Exercise induces an increase in muscle UCP3 as a component of the increase in mitochondrial biogenesis

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Jones, Terry E., Keith Baar, Edward Ojuka, May Chen, and John O. Holloszy. Exercise induces an increase in muscle UCP3 as a component of the increase in mitochondrial biogenesis. Am J Physiol Endocrinol Metab 284: E96–E101, 2003. First published September 17, 2002; 10.1152/ajpendo.00316.2002.—Previous studies have indicated that exercise acutely induces large increases in uncoupling protein-3 (UCP3) in skeletal muscle, whereas endurance training results in marked decreases in muscle UCP3. Because UCP3 expression appears to be regulated by the same mechanism as other mitochondrial constituents, it seemed unlikely that exercise would result in such large and divergent changes in mitochondrial composition. The purpose of this study was to test the hypothesis that major changes in UCP3 protein concentration do not occur independently of mitochondrial biogenesis and that UCP3 increases as a component of the exercise-induced increase in mitochondria. We found a large increase in UCP3 mRNA immediately and 3 h after a bout of swimming. UCP3 protein concentration was increased 35% 18 h after a single exercise bout, 63% after 3 days, and 84% after 10 days of exercise. These increases in UCP3 roughly paralleled those of other mitochondrial marker proteins. Our results are consistent with the interpretation that endurance exercise induces an adaptive increase in mitochondria that have a normal content of UCP3.

The mitochondrial protein UCP3 was identified on the basis of its homology with the uncoupling protein-1 (UCP1) present in brown adipose tissue (14). It has been proposed on the basis of its homology with UCP1 that UCP3 also functions as an uncoupling protein (5, 14). Furthermore, some investigators have interpreted the results of studies of the heterologous expression of UCP3 in yeast (36) and of overexpression or knockout of UCP3 in transgenic mice (8, 9, 34) as providing evidence for uncoupling.

UCP3 is expressed primarily in skeletal muscle (14). Because of its possible role in energy metabolism, a number of investigators have studied the adaptive response of UCP3 in skeletal muscle to exercise (11, 23, 29, 33, 37). Tsuboyama-Kasaoka et al. (33) reported that UCP3 mRNA was increased sevenfold in skeletal muscle of mice 3 h after a single bout of treadmill running and 16-fold 3 h after exercise in mice that had been trained for 3 wk by swimming. They speculated that “upregulation of UCP3 mRNA may be a defense mechanism against extra energy supply to consume extra energy in skeletal muscle.” Similarly, Zhou et al. (37) reported that UCP3 mRNA was increased approximately sevenfold immediately after a 200-min-long bout of swimming. A similar response was seen after a bout of treadmill running. They also reported that UCP3 protein concentration was increased 3.5-fold immediately after 100 min of treadmill running and 5.6-fold immediately after 200 min of running (37). In studies in humans, Pilegaard et al. (23) found an ~2.5-fold increase in UCP3 mRNA 4 h after a 4-h bout of cycling, and Schrauwen et al. (29) reported an approximately twofold increase in UCP3 mRNA 4 h after exercise in men who performed cycle exercise for 2 h in the fasting state.

Although there were differences in the time course and magnitudes of the increases, all of the studies on the acute effect of exercise have shown that UCP3 mRNA is increased after a bout of exercise. However, Boss et al. (6) reported that, in contrast to the acute effects of exercise, endurance exercise training by means of an 8-wk-long program of treadmill running resulted in 76 and 59% decreases in UCP3 mRNA in tibialis anterior and soleus muscles of rats. They speculated that “a need for high metabolic efficiency is associated with decreased mRNA expression of UCPs in skeletal muscle which would decrease energy dissipation” in the trained state. Similarly, Schrauwen et al. (29) reported that endurance-trained men had significantly reduced UCP3 mRNA levels in quadriceps muscles compared with untrained men and that UCP3 mRNA was negatively correlated with maximal oxygen uptake capacity in these subjects.

Taken together, the results of these studies have led to the hypotheses that the “acute regulation of UCP3 gene expression has immediate and functionally important consequences” (37), whereas the decrease in UCP3 mRNA with long-term training results in an increase in the metabolic efficiency of muscle (6, 30). These hypotheses imply that exercise results in major

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changes in mitochondrial composition, with a great short-term increase in UCP3 and a large long-term decrease in the amount of UCP3 relative to the other mitochondrial constituents. Because UCP expression appears to be regulated by the same mechanisms as other mitochondrial constituents (25, 35), it seemed improbable to us that major changes in UCP3 protein concentration would occur independently of mitochondrial biogenesis. Therefore, the purpose of the present study was to test the hypothesis that UCP3 protein concentration increases as a component of the increase in skeletal muscle mitochondria induced by exercise (4, 18).

