The Basal Proton Conductance of Skeletal Muscle Mitochondria from Transgenic Mice Overexpressing or Lacking Uncoupling Protein-3*

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The ability of native uncoupling protein-3 (UCP3) to uncouple mitochondrial oxidative phosphorylation is controversial. We measured the expression level of UCP3 and the proton conductance of skeletal muscle mitochondria isolated from transgenic mice overexpressing human UCP3 (UCP3-tg) and from UCP3 knockout (UCP3-KO) mice. The concentration of UCP3 in UCP3-tg mitochondria was −3 μg/mg protein, 20-fold higher than the wild type value. UCP3-tg mitochondria had increased nonphosphorylating respiration rates, decreased respiratory control, and 4-fold increased proton conductance compared with the wild type. However, this increased uncoupling in UCP3-tg mitochondria was not caused by native function of UCP3 because it was not proportional to the increase in UCP3 concentration and was neither activated by superoxide nor inhibited by GDP. UCP3 was undetectable in mitochondria from UCP3-KO mice. Nevertheless, UCP3-KO mitochondria had unchanged respiration rates, respiratory control ratios, and proton conductance compared with the wild type under a variety of assay conditions. We conclude that uncoupling in UCP3-tg mice is an artifact of transgenic expression, and that UCP3 does not catalyze the basal proton conductance of skeletal muscle mitochondria in the absence of activators such as superoxide.

Uncoupling protein-1 (UCP1) in brown adipose tissue plays a key role in adaptive thermogenesis (1–3). It allows protons that have been pumped by electron transport to return to the mitochondrial matrix and thus uncouples mitochondrial respiration from ATP synthesis, leading to rapid respiration and heat production. Uncoupling protein-3 (UCP3) is a UCP1 homologue, which is expressed predominantly in skeletal muscle and brown fat (4–6). It has been proposed that UCP3, like UCP1, increases mitochondrial proton conductance and so might play an important role in the regulation of thermogenesis, energy expenditure, and body mass (4–10).

There is a great deal of evidence implicating UCP3 in mitochondrial uncoupling: UCP3, like UCP1, uncouples when expressed in yeast (6, 7, 11–13), mammalian cells (9), or proteoliposomes (8, 14). However, there is strong evidence that the uncoupling seen in yeast is an expression artifact (13, 15–18). Moreover, experimental evidence from studies where the expression levels of UCP3 have been physiologically altered do not support an uncoupling role for UCP3 (19–27). For example, in starvation, when thermogenesis in skeletal muscle is depressed (28), UCP3 protein concentration in isolated rat muscle mitochondria increases (6, 9, 19) but the proton conductance does not (19).

Uncoupling by UCP1 is activated by fatty acids and potently inhibited by purine nucleotides, whereas uncoupling caused by expression artifacts is not, providing a clear-cut way to identify native UCP1 uncoupling function (13, 17, 18). Following our recent demonstration that uncoupling through UCP3 (and other UCPs) is activated by superoxide and inhibited by purine nucleotides such as GDP (29), the same test is now available to distinguish native from artifactual uncoupling by UCP3 (13).

Genetic manipulation using transgenic animals can provide a powerful way to study the function of UCP1 homologues in mammals. Mice overexpressing human UCP3 in skeletal muscle (UCP3-tg mice) are hyperphagic but weigh less than wild type controls (30). Mitochondria isolated from their skeletal muscles have several features that indicate uncoupling: decreased respiratory control ratio, increased state 4 respiration rate, and decreased membrane potential (30). However, it is unclear what expression levels were achieved and whether this uncoupling is a native function of UCP3. Two recent studies using UCP3 knockout mice show that the lack of UCP3 is not associated with obesity or with decreased metabolic rate (31, 32). Proton conductance was decreased in skeletal muscle mitochondria (31), and the respiratory control ratio was increased (32), suggesting that UCP3 accounts for some of the basal proton conductance in skeletal muscle mitochondria. However, the basal proton conductance of muscle mitochondria is insensitive to GDP (29, 33), whereas all validated proton conductance by UCP3 is GDP-sensitive (14, 29), raising some doubts...
about the validity of the conclusion that UCP3 contributes to the basal proton conductance of skeletal muscle mitochondria. In the present study, we relate the concentration of UCP3 protein in skeletal muscle mitochondria of UCP3-tg, UCP3 knockout, and wild type mice to mitochondrial proton conductance and we analyze whether the uncoupling in UCP3-tg mouse mitochondria is native or artificial. Our results indicate that UCP3 does not contribute to the basal proton conductance of skeletal muscle mitochondria.

