Role of UCP3 in state 4 respiration during contractile activity-induced mitochondrial biogenesis

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Ljubicic, Vladimir, Peter J. Adhihetty, and David A. Hood. Role of UCP3 in state 4 respiration during contractile activity-induced mitochondrial biogenesis. J Appl Physiol 97: 976–983, 2004. First published May 14, 2004; 10.1152/japplphysiol.00336.2004.—In an effort to better characterize uncoupling protein-3 (UCP3) function in skeletal muscle, we assessed basal UCP3 protein content in rat intermyofibrillar (IMF) and subsarcolemmal (SS) mitochondrial subfractions in conjunction with measurements of state 4 respiration. UCP3 content was 1.3-fold (P<0.05) greater in IMF compared with SS mitochondria. State 4 respiration was 2.6-fold greater (P<0.05) in the IMF subfraction than in SS mitochondria. GDP attenuated state 4 respiration by ∼40% (P<0.05) in both subfractions. The UCP3 activator oleic acid (OA) significantly increased state 4 respiration in IMF mitochondria only. We used chronic electrical stimulation (3 h/day for 7 days) to investigate the relationship between changes in UCP3 protein expression and alterations in state 4 respiration during contractile activity-induced mitochondrial biogenesis. UCP3 content was increased by 1.9- and 2.3-fold in IMF and SS mitochondria, respectively, which exceeded the concurrent 40% (P<0.05) increase in cytochrome-c oxidase activity. Chronic contractile activity increased state 4 respiration by 1.4-fold (P<0.05) in IMF mitochondria, but no effect was observed in the SS subfraction. The uncoupling function of UCP3 accounted for 50–57% of the OA-induced increase in state 4 respiration in IMF mitochondria, which was independent of the induced twofold difference in UCP3 content due to chronic contractile activity. Thus modifications in UCP3 function are more important than changes in UCP3 expression in modifying state 4 respiration. This effect is evident in IMF but not SS mitochondria. We conclude that UCP3 at physiological concentrations accounts for a significant portion of state 4 respiration in both IMF and SS mitochondria, with the contribution being greater in the IMF subfraction. In addition, the contradiction between human and rat training studies with respect to UCP3 protein expression may partly be explained by the greater than twofold difference in mitochondrial UCP3 content between rat and human skeletal muscle.

skeletal muscle; exercise; mitochondria; uncoupling protein-3

THE UNCOUPLING PROTEINS (UCPs) represent a family of mitochondrial carrier proteins, including UCP1, UCP2, and UCP3, which are localized in the inner mitochondrial membrane. The physiological function of UCP1, which is native to brown adipose tissue mitochondria, is to uncouple the electrochemical proton gradient from ATP synthesis, thereby generating heat (35). Whether UCP2, like UCP1, acts similarly to uncouple the events of the electron transport chain from oxidative phosphorylation is still unclear (12, 17). Evidence points to the possible involvement of UCP2 in diabetes (61) and to reactive oxygen species (ROS) regulation (1). UCP3 is 57 and 73% homologous to UCP1 and UCP2, respectively, and is found predominantly in skeletal muscle (4, 55). Despite this similarity in primary structure, the function of UCP3 remains a matter of debate. Some studies suggest that the protein behaves as a mediator of ROS production (6, 56), whereas others point to a role for UCP3 in fatty acid (FA) transport and metabolism (24, 45, 46). There is considerable discussion concerning whether UCP3 plays a role in mitochondrial proton leak, which potentially accounts for ∼50% of the oxygen consumption rate of skeletal muscle (40). Some studies in which UCP3 expression has been reduced in knockout animals (7), or upregulated via transgenic overexpression (7, 21), show no substantial change in the resting (non-ADP stimulated) state 4 rate of mitochondrial respiration. However, these results are countered by other studies that have established a substantial contribution of UCP3 to state 4 respiration (8, 9, 19, 34, 56).

In skeletal muscle, mitochondria consist of the functionally and biochemically distinct intermyofibrillar (IMF) and subsarcolemmal (SS) subfractions, which are localized to discrete cellular compartments. IMF mitochondria are interspersed within the myofibrils, whereas SS mitochondria are situated beneath the muscle plasma membrane, in proximity to peripherally located myonuclei. It is well established that these mitochondrial subfractions adapt differently to conditions of chronic muscle use or disuse (26). This mitochondrial heterogeneity in composition and adaptation within muscle could be due, in part, to differential rates of protein and lipid import (52), protein synthesis or degradation (11), or mitochondrial DNA expression between the two fractions. Thus, to advance a comprehensive understanding of UCP3 function in skeletal muscle, an analysis of UCP3 function within IMF and SS mitochondrial subfractions is essential.