**MATERIALS AND METHODS**

*Materials.* Reagents for enhanced chemiluminescence (ECL) were obtained from Amersham Pharmacia Biotech. Reagents for SDS-polyacrylamide gel electrophoresis and Zeta-Probe membranes were from Bio-Rad, TRIZol reagent, for isolation of RNA, was purchased from Invitrogen. [α-32P]dATP was purchased from NEN Life Science Products. ULTRAhyb and the Strip EZ DNA labeling kit were obtained from Amersham. A rabbit polyclonal antibody directed against the 20 carboxy-terminal amino acids of citrate synthases was generated by Alpha Diagnostic International. A mouse anti-human cytochrome oxidase (COX) subunit I monoclonal antibody was purchased from Molecular Probes. A mouse anticytochrome c monoclonal antibody was purchased from Pharmingen International. Horseradish peroxidase-conjugated secondary antibodies were from The Jackson Laboratory. All other reagents were purchased from Sigma Chemical.

*Animals.* Male wistar rats (~100 g) were purchased from Charles River Laboratories. Purina chow and water were provided ad libitum. This study was approved by the Animal Studies Committee of Washington University School of Medicine.

*Exercise.* The rats were accustomed to swimming for 2 days, 10 min/day, before performing the exercise protocol of swimming for two 3-h-long periods separated by a 45-min rest period, as described previously (24, 26). One group of rats performed the exercise protocol for 1 day, one group of rats performed three daily bouts of exercise, and a third group performed 10 daily bouts of exercise. Animals were anesthetized with pentobarbital sodium (5 mg/100 g body wt) immediately, 9 h, or 18 h after the last bout of swimming, and triceps and epitrochlearis muscles were collected. In a separate experiment, soleus and white and red portions of quadriceps muscles were harvested from sedentary rats. All muscles were clamp frozen and stored at ~80°C. The anesthetized rats were killed by exsanguination.

*Northern blot analysis.* Triceps muscle was homogenized in TRIZol. Total RNA was precipitated using chloroform and isopropanol. Total RNA (25 μg) was size fractionated in a 1% formaldehyde agarose gel and transferred to Zeta-Probe membrane. Hybridization was carried out overnight in ULTRAhyb containing a cDNA probe with specific radioactivity of 106 cpm/ml. The cDNA utilized in this study was rat UCP3 (a generous gift from G. Lynis Dohm), labeled by use of a Strip-EZ DNA kit and α-32PdATP. Autoradiographs of the membranes were made using Kodak Biomax MR film.

*Western immunoblotting.* Epitrochlearis, triceps, soleus, white quadriceps, or red quadriceps muscle was homogenized in a buffer containing 20 mM HEPES, 1 mM EDTA, and 250 mM sucrose, pH 7.4. Protein content was measured using bicinchoninic acid (Pierce). Aliquots of homogenate were solubilized in Laemmli sample buffer and subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes. Membranes were blocked in PBS or TBS containing 5% nonfat dry milk. Blots were probed with antibodies directed against UCP3, cytochrome c, citrate synthase, and COX subunit I. Blots were then incubated with secondary antibody conjugated to horseradish peroxidase. Antibody bound protein was detected by ECL. Films were scanned and analyzed using Sigma Scan (Jandel Scientific).

*Statistical analyses.* Results are expressed as means ± SE. Statistical comparisons were made using unpaired t-tests with the level of statistical significance set at P < 0.05.

**RESULTS**

*Response of UCP3 to a single bout of exercise.* We were unable to quantify the magnitude of the increase of UCP3 mRNA, as a UCP3 mRNA signal was not detected in nonexercised muscles. However, Fig. 1A clearly shows that UCP3 mRNA expression was increased in triceps muscles of rats immediately after, and 3 h after, a bout of swimming. This finding confirms the results of Zhou et al. (37). However, in contrast to the finding of Zhou et al. that UCP3 protein was increased approximately fivefold immediately after 200 min of exercise, there was no increase in UCP3 protein in muscles of our rats ~10 min after 6 h of exercise (Fig. 1B).

*Adaptive response of UCP3 and mitochondrial marker enzymes to a program of daily swimming.* Eighteen hours after a bout of swimming, UCP3 protein concentration was increased ~35% in epitrochlearis muscle (Figs. 1B and 2). There was a further progressive increase in UCP3 protein concentration in the epitrochlearis muscle in response to 3 and 10 days of daily swimming (Fig. 2). This pattern of increase was similar to that of COX subunit I. Except for a more rapid initial increase, the increments in UCP3 also paralleled those of two other mitochondrial markers, cytochrome c and citrate synthase. A similar pattern of increase of mitochondrial marker enzymes was seen in triceps muscle (data not shown). Thus it appears that the adaptive increase in UCP3 in response to exercise reflects the overall increase in mitochondrial biogenesis.

