MINIREVIEW

Physiological Role of UCP3 May Be Export of Fatty Acids from Mitochondria When Fatty Acid Oxidation Predominates: An Hypothesis

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This hypothesis proposes a physiological role for uncoupling protein-3 (UCP3) in the export of fatty acid anions from muscle and brown adipose tissue (BAT) mitochondria when fatty acids are the predominant substrate being used. It proposes that excess acyl CoA within the mitochondria is hydrolyzed by a mitochondrial acyl CoA thioesterase, yielding fatty acid anion and CoASH. The fatty acid anion is exported to the cytosol by being carried across the inner mitochondrial membrane by UCP3. The CoASH is conserved within the mitochondrion to participate in other reactions for which it is needed during fatty acid oxidation in the β-oxidation cycle and in the tricarboxylic acid cycle. The export of the fatty acid anion thus permits continued rapid fatty acid oxidation in the face of an oversupply. The hypothesis provides a logical explanation for the observed up-regulation of gene expression for UCP3 in muscle when there is a switch to fatty acid oxidation, as during fasting, and in BAT when fatty acid oxidation is stimulated, as during exposure to cold. It provides a plausible physiological role for UCP3 as a transporter protein, not as an uncoupling protein. [E.B.M. 2001, Vol 226:78–84]

Key words: uncoupling proteins; fatty acids; skeletal muscle; brown adipose tissue; acyl CoA thioesterase; thermogenesis; cold; mitochondrial carrier proteins

The Original Uncoupling Protein, UCP1

The discovery of cold-induced nonshivering thermogenesis and its location in brown adipose tissue (BAT) in the 1950s to 1970s was soon followed by purification of the first uncoupling protein (UCP1) and later by cloning of the gene for UCP1 (1, 2). UCP1 is expressed exclusively in BAT. It exerts its uncoupling function only when BAT is stimulated via its sympathetic nerve supply. Uncoupling does not occur without such stimulation. Thus, the function of the UCP1 is tightly regulated (3).

The molecular mechanism by which UCP1 exerts its uncoupling function is still under intense investigation (3–12). There are two current hypotheses. One suggests that UCP1 acts directly as an inward proton translocator (10, 11). The other suggests that UCP1 acts as an outward fatty acid anion carrier, with the fatty acid anions picking up a proton and re-entering directly across the lipid bilayer of the inner membrane, delivering the proton to the matrix ("flip-flop" acidification of the matrix) (6–9). In both hypotheses, the end result is entry of protons into the matrix, dissipation of the proton gradient, and uncoupling of oxidative phosphorylation, which leads to increased oxidation of substrates. Fatty acids are the predominant substrate for thermogenesis in BAT, mobilized by stimulated lipolysis from the triacylglycerol reserves in the BAT.

The vital role that uncoupling of oxidative phosphorylation by UCP1 in BAT plays in protection against cold is illustrated by the marked cold-intolerance of UCP1 knockout mice (13). The idea that thermogenesis in BAT mediated by UCP1 might be a major component of energy expenditure under certain circumstances, and that a defect in this function might, therefore, be conducive to obesity, has been discussed over many years (2, 14). However, transgenic mice deficient in UCP1 do not become obese and presumably compensate in some way for the absence of UCP1-mediated thermogenesis when they live at room temperature.

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Newer UCPs

In contrast to the sequence of events outlined above, in which a physiological function was known first and a protein that mediated this function later identified and cloned, the last few years have seen the opposite sequence of events (15). Four more homologous proteins have been cloned, but they remain in search of the physiological functions that they mediate. The recent cloning of UCP2 (16–18), of UCP3 (18–20), of BMCP-1 (21, 22) (also referred to as UCP5 by some authors [23, 24]), and of UCP4 (23, 24) has stimulated much research. However, their functions under physiological conditions are not understood as yet. Each has its own tissue specific expression, but all have a much wider tissue distribution than UCP1. These new members of the mitochondrial carrier protein superfamily all bear homology to UCP1 and to other members of the family, such as the adenine nucleotide transporter (ANT) and the phosphate carrier (25). Up to 29 members of this family probably exist, but most of these have not yet been identified (7, 8).