Debate also surrounds the adaptive response of UCP3 protein levels to chronic physical activity. Contractile activity, performed regularly in the forms of endurance training or chronic electrical stimulation, produces a well-established adaptation in skeletal muscle termed mitochondrial biogenesis (26). Jones et al. (33) have recently shown that swim training increases UCP3 protein content in rat muscle as a component of this adaptation. In addition, Putman and colleagues (39) demonstrated that chronic injections of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside elicited increased UCP3 mRNA and protein expression concurrent with enhanced mitochondrial enzyme activities. However, a number of studies in humans have reported that UCP3 protein expression was significantly lower in muscle of trained individuals, compared
with untrained controls (42), or compared with pretrained values (41, 46). Although these studies have demonstrated that UCP3 expression can be altered by chronic physical activity, few have investigated the functional outcomes of the changes in UCP3 content. Fernström et al. (16) have recently shown that both UCP3 and state 4 respiration were decreased in humans after a period of endurance training. Indeed, a better understanding of the role of UCP3 in muscle requires the measurement of changes in UCP3 protein, along with their physiological consequences. Therefore, the purposes of the present study were 1) to establish whether chronic contractile activity induces a differential response in UCP3 protein expression between SS and IMF mitochondria and 2) to relate changes in UCP3 expression during contractile activity-induced mitochondrial biogenesis to alterations in state 4 respiration. On the basis of the literature, we hypothesized that UCP3 would contribute, at least in part, to state 4 respiration in IMF and SS mitochondria and that the contribution of UCP3 would be augmented as a result of chronic contractile activity, if UCP3 protein expression were enhanced by this treatment. In addition, because the UCP3 response to exercise appears to differ between humans and rodents, we provide a preliminary analysis of some potential reasons for this difference.

METHODS

Materials. Medwire (Leico Industries, New York, NY) was used to fashion stimulating electrodes for the in vivo contractile activity protocol. Cytochrome c from horse heart, glutamate, GDP, and oleic acid (OA) were purchased from Sigma Chemical (Oakville, ON). The UCP3 antibody, raised against a 14-residue peptide near the COOH terminus of human UCP3, was obtained from Chemicon (AB3046; Temecula, CA). This antibody has been used to measure UCP3 protein content in rat, mouse (21), and human (16) muscle. Nitrocellulose membrane (Hybond N), horseradish peroxidase-conjugated secondary antibodies, and reagents for enhanced chemiluminescence employed for Western blot analyses were obtained from Amersham Pharmacia Biotech (Baie D’Urfe, PQ). All other chemicals were purchased from Sigma Chemical.

In vivo chronic contractile activity protocol. Male Sprague-Dawley rats (n = 53; Charles River, St. Constant, PQ) were housed individually and were given food and water ad libitum. The procedure as outlined previously (51) was followed for implantation of electrodes and chronic low-frequency electrical stimulation of animals. Briefly, rats were anesthetized with pentobarbital sodium (60 mg/kg), and under aseptic conditions, two platinum stimulating electrodes were sutured unilaterally flanking the common peroneal nerve of the left hindlimb. Electrode wires were passed subcutaneously from the thigh to the base of the neck, where they were exteriorized and secured to an external stimulator unit fastened to the back of the animal with cloth tape. Stimulation was adjusted at the time of electrode implantation to result in palpable contractions of the tibialis anterior (TA) and extensor digitorum longus (EDL) muscles. After a 1-wk recovery period, the TA and EDL muscles were chronically stimulated (10 Hz, 0.1 ms duration) 3 h/day for 7 days. The contralateral limb was used as a nonstimulated internal control in all animals. EDL muscles were employed for cytochrome-c oxidase (COX) enzyme activity measurements, whereas all other analyses were performed using the TA muscles.

For the comparison of UCP3 expression in rat, mouse, and human muscle, muscle samples were obtained from normal human vastus lateralis (kindly provided by Dr. Tanja Taivassalo and Dr. Ronald G. Haller, University of Texas Southwestern Medical Center, Dallas, TX) and from rat and mouse fast-twitch hindlimb muscle. Extracts were then prepared for immunoblotting, as described below.