**EXPERIMENTAL PROCEDURES**

**Isolation of Skeletal Muscle Mitochondria**—Transgenic mice (10–15 weeks old) were from the UCP3-tg (30) and UCP3-KO (29) strains, with age-paired controls from the parental strain (UCP3-tg) or from the same litters (UCP3-KO), generated at SmithKline Beecham Pharmaceuticals. They were killed by stunning followed by cervical dislocation. The skeletal muscle was immediately dissected from the hindlimbs, weighed, and placed in isolation medium containing 100 mM KCl, 50 mM Tris-HCl, 2 mM EGTA, pH 7.4, on ice. Mitochondria were isolated from 4–8 mice/preparation as described previously (34, 35), with all steps at 4 °C. Tissue was shredded with a sharp blade, minced with sharp scissors, rinsed with isolation medium four or five times, stirred for 2 min in a medium containing 100 mM KCl, 50 mM Tris-HCl, 2 mM EGTA, 1 mM ATP, 5 mM MgCl₂, 0.5% (w/v) bovine serum albumin (BSA), and 18.7 units of protease/o of tissue (nagarse or Sigma protease type VIII), pH 7.4, and gently homogenized using a Polytron tissue homogenizer. The homogenate was stirred for 3 min, then centrifuged at 490 × g for 10 min. The supernatant was filtered through muslin and centrifuged at 10,368 × g for 10 min. Mitochondrial pellets were resuspended in isolation medium, combined, and centrifuged at 10,368 × g for 10 min and then at 3841 × g for 10 min, and resuspended in isolation medium. Protein concentration was determined by the biuret method.

**Western Blots**—Chemicon-3046 antibody, raised to a 14-amino acid peptide near to the C terminus of human UCP3, was used at a dilution of 1:1,000 to detect UCP3 in mouse skeletal muscle mitochondria as described previously (13). Human UCP3 expressed in Escherichia coli inclusion bodies was used for calibration (13).

**Measurement of Oxygen Consumption**—Oxygen consumption was measured using a Clark-type oxygen electrode (Rank Brothers Ltd., in the same litters). The electrode was calibrated with air-saturated medium, combined, and centrifuged at 10,368 × g for 10 min and then at 3841 × g for 10 min, and resuspended in isolation medium. Protein concentration was determined by the biuret method.

**Measurement of Proton Conductance**—The respiration rate of mitochondrial proton conductance was measured using a Clark-type oxygen electrode (Rank Brothers Ltd., in the same litters). The electrode was calibrated with air-saturated medium, combined, and centrifuged at 10,368 × g for 10 min and then at 3841 × g for 10 min, and resuspended in isolation medium. Protein concentration was determined by the biuret method.

**RESULTS**

**Body Weight**—Mice overexpressing human UCP3 in skeletal muscle (UCP3-tg) weighed 19% less than paired wild type mice of the same age (Table I). This was true for both males (18%) and females (19%). UCP3 knockout mice (UCP3-KO) weighed the same as wild type mice (Table I).

**Concentration of UCP3 in Skeletal Muscle Mitochondria**—The concentrations of UCP3 in mitochondria isolated from skeletal muscle of wild type, UCP3-tg, and UCP3-KO mice were determined by Western blot calibrated using partially purified recombinant UCP3. An example is shown in Fig. 1. UCP3 concentration in UCP3-tg mouse mitochondria was variable, but on average it was expressed at 3200 ng/mg mitochondrial protein (Table II), ~20 times higher than the concentration in wild type mouse mitochondria (Table II and Ref. 13). UCP3 levels were undetectable in skeletal muscle mitochondria from knockout mice, confirming the knockout status of the animals and the specificity of the antibody (13, 29). A small amount of UCP3 (~10 ng/mg protein) was measured in mitochondria from littermate UCP3 (+/−) mice (Fig. 1).

**Oxygen Consumption**—Respiration rates with succinate as substrate in the presence of oligomycin to inhibit any ATP synthesis (state 4) were significantly higher in mitochondria from UCP3-tg mice than controls (Table III), but state 2 and state 3 rates were not significantly different. The respiratory control ratio was lower in mitochondria from UCP3-tg mice (Table III).

