due to macrophages having acquired a higher than normal killing potential. In light of these observations, we propose that the uncoupling of kidney mitochondria by $O_2^-$ is mediated by UCP2, but this proposal requires further investigation.

The help of Stephen Roebuck in the preparation of mitochondria is gratefully acknowledged.

The physiological function of uncoupling protein

**References**


Received 11 June 2001

---

**UCP3 and its putative function: consistencies and controversies**

M.-E. Harper*, R. M. Dent†, V. Bezaire*, A. Antoniou*, A. Gauthier*, S. Monemdjou* and R. McPherson*

*Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, 451 Smyth Road, Ottawa, ON, Canada K1H 8M5, and †Department of Psychiatry, Faculty of Medicine, University of Ottawa, 451 Smyth Rd, Ottawa, ON, Canada K1H 8M5

**Abstract**

The physiological function of uncoupling protein 3 (UCP3) is as yet unknown. Based on its 57% homology to UCP1 whose physiologic function is uncoupling and thermogenesis, UCP3 was attributed with the function of mitochondrial uncoupling through proton-leak reactions. UCP3 is expressed selectively in muscle, a tissue in which it has been estimated that proton leak accounts for approx. 50% of resting energy metabolism. Genetic linkage, association and variant studies suggest a role for UCP3 in obesity and/or diabetes. Studies of the heterologous expression of UCP3 in yeast
provide support for the idea that UCP3 can un-couple mitochondrial oxidative phosphorylation, but the physiological relevance of these results is questionable. In vitro studies of mitochondria from Ucp3\(^{-}\) mice provide support, but there are no changes in resting metabolic rate (RMR) of mice. In vivo studies demonstrate increased ATP synthesis, but estimates of substrate oxidation rate indicate no change. Mice that greatly overexpress Ucp3 in muscle have increased RMR. Inconsistent with the function of uncoupling are the observations that fasting results in increased expression of UCP3, but no change in muscle proton leak. Moreover, fasting decreases energy expenditure in muscle. Expression patterns for Ucp3 and lipid-metabolism genes support a physiological role in fatty acid oxidation. Overall, findings support a role for Ucp3 in fatty acid metabolism that may have implications for obesity and/or Type II diabetes.

**Introduction**

Uncoupling protein (UCP) 3 is a mitochondrial carrier protein of as-yet undefined physiological function. The gene is found adjacent to that for Ucp2 on chromosome 7 in mice and on chromosome 11 in humans [1]. Alternative splicing results in two mRNA transcripts, referred to as the short (Ucp3\(_s\)) and long (Ucp3\(_l\)) forms [1]. The short form does not include the sequences corresponding to the last (sixth) transmembrane domain. In mice, findings suggest that only the long form is expressed. Very recent findings suggest that this is also the case in human muscle [2]. The short and long forms of UCP correspond to proteins of 275 and 312 amino acids in length, respectively [1], but again, there is some doubt as to whether the short form is translated into a functional protein. The gene is expressed predominantly in skeletal muscle in humans, and also in brown adipose tissue of mice and rats [3,4].

Since the cloning of its gene in 1997 [3,4] there has been an enormous amount of interest in Ucp3 and over 200 papers have been published. Initial hypotheses have linked UCP3 with the function of mitochondrial proton leak. The prospect that this protein might be responsible for the substantial amount of energy expenditure attributed to mitochondrial proton leak has been at the root of the groundswell of interest. Proton leak has been estimated to account for 20–50\% of cellular energy expenditure, and roughly 20\% of the standard metabolic rate (in rats; see [5]). Thus the possibility that this process could be exploited as a means to treat obesity is being explored.

Various approaches have been used to address the question: does UCP3 cause mitochondrial proton leak? These have included heterologous expression in yeast, the use of liposome systems into which recombinant protein is inserted, and the creation of Ucp3\(^{-}\) mice and of mice in which Ucp3 is overexpressed. The effects of different physiological stimuli where the expression of Ucp3 is altered (e.g. responses to fasting and hyperthyroidism) have also been explored. Despite the high degree of scientific attention directed at UCP3, its primary physiological function is still however unknown.

