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Regulation of skeletal muscle UCP-2 and UCP-3 gene expression by exercise and denervation

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[1]*Department of Biochemistry, East Carolina University School of Medicine, Greenville, North Carolina 27858–4354;* [2]*Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan 48201; and* [3]*Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215*

Cortright, Ronald N., Donghai Zheng, Jared P. Jones, James D. Fluckey, Stephen E. DiCarlo, Danica Grujic, Bradford B. Lowell, and G. Lynis Dohm. Regulation of skeletal muscle UCP-2 and UCP-3 gene expression by exercise and denervation. *Am. J. Physiol.* 276 (*Endocrinol. Metab.* 39): E217–E221, 1999.—The factors that regulate gene expression of uncoupling proteins 2 and 3 (UCP-2 and UCP-3) in skeletal muscle are poorly understood, but both genes are clearly responsive to the metabolic state of the organism. Therefore, we tested the hypothesis that denervation and acute and/or chronic exercise (factors that profoundly affect metabolism) would alter UCP-2 and UCP-3 gene expression. For the denervation studies, the sciatic nerve of rat and mouse hindlimb was sectioned in one leg while the contralateral limb served as control. Northern blot analysis revealed that denervation was associated with a 331% increase ($P < 0.001$) in UCP-3 mRNA and a 200% increase ($P < 0.01$) in UCP-2 mRNA levels in rat mixed gastrocnemius (MG) muscle. In contrast, denervation caused a 53% decrease ($P < 0.001$) in UCP-3 and a 63% increase ($P < 0.01$) in UCP-2 mRNA levels in mouse MG. After acute exercise (2-h treadmill running), rat UCP-3 mRNA levels were elevated (vs. sedentary control) 252% ($P < 0.0001$) in white gastrocnemius and 63% ($P < 0.05$) in red gastrocnemius muscles, whereas UCP-2 levels were unaffected. To a lesser extent, elevations in UCP-3 mRNA (22%; $P < 0.01$) and UCP-2 mRNA (55%; $P < 0.01$) levels were observed after acute exercise in the mouse MG. There were no changes in either UCP-2 or UCP-3 mRNA levels after chronic exercise (9 wk of wheel running). These results indicate that acute exercise and denervation regulate gene expression of skeletal muscle UCPs.

uncoupling proteins; thermogenesis; rodent bioenergetics

UNCOUPLING PROTEINS (UCP) are members of the mitochondrial carrier protein family, which includes UCP-1, UCP-2, and UCP-3 (4, 8, 17). UCP-1 is found exclusively in mammalian brown adipose tissue, where it performs the function of generating heat by uncoupling oxidative phosphorylation (19). This process protects against hypothermia and regulates energy balance (7). UCP-1 is highly regulated; for example, its activity is decreased by purine nucleotides, diphosphates, or triphosphates and is increased by fatty acids (13). Two new uncoupling proteins, UCP-2 and UCP-3, have recently been discovered (4, 8, 9, 24). UCP-2 is 56% identical to UCP-1 and is expressed ubiquitously throughout body tissues, with the highest levels found in white adipose tissue (11). UCP-3 is 57 and 73% identical to human UCP-1 and UCP-2, respectively, and is found predominantly in skeletal muscle (4).

The factors that regulate UCP-2 and UCP-3 gene expression or clearly define their metabolic role(s) are poorly understood. However, both UCP-2 and UCP-3 have been shown to uncouple mitochondrial respiration, at least partially, both in transfected yeast (8) and C_2C_{12} myoblasts (3). Therefore, their presence in skeletal muscle is of great interest, because this tissue is an important site of thermogenesis and energy homeostasis in mammals (1, 23). Some physiological stimuli have been shown to alter UCP gene expression. Unlike UCP-1, UCP-2 mRNA is not increased by cold exposure, but gene expression is sensitive to dietary factors such as high-fat content (8). Starvation (48 h) in rats results in an eightfold increase in UCP-3 mRNA (with no change in UCP-2), whereas refeeding is associated with a marked reduction in UCP-3 below basal (10). As suggested by Gong et al. (10), the starving animal, despite loss of body tissue and compromised energy substrate supply, is still required to maintain body core temperature for survival. UCP-3 may serve this function, suggesting a possible shift in the role of thermogenic regulation from brown adipose tissue (BAT) to skeletal muscle. In support of this concept, UCP-1 and UCP-3 gene expression in BAT is reduced under starvation conditions, suggesting that energy conservation may still operate despite elevations in muscle UCP-3 expression (10). Similarly, Boss et al. (3) demonstrated that acute fasting (24–48 h) is associated with an increase in UCP-3 expression in mouse and rat muscle, an observation consistent with a thermogenic role for UCP-3 in skeletal muscle during energy deprivation. Most recently, however, it has been reported that elevations in blood free fatty acids by lipid infusion

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(similar to starvation) also elevate UCP-3 gene expression, indicating that the seemingly paradoxical induction of UCP-3 with starvation may be linked to the use of free fatty acid as a fuel rather than an increased need of the organism to dissipate energy (25).

The above discussion clearly indicates that UCP gene expression is responsive to the metabolic state of the organism. Muscle activity by exercise and muscle inactivity by surgical denervation are well established models used to profoundly alter the metabolic state of skeletal muscle. For example, protracted physical activity results in elevated metabolic oxidative capacity (14) (e.g., availability of free fatty acids for oxidation), whereas the absence of muscle innervation severely reduces muscle oxidative enzyme capacity (21). Therefore, we investigated the effects of physical activity by muscle contractions (acute or chronic running) and inactivity by denervation on UCP gene expression in mouse and rat gastrocnemius muscle.