Respiration rates and the respiratory control ratios in mitochondria from UCP3-KO mice were not different from controls, either in the presence or the absence of 0.2% BSA (Table IV). State 4 respiration rates in the absence of BSA were faster, and respiratory control ratios were lower, in both wild type and knockout mice compared with the values in the presence of 0.2% BSA, because of the uncoupling effect of fatty acids (Table IV).

**Mitochondrial Proton Conductance**—The kinetic response of the proton leak rate to its driving force, membrane potential, in skeletal muscle mitochondria from UCP3-tg mice was different from controls. At any potential, the proton leak rate was 3–5-fold greater compared with wild type (Fig. 2), demonstrating an approximately 4-fold increase in mitochondrial proton conductance in mitochondria from the UCP3-overexpressing mice. We have shown previously that the activation of skeletal muscle mitochondrial proton conductance by superoxide is de-
Role of UCP3 in Mitochondrial Basal Proton Conductance

To determine whether the extra proton conductance in skeletal muscle mitochondria from UCP3-tg mice was activable by superoxide, we repeated the measurements in the presence of xanthine plus xanthine oxidase, a superoxide-generating system (29), in the presence of endogenous fatty acids. Superoxide stimulated the proton conductance in skeletal muscle mitochondria from both wild type (Fig. 3a) and UCP3-tg mice (Fig. 3b). Superoxide stimulation was fully prevented by addition of 500 μM GDP to inhibit UCP3 function. The GDP-sensitive activation of proton conductance by superoxide was the same in UCP3-tg and control mitochondria (Fig. 3), showing that the increased proton conductance in mitochondria from UCP3-tg mice was not further stimulated by a known activator (29) of native UCP3.

To determine whether the extra proton conductance in skeletal muscle mitochondria from UCP3-tg mice was inhibited by purine nucleotides, we repeated the measurements in the presence of GDP and endogenous fatty acids. GDP (500 μM) had no effect on the proton conductance in either UCP3-tg or control mitochondria (Fig. 3c), showing that the increased proton conductance in mitochondria from UCP3-tg mice was not sensitive to a known inhibitor (8, 14, 29) of native UCP3.

To examine whether native UCP3 makes any contribution to the basal proton leak of isolated mouse skeletal muscle mitochondria, we compared the proton conductance of mitochondria from wild type and UCP3 knockout mice. In our standard assay medium, containing BSA to chelate endogenous fatty acids (43) and magnesium to decrease unphysiological proton leak pathways (33), using succinate as substrate and varying the mitochondrial membrane potential by titration with malonate to inhibit succinate oxidation, the proton conductance of mitochondria from UCP3 knockout mice was indistinguishable from that of wild type mice (Fig. 4a).

This result, showing no contribution of UCP3 to the basal proton conductance of mouse skeletal muscle mitochondria, differs from that published by others (31, 32). We therefore explored the effect of UCP3 knockout under a wide variety of different assay conditions (Fig. 4). There was no difference between wild type and knockout mitochondrial proton conductance in the presence of lauric acid under conditions in which lauric acid clearly stimulated the basal proton conductance (Fig. 4b). There was no difference between wild type and knockout mitochondrial proton conductance in the absence of BSA (Fig. 4c), in the absence of both BSA and magnesium (Fig. 4d), in the absence of BSA when membrane potential was titrated with cyanide (Fig. 4e) or with succinate (Fig. 4f) instead of malonate, or in the presence of palmitate when membrane potential was titrated with cyanide (Fig. 4f) or with succinate (Fig. 4h).

**DISCUSSION**

Transgenic mice overexpressing human UCP3 in skeletal muscle are lean despite being hyperphagic (30). In the present study, we have confirmed that UCP3-tg mice (both male and female) weigh less than the wild type (Table I). We reported previously that skeletal muscle mitochondria from UCP3-tg mice are uncoupled as shown by decreased respiratory control ratio, increased state 4 respiration rate, and decreased membrane potential (30). Table III confirms these observations. In the present study we show directly that skeletal muscle mitochondria from UCP3-tg mice have ~4-fold greater proton conductance than wild type controls (Figs. 2a and 3c).