Current explanations for the functions of the newer UCPs are based on the assumption that they do indeed serve to uncouple oxidative phosphorylation, as does UCP1, and that they might therefore influence energy expenditure and energy balance. The most obvious candidate for a physiological phenomenon that might be mediated by one or more UCPs is the proton leak in mitochondria in all tissues. However, there is so far no evidence that any UCP mediates the proton leak under physiological conditions (26, 27). Recent reviews have concluded that these novel "uncoupling" proteins might have an entirely different function (3, 28–30), and they have been listed as transporters of unknown function (28). In agreement with this view, recent studies of UCP3 knockout mice have not revealed any major role of this protein in energy balance and have suggested a role other than uncoupling (31, 32).

This review proposes a role of UCP3 in fatty acid metabolism that does not involve an uncoupling function, although it does involve an accepted property of this protein, namely, the ability to transport fatty acid anions (see below).

Expression of UCP3 in Muscle Is Strongly Correlated with Fatty Acid Oxidation

Expression of UCP3 is confined to tissues that are heavily dependent on fatty acid oxidation, namely, BAT and skeletal muscle. The hypothesis presented here applies specifically to these tissues.

Studies of changes in expression of UCP3 in skeletal muscle indicate a close relationship to situations in which there is increased oxidation of fatty acids by the muscle. The marked increase in mRNA level for UCP3 induced by fasting in both mice and rats (reviewed in [25, 33]) initially sparked speculation about a thermogenic role for this protein in muscle. However, the well-known reduction in energy expenditure observed in muscle during fasting speaks against any increase in uncoupling and suggests the possibility of some other function of UCP3. Many studies have revealed that any physiological situation in which plasma FFA level is increased is associated with increased mRNA level for UCP3 in muscle. There is a transient increase in mRNA level for UCP3 in muscle in the early stages of exposure to cold. Thus, in shivering skeletal muscle there is a transient increase between 3 and 24 hr (34, 35), followed by a return to normal levels (34, 35), and then a reduction to lower than normal levels by 6 to 10 days (35, 36). These changes are associated with a transient rise in plasma FFA level (37). An increase is also seen in muscle of rats with acute STZ-induced diabetes and a high level of FFA in their blood (38–40). An increase is also seen in animals with cancer cachexia (41, 42). The transient increase seen after a bout of exercise (43–46) is probably associated with the known increase in FFA level at this time that is due to the mismatch between the abrupt reduction in utilization of FFA and the slower reduction in FFA mobilization from WAT. UCP3 mRNA appears in muscle of newborn rats only after birth, and this is correlated with the rise in blood FFA at that time (47). More convincing than the many associations between plasma FFA concentration and muscle UCP3 mRNA is the finding that a simple increase in the FFA level in blood produced by administration of a lipid emulsion increases mRNA level for UCP3 in muscle (48, 49). Not only mRNA for UCP3 but also mRNAs for other proteins involved in mitochondrial fatty acid oxidation are increased by fasting (e.g., CPT1, LCAD) (45). Thus, appearance of UCP3 mRNA in muscle correlates with a switch to oxidation of fatty acids in this tissue, whether thermogenesis is increased (as during shivering or exercise) or reduced (as during fasting) (50, 51).

That the fasting-induced increase still occurs in rats at thermoneutrality (29°C) (50) indicates the lack of thermoregulatory implications of the process. (This contrasts with the fasting-induced increase in mRNA for UCP1 in BAT of rats at 23°C which is prevented by keeping the animals at thermoneutrality [28°C] [52] and clearly is activated for thermoregulatory purposes.) Muscle mitochondria of UCP3 knockout mice have a higher membrane potential but no change in state 4 respiration, suggesting no reduction in proton leak but some change in their bioenergetic processes (31). Despite an increase in UCP3 protein in muscle of fasting rats (53, 54), no increase in proton leak and no change in membrane potential are detectable in the muscle mitochondria of these animals (53). Thus, either any potential uncoupling function of the UCP3 is not switched on in isolated mitochondria or UCP3 does not mediate uncoupling in muscle mitochondria.