MATERIALS AND METHODS

Animals. Male C57 black mice and male or female Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were provided food and water ad libitum and were maintained at constant room temperature (20–22°C) under controlled lighting conditions (12:12-h light-dark cycle). Animal care was in accordance with the guidelines set forth in the National Research Council's *Guide for the Care and Use of Laboratory Animals* (16).

Acute exercise. Animals (8 wk old) were allowed to eat ad libitum overnight; then, before treadmill running, food was withheld for both the exercise and sedentary control groups for the remainder of the experiment. Exercised rats ($n = 8$) and mice ($n = 8$) completed one bout of treadmill running (2 h, 0% grade; mice, 12–14 m/min; rats, 28–32 m/min). One hour postexercise, treatment and control mice ($n = 8$) and rats ($n = 8$) were stunned and killed via cervical dislocation, and gastrocnemius muscles (red and white or mixed muscle for rats and mice, respectively) were harvested, quickly frozen with liquid nitrogen-cooled metal tongs, and stored at −70°C. Muscles were harvested for RNA isolation and Northern analysis.

Chronic exercise. Sixteen male and 16 female, weanling (21 day) rats were randomly assigned to either a sedentary control (males = 7; females = 7) or a daily exercise group (males = 9; females = 9) and were allowed to run spontaneously in running wheels while sedentary rats remained in standard rat cages for 9 wk. This model of endurance exercise was chosen to eliminate potential alterations in feeding and activity behavior known to occur (22) with compulsive exercise (e.g., food intake of rats is very sensitive to any deviation from normal routine, such as changes in lighting and noise). Body mass and running distance were measured over the 9-wk period. Previous results from our laboratory confirmed that animals will run between 5 and 10 km per day, which is more than sufficient to induce an endurance training effect (5, 6). Animals were anesthetized with a ketamine (18 mg/ml)-xylazine (2 mg/ml) mixture (0.1 ml/100 g body wt ip) and killed at the end of their dark cycle (animals run predominantly during the dark cycle). Muscles were harvested for RNA isolation and Northern analysis.

Denervation. Eight male rats and 8 male mice were anesthetized as just described, followed by denervation of the right hindlimb via sciatic nerve section. The contralateral limb was sham operated and served as control. Seventy-two hours after surgery, rats and mice were stunned and killed by cervical dislocation. Gastrocnemius muscles were harvested for RNA isolation and Northern analysis.

Determination of mRNA levels. RNA was isolated from skeletal muscle using TRIzol (GIBCO-BRL) reagent (12) and subjected to Northern blot analysis, as previously described (18). Briefly, total RNA (20 μg/sample) was size-fractionated on a 1.25% agarose, 2 M formaldehyde gel and electrotransferred to Hybond N membrane (Amersham, Arlington Heights, IL). Blots were hybridized with a random primed [α-^{32}P]dATP-labeled probe for mUCP-2 and mUCP-3 mRNA and 18 S RNA. Hybridization probes were generated by random priming from the following mouse cDNA templates: mUCP-3, a 1,153-bp fragment (from 166 bp 5′ of start codon to 987 bp 3′ of start codon) and mUCP-2, a 1,054-bp fragment (from 26 bp 5′ of start codon to 1,028 bp 3′ of start codon). Northern blots were visualized by phosphorimaging and quantitated using Imagequant software (Molecular Dynamics, Sunnyvale, CA). The level of 18 S rRNA was used to normalize variability in RNA loading.

Statistical analysis. Differences in mRNA levels between denervated and contralateral control limbs were tested using a (two-tailed) Student's paired *t*-test. Statistical comparisons between exercised and sedentary groups were made using an unpaired (two-tailed) *t*-test. Results are expressed as means ± SE, and level for statistical significance was established a priori at $P < 0.05$.

RESULTS

Results of the Northern blot analysis of UCP-3 and UCP-2 for control and denervation treatments are summarized in Table 1 (absolute numbers as arbitrary units are presented only for conditions where statistical differences were observed). When expressed as a percentage of control, results demonstrate that, in rat mixed gastrocnemius (MG), denervation increases UCP-3 mRNA 331% ($P < 0.001$), but effects are opposite ($P < 0.001$) in the mouse (53% decrease in mRNA levels). UCP-2 mRNA levels were also increased (200%; $P < 0.01$) above control by denervation in the rat, whereas the increase was similar but to a lesser extent (63%; $P < 0.01$) in the mouse MG.

Northern blot analysis for UCP-3 and UCP-2 after acute and chronic exercise is also summarized in Table 1. An acute bout of endurance exercise increased rat UCP-3 mRNA 252% ($P < 0.0001$) and 63% ($P < 0.05$) above that of sedentary animals in white and red gastrocnemius, respectively. UCP-3 mRNA was elevated ($P < 0.01$) by exercise to a lesser extent (22%) above sedentary animals in mouse MG. No significant effect of exercise was observed for UCP-2 in the rat, whereas exercise increased ($P < 0.01$) UCP-2 mRNA levels by 55% in the mouse. There was no significant effect of chronic exercise on UCP-2 or UCP-3 mRNA in either female or male rats.

DISCUSSION

The purpose of the present study was to examine the effects of muscle activity (acute and chronic exercise) or inactivity (denervation) on the control of UCP gene expression. The major findings of the study are that *1*) skeletal muscle denervation increases UCP-3 gene expression in the rat but decreases mRNA levels in the

Table 1. *Effects of denervation and acute and chronic exercise on skeletal muscle uncoupling protein gene expression*

	Rat			Mouse	
	Denervation	Acute exercise	Chronic exercise	Denervation