are oxidized in the inner mitochondrial membrane, through a passage of the proteins in the respiratory chain. These proteins change their conformation when absorbing electrons and protons are expelled. The protons flow back through specific sites, as the inner mitochondrial membrane is a tight membrane. The flow of protons occurs through the F1F0-ATPase, which thereby gets energy to drive the formation of ATP. Peter Mitchell therefore proposed that the oxidation of energy substrates is coupled to ATP synthesis through this proton flow (Reid *et al.* 1966). Hence, consumption of oxygen, i.e. respiration, is coupled to the formation of ATP from ADP and P.

However, this coupling is not perfect (Ricquier & Bouillaud 2000a). Mitochondria may still use oxygen without producing ATP, and mitochondria may respire without producing ATP. The coupling is imperfect because of a partial uncoupling, as evidenced by several studies. The question is then how this uncoupling occurs. Theoretically there are three ways through which an uncoupling might occur: (1) a redox slip; (2) a proton leakage; and (3) inhibition of F1F0-ATPase. A redox slip is defined as the failure of the respiratory

chain to extrude protons out of the membrane (Fig. 1). Although electrons are passing through the respiratory chain proteins, a reduced number of protons are extruded. In this way a lower number of protons reach the F1F0-ATPase. Therefore, a higher respiratory rate is needed for a certain ATP synthesis. A proton leakage means that there will be a lower number of protons flowing through the F1F0-ATPase, even at a normal proton extrusion rate. In this way again, a higher respiratory rate is needed for a certain ATP synthesis. A third reason for an uncoupling phenomenon is a failure of the F1F0-ATPase to respond to the proton flow, because of a disturbance in the enzyme itself by various factors, such as the F1-inhibitor (Walker 1994). Whatever the mechanism, a partial coupling means that energy metabolism is less efficient and that energy will be wasted as heat. Heat may be important to maintain body temperature, but there may be other reasons for having a partial uncoupling. Reasons suggested for this partial uncoupling may be the regulation of the energy balance during overfeeding, the regulation of ATP-dependent processes and the control of free oxygen radicals.

Uncoupling proteins are proteins that can uncouple ATP production from mitochondrial respiration, by causing a proton leakage (Ricquier & Bouillaud 2000b). Thereby energy is dissipated as heat and the energy metabolism becomes less efficient. To date five different uncoupling proteins have been identified, namely UCP1, UCP2, UCP3, UCP4 and UCP5/BMCP1, with different tissue distribution and different roles (Boss *et al.* 2000, Erlanson-Albertsson 2002). It is unexpected that the organism, indeed, has mechanisms that may enhance energy wasting, as food seeking and energy conservation are such evolutionary important matters. In this article, discussion is focused on what is known today about the role of these proteins, with emphasis on UCP1, UCP2 and UCP3, and their role in energy metabolism.

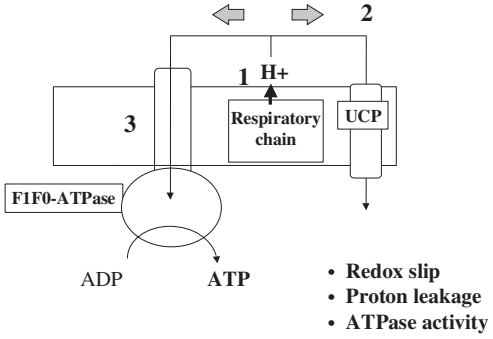

Figure 1 In the mitochondria the energy derived from the respiratory chain is converted into a proton electrochemical gradient that is dissipated through the mitochondrial F_1F_0-ATPase enzyme, producing ATP. In fact, mitochondrial ATP synthesis is not 100% coupled to respiration. The partial uncoupling may be because of the presence of (1) a redox slip; (2) a proton leakage through uncoupling proteins UCP; and (3) the inhibition of F_1F_0-ATPase activity. The UCP family contains five different proteins, UCP1–5.