Mitochondrial isolation. After a 21-h recovery period after the cessation of the final stimulation bout, the animals were anesthetized with pentobarbital sodium (60 mg/kg), and the TA and EDL muscle specimens were quickly excised from the chronically stimulated and the contralateral limbs. The TA muscles were immediately placed into ice-cold buffer and briefly minced, and the IMF and SS mitochondria were fractionated by differential centrifugation as described previously in detail (10, 52). Mitochondria were resuspended in resuspension medium (100 mM KCl, 10 mM MOPS, and 0.2% BSA), and an aliquot of the suspension was taken for measurements of protein content (5). The EDL muscles from stimulated and control legs were frozen in liquid nitrogen and subsequently processed for enzyme activity determination.

Mitochondrial respiration. Samples of isolated IMF and SS mitochondrial subfractions were incubated with 2 ml of VO2 buffer (250 mM sucrose, 50 mM KCl, 25 mM Tris·HCl, 10 mM K2HPO4, and 0.2% BSA, pH 7.4) at 30°C in a water-jacketed respiratory chamber with continuous stirring. Experiments conducted in the presence of GDP alone were done without added BSA in the VO2 buffer. Respiration rates (ng atom O2/mg) were measured and revealed rates similar to those of previous experiments (977). Furthermore, the addition of NAAD during state 3 measurements had no effect on the respiration rate (data not shown), indicating excellent mitochondrial membrane integrity. Mitochondria respiring in state 4 were subjected to the addition of 2.5 mM GDP in the presence or absence of 300 μM OA, allowing the relative contribution of UCP3 to state 4 respiration to be assessed. The UCP3-mediated OA-induced increase in state 4 respiration was identified by supplementing the respiration medium with OA, followed by a successive addition of GDP. The specific effect of OA on UCP3 is represented by the extent of the GDP-mediated inhibition of the OA-induced respiration.

Immunoblotting. Frozen rat, mouse, and human muscles were pulverized to a fine powder with a stainless steel mortar that was cooled to the temperature of liquid nitrogen. Powdered tissues were diluted 1:20 (wt/vol) in 100 mM Na-K-phosphate extraction buffer (pH 7.2) containing 2 mM EDTA. The suspension was sonicated (3 × 5 min) with ice, and the extraction was performed as previously described (27). Whole muscle and isolated IMF and SS mitochondrial protein extracts were separated by 12% SDS-PAGE and subsequently electroblotted to nitrocellulose membranes. After transfer, membranes were blocked (1 h) with 5% skim milk in 1× TBST [Tris-buffered saline-Tween 20: 25 mM Tris·HCl (pH 7.5), 1 mM NaCl, and 0.1% Tween 20] solution. Blots were then incubated in blocking buffer with antibody directed against UCP3 (1:500) overnight at 4°C. After three 5-min washes with TBST, blots were incubated at room temperature (1 h) with the appropriate secondary antibody coupled to horseradish peroxidase and washed again 3 × 5 min with TBST. Antibody-bound protein was revealed by using the enhanced chemiluminescence method. Films were scanned and analyzed by using SigmaGel software (Jandel Scientific, San Rafael, CA).

COX activity. COX activity was determined as described previously (20). Enzyme activity was determined as the maximal rate of oxidation of fully reduced cytochrome c, measured by the change in absorbance at 550 nm in a Beckman DU-64 spectrophotometer.

Statistical analysis. Data are expressed as means ± SE. Experiments employing both OA and GDP were analyzed by using a two-way, repeated-measures ANOVA, followed by Tukey’s post hoc assessment to determine individual differences. Paired Student’s t-tests were used for comparison of data obtained for the chronically stimulated and contralateral nonstimulated muscles, as well as for comparisons between IMF and SS mitochondria isolated from control muscle. Statistically significant distinctions between groups represented in the graphs depicted as fold differences are computed using...
the raw data sets before conversion to the fold difference values. Unpaired Student’s t-tests were used for interspecies comparisons. Statistical differences were considered significant if $P < 0.05$.