The concentration of UCP3 in wild type mouse muscle mitochondria is very low, ~140 ng of UCP3/mg of mitochondrial protein (13), which is 400 times less than the concentration of UCP1 in brown adipose tissue mitochondria from cold-adapted mice (reviewed in Ref. 17). We find that skeletal muscle mito-
Role of UCP3 in Mitochondrial Basal Proton Conductance

Fig. 2. Proton leak kinetics of mitochondria isolated from age-paired wild type and UCP3-tg mice. Rate of proton leak (expressed as the respiration rate that drives the leak) is plotted as a function of mitochondrial membrane potential. For details see “Experimental Procedures.” Potential was varied by titration with malonate. a, skeletal muscle mitochondria from wild type (■) and UCP3-tg mice (○) incubated in medium containing 0.3% BSA. b, mitochondria from wild type (■, ■) and UCP3-tg mice (○, ○) incubated in medium containing 0.3% BSA and lacking (■, ○) or containing 250 μM lauric acid (■, ○). Values are means ± S.E. of 12 (a) or 3 (b) independent experiments.

Fig. 3. Effects of superoxide and GDP on the proton leak kinetics of mitochondria isolated from age-paired wild type and UCP3-tg mice. For details see “Experimental Procedures.” Mitochondria were incubated in medium without BSA containing 4 mM succinate. Potential was varied by titration with cyanide up to 100 μM. a, skeletal muscle mitochondria from wild type mice with no additions (■), with xanthine and xanthine oxidase (■), or with xanthine and xanthine oxidase plus 500 μM GDP (■). b, mitochondria from UCP3-tg mice with no additions (■), with xanthine and xanthine oxidase (○), or with xanthine and xanthine oxidase plus 500 μM GDP (▲). c, mitochondria from wild type (■, ■) and UCP3-tg mice (○, ▲) with no additions (■, ○) or with 500 μM GDP (▲, ▲). Values are means ± S.E. or range of four (a), two (b), and (c, controls), or 1 (c, plus GDP) independent experiments.

The mitochondria from UCP3-tg mice contain ~3 μg of hUCP3/mg of protein (Fig. 1 and Table II), which is ~20 times the endogenous mUCP3 background, and corresponds quite well to the 66-fold increase in UCP3 mRNA in these animals (30).

There are two lines of evidence that lead to the conclusion that the greater mitochondrial proton conductance in UCP3-tg mouse mitochondria compared with controls is not a reflection of any native uncoupling activity of UCP3. First, UCP3 protein concentration was 20-fold higher in UCP3-tg mitochondria (Table II), but proton conductance increased only ~4-fold (Figs. 2a and 3c). Either endogenous mUCP3 catalyzes 20% or less of the basal proton conductance of mitochondria from mouse skeletal muscle (so increasing this small component 20-fold increased the overall conductance only 4-fold), or the exogenous hUCP3 was substantially less active in proton translocation than the endogenous mUCP3. Second, there was no increase in native activity of UCP3 in skeletal muscle mitochondria from UCP3-tg mice (Fig. 5). Native function of UCP1 in brown adipose tissue mitochondria or heterologous expression systems can be tested by its sensitivity to purine nucleotides such as GDP (17). We have shown recently that uncoupling by native UCP3 in skeletal muscle mitochondria can be activated by superoxide in the presence of fatty acids, and that this activation is completely inhibited by low concentrations of purine nucleotides (29). This provides an assay for native UCP3 function analogous to that for UCP1: uncoupling caused by UCP3 expression that can be stimulated by superoxide and inhibited by GDP is native, and uncoupling that cannot be manipulated in this way is a non-native expression artifact. We have used this assay to show that uncoupling by hUCP3 expressed in yeast mitochondria is an expression artifact (13). We conclude from the present results (Fig. 3) that the uncoupling observed in skeletal muscle mitochondria from UCP3-tg mice, like the uncoupling caused by mammalian UCP3 in yeast mitochondria, is some sort of UCP3 expression artifact that does not reflect any native proton conductance function of UCP3.

Artificial uncoupling associated with UCP3 overexpression might be caused by non-native insertion, folding, or interactions of the protein in the inner membrane, leading to compromised membrane integrity. There are several examples of artifactual proton conductance caused by overexpression of UCP1 and related mitochondrial carrier proteins; UCP1 (17), UCP2 (18), UCP3 (13, 15, 16), and the oxoglutarate carrier (44) can all cause artifactual uncoupling in yeast or mammalian cells. Conversely, of the mammalian mitochondrial carrier family proteins, only UCP1 (18, 29, 45) and a yeast-bovine hybrid of the adenine nucleotide carrier (46) have been shown to have significant native function following expression in other organisms. The inability of overexpressed UCP3 to catalyze superoxide-stimulated proton conductance is probably because of poor protein insertion or folding, although limitation by some unrecognized endogenous cofactor also remains a formal possible explanation.