The role of UCP1 – the old one

UCP1 was discovered 30 years ago, being present in brown adipose tissue and responsible for thermogenesis in this tissue, and being particularly important in hibernating animals. UCP1 contains 306 amino acids, has a molecular weight of 32 kDa and an isoelectric point of 9.3. The amino acid sequence has been found highly conserved in a comparison of UCP1 from rat, mouse and man.

The function of UCP1 in energy metabolism was originally described to be mediation of non-shivering thermogenesis (Nicholls & Locke 1984). UCP1 is characterized by being expressed in one tissue only, namely, in mammalian brown adipose tissue. Brown adipose tissue is innately uncoupled, i.e. shows a spontaneously high thermogenesis without accompanying ATPsynthesis (Nedergaard *et al.* 1999). It is also known that the thermogenesis can be inhibited by di- and triphosphate nucleotides, normally demonstrated by the use of guanosine diphosphate (GDP) (Arechaga *et al.* 2001). That the uncoupling in brown adipose tissue is because of the presence of UCP1 has been demonstrated in experiments with UCP1-ablated mice (Matthias *et al.* 1999). The mitochondria in brown adipose tissue from UCP1-deficient mice demonstrate a low rate of thermogenesis, which could not be influenced by GDP (Monemdjou *et al.* 1999). It also appears that the tissue has been adapted to become thermogenic in that the F1F0 ATPsynthase has a low activity, the c subunit of the ATPsynthase being repressed in brown adipose tissue (Cannon & Vogel 1977). Another characteristic feature of the UCP1 protein is the specific activation by fatty acids. Direct addition of fatty acids to isolated brown adipocytes results in a 10-fold increase in thermogenesis. This response is not seen when fatty acids are added to UCP1-ablated brown fat cells (Matthias *et al.* 2000). The mechanism for this activation by fatty acids is a matter of debate, as fatty acids by themselves might cause a non-specific uncoupling of the mitochondria (Klingenberg 1999). It has been suggested that UCP1 works as a fatty acid cycler, returning anionic fatty acids to the intermembrane space (Garlid *et al.* 2000).

Which effects are observed in the organism upon ablation of the UCP1-gene? The answer is that the basal metabolic rate is not changed (Enerback *et al.* 1997), indicating that the high metabolic rate of small animals

occurs even in the absence of an activated thermogenesis in brown adipose tissue. The UCP1 knockout mice, however, demonstrate certain heat lability when exposed to low ambient temperature (Enerback *et al.* 1997). In the analysis of this heat lability it was found that the UCP1-ablated mice could be induced to tolerate cold exposure for months, but the heat necessary for adaptation originated from shivering thermogenesis, i.e. from muscle tissue shivering and not from non-shivering thermogenesis (Golozoubova *et al.* 2001). Hence, the conclusion is that UCP1 mediates a thermogenic response upon cold exposure.

Another function of UCP1 was a diet-induced thermogenesis observed after cafeteria feeding (Rothwell & Stock 1979). This was postulated to be a defence mechanism against obesity induced by a high-fat diet. Several investigators have found an up-regulation of UCP1 during high-fat feeding in rodents (Portillo *et al.* 1998, Rippe *et al.* 2000), acting to defend the animals against diet-induced obesity. It was thus a surprise that the UCP1-ablated mice failed to develop obesity on a high-fat diet (Enerback *et al.* 1997). Body weight and fat pad weight were similar in ablated and wild-type animals, respectively. Thus, it seems that the mechanisms that conserves a normal body mass is highly efficient when energy intake is varied. Other compensatory thermogenic responses, such as futile cycling or as yet undiscovered thermogenic tools, may be needed to increase thermogenesis (Monemdjou *et al.* 2000).

