**The uncoupling proteins, a review**

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**Emergence of a new uncoupling protein family**

The uncoupling protein-1 (UCP1), cloned in 1985 (1–4) and called UCP until 1997, is an inner mitochondrial membrane protein (5) expressed exclusively in the brown adipocyte (6–8). It dissipates the mitochondrial proton (H+) gradient generated by the respiratory chain, producing heat instead of ATP (8, 9).

In rodents the brown adipose tissue (BAT) contributes to both the maintenance of body temperature in a cold environment through nonshivering thermogenesis and the control of body weight through the regulatory (or facultative) part of diet-induced thermogenesis (10, 11). It has been shown that during cold acclimation the capacity of brown adipocytes to produce heat is determined by the UCP1 content of their mitochondria (12–14).

Several observations led to the hypothesis that there could exist uncoupling proteins in tissues other than BAT. First, adult humans, who possess very little active BAT (15, 16), produce heat in their skeletal muscle in response to glucose or catecholamine administration (17–20). Secondly, mitochondrial H+ leaks have been observed in tissues devoid of UCP1 (21–23). They may account for up to 50% of the oxygen consumption of some tissues (24, 25), and up to 30% of whole body metabolic rate in the rat (24–26). It was suggested that the H+ leak was related to resting metabolic rate (27, 28).

Supporting the previous observations, several groups independently cloned novel UCPs, i.e. UCP2 (29–31) and UCP3 (31, 32). UCP2 is expressed in most tissues studied in humans and rodents, and UCP3 mainly in skeletal muscle in humans, and BAT and skeletal muscle in rodents (31). UCP1, which was thought to be a protein unique to BAT, is in fact a member of an emerging family of uncoupling proteins expressed in humans and animals, and even in plants (33, 34).

**Regulation of UCP1, UCP2 and UCP3 gene expression**

**What is known about UCP1**

The expression of UCP1 is regulated at the transcriptional level (43), and its control has been extensively studied (35, 44) (Table 1). Norepinephrine is a strong physiological activator of UCP1 expression (45, 46), and activation of the β1-, β2-, β3- and α1-adrenergic receptors (46–48) as well as inhibition of the α2-adrenergic receptor (49) has been shown to increase the expression of UCP1. The thyroid hormone tri-iodothyronine (T3) has been reported to act as a permissive factor for the full induction of UCP1 gene expression by norepinephrine (50, 51).

The expression of UCP1 is also increased by retinoic acid (52–54) and by peroxisome proliferator-activated receptor (PPAR) agonists like thiazolidinediones (55–57).
The expression of UCP3 varies like that of UCP1 in BAT

As shown in Tables 1 and 2, changes in environmental temperature (42, 58, and O Boss, H Ghezraoui, J Seydoux, J-P Giacobino & P Muzzin, unpublished observations), variations in food intake (41, 42, and I Cusin, K E Zakrzewska, O Boss, P Muzzin, J P Giacobino, D Ricquier, B Jeanrenaud & F Rohner-Jeanrenaud, unpublished observations), and administration of T₃ (41, 58) or glucocorticoids (41) affect identically UCP3 mRNA expression in BAT. One exception is the absence of effect of the β₂-adrenergic agonist CL 316,243 on UCP3 mRNA expression in BAT reported by Gong et al. (41).

In the BAT of hereditary obese ob/ob mice (41) and fa/fa rats (42, 46, 47) both UCP1 and UCP3 mRNA expressions are decreased compared with lean controls. The administration of leptin to ob/ob mice increases BAT UCP1 and UCP3 mRNA levels (41).

These observations support the hypothesis that both UCP1 and UCP3 contribute to thermogenesis in BAT.

As shown in Table 3, UCP2 in BAT differs from UCP1 and UCP3 in the way its expression varies with metabolic changes. For instance UCP2 mRNA expression in BAT is insensitive to fasting (41, 59), refeeding (41) and administration of glucocorticoids (41).

In BAT as well as in white adipose tissue (WAT) UCP2 mRNA expression is increased in all rodent models of hereditary obesity tested so far (30, 41, and O Boss O, P Muzzin, F Assimacopoulos-Jeannet & J P Giacobino, unpublished observations), and a significant positive correlation has been reported between UCP2 mRNA levels in WAT and body mass index in humans (60). The pattern of regulations of UCP2 expression supports the idea that UCP2 might have functions other than that of thermogenesis.

The expression of UCP3 often varies in opposite directions in skeletal muscle vs BAT

In contrast to what is observed in BAT, changes in environmental temperature do not affect UCP3 mRNA expression in skeletal muscle (42). These results are in line with the notion that muscle is not involved in nonshivering thermogenesis in rodents.