In this brief review we examine what the recent literature is suggesting with regard to the physiological function of UCP3, and how UCP3 may be related to energy metabolism. Regardless of the specific mechanism of action of UCP3, there are several reports in the literature that suggest a role for Ucp3 in fat metabolism and in the development of obesity and/or Type II diabetes.

**Mitochondrial proton leak and energy expenditure**

Mitochondrial proton leak has been studied extensively by Brand and colleagues for over a decade [6], and there are some excellent recent reviews [5,7]. Mitochondrial proton leak refers to the process(es) by which protons return to the mitochondrial matrix from the mitochondrial inter-membrane space through a route other than ATP synthase. A major consequence of proton leak is the loss of some of the potential energy stored in the electrochemical gradient referred to as proton-motive force (PMF). The energy is thus not captured through ATP synthesis, but is lost as heat. Proton leak results in a drop in PMF and this, in turn, results in increased substrate oxidation to fuel the pumping of protons by complexes of the electron transport chain, which in turn restores PMF.

Mitochondrial proton leak contributes significantly to energy expenditure. This has been demonstrated in studies of isolated mitochondria and cells, and also in intact tissues. Estimates of the contribution of leak to resting cellular energy expenditure are in the order of 25\% and 50\% for rat hepatocytes and hind-limb muscle, respectively [8,9]. Under non-resting conditions, findings suggest that leak continues to make a smaller but still very significant contribution to cellular energy expenditure.
Cloning of Ucp3: the missing leak protein?

In 1997 the Ucp3 gene was cloned based on its homology to the well-known uncoupling protein, Ucp1 [3,4]. Given its homology to UCP1 (57\%), the pronounced capacity for UCP1-mediated uncoupling in brown adipose tissue and the results of initial analyses of UCP3 function, it was logical and very exciting to consider that UCP3 might be responsible for mitochondrial proton leak in muscle of humans.

Uncoupling-mediated thermogenesis is the primary function of brown adipose tissue and it is of major importance for thermoregulation in newborn humans and in rodents. The fact that the Ucp1-deficient mouse is cold-intolerant confirmed the long-standing belief amongst researchers that the physiological function of UCP1 was indeed heat production [17]. On account of its ability to uncouple oxidative phosphorylation and its thermoregulatory importance, it was however a surprise to many that the Ucp1-deficient mouse was not more prone to obesity than wild-type control mice [17].

Initial results published at the time of the cloning of Ucp3, or shortly thereafter, support the idea that UCP3, similar to UCP1, is an effective uncoupler. Ucp3 has been expressed in yeast systems, and most results have shown that mitochondrial PMF is decreased and that State 4 respiration is increased (e.g. [3,4,18,19]). While the latter findings are consistent with a role for UCP3 as an uncoupling protein, many findings could possibly be explained as a result of the overexpression of a heterologous protein. Stuart et al. [5] describe several potential problems associated with this sort of approach for the study of UCP3 function. The principal concerns are 3-fold, and relate to (1) the fact that, in many of the studies, the heterologous expression of Ucp3 in yeast results in a decrease in FCCP (carbonyl cyanide p-trifluoromethoxyphenylhydrazone)-induced maximal rates of respiration (not an increase as might be expected with significant uncoupling), (2) the inference in some studies of the presence and activity of the heterologous protein based simply on the phenotype of uncoupling and (3) unknown levels of protein expressed [5].

Notwithstanding the above criticisms, a role for Ucp3 and/or Ucp2 in total body energy expenditure has been supported by genetic linkage, association, and variant studies. Bouchard et al. [20] demonstrated that the chromosomal locations of Ucp2 and Ucp3 are linked to basal metabolic rate. It must be remembered that as the genes for Ucp2 and Ucp3 are only 6000 bp apart, the linkage between Ucp3 and energy expenditure or obesity applies equally to Ucp2, or to another closely linked gene. Several variants in the coding region of Ucp3 have been identified (e.g. [21–25]). Overall, results suggest that variation in the coding region is not a common factor in the development of obesity or insulin resistance. However, mRNA expression of Ucp3 was found to correlate negatively with body-mass index and positively with sleeping metabolic rate in studies of Pima Indians [26]. Moreover, an association has been reported between a polymorphism in the splice-donor junction site of exon 6 of Ucp3, and respiratory quotient in a population of African Americans [23]. Chung et al. [25] studied the same polymorphism in a larger population in Maywood, IL, U.S.A., and were not able to detect the differences in fatty acid oxidation that were reported by Argyropoulos et al. [23]. Unfortunately, Chung et al. were limited to analyses of two individuals.
for their analyses of respiratory quotients, fat oxidation, resting metabolic rate and muscle mitochondrial characteristics. Altogether, results suggest that Ucp3 may be an important obesity/Type II diabetes gene.