As to the mechanism of action, UCP1 has clearly been shown to shunt protons through the inner mitochondrial membrane of brown adipose tissue, hence generating heat (Nicholls & Locke 1984). In addition to a direct control by tissue metabolites, such as the di- and triphosphate nucleotides and fatty acids mentioned above, brown fat thermogenesis is subjected to hormonal as well as neuronal control. The sympathetic nervous system and noradrenaline have been suggested to be one key factor in the regulation of UCP1 expression (Himms-Hagen *et al.* 1994). In man, brown adipose tissue is very active in the neonate, but gradually loses its importance upon aging, being present in adult humans to a variable extent (Lean *et al.* 1986). Outdoor workers have an increased level of brown adipose tissue as have patients with pheochromocytoma, a tumour producing adrenalin that increases the production of UCP1 (Ricquier *et al.* 1982). This suggests a role of UCP1 in regulating thermogenesis under certain circumstances (see Fig. 2).

The role of UCP2 – the first newcomer

The identification of UCP2 started a turbulent period of interest in energy expenditure for the regulation of the energy balance (Fleury *et al.* 1997, Gimeno *et al.* 1997, Ricquier & Bouillaud 2000b). The discovery came soon after the discovery of the leptin gene and its role in energy intake, and when the time had come to introduce genes for regulating energy expenditure. UCP2 in man consists of 308 amino acids, has a molecular weight of 33 kDa and an isoelectric point of 9.7. At the amino acid level, UCP2 is identical to 56% UCP1. The most spectacular finding concerning the UCP2 gene was its wide distribution in tissue, UCP2 in contrast to UCP1 being expressed not only in brown adipose tissue, but also in white adipose tissue (WAT), skeletal muscle, heart, kidney, lung, spleen, macrophages, thymus and bone marrow (Fleury *et al.* 1997), brain (Richard *et al.* 1998, Horvath *et al.* 1999), islets of Langerhans (Chan *et al.* 2001) and in the gastrointestinal tract (Wang *et al.* 1999, Rippe *et al.* 2000). The recognition of the wide distribution in tissue was based on mRNA determinations. The localization of the UCP2 protein was more problematic, mainly because of the lack of tools sufficiently sensitive to detect the protein, which is present at 1% of the levels of UCP1 in brown adipose tissue. Using specific antibodies against human UCP2, UCP2 has been demonstrated in spleen, lung, stomach and white adipose tissue (Pecqueur *et al.* 2001). Another spectacular finding concerning UCP2 was the up-regulation by a high-fat diet, suggesting UCP2 to be important for determining basal metabolic rate and possibly resistance to obesity. These conclusions were based on the fact that UCP2 mRNA was upregulated during high-fat feeding, in the obesity-resistant A/J mouse, whereas the obesity-prone B6 mouse failed to change its UCP levels and developed obesity (Fleury *et al.* 1997, Surwit *et al.* 1998).

However, today there are no data to demonstrate a role of UCP2 in maintaining an energy balance. UCP2-deficient mice generated by homologous recombination in embryonic stem cells had a normal body weight

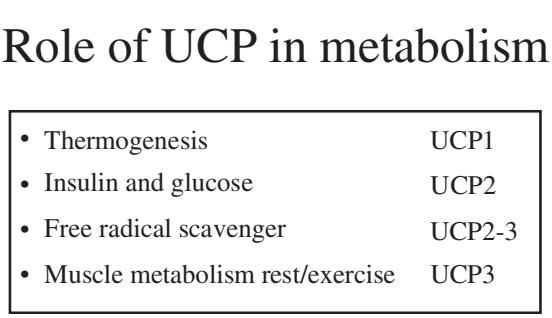

Figure 2 Putative roles of uncoupling proteins UCP1–3 in energy metabolism. UCP1 has a thermogenic role, whereas UCP2 may be important for the regulation of insulin secretion. UCP3 is probably important in the regulation of energy handling in skeletal muscle during rest and exercise. Both UCP2 and UCP3 may be scavengers of reactive oxygen species.