It has been shown that after 10 days of food restriction (50%) and during the refeeding phase thereafter the metabolic efficiency of rats is increased and remains elevated until the animals have recovered their body fat stores (61). There are two components in
<table>
<thead>
<tr>
<th>Condition</th>
<th>Skeletal muscle</th>
<th>BAT</th>
<th>WAT</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold (48h)</td>
<td>(59), ↑ SO (59)</td>
<td>(59), → (29)</td>
<td>—</td>
<td>(59)</td>
</tr>
<tr>
<td>Fasting (24–48h)</td>
<td>(59), → (41), ↓ (67)</td>
<td>(59, 41)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Severe food restriction (90%)</td>
<td>(60)</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Food restriction (40%)</td>
<td>—</td>
<td>(Cusin et al., unpublished)</td>
<td>—</td>
<td>(Cusin et al., unpublished)</td>
</tr>
<tr>
<td>Refeeding (24h)</td>
<td>(60)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>High fat diet</td>
<td>(133)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Endurance training</td>
<td>(63)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Obesity</td>
<td>(fa/fa (Boss et al., unpublished), ob/ob (41))</td>
<td>(Cusin et al., unpublished), ob/ob (41)</td>
<td>(97, Cusin et al., unpublished)</td>
<td>(134)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>(41), (135)</td>
<td>(41)</td>
<td>(136)</td>
<td>(135, 136)</td>
</tr>
<tr>
<td>Thyroid hormone (T3)</td>
<td>(135, 136)</td>
<td>(41)</td>
<td>(136)</td>
<td>(135, 136)</td>
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<tr>
<td>Insulin (3h)</td>
<td>(60)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ro 16–8714 (30h)</td>
<td>(fa/fa (Boss et al., unpublished), ob/ob (41))</td>
<td>(59, → fa/fa (Boss et al., unpublished), ob/ob (41))</td>
<td>—</td>
<td>(29, 41)</td>
</tr>
<tr>
<td>CL 316.243 (10 days)</td>
<td>(29, 41)</td>
<td>(29, 41)</td>
<td>(29, 41)</td>
<td>(41)</td>
</tr>
</tbody>
</table>

SO = soleus muscle, fa/fa = obese fa/fa Zucker rats, ob/ob = obese ob/ob C57BL mice, db/db = obese db/db C57BL mice.

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In this study, the regulation of UCP2 mRNA expression was examined under various conditions, including fasting, food restriction, endurance training, obesity, hypothyroidism, thyroid hormone (T3) treatment, insulin, Ro 16-8714, and CL 316.243. The table shows the changes in UCP2 mRNA expression in skeletal muscle, BAT, WAT, and heart tissues under these conditions. Notably, fasting and severe food restriction led to an increase in UCP2 mRNA expression, while hypothyroidism and insulin treatment resulted in a decrease. The mechanisms behind these changes are complex, involving factors such as free fatty acids, thyroid hormone, and insulin, which play roles in the regulation of energy metabolism. Further studies are needed to fully understand these regulatory mechanisms and their implications in metabolic diseases and obesity.
Physiological relevance of UCP1, UCP2 and UCP3, and their potential role in human disease

Possible role of UCP2 in the maintenance of low levels of oxygen and oxygen radicals

Under resting conditions energy consumption is low and the availability of ADP for phosphorylating respiration decreases, which may result in an increase in intracellular levels of $O_2$ and one-electron $O_2$ reductants (93). These conditions could enhance the formation of reactive oxygen species (ROS), e.g. superoxide anion, hydrogen peroxide and hydroxyl radical, and then cause oxidative damage within the cell. It has been proposed by Skulachev (93) that nonphosphorylating (uncoupled or noncoupled) mitochondrial respiration allows the maintenance of low levels of both $O_2$ and ROS when phosphorylating respiration fails to do so due to a lack of ADP. An increase in the $H^+$ leak of the mitochondrial membrane in State 4 (without available ADP) would thus stimulate $O_2$ consumption and decrease the formation of ROS (93).

A role for UCP2 as a regulator of mitochondrial hydrogen peroxide generation has been proposed by Ne`gre-Salvayre et al. (94). The authors showed that addition of the UCP1 inhibitor GDP to mitochondrial fractions from liver nonparenchymal cells or from BAT, which both highly express UCP2 mRNA, raises both the mitochondrial membrane potential and hydrogen peroxide production. GDP was completely ineffective on mitochondria from hepatocytes, which are deficient in UCP2 (94). The authors suggested that UCP2 activity is inhibited by GDP and that UCP2 is able to modulate hydrogen peroxide formation, supporting a role for UCP2 in the control of cellular processes involving free radicals generated by mitochondria, such as oxidative damage, inflammation, or apoptosis (94).