Even if uncoupling of skeletal muscle mitochondria by UCP3 in UCP3-tg mice is not physiological, observations that uncoupling of muscle mitochondria in vivo leads to hyperphagia (30)
Skeletal muscle mitochondria from littermate wild type (\(H18554\), \(H12135\) (\(H18554\)), titrated with malonate in medium containing 0.3% BSA without (\(a\)) titrated with malonate in medium containing 0.3% BSA (\(b\)), titrated with malonate in medium without BSA (\(c\)), titrated with malonate in medium without BSA and without Mg\(^{2+}\) (\(d\)), titrated with CN (up to 100 \(\mu\)M) in medium without BSA (\(e\)), or titrated with CN in medium containing 0.3% BSA and 300 \(\mu\)M palmitate (\(f\)). Alternatively, they were incubated in medium lacking substrate and titrated with succinate (up to 1 m M) in medium without BSA (\(g\)) or titrated with succinate in medium containing 0.3% BSA and 300 \(\mu\)M palmitate (\(h\)). Values are means ± S.E. (or range) of six (\(a\)), two (\(b\), one (\(c\), one (\(d\), three (\(e\), two (\(f\)), three (\(g\), or two (\(h\) independent experiments.

![Proton leak kinetics of mitochondria isolated from wild type and UCP3-KO mice.](https://www.jbc.org/content/2777/6/2777/F4.large.jpg)

**FIG. 4.** Proton leak kinetics of mitochondria isolated from wild type and UCP3-KO mice. For details, see “Experimental Procedures.” Skeletal muscle mitochondria from littermate wild type (\(\bullet\), \(\circ\)) and UCP3-KO mice (\(\triangle\), \(\square\)) were incubated with 4 \(\mu\)M succinate as substrate and titrated with malonate in medium containing 0.3% BSA (\(g\)) or titrated with malonate in medium containing 0.3% BSA without (\(\triangle\), \(\checkmark\)) (\(c\)), and with (\(\bullet\), \(\circ\)) 250 \(\mu\)M lauric acid (\(b\)), titrated with malonate in medium without BSA (\(c\)), titrated with malonate in medium without BSA and without Mg\(^{2+}\) (\(d\)), titrated with CN (up to 100 \(\mu\)M) in medium without BSA (\(e\)), or titrated with CN in medium containing 0.3% BSA and 300 \(\mu\)M palmitate (\(f\)). Alternatively, they were incubated in medium lacking substrate and titrated with succinate (up to 1 m M) in medium without BSA (\(g\)) or titrated with succinate in medium containing 0.3% BSA and 300 \(\mu\)M palmitate (\(h\)). Values are means ± S.E. (or range) of six (\(a\)), two (\(b\), one (\(c\), one (\(d\), three (\(e\), two (\(f\)), three (\(g\), or two (\(h\) independent experiments.

and reduced body mass (30, 47) remain an important demonstration that uncoupling of skeletal muscle mitochondria may be an excellent target for the development of anti-obesity pharmaceuticals. These experiments would be a modern tissue-specific demonstration of the anti-obesity effects of mild uncoupling that were first seen with chemical uncouplers like dinitrophenol (see Ref. 48). Proton leak in muscle is a significant contributor to standard metabolic rate (49), and modulation of mitochondrial proton conductance in muscle constitutes a mechanism of regulation of energy dissipation and standard metabolic rate. Whether the uncoupling caused by transfection of UCP3 into myotubes and cardiac muscle cells (50) or by transgenic expression of UCP1 in mouse muscle (47) is a result of native function of the UCPs or an expression artifact remains to be determined by direct tests of the GDP and superoxide sensitivity of mitochondria isolated from the relevant cells. In principle, carefully interpreted experiments using gene knockouts can be more reliable than those using overexpression. Overexpression of a protein can lead to artifacts, whereas disruption of the gene for a native protein can lead to unambiguous conclusions; if a phenotype remains after a gene is knocked out, then that gene cannot be solely responsible for the phenotype. Two recent studies showed greatly reduced proton conductance (31 and increased respiratory control ratio (32) in skeletal muscle mitochondria isolated from UCP3 knockout mice. There was also increased production of ROS in UCP3 knockout mitochondria (32) as predicted if coupling was improved (51). No changes in whole body energy metabolism were detected. However, UCP3 knockout mice were subsequently reported to have increased efficiency of cellular ATP production, suggesting uncoupling by UCP3 in skeletal muscle in vivo (52).