(Arsenijevic *et al.* 2000). Also after challenging the mice, either with cold exposure for 24 h or with a high-fat diet for 8 weeks there was no detectable change in body weight or fat pad weight compared to wild-type animals. Hence, the knockout animals failed to support a role of UCP2 in temperature regulation or in thermogenesis induced by a high-fat diet (Arsenijevic *et al.* 2000). The absence of major alterations in energy balance in the UCP2-ablated mice could be related to a low expression of UCP2 in mitochondria in wild-type animals or an inability of these mitochondria to cause an uncoupling. The UCP2-ablated mice were, however, more resistant to infection by *Toxoplasma gondii*, an intracellular parasite that may infect the brains of normal mice and cause death (Arsenijevic *et al.* 2000). The tentative conclusion of these experiments is that the macrophages in the absence of UCP2 have a greater capacity to produce free oxygen radicals, which can come into play in killing the infectious agents. The function of UCP2 would thus be to decrease the number of free oxygen radicals, i.e. act as an antioxidative agent. It is known that the mitochondrial production of reactive oxygen species (ROS) is modulated by the value of the mitochondrial potential, which is itself controlled by the level of coupling of respiration to ADP phosphorylation. So, when the level of coupling decreases, the potential decreases and thus the production of free oxygen radicals. Ubisemiquinone is an important producer of reactive oxygen species. When UCP2 is disrupted the half-life of ubisemiquinone is increased and consequently the production of ROS. A beneficial role of mild uncoupling is thus to prevent excess formation of ROS.

The failure of UCP2 to correct the energy balance during high-fat feeding has been demonstrated by several groups (Surwit *et al.* 1998, Rippe *et al.* 2000). The role of UCP2 seems to be tissue-specific, based on tissue-related regulation. Thus, high-fat feeding has been shown to cause an upregulation of UCP2 in white adipose tissue, brown adipose tissue and skeletal muscle (Fleury *et al.* 1997, Matsuda *et al.* 1997, Surwit *et al.* 1998). At the same time, a down-regulation of UCP2 in the stomach and intestine was observed in mice fed a high-fat diet (Rippe *et al.* 2000), suggesting adipose tissue UCP2 and gastro-intestinal UCP2 to be differently regulated. It is true that adipose tissue and the gastro-intestinal tract are involved in two different physiological situations, the gastro-intestinal tract being necessary for the delivery and uptake of dietary fat, the adipose tissue being important for the storage of fat as well as for its utilization. It may be that the down-regulation of intestinal UCP2 during high-fat feeding is a way for the organism to optimize fat intake and fat absorption, ATP being necessary, in particular, for the re-esterification of the absorbed fatty acids with glycerol and for the formation of chylomicrons. The adipose tissue, on the other hand, will perhaps need UCP2 in the same situation, perhaps to increase the formation of reduced equivalents, NADH, necessary for lipogenesis.

In the search for an alternative function, UCP2 has been found to regulate insulin secretion (Chan *et al.* 1999, Polonsky & Semenkovich 2001, Zhang *et al.* 2001). Overexpression of UCP2 in insulin-producing cells (INS-1) reduced the glucose-induced insulin secretion (Chan *et al.* 1999). On the other hand, UCP2-deficient mice were found to secrete more insulin in response to glucose than wild-type animals (Zhang *et al.* 2001). Thus, UCP2, by virtue of its proton leakage activity and the consequent decrease in ATP-production, down-regulated the ability of beta cells to secrete insulin. This relationship has led to speculations that the increased level of UCP2, which is observed in obesity, contributes to the development of type 2 diabetes. In fact, induction of UCP2-deficiency in ob/ob mice can partially hamper the development of diabetes and insulin resistance (Zhang *et al.* 2001).

In summary, the role of UCP2 in the energy balance is not clear. It seems that there is actually no role for UCP2 in establishing a metabolic balance as concerns energy expenditure during overfeeding. Rather, the role of UCP2 is to protect the organism against free oxygen radicals, a role that may be particularly important during aging and degenerative disease, conditions related to an excessive production of free radical species.