Changes in muscle UCP3 mRNA are thus not related to thermoregulatory needs, even in animals shivering during exposure to cold, and not to the energy expenditure of the muscle, which can be low or high, but rather to the switch to use of FFA as a substrate. A role for UCP3 in fatty acid

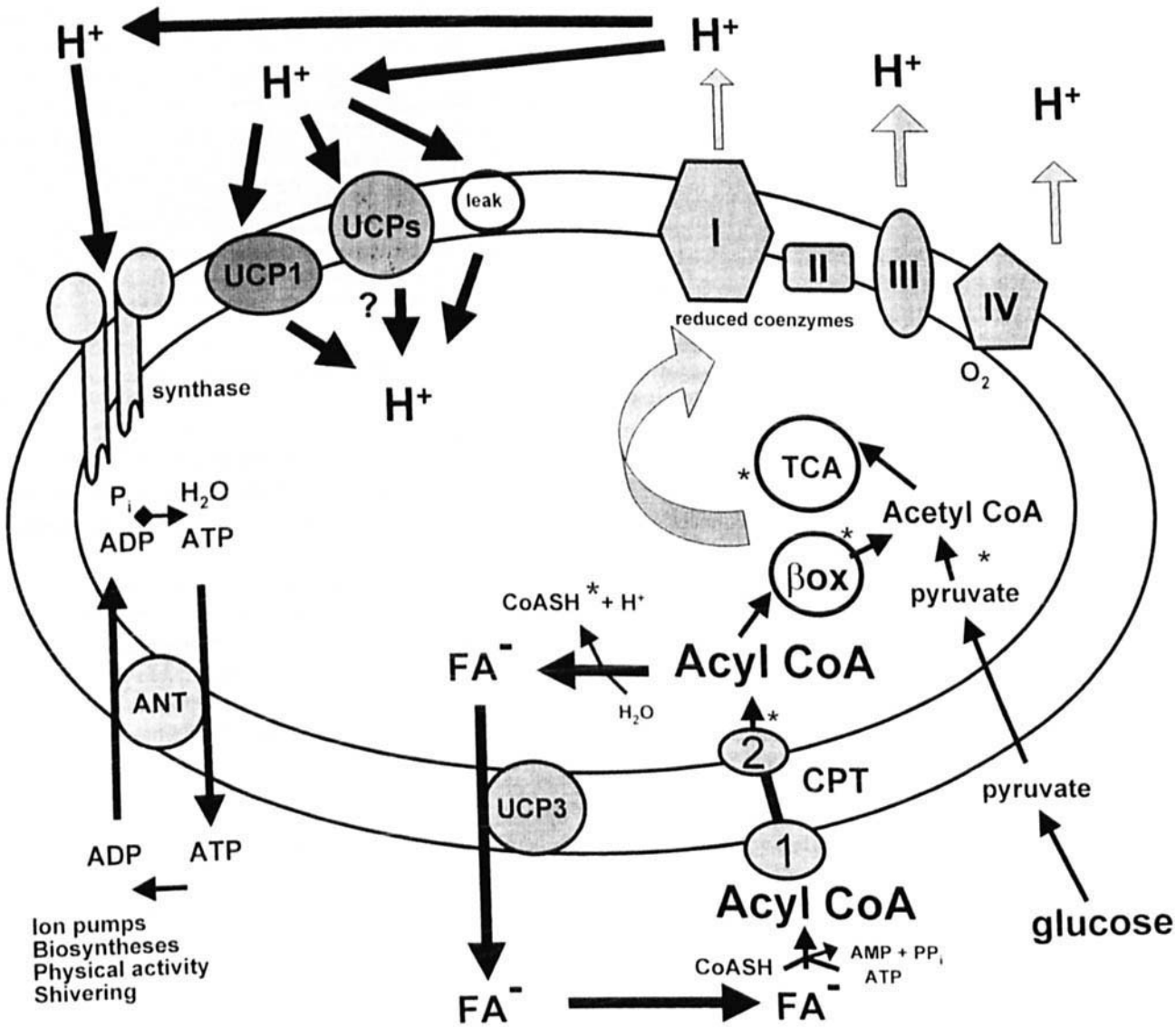
metabolism has frequently been suggested, but the exact nature of this role has not been clarified (3, 28–30, 55).

In BAT, UCP3 mRNA levels are increased when fatty acid oxidation is stimulated, as during exposure to cold (34, 36, 56, 57), and is reduced when it is inhibited, as in animals at thermoneutrality (57). In BAT, the cold-induced increase is mediated by the sympathetic nerves to the tissue (56). In contrast to muscle, in which raised concentration of fatty acids in blood is associated with increased UCP3 mRNA levels, BAT exhibits reduced UCP3 mRNA levels during fasting (20, 34, 50, 54, 58), probably secondary to the known suppression of sympathetic nervous system activity under these conditions. These changes in UCP3 mRNA level would be consistent for a role of UCP3 in BAT thermogenesis, as they are the same as those for UCP1. However, lack of a role for UCP3 in thermogenesis in BAT is indicated by the lack of thermogenic response to noradrenaline by brown adipocytes of UCP1 knockout mice (5). These adipocytes would have UCP3 in their mitochondria and control mechanisms, including stimulated lipolysis, appropriate for thermogenic functioning of UCP1, but UCP3-mediated uncoupling is not evoked. Lack of a role for UCP3 in BAT thermogenesis is also indicated by the cold-intolerance of the UCP1 knockout mice, in which UCP3 does not substitute for UCP1 in cold-induced nonshivering thermogenesis (13).