Our results (Fig. 4, Table IV) do not confirm the published studies (31, 32). We found that the basal proton conductance of skeletal muscle mitochondria isolated from UCP3 knockout mice was indistinguishable from wild type, showing that UCP3 does not contribute to the basal proton conductance of isolated mitochondria under our conditions, although we showed previously that UCP3 is required for the inducible proton conductance that can be activated by superoxide, because the superoxide effect was absent in UCP3 knockouts (29). It is not clear why our results differ from the previous studies. Our knockouts were generated independently, and we have confirmed that UCP3 mRNA (29) and UCP3 protein (13, 29) (Fig. 1, Table II) are not expressed. We suspect that the absence of serum albumin in the assays conducted in the published studies allowed different levels of uncoupling by different levels of contaminating fatty acids in mitochondria from the knockouts and the wild type, or perhaps superoxide or some other activator allowed inducible proton conductance by UCP3, which was lost in the knockouts. Because we could not replicate the published studies, even by including or omitting BSA in our assays (Fig. 4), we could not test these suspicions by repeating the observations in the presence of BSA, BSA and added fatty acid, GDP, or superoxide dismutase. In any event, our observation of unchanged basal proton conductance in UCP3 knockouts shows unambiguously that UCP3 is not solely responsible for that basal proton conductance.

What conclusions can now be drawn concerning the physiological involvement of UCP3 in mitochondrial proton conductance? UCP3 has strong sequence homology to UCP1, supporting suggestions that it too is an uncoupling protein. However, the uncoupling of yeast mitochondria by mammalian UCP3 (6, 7, 11–13) is an expression artifact (13, 15–18) that provides no evidence for a physiological uncoupling function in mammalian...
cells. Uncoupling following UCP3 expression in transgenic mammalian systems (9, 30, 50) may also be an expression artifact that provides no evidence for physiological uncoupling by UCP3, as shown for the uncoupling of mouse skeletal muscle in UCP3-tg mice in the present paper. Experiments in which UCP3 concentrations have been changed by physiological manipulations (19–27), and the lack of effect of UCP3 knockout on whole animal energy metabolism and basal metabolic rate (31, 32) despite the importance of muscle proton leak to physiological energetics (49), support the conclusion that UCP3 is not a significant contributor to the basal proton conductance of muscle mitochondria. The reported decrease in proton conductance of mitochondria from UCP3 knockout mice (31, 32) is a strong argument in favor of the hypothesis that UCP3 contributes significantly to basal proton conductance, but we have not been able to confirm this result (Fig. 4), raising serious questions about its validity. The report that UCP3 knockout mice have increased efficiency of ATP production in vivo (52) requires the unidirectional (forward) rate of ATP synthesis to be similar to the net (forward minus backward) rate, which is unlikely to be true, bearing in mind the rapid P-i-ATP exchange through glycolytic glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase (see Ref. 53).

However, UCP3 does catalyze GDP-sensitive anion transport and net proton transport in liposomes (8, 14, 54) and isolated skeletal muscle mitochondria (29, 55) in the presence of suitable activators (fatty acids, ubiquinone, or superoxide). Such inducible proton conductance does not appear to make a significant contribution to metabolic rate and thermogenesis, because these parameters are unchanged in UCP3 knockout mice (31, 32). It is possible that UCP3 causes significant inducible proton leak in particular cells or under particular conditions that have yet to be described, as may be the case for UCP2 in thymocytes (56) and pancreatic β-cells (57, 58). However, it seems more likely that UCP3 has no significant physiological uncoupling function, but instead transports superoxide anions and acts as a vital part of the ROS defense system of muscle mitochondria, as we have postulated elsewhere (29).

We conclude that the uncoupling effect found in UCP3-tg mice is not a native activity of UCP3 but an expression artifact that causes uncoupling in vivo. Results obtained in UCP3 knockout mice confirm this view, as the lack of UCP3 does not cause obesity or decreased mitochondrial proton conductance. UCP3 is not responsible for the basal proton conductance of skeletal muscle mitochondria, although it can catalyze an inducible proton conductance when activated by superoxide. This interaction could be important in decreasing mitochondrial ROS, which may lead, in the long term, to aging and age-related diseases.

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