The role of UCP3 – the second newcomer

Shortly after the discovery of the UCP2 gene, the third member in the family of uncoupling proteins, the UCP3, was discovered (Boss *et al.* 1997, Vidal-Puig *et al.* 1997). The novel UCP had a gene sequence, which was 57% identical to the human UCP1 gene and 73% identical to the human UCP2 gene, the non-identical residues being mainly conservative substitutions. The UCP3 gene was, furthermore, mapped to the distal segment of human chromosome 11q13, adjacent to the UCP2 gene (Solanes *et al.* 1997).

In contrast to the UCP2 gene, the expression of the UCP3 gene has a more specific distribution in the tissue, being restricted mainly to skeletal muscle, in rodents (Vidal-Puig *et al.* 1997, Boss *et al.* 1998, Cadenas *et al.* 1999) as well as in man (Bao *et al.* 1998). The expression of the UCP3 protein was confirmed by Western blot in the mitochondria of skeletal muscle in mice (Harper *et al.* 2002). The protein was not detected in UCP3-ablated mice, verifying the specificity of immunodetection. Calibration against purified UCP3 showed that the concentration of UCP3 was extremely low in the muscle mitochondria, amounting to 0.14 μg mg^{-1} mitochondrial protein. This should be

compared to the concentration of UCP1 found in brown adipose tissue mitochondria, which was about 25–80 μg mg^{-1} mitochondrial protein. Thus, the expression of UCP3 is at least 200–700-fold lower than the expression of UCP1. The low expression of UCP3 is important in the discussion of the function of UCP3 in energy metabolism.

The highly specific expression of UCP3 in skeletal muscle suggested that the gene would be important in the regulation of energy expenditure in this organ. Experiments with UCP3-knockout mice failed to show any important role for UCP3 in regulating whole body energy metabolism, evidenced by at least three different studies (Gong *et al.* 2000, Vidal-Puig *et al.* 2000, Cline *et al.* 2001). These mice did not show any alteration in their metabolic rate compared to wild-type animals. They were not obese, when tested with chow diet or with a high-fat diet. This suggests that the function of UCP3 under normal conditions would not be to protect against diet-induced obesity. The thermoregulation was also normal, even when the animal was challenged with a low ambient temperature. Most surprising in the UCP3-ablated animals was the finding that the tolerance for exercise was unchanged (Vidal-Puig *et al.* 2000). Thus, the weight of the skeletal muscle as well as the resting levels of ATP, ADP and creatine phosphate were similar in the UCP3-ablated animals compared with the wild-type animals. Mitochondrial oxygen consumption in isolated skeletal muscle was, however, significantly reduced in the UCP3-ablated animals compared to the wild-type animals, suggesting the skeletal muscle mitochondria of the KO-animals to be more coupled. Such an observation supports a true proton transport activity of UCP3 and the function of UCP3 to be a genuine uncoupler. That UCP3 is a significant contributor to proton leakage in muscle was verified by experiments on other UCP3-ablated mice (Gong *et al.* 2000, Cline *et al.* 2001), suggesting a true uncoupling activity of UCP3 in skeletal muscle *in vivo*. Using an UCP3-ablated mouse it was found that the rate of ATP synthesis from ADP and phosphate was fourfold higher than in the wild-type animal, suggesting an increased efficiency of ATPsynthesis in the UCP3-ablated animals (Cline *et al.* 2001). Again, at the whole body level there was no change in energy expenditure.

While the uncoupling or proton leakage caused by UCP3 thus does not affect energy metabolism in general there may be a more specific role of UCP3 in muscle metabolism. Such a role would be to regulate proton flow and ATP production in skeletal muscle during rest and during exercise.