All the foregoing strongly suggests that UCP3 plays a role in mitochondrial fatty acid oxidation in both muscle and BAT, but that this role is not that of an uncoupler. Elucidating the nature of this role requires detailed consideration of the metabolic pathways of fatty acid oxidation in muscle and BAT, both in cytosol and mitochondria, and of how UCP3 might fit into them.

Pathways of Fatty Acid Metabolism in Muscle

Metabolic pathways involved in fatty acid oxidation and their compartmentalization in cytosol and mitochondrion are well described in textbooks. Plasma FFA enter the



muscle or are produced by stimulated lipolysis in the brown adipocyte and are activated to acyl CoA in the cytosol. Figure 1 illustrates the principal features of fatty acid oxidation in these tissues. The acyl group in the cytosol is then transferred to carnitine by CPT1 (carnitine palmitoyl transferase 1) at the outer surface of the mitochondrial inner membrane. After traversing the inner membrane, the acyl group is transferred back to coenzyme A by CPT2 (carnitine palmitoyl transferase 2) and the carnitine returns across the inner membrane to the outside. (The carnitine shuttle is needed because coenzyme A itself does not cross the inner membrane readily. There are thus two pools of cellular coenzyme A, one in the mitochondrial matrix, the other in the cytosol, and these pools are separated by the mitochondrial inner membrane.) The acyl CoA formed in the matrix of the mitochondrion then enters the pathway of β-oxidation, for which the enzymes are located on the inside of the inner membrane or in the matrix. The acetyl CoA produced in this cycle is oxidized in the tricarboxylic acid cycle, with enzymes also on the inside of the inner membrane or in the matrix. The reduced coenzymes, NADH and $FADH_2$, produced in these pathways are then oxidized in the electron transport system, entering at Complex I or at Complex II, respectively. The proton gradient across the mitochondrial inner membrane produced during their oxidation drives the synthesis of ATP by the proton-translocating ATP synthase.

Note that fatty acids do not appear in the mitochondrial pathway. Mitochondria do not import fatty acids from the cytosol and are unable to convert fatty acids to acyl CoA for subsequent oxidation. Possibly mitochondria need to be protected from fatty acids. At first glance there would not appear to be any role for UCP3 in these pathways.

Postulated Role of UCP3 in Fatty Acid Oxidation: Export of Fatty Acid Anions from Mitochondria. Could the physiological function of UCP3 be to export fatty acid anions from mitochondria? As noted above, transport of fatty acid anions is an accepted property of UCPs, including UCP1 and UCP3. If UCP3 had this function, what could be the source for these anions? This source would be related to increased fatty acid oxidation. Metabolic pathways for fatty acid oxidation do not show any fatty acid anion other than the one that is converted to acyl CoA in the cytosol (Fig. 1).

Thinking outside the usual textbook box, however, reveals that a potential source of fatty acids in mitochondria could be the hydrolysis of acyl CoA by a thioesterase. Mitochondrial thioesterases have been studied for many years, but no physiological role in metabolism has hitherto been assigned to them and no thought seems to have been given to the fate of the fatty acids produced by them at a location where these fatty acids cannot be metabolized. Two forms of mitochondrial acyl CoA thioesterase have recently been cloned (59–62), and these differ from the better-known cytosolic and peroxisomal isoforms of this class of enzyme. One form is widely expressed in heart, skeletal muscle, BAT, WAT, and kidney (59–61). This enzyme is not constitutively expressed in liver, but its expression there is markedly up-regulated by fasting and by the peroxisome