UCP2 and insulin secretion

Glucose-induced insulin secretion by pancreatic islet cells has been shown to require mitochondria (95), and to involve an increase in the cellular ATP/ADP ratio (96). UCP2 is expressed in pancreatic islets (97) and might, by decreasing the cellular ATP levels, blunt glucose-induced insulin secretion. Leptin, which decreases the insulin response to glucose and other fuels (98), was found to increase UCP2 mRNA expression in pancreatic islets (97). It can be hypothesized that a high level of UCP2 could decrease insulin secretion.

In obese diabetic rodents defects in insulin secretion are often associated with hyperlipidemia and increased triglyceride content in pancreatic islets (97, 99). It can
be hypothesized that increased levels of FFAs in pancreatic islet cells may enhance UCP2 expression, and in turn, impair insulin secretion. The effect of FFA on UCP2 gene expression might be mediated by PPARγ since troglitazone has been shown to increase UCP2 mRNA levels in pancreatic islets (68).

**UCP1, UCP2 and UCP3 as obesity gene candidates**

In most genetically obese rodents BAT is poorly active (35) and the level of UCP1 mRNA and/or UCP1 is generally lowered (46).

Lowell et al. (100) obtained transgenic mice with severely reduced BAT mass using a suicide transgene encoding the diphtheria toxin A chain (DTA) downstream from the UCP1 promoter. The transgenic UCP1-DTA mice developed obesity and then hyperphagia, diabetes and hyperlipidemia (100, 101). On the other hand, Kopecky et al. (102) observed in obese transgenic mice expressing UCP1 in their WAT (aP2-UCP1 transgene) a decrease in body weight and in WAT. UCP1-knockout mice were found to be cold-sensitive but neither hyperphagic nor obese (103). This suggests that UCP1 plays an essential role in cold-induced thermogenesis. The fact that UCP1-knockout mice did not become hyperphagic and obese suggests either that UCP1 is not essential for the control of body weight or that its absence can be compensated for by other dissipating pathways. An increase in UCP2 mRNA expression was observed in BAT of UCP1-knockout mice (103), and a possible compensatory role of UCP3 in BAT and/or in skeletal muscle has not yet been tested. These studies suggest that a functional BAT, but not UCP1, is essential to prevent the development of obesity in rodents. On the other hand, ectopic expression of UCP1 in WAT induces body fat loss.

In humans, a polymorphic BclI site (104) resulting from an A/G mutation (105) has been described in the 5'-flanking region of the UCP1 gene. This polymorphic site was reported to correlate significantly with the percentage of body fat gain over time (104), to be a predictive factor associated with high weight gain during adult life in morbidly obese subjects (106), and to be associated with a low body weight loss after caloric restriction (107). These studies suggest that UCP1 may play a role in energy balance and weight gain in humans.

In addition, five different amino acid polymorphisms in UCP1 (R/W40, A/T64, V/M137, M/L229 and K/N257) and two nucleotide substitutions in the nontranslated region of exon 1 have been reported (108). However, none of these polymorphisms seems to be a common factor contributing to obesity in the population studied (108).

Because UCP2 and UCP3 might be involved in energy dissipation they might participate in weight regulation. Mutations in their genes could play a role in the development of obesity and/or noninsulin-dependent diabetes. A significant linkage between flanking markers in the vicinity of the UCP2 gene and resting metabolic rate in a Canadian population has been reported by Bouchard et al. (109), whereas no significant linkage was found with body mass index or central adiposity in Northern European, type 2 diabetic patients (110). A common amino acid polymorphism (A/V55) in UCP2 has been found which does not seem to be implicated in the pathogenesis of obesity or insulin resistance in Caucasians of Danish ancestry (111).

The UCP3 gene was shown to lie in the same region as the UCP2 gene, i.e. in 11q13 between markers D11S916 and D11S911 or D11S3966 (37, 38, 41). Thus, the linkage observed by Bouchard et al. (109) between these markers and resting metabolic rate may be due to variations in the UCP2, UCP3 or another gene in the vicinity. The identification of polymorphic sequences in the UCP2 and UCP3 genes will allow for more specific linkage studies.

**UCP2 and UCP3 as novel targets for anti-obesity and anti-diabetes drugs**

The recent discovery of these novel uncoupling proteins brings new hope to obesity research, as the expression level and/or the activity of these proteins may play a role in the differences in metabolic rates (112) and body fat mass (113) observed between individuals.

Even if no defects in the UCP2 and UCP3 genes can be associated with human obesity, these proteins could still represent good targets for drugs aimed at increasing energy expenditure. The maintenance of skeletal muscle UCP3 expression during food restriction and refeeding might prevent the observed increase in food efficiency and might help lose or not regain weight. It could therefore break the vicious circle of what is called the 'yo-yo' phenomenon.

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