**RESULTS**

**Relationship of UCP3 protein expression to state 4 respiration in mitochondrial subfractions.** State 4 mitochondrial respiration rates were 2.6-fold greater in IMF mitochondria than those observed in the SS mitochondrial subfraction ($P < 0.05$; Fig. 1A), in agreement with previous studies (10, 28, 37). In contrast, UCP3 protein levels were only 1.3-fold greater in IMF mitochondria, compared with the SS subfraction ($P < 0.05$; Fig. 1B).

**Effect of chronic contractile activity on protein expression and state 4 respiration.** Chronic contractile activity results in an increase in mitochondrial content and oxygen consumption per unit of muscle mass (26). In nonstimulated EDL muscle, COX activity was 6.04 ± 0.79 U/ g muscle. Seven days of contractile activity resulted in a 1.4-fold increase in COX activity, a marker of mitochondrial biogenesis ($P < 0.05$; Fig. 2A). Chronic contractile activity also resulted in a 44% increase in the rate of state 4 respiration in IMF mitochondria ($P < 0.05$; Fig. 2B). In contrast, no effect of chronic contractile activity was observed on the state 4 respiration rate of SS mitochondria (Fig. 2B). UCP3 protein content was increased by 1.9- and 2.3-fold in IMF and SS mitochondria, respectively ($P < 0.05$; Fig. 2C), after 7 days of chronic contractile activity.

**Effect of UCP3 ligands on state 4 respiration.** To develop an understanding of UCP3 function in mitochondria, we measured state 4 respiration rates in isolated mitochondria after the additions of OA and GDP. OA stimulates, whereas GDP inhibits UCP3 activity (13, 54). Supplementation of the respiration medium with GDP reduced state 4 respiration rates in nonstimulated control SS and IMF mitochondrial subfractions by 38 and 44% ($P < 0.05$; Fig. 3A), respectively. Similar GDP-mediated reductions in respiration rates were also observed from mitochondria isolated from chronically stimulated muscle ($P < 0.05$; Fig. 3A). To further evaluate the contribution of UCP3 to state 4 respiration, mitochondrial oxygen consumption was measured in the presence of OA, followed by the addition of GDP. OA significantly increased state 4 respiration by 2.3- and 2.1-fold in IMF mitochondria isolated from control and chronically stimulated muscle, respectively ($P < 0.05$; Fig. 3B). The addition of GDP to IMF mitochondria from nonstimulated muscle produced a subsequent 50% reduction ($P < 0.05$) of the OA-induced increase in uncoupled respiration, whereas in IMF mitochondria from chronically stimulated muscle, the GDP effect resulted in a 57% decrease. These reductions represent the specific UCP3-mediated contribution to the OA-induced increased rate of respiration, equal to 16.3 ± 2.4 and 25.2 ± 7.4 ng atom O min$^{-1}$ mg$^{-1}$ in IMF mitochondria isolated from control and chronically stimulated muscle.

![Fig. 1. State 4 respiration and uncoupling protein-3 (UCP3) protein content in intermyoﬁbrillar (IMF) and subsarcolemmal (SS) mitochondrial subfractions. A: mitochondrial state 4 respiration rates ($n = 18$). B: SS and IMF mitochondria (50 μg protein/lane) from rat tibialis anterior muscle were subjected to Western blot analyses. UCP3 protein content was quantitated, and a summary of repeated experiments ($n = 21$ animals) is shown. A typical Western blot is illustrated. Values are means ± SE. *$P < 0.05$, IMF vs. SS.](http://jap.physiology.org/doi/10.1152/jappl.00767.2003)

![Fig. 2. Contractile activity-induced mitochondrial adaptations. A: effect of chronic contractile activity on skeletal muscle cytochrome-c oxidase (COX) activity ($n = 6$) of whole muscle tissue after 7 days of chronic stimulation. B: state 4 respiration rates of IMF and SS mitochondria isolated from chronically stimulated and contralateral control tibialis anterior ($n = 18$ animals). C: effect of chronic contractile activity on UCP3 protein content ($n = 8$ animals), and typical Western blots (50 μg protein/lane) of UCP3 protein levels in the mitochondrial subfractions. Values are means ± SE. C, control; S, stimulated. *$P < 0.05$, stimulated vs. control, nonstimulated muscle.](http://jap.physiology.org/doi/10.1152/jappl.00767.2003)
muscle, respectively (Fig. 3B, inset). In contrast, respiration rates of SS mitochondria isolated from both chronically stimulated and contralateral nonstimulated control muscle were unchanged in the presence of OA, and they remained unaffected by the addition of GDP (Fig. 3C).