*Relationship between UCP3 and other mitochondrial constituents in the three muscle fiber types.* It has been reported that expression of UCP3 protein is most abundant in type IIb fibers, less in type IIA fibers, and lowest in type I fibers (17). This report appears to be in conflict with our hypothesis that the UCP3 content of skeletal muscles is proportional to their content of mitochondria, because type IIB fibers have a lower content of mitochondria than type IIA or type I fibers. We therefore compared the pattern of UCP3 expression with that of two mitochondrial marker proteins, COX subunit I and cytochrome c, in the superficial white and deep red portions of the quadriceps muscle and in the soleus muscle. The superficial, white portion of the quadriceps contains a higher proportion of type IIB fibers, the deep, red portion of the quadriceps is made up...
predominantly of type IIa fibers, whereas the soleus has a high proportion of type I fibers (1). As shown in Fig. 3, the concentration of UCP3 protein roughly parallels that of two other mitochondrial marker proteins.

**DISCUSSION**

The results of this study support our hypothesis that the increase in UCP3 protein that occurs in response to exercise does not represent an increase in UCP3 protein per se. Instead, it appears that UCP3, a mitochondrial constituent, increases as a component of the exercise-induced increase in muscle mitochondria. Previous studies have shown that mitochondria from endurance-trained muscle are tightly coupled (2, 18, 22). An increase in tightly coupled muscle mitochondria of normal composition, such as occurs with endurance training, does not result in an increase in resting metabolic rate (31), because there is no increase in the energy requirement of trained resting muscle or, therefore, in the availability of ADP and inorganic phosphate (10, 12).

Previous studies of the acute effects of exercise showed that UCP3 mRNA is increased immediately or shortly after a bout of exercise (23, 33, 37). This finding led to speculation regarding the functional significance of increased UCP3 expression. Tsuboyama-Kasaoka et al. (33) hypothesized that the large upregulation of UCP3 mRNA may play a role in “fine adjustments in energy expenditure that may be a defense mechanism against extra supply to consume extra energy in skel-

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**Fig. 1.** Effects of 1 bout of exercise on triceps muscle uncoupling protein-3 (UCP3) mRNA and protein content. A: Northern blots were performed on RNA from triceps muscle of sedentary rats and on muscles of exercised rats obtained ∼10 min or 3 or 18 h after exercise, as described in MATERIALS AND METHODS. B: Western blots were performed on homogenates of epitrochlearis muscles from sedentary rats and on muscles of exercised rats obtained ∼10 min and 18 h after exercise, as described in MATERIALS AND METHODS. Each bar represents the mean ± SE for muscles from 6–11 rats.

**Fig. 2.** Time course of the increases in UCP3 protein and a number of other mitochondrial proteins in response to exercise. Epitrochlearis muscles were obtained from sedentary rats and from rats exercised by swimming once for either 3 or 10 days. Muscles were taken ∼18–20 h after the last exercise bout. Enzyme protein levels were determined by Western blot analysis as described in MATERIALS AND METHODS. Each point represents the mean obtained on muscles from 3–12 rats.

**Fig. 3.** Comparison of UCP3, cytochrome c, and cytochrome oxidase (COX) subunit I levels in the soleus and the deep red and superficial white portions of the quadriceps muscle. Muscle homogenates were used to determine UCP3, cytochrome c, and COX protein levels, as described in MATERIALS AND METHODS. Bars are means ± SE for muscles from 3–4 rats.
et al. muscles.” They also speculated that this increase in UCP3 expression might play a role in the increase in glucose uptake seen 3 h after exercise.

Although increases in mRNA are frequently referred to as increases in gene expression, mRNA only provides the information that can lead to increased gene expression. Gene expression can be controlled at various points beyond transcription in the sequence of events leading to an increase in the concentration of a protein and modification of the phenotype. Increased gene expression and its physiological consequences do not occur until there is an increase in the concentration of the protein encoded by the gene. As illustrated by the response of UCP3 to exercise, the magnitude of an adaptive increase in the concentration of a protein can be very different from that of the increase in its mRNA, i.e., 7- to 16-fold increases in mRNA after a bout of exercise (33, 37) vs. an ∼35% increase in UCP3 protein concentration 18 h after one bout and an ∼84% increase after 10 daily exercise bouts (Fig. 2).