**Ucp3 expression: paradoxical findings**

There is significant debate on the question of the true physiological function of UCP3. Much of the debate has arisen from observations that Ucp3 mRNA expression in muscle increases during fasting and severe food restriction in rats and mice [27,28], physiological situations where muscular energy expenditure is known to decrease [29]. The uncoupling of oxidative phosphorylation by UCP3 would thus run counter to adaptive decreases in energy expenditure. These findings are much more consistent with a role for UCP3 in the regulation of fatty acid metabolism than in thermogenesis and energy balance. The latter paradox, and a hypothesized role for UCP3 in lipid handling, have been championed by Dulloo and colleagues [30].

The findings of Cadenas et al. [31] are also significant in countering the idea that the primary physiological function of UCP3 is mitochondrial proton leak. Their studies in rats showed that a 24 h fast resulted in increased expression of Ucp2 and Ucp3 mRNA and their corresponding proteins in muscle of rats, but no change in mitochondrial proton leak.

These and other inconsistencies, i.e. with the original hypothesis that UCP3 uncouples oxidative phosphorylation under physiological situations, will be further discussed below.

**Life without Ucp3**

Transgenic technologies have allowed the creation of mice that are deficient in Ucp1 [17], Ucp2 [32], Ucp3 [33,34] and both Ucp1 and Ucp3 [33]. In not one of these transgenic mouse models has a difference in metabolic rate been detected. Even in the cold-intolerant Ucp1-- mouse, no difference has been reported as yet. However, the detection limit of indirect calorimetry is approx. 7 O0 at best, and thus in some of these transgenic mouse models, any difference in the metabolic rates between transgenic and wild-type mice may be hidden in the ‘noise’ and hence undetectable. Food intake and levels of adiposity (even following the feeding of a high-fat diet) are also similar between Ucp-deficient mice and controls. The interpretation of results of knockout mouse studies need however to be tempered by the recognition that there may be many adaptive ‘secondary’ responses to the genomic manipulations.

Two groups simultaneously provided the first reports on the Ucp3-- mouse [33,34]. Vidal-Puig et al. [34] found that skeletal-muscle mitochondria lacking UCP3 are better coupled (improved respiratory control ratio), and produce higher amounts of reactive oxygen species than control mitochondria. Based on the hypothesized role for UCP3 in the metabolism of the fatty acids, it was expected that the mice might demonstrate abnormal oxidation of lipids, as assessed by indirect calorimetry, and perhaps by abnormal serum lipid profiles. Respiratory quotients were studied under fed and fasting states, but no differences were detected (see below). However, significantly higher levels of free fatty acids were noted in older (18–24 week) mice when fed a high-fat diet.

Similarly, Gong et al. [33] reported that the absence of UCP3 in mice results in a phenotype at the whole-body level that is not different than that of controls. Studies of the overall kinetics of the mitochondrial proton-leak reactions showed significantly higher mitochondrial PMF values in skeletal-muscle mitochondria of Ucp3-- mice compared with control mice. This is consistent with significantly lower rates of mitochondrial proton leak in Ucp3-- mice, or with another electrophoretic role for UCP3. It is of interest that Gong et al. [33] report no significant change in State 4 respiration, which represents the maximum leak-dependent respiration. The latter findings have been corroborated in further studies in our laboratory. Moreover, in our more recent studies we were able to detect significant impairments in fatty acid oxidation at the whole-animal level, as determined by indirect calorimetric measures of respiratory quotients [35]. These findings and the patterns of Ucp3 expression under conditions where fatty acid oxidation is high lead us to believe that UCP has some dissipative effects on PMF, and plays an important role in fatty acid metabolism.