**Species comparison of UCP3 protein expression.** To identify some potential reasons for the apparent species-specific difference in UCP3 expression in response to exercise, we measured UCP3 levels in whole muscle extracts derived from human, rat, and mouse muscle. UCP3 protein content was expressed per unit of COX activity to correct for inherent variations in mitochondrial density between species. Compared with human muscle, COX activity was 2.4- and 3.9-fold greater in rat and mouse muscle, respectively (Fig. 4A). When UCP3 protein levels (Fig. 4B) were expressed per unit of COX activity, a 2.4-fold greater UCP3 content was observed in rat compared with human skeletal muscle ($P < 0.05$; Fig. 4C). There was no difference in UCP3 content between rat and mouse skeletal muscle, as noted previously (21).

**DISCUSSION**

UCP3, a member of the mitochondrial transporter superfamily, is a protein for which several possible functions have been proposed (6, 18, 24, 46, 56). A role for UCP3 in FA metabolism has been suggested, where UCP3 expedites rates of FA oxidation by acting as a protein that facilitates mitochondrial FA anion efflux (24, 45, 46). Accordingly, a lack of UCP3 would eventually lead to accumulation of FA anions inside the matrix, and to disturbances in FA oxidation, which is consistent with reported abnormalities in fat oxidation in UCP3 knockout mice (2). The role of UCP3 in the regulation of mitochondrial state 4 respiration has also been the subject of considerable debate (7, 9, 34, 38). In addition, the nature of the adaptation of this protein to stressors such as chronic physical activity remains contested (16, 33, 41, 46). Therefore, the main purposes of the present study were to characterize UCP3 protein content and function in skeletal muscle IMF and SS mitochondria and to relate chronic contractile activity-induced adaptations in UCP3 content to state 4 respiration in the mitochondrial subfractions.

We observed that state 4 respiration in IMF mitochondria was significantly higher compared with the SS subfraction,
GDP had a pronounced effect (by UCP3 function are accompanied by alterations in state 4 uncoupling activity is supported only if agents that modify ligand-gating manipulations. The interpretation that UCP3 has mitochondrial subfractions.

Analysis of the distribution of UCP3 protein in skeletal muscle with functional consequences, provides a more complete analysis. However, our combination of protein expression data, along with functional consequences, provides a more complete analysis. Note the marked stimulation of state 4 respiration in IMF (2.3-fold), but not SS mitochondria, in the presence of OA. In contrast, the inhibitory influence of GDP had a pronounced effect (by ~40%) on both mitochondrial subfractions. Chronic contractile activity elicited an ~2-fold increase in UCP3 content in both mitochondrial subfractions. This corresponded with a 1.4-fold increase in state 4 respiration in the IMF subfraction but no change in the SS mitochondria. In the presence of OA, state 4 respiration was significantly augmented in IMF mitochondria by 2.1-fold, whereas the SS subfraction remained unresponsive. The inhibitory effect of GDP on state 4 respiration remained at ~45% after chronic contractile activity in both mitochondrial subfractions. This summary reveals that UCP3 contributes significantly (40–50%) to the rate of state 4 respiration. However, a marked increase in UCP3 expression was not accompanied by parallel increments in state 4 respiration, suggesting that changes in UCP3 activity, rather than alterations in gene expression, are more important in modifying state 4 respiration in skeletal muscle.

Fig. 5. Theoretical relationship between UCP3 content and state 4 respiration in SS (dashed lines) and IMF (solid lines) mitochondria, on the basis of acquired data. Basal UCP3 protein expression and state 4 respiration for IMF mitochondria are assigned a value of 1.0. Basal levels of UCP3 in SS mitochondria were ~30% lower than in IMF mitochondria; therefore, SS UCP3 expression in control muscle is given a value of 0.7. State 4 respiration rates of SS mitochondria were significantly lower compared with the IMF subfraction. The activity of basal levels of UCP3 was enhanced with OA (+OA), calculated as the effect of OA that is GDP inhibitable, or specifically inhibited with GDP (+GDP) resulting in alterations in state 4 respiration. Note that the marked stimulation of state 4 respiration in IMF (2.3-fold), but not SS mitochondria, in the presence of OA. In contrast, the inhibitory influence of GDP had a pronounced effect (by ~40%) on both mitochondrial subfractions. Chronic contractile activity elicited an ~2-fold increase in UCP3 content in both mitochondrial subfractions. This corresponded with a 1.4-fold increase in state 4 respiration in the IMF subfraction but no change in the SS mitochondria. In the presence of OA, state 4 respiration was significantly augmented in IMF mitochondria by 2.1-fold, whereas the SS subfraction remained unresponsive. The inhibitory effect of GDP on state 4 respiration remained at ~45% after chronic contractile activity in both mitochondrial subfractions. This summary reveals that UCP3 contributes significantly (40–50%) to the rate of state 4 respiration. However, a marked increase in UCP3 expression was not accompanied by parallel increments in state 4 respiration, suggesting that changes in UCP3 activity, rather than alterations in gene expression, are more important in modifying state 4 respiration in skeletal muscle.