Zhou et al. (37) hypothesized that the rapid increase in UCP3 expression induced by exercise functions to decrease production of reactive oxygen species (ROS) in contracting muscles. This hypothesis seemed reasonable in the context of their finding that the amount of UCP3 protein was increased 5.6-fold in leg muscles of rats immediately after a single, 200-min-long bout of treadmill running. This large and very rapid increase in protein concentration, which paralleled the increase in UCP3 mRNA, is, to the best of our knowledge, unprecedented. The largest and most rapid increases in mitochondrial proteins in skeletal muscle in response to exercise reported previously are probably those of GLUT4 and δ-aminolevulinic acid synthetase, both of which can increase as much as twofold during an 18-h postexercise period (19, 26). In any case, we were unable to detect an increase in UCP3 protein immediately postexercise and found only a 35% increase 18 h after one bout of exercise. We have no explanation for this discrepancy.

Boss et al. (6) reported that a strenuous 13-wk-long program of treadmill exercise training resulted in decreased expression of UCP3 (an average decrease of ∼65%) in rat skeletal muscles. They concluded that, in keeping with their hypothesis, this “decreased expression of uncoupling protein in skeletal muscle would allow for a higher level of metabolic efficiency . . . which would . . . favor energy storage . . . recovery.” Similarly, Schrauwen et al. (29) reported that UCP3 expression was significantly lower in muscles of trained compared with untrained men and that maximal oxygen uptake capacity was negatively correlated with UCP3. In these studies, UCP3 mRNA, rather than actual UCP3 expression, was measured. Nevertheless, the finding of a decrease in UCP3 mRNA concentration is puzzling and seems incompatible with our finding of a progressive increase in UCP3 protein concentration during 10 days of exercise training (Fig. 2). This finding of Boss et al. is also in disagreement with the results of Tsuboyama-Kasaoka et al. (33), who found a large increase in UCP3 mRNA after 3 wk of exercise training. Our results provide evidence that exercise does not result in major changes in the ratio of UCP3 to other mitochondrial constituents. This is in contrast to the picture provided by previous studies of marked alterations in mitochondrial composition, with an initial large disproportional increase in UCP3 with one exercise bout (33, 37) and a large decrease in UCP3 with training (6, 29).

There is considerable controversy regarding the functional role of UCP3. UCP1 mediates adaptive thermogenesis in brown adipose tissue by uncoupling mitochondrial respiration from ATP synthesis (27). Because UCP3 is a homolog of UCP1, it has been proposed that UCP3 also functions as an uncoupling protein that plays a role in skeletal muscle thermogenesis and energy expenditure (6, 9, 14, 20).

The results of two studies on UCP3 knockout mice were interpreted to indicate that mitochondria were more tightly coupled, with an increased state 3-to-state 4 ratio in one study (34) and, in another study (9), a fourfold increase in the rate of ATP synthesis with no change in citrate cycle flux, CO2 production, or energy expenditure. The latter finding is surprising, because the theoretical maximum P/O ratio (i.e., number of ATPs generated per oxygen atom) is 3, whereas more recently values of ∼2.5 have been obtained (3). These findings also do not seem to fit with the apparent absence of an altered phenotype in UCP3 knockout mice. They do not have a decreased metabolic rate and are not obese (7, 9, 15, 34), and they respond normally to thyroid hormone (15). A subsequent study on UCP3 knockout mice by Cadenas et al. (7) failed to confirm the earlier findings of more tightly coupled mitochondria (9, 34); instead, they found that respiratory control and proton conductance were unchanged in mitochondria lacking UCP3. There is evidence that UCP3 can uncouple mitochondria when expressed in yeast (36) or overexpressed in mouse muscle (7, 8). However, as discussed by Brand and coworkers (7, 16), this uncoupling appears to be an expression artifact due to disruption of mitochondrial function by the enormous, 20- to 66-fold, unphysiological increases in UCP3. Although studies in which UCP3 expression was altered by physiological interventions also do not support an uncoupling role for UCP3 (21, 28, 32), further research is needed to firmly establish whether or not UCP3 functions as an uncoupling protein in muscle under some physiological conditions such as, for example, cold exposure.

Another hypothesis regarding the biological role of UCP3 in skeletal muscle is that it has no significant uncoupling function under physiological conditions but serves to transport superoxide anions as a component of the defense mechanism against ROS (5, 13). If this concept is correct, our results would indicate that endurance exercise training results in an increase in skeletal muscle mitochondria that have a normal capacity to protect against ROS by the UCP3-mediated transport of superoxide anions.

In conclusion, our results provide evidence that endurance exercise results in an increase in UCP3 pro-
tein in skeletal muscle as a component of the exercise-induced increase in mitochondrial biogenesis. In contrast to the results of previous studies, they indicate that this adaptive response results in mitochondria with a normal content of UCP3 rather than in mitochondria with a markedly increased or decreased compliment of UCP3.

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