As the uncoupling effect of UCP3 is not significantly thermogenic, other roles for such an uncoupling have been looked for. In line with an earlier suggestion, there is now evidence of a role for UCP3 in the protection of tissues against oxidative damage by a decrease in the mitochondrial production of reactive oxygen species (Echtay *et al.* 2002). The superoxides themselves produce a proton leakage in all types of mitochondria, including the muscle mitochondria. However, in the UCP3-knockout mice there was no proton leakage induced by superoxides. This suggests that the superoxides caused proton leakage by interacting with UCP3. How this occurs is unknown, as is the mechanism for proton leakage caused by UCP3. There are at least two schools of thought. One is that UCP3 is a true proton transporter. The other is that UCP3 acts as a fatty acid cycler; as was discussed above for UCP1, fatty acids are the true proton transporters (Garlid *et al.* 2000).

A role of UCP3 as a fatty acid cycler is supported by the fact that UCP3 is upregulated during situations where fatty acids are important as energy substrate molecules, i.e. during starvation (Cadenas *et al.* 1999, Hildebrandt & Neufer 2000) and during high-fat feeding (Samec *et al.* 1999). This regulation by lipid fuel is more pronounced in fast glycolytic 'white' muscles than in the slow oxidative muscles (Hildebrandt & Neufer 2000). Thus, UCP3 would promote fatty acid oxidation by acting as a mitochondrial fatty acid anion transporter, transporting the fatty acids out of the matrix (Dulloo & Samec 2000). This would be helpful in situations in which the delivery of fatty acids into the mitochondria exceeds the capacity to oxidize fat. A role for UCP3 in skeletal muscle is supported by the fact that UCP3 expression is severely decreased in denervated muscle, which could lead to an accumulation of excess energy as fat in the muscle and a decreased metabolic capacity (Kontani *et al.* 2002). Some studies support a role of UCP3 in regulating basal metabolic rate in humans. Thus, in the Pima Indians the expression of UCP3 in skeletal muscle was inversely correlated with body mass index (Schrauwen *et al.* 1999). Also, a strong genetic linkage was found between the UCP2/3 locus and basal metabolic rate in man (Bouchard *et al.* 1997). Furthermore, an overexpression of UCP3 in mouse skeletal muscle resulted in mice that were hyperphagic but weighed less than their wild-type littermates (Clapham *et al.* 2000). Lower levels of fasting glucose and insulin were also measured in these animals. This provides evidence that skeletal muscle UCP3 has the potential to influence the metabolic rate and glucose homeostasis in the whole body.

In conclusion, the exact physiological function of UCP3 is not known. It has been speculated that UCP3 affects energy metabolism, in particular muscle energy metabolism, where it regulates the utilization of fatty acids as energy substrate and also acts as a protector against reactive oxygen species.

The role of UCP4 and UCP5 – the counting downs

UCP4 and UCP5 (Sanchis *et al.* 1998, Mizuno *et al.* 2000, Yu *et al.* 2000) are the two members of the uncoupling protein family most recently described. They are located mainly in the brain and bear sequence similarities, in particular, to the UCP3 gene (Mao *et al.* 1999, Kondou *et al.* 2000). The role of these proteins in energy metabolism is not known, but they have been suggested to affect regulatory centres in the brain as also to provide protection against free-radical oxygen species during aging.

General conclusions

Mitochondrial uncoupling proteins have the potential to dissipate energy as heat by uncoupling oxidative phosphorylation. It has been speculated that these proteins play important roles in energy metabolism. However, as judged by disruption of genes, neither of these proteins affect whole body energy metabolism. The proteins seem to have more specific functions. UCP1 is expressed in brown fat and in rodents is crucial for tolerance to cold. UCP2 may be important for the regulation of insulin secretion from the islets of the pancreas, whereas UCP3 has been speculated to play a role for the regulation of fatty acid metabolism in skeletal muscle. In addition, all three proteins act as free oxygen radical scavengers. It cannot be excluded that experimental induction of the proteins may affect energy expenditure and whole body metabolism.

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