Figure 1. Outline of mitochondrial metabolism to illustrate functions of uncoupling proteins, and the hypothesized role of UCP3 in export of fatty acids anions in muscle and BAT mitochondria. (Bottom right) Formation of reduced coenzymes during fatty acid and glucose metabolism. Fatty acids are converted to acyl CoA in the cytosol, a reaction that requires CoASH and converts ATP to AMP plus PP_i. The carnitine shuttle, with participation of carnitine palmitoyl transferases 1 and 2 (CPT) and a CoASH transacylase results in appearance of acyl CoA in the mitochondrial matrix. The β-oxidation cycle (βox) converts the acyl CoA to acetyl CoA. (Acetyl CoA can also be derived from glucose metabolism via pyruvate.) Acetyl CoA from fatty acid β-oxidation (and from pyruvate oxidation) are then oxidized in the tricarboxylic acid cycle (TCA). (Top right) Oxidation of the reduced coenzymes NADH + H^+ and $FADH_2$ and creation of a proton gradient. NADH+ H^+ is produced in one reaction of the β-oxidation cycle, by pyruvate dehydrogenase and by three reactions of the TCA cycle (isocitrate dehydrogenase, ketoglutarate dehydrogenase, malate dehydrogenase) and is oxidized at Complex I of the electron transport system. $FADH_2$ is produced by acyl CoA dehydrogenase (in the β-oxidation cycle), succinate dehydrogenase (in the TCA cycle), and glycerol-3-phosphate dehydrogenase (reoxidation of NADH+ H^+ produced in glycolysis by the glycerol-3-phosphate shuttle). These enzymes form part of the inner mitochondrial membrane in close association with Complex II. As oxidations occur, energy is used by Complexes I, II, and IV to pump protons out of the mitochondrion to create a proton gradient across the inner membrane. *Use of the proton gradient to drive ATP synthesis.* In most mitochondria most of the time the energy of the proton gradient is used to drive the synthesis of ATP by the proton-translocating ATP synthase of the inner membrane. Oxidation and phosphorylation are coupled. This is true of muscle mitochondria most of the time and of BAT mitochondria when the BAT is not stimulated. *Uses for ATP (increased usage results in increased thermogenesis).* Use of ATP for a variety of purposes regenerates ADP and permits further phosphorylation reactions. Increased usage for physical activity or for shivering is the basis for exercise-induced thermogenesis and cold-induced shivering thermogenesis. *Mediation of cold-induced nonshivering thermogenesis in BAT by UCP1.* UCP1 mediates a specific proton leak process (see text for discussion). Stimulation of function of this protein allows an increase in heat production by brown adipocytes of up to 100× as uncoupling occurs. The principal fuel for the increased thermogenesis is fatty acids. *Other leaks, mediated by proteins or not.* All mitochondria allow protons to leak back into the matrix. It is not clear whether this proton leak in general is mediated by any of the known UCPs (designated as "leak" in the Figure). This proton leak dissipates up to 50% of the energy of the proton gradient. In other tissues, as well as BAT, the presence of other UCPs (UCP2, UCP3, BMCP1, UCP4) has been postulated to also allow a proton leak and some uncoupling. Evidence for this function of these proteins is so far not conclusive and other functions for them are being sought (see text for discussion). *Hypothesized role of UCP3 in fatty acid anion export.* This is an overflow route for fatty acid anions when acyl group entry is in excess of its removal by oxidation. This hypothetical function of UCP3 in muscle occurs when the entry of acyl groups into the mitochondrion is accelerated (as during fasting, exercise, shivering) and in BAT when fatty acid oxidation is stimulated by uncoupling (by UCP1), as during cold-induced nonshivering thermogenesis. The function is to regenerate CoASH for use in other reactions. These other reactions in the mitochondrial matrix are designated by the asterisk (*). They are pyruvate dehydrogenase, ketoglutarate dehydrogenase, and 3-ketoacyl CoA thiolase. The operation of these reactions, vital to continued fatty acid oxidation, might be impaired if the mitochondrial pool of CoASH were tied up as acyl CoA. Removal of CoA from acyl CoA by a thioesterase produces a FA anion (FA^-), which is then transported out of the mitochondrion by UCP3. The same hypothesis can be applied to BAT when fatty acid oxidation is accelerated (stimulation of lipolysis and accelerated fatty acid oxidation occurs due to activation of the uncoupling function of UCP1). Protection against depletion of CoASH in the matrix is provided by the action of the thioesterase and export of the FA^- by UCP3.

proliferator, clofibrate (61). There are no reports on its expression in muscle during fasting, but an up-regulation would be predicted by analogy with the known role of PPARα in its expression in liver (61) and the role of this nuclear receptor, as well as its co-activator, PGC-1, in fatty acid-mediated up-regulation of expression of other enzymes of fatty acid oxidation (63) as well as of UCP3 (64) in muscle. A second, different, form of mitochondrial acyl CoA thioesterase has also been cloned (62). Both isoforms of this enzyme are expressed in all tissues examined, including muscle. BAT mitochondria contain several thioesterases and the activity of at least one of these increases in response to cold (65, 66).