similar to values previously reported (3, 10, 37). State 4 respiration represents one of a number of functional and biochemical parameters that are distinct between the two mitochondrial subpopulations (3, 10, 28, 37). Our data show that the differential expression of UCP3 contributes further to the unique constitution of each mitochondrial subfraction. In contrast to our findings, Jimenez et al. (32) have detailed a higher basal UCP3 expression in the SS subfraction isolated from mouse TA, whereas Iossa et al. (28) found no difference in rat skeletal muscle UCP3 content between IMF and SS mitochondria. We cannot explain these discrepant results other than to propose either species (32) or methodological differences (28).

However, our combination of protein expression data, along with functional consequences, provides a more complete analysis of the distribution of UCP3 protein in skeletal muscle mitochondrial subfractions.

A main objective of this study was to measure UCP3 content along with an assessment of its uncoupling behavior through ligand-gating manipulations. The interpretation that UCP3 has uncoupling activity is supported only if agents that modify UCP3 function are accompanied by alterations in state 4 respiration. Similar to UCP1, long-chain FAs augment, whereas purine nucleotides arrest, UCP3-mediated H+ translocation (13, 60). The nucleotide binding domain of brown adipose tissue-restricted UCP1, which is inhibited by GDP, is highly conserved in UCP3 (31), the only isoform expressed at significant levels of protein in skeletal muscle. On the basis of the ~40% inhibition of state 4 respiration by GDP in SS and IMF mitochondria, our data suggest that UCP3 contributes significantly to state 4 respiration, in both mitochondrial subfractions. These data are supported by experiments in which GDP was used to inhibit the FA-induced increase in state 4 respiration (60). It is known that proton conductance through UCP3 is enhanced in the presence of cofactors such as FAs (e.g., OA), coenzyme Q, and ROS (13, 14, 30). However, FAs have protein- and phospholipid-mediated uncoupling properties independent of their influence on UCP3 (22, 49, 58). The specific effect of OA on UCP3 can be identified by the extent of the GDP-mediated attenuation of the OA-induced respiration. The successive additions of OA followed by GDP revealed that UCP3 accounts for 50% of the FA-induced uncoupling in IMF mitochondria, similar to the fractional contribution estimated with GDP alone. Interestingly, this effect was only observed in IMF mitochondria, because OA failed to influence state 4 respiration in the SS subfraction. It is noteworthy that the inhibitory effect of GDP on state 4 respiration in SS mitochondria (Fig. 3A) was abrogated when GDP was added subsequent to OA. This suggests a more complex interaction of these ligands with UCP3 in the SS subfraction, and it underscores the importance of distinguishing between the IMF and SS subfractions when assaying functional characteristics of mitochondria in striated muscles. The differential response of SS and IMF mitochondria is supported by other evidence that the two mitochondrial subfractions respond differently to FA-induced uncoupling (28–30) and to other environmental stimuli (3, 28, 30, 37, 59). This could be due to variations in membrane architecture and enzyme activity (10), net protein synthesis (11), and/or rates of protein import (52).

In addition, our data obtained from rat IMF mitochondria

Table 1. Comparison of UCP3 expression under various physiological conditions

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<td>41</td>
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UCP3, uncoupling protein-3; NM, not measured; ↑, increase; ↓, decrease; ⇧, no change. Long-chain fat diet. Endurance training. Sprint training. Type 2 diabetes in humans and posttrauma-induced in rodents. Gastrocnemius. Soleus.
appear to differ from those acquired from human muscle mitochondria, because Tonkonogi et al. (54) did not observe a significant GDP-induced decrement in respiration rate in the presence of oleate. This may be a consequence of the lower UCP3 protein concentration within human skeletal muscle mitochondrial membranes compared with the rat (Fig. 4) and/or a species-specific difference in UCP3 function. Nonetheless, the data from our experiments employing ligands that modify UCP3 activity substantiate the interpretation that UCP3 can account for a considerable portion of state 4 respiration in IMF and SS mitochondria from rat muscle, with its contribution to state 4 respiration being greater in the IMF compared with the SS subfraction.