**Life with abundant Ucp3**

To address the question of phenotypic effects of increased levels of UCP3 in muscle, Clapham et al. [36] generated transgenic mice that overexpress human Ucp3 (hUcp3). hUcp3 transcription was driven by the mouse z-actin promoter, and levels of expression were found to be approx. 66-fold greater than the endogenous expression of Ucp3.
Overexpression at the level of protein has not been reported. Mice were found to have significantly lower body weights, despite increased food intake, compared with controls. Following the feeding of a palatable diet (condensed milk diet) the hUcp3 mice still displayed lower body weights despite higher energy intakes. Muscle mitochondrial proton conductance is increased in hUcp3 mice compared with wild-type controls. Mice overexpressing hUcp3 were also more insulin-sensitive, and had lower plasma total cholesterol than wild-type mice. While this level of overexpression is unlikely to be achieved physiologically, these mice provide a very important model in which to address the mechanisms, and supporting pathways through which metabolism is affected (see the discussion below of recent findings from hUcp3 mice).

**Ideas on the physiological function of UCP3**

Despite the large numbers of papers published over the last 4 years, it is not clear what the physiological function of UCP3 is. Why does gene expression increase during fasting and under all situations where there are increased circulating levels of fatty acids? These are most often situations where efficiency in energy metabolism would be a priority, not situations where muscular thermogenesis and energy wastage would be desirable. It is interesting and relevant that while the physiological function of UCPI is well recognized as thermogenesis, mechanistic aspects of its function are still debated [37,38]. Ucp3 may cause mitochondrial proton leak under some situations, but it may have more important functions in the transport of other metabolites such as those related to fatty acid oxidation, as suggested several years ago by Dulloo and colleagues [30].

However, a mechanism to explain how UCP3 might enhance fatty acid oxidation was not proposed until recently [39]. This hypothesis outlines how UCP3 could facilitate rapid rates of fatty acid oxidation by acting as a mitochondrial fatty acid efflux protein. It was proposed that UCP3 functions in concert with mitochondrial thioesterase(s) (MTE) to remove free fatty acid (produced by MTE) from the matrix and liberate CoA. The latter is in relatively high demand during fatty acid oxidation.

Some support for the idea that UCP3 may function as a fatty acid efflux protein was recently gleaned in further studies of the hUcp3-overexpressing mouse (described above). Moore et al. [40] studied the expression of genes related to fatty acid metabolism in muscle. Genes included those for carnitine palmitoyl transferase, lipoprotein lipase, MTE-1 and CD36, amongst others. The most marked change in gene expression was a 3-fold increase in MTE-1 mRNA. Lipoprotein lipase expression also increased, but to a lesser extent (50%).

We and others have been exploring muscle Ucp3 expression in relation to weight loss under carefully defined clinical conditions. The recent findings of Schrauwen and colleagues [41] are of particular interest in light of the above discussion. The effect of weight loss on Ucp2 and Ucp3 expression and UCP3 protein was examined in the muscle of seven obese men with Type II diabetes. Subjects were treated with 10 weeks of a hypo-caloric diet which consisted of a 2000 kJ/day meal-replacement program (Modifast, Novartis) for 4 weeks, followed by the gradual addition of energy-restricted meals to achieve a stable weight condition in the last 2 weeks of the programme. Ucp3 mRNA decreased from pre-diet measurements, and the changes in Ucp3 mRNA and protein were negatively associated with changes in body weight and body-mass index. Moreover, these changes were positively correlated with changes in cytosolic fatty acid-binding protein. Authors conclude that their findings support a role for Ucp3 (and Ucp2) in the handling of lipids as a fuel.

**Summary and conclusion**

Altogether, findings support a role for Ucp3 in fatty acid metabolism that may have implications for obesity and/or Type II diabetes. While there are many findings in the literature that are consistent with a role for UCP in mitochondrial proton leak, there are a number of significant controversial findings which suggest that the primary physiological role of UCP3 is in the regulation of fatty acid metabolism.

**References**


© 2001 Biochemical Society
Mitochondrial Uncoupling Proteins


Received 30 May 2001

773 © 2001 Biochemical Society