Thus, mitochondrial acyl CoA can be hydrolyzed by one or more thioesterases present in the mitochondrial matrix (Fig. 1). Since mitochondria lack any activating enzyme that converts fatty acid to acyl CoA, the existence of a carrier that would export the fatty acid anions outward across the inner membrane would be predicted. Until 1997 no candidates for this export function existed. However, the recent cloning of four homologues of UCP1 (see above) has provided four plausible candidates for this function.

The hypothesis presented here thus proposes that under physiological conditions an acyl CoA thioesterase hydrolyzes acyl CoA and produces a fatty acid anion in the mitochondrial matrix. This occurs when fatty acids are the principal substrate being oxidized, either in skeletal muscle or in BAT (Fig. 1). The fatty acid anion is transported across the inner membrane by UCP3, returning to the pool of fatty acid anions in the cytosol. It can be noted that complete recycling of fatty acids might occur when their supply was excessive. That is, conversion of a fatty acid anion to acyl CoA outside the mitochondrion, transport of the acyl group across the membrane as acylcarnitine, transfer of the acyl group to CoASH inside the mitochondrion, hydrolysis by acyl CoA thioesterase, and export of the fatty acid anion to the outside again by UCP3. Such a futile cycle would result in hydrolysis of ATP (two per fatty acid anion cycled) and liberation of one proton in the matrix. Energy expenditure due to operation of this cycle would be due mainly to the ATPase effect rather than uncoupling via proton entry. Such futile cycling could also occur when the level of UCP3 is vastly increased by over-expression in transgenic mice (67) and account for at least part of the increased energy expenditure in these animals.

What is the function of this mitochondrial fatty acid export system under physiological conditions? The hypothesis offered by the researchers who first cloned the thioesterase is that removal of coenzyme A from the acyl CoA regenerates the supply of CoASH needed for other mitochondrial reactions involved in fatty acid oxidation (59, 61). These reactions are ketoglutarate dehydrogenase in the tricarboxylic acid cycle and 3-ketoacylthiolase in the β-oxidation cycle. Demand for CoASH by these reactions is high, and would be 15 times that needed for the transfer of a palmitoyl group from palmitoyl carnitine to CoASH by CPT-2 during the complete oxidation of palmitate to CO_2 and water. If transport of fatty acid into the mitochondrion via the carnitine shuttle were markedly accelerated, there would be the risk of depriving the other CoASH requiring reactions of their substrate and of impairing fatty acid oxidation. Thus, the function of the fatty acid export cycle is to liberate CoASH for other uses at times of dependence on fatty acid oxidation as an energy source so as to enhance fatty acid oxidation as well as to free the mitochondrion of a potentially deleterious substance, free fatty acid, that it is unable to metabolize.

Does the Fatty Acid Export Hypothesis Also Apply to Other UCPs?

The hypothesis presented here is that UCP3 is an important player in the export of fatty acid anions from muscle and BAT mitochondria and that it is a component of fatty acid oxidation. The two individual players in this pathway are known, that is, UCP3 to export fatty acid anions and an acyl CoA thioesterase to hydrolyze acyl CoA. The association of increased UCP3 expression in muscle and BAT at times when fatty acid oxidation predominates has also been noted before. The present hypothesis puts these hitherto disparate observations into a coherent story that provides a logical function for UCP3 in fatty acid oxidation in muscle and BAT. This role is not that of an uncoupler under physiological conditions.

It is likely that other UCPs may play a similar role in other tissues under other circumstances, for example in liver, in brain, or in β-cells of pancreatic islets. It is possible that no UCP, other than UCP1 in BAT, acts as an uncoupler under physiological circumstances. The name given to these carrier proteins of unknown function was based on structural homology with the long-known BAT UCP1. This name has focused most research on these proteins, now reported in almost 500 publications, on the possibility of their having a thermogenic function and hence a potential role in energy balance and obesity. It may now be time to give them another, less misleading, name, such as mitochondrial carrier proteins (MCPs), without specifying the substance they carry or their function until these have been elucidated.

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