We also hypothesized that increasing UCP3 protein content via chronic contractile activity would enhance its fractional contribution to state 4 respiration and thus would result in an exaggerated response to the effects of OA and GDP. Similar to Tonkonogi et al. (54), we found that mitochondria isolated from chronically active muscle exhibited an increased response to OA, but this was only evident in the IMF subtraction. Surprisingly, the UCP3-mediated response, on the basis of the extent of GDP inhibition of the OA effect, was not significantly higher in IMF mitochondria from chronically stimulated muscle, despite the twofold greater UCP3 content induced by chronic contractile activity. These data suggest that the role of UCP3 in mediating changes in proton leak, and thus state 4 respiration, is already maximal at endogenous levels of UCP3 in IMF mitochondria. In support of this, others (28) have shown that fasting induces UCP3 expression in IMF mitochondria but that mitochondrial proton leak remains constant. This dissociation between induced levels of UCP3 and state 4 respiration is also evident from the fact that chronic contractile activity markedly increased UCP3 content in SS mitochondria, whereas state 4 respiration remained unchanged. These data imply a disproportionate relationship between UCP3 content and state 4 respiration during conditions of contractile activity-induced mitochondrial biogenesis. Thus it appears that the modulation of UCP3 activity, rather than the induction of a change in gene expression, is more important in determining the role of UCP3 in proton leak and state 4 respiration. Figure 5 summarizes the role of UCP3 in modulating state 4 respiration in SS and IMF mitochondria, on the basis of our data.

One of the unique features of skeletal muscle is its ability to undergo mitochondrial biogenesis in response to chronic contractile activity (26). The 40% increase (P < 0.05) in COX activity indicates that mitochondrial biogenesis was elicited by the 7-day chronic stimulation protocol. Furthermore, changes in mitochondrial phenotype were elicited as noted by the approximately twofold increase in UCP3 content in both SS and IMF mitochondrial subfractions (Fig. 2C). The contractile activity-induced adaptations of UCP3 seen in the present study, along with the results of Jones et al. (33), who used swim training in rodents, are in contrast to the findings of studies using human subjects (16, 41, 46) that show a decreased UCP3 protein content after endurance training. Indeed, inspection of the literature reveals marked differences in the expression of UCP3 between rodents and humans in response to identical physiological conditions (Table 1). Assuming that these diverse conditions result in similar cellular environments when imposed on both rodents and humans, the resulting differences in UCP3 gene expression might be explained by the reported divergence in the human and rodent promoter regions upstream of the UCP3 gene (15), which could lead to different transcriptional activation among species. However, we also speculate that the two- to threefold lower density of UCP3 in the inner mitochondrial membrane of human muscle (Fig. 4C) may lead to a quantitatively different cellular signal for transcription, as a result of subtle variations in mitochondrial membrane potential, ROS production, ATP-to-ADP ratio, or FA metabolism between the two species. These differences may become more prominent in a contracting muscle cell, compared with at rest, and may establish an environment in which UCP3 expression is differentially affected in a species-specific manner.

In summary, the present study has demonstrated that UCP3 contributes meaningfully to the rate of state 4 respiration in skeletal muscle mitochondria. On the basis of the divergent UCP3 content and responsiveness of IMF and SS mitochondria, our data suggest that IMF mitochondria play an important role in the regulation of energy coupling in muscle cells, whereas SS mitochondria exert a more stringent control over their metabolic efficiency, and are less susceptible to changes in UCP3 expression and function. It is known that the proton leak across the mitochondrial inner membrane accounts for ~50% of the oxygen consumption rate of resting, perfused rat skeletal muscle (40). Because this leak dissipates significant amounts of potential energy, increasing mitochondrial uncoupling would appear to be an effective way to substantially increase resting energy expenditure. Thus we speculate that augmenting UCP3 activity, rather than altering UCP3 gene expression, may represent a goal for potential pharmacological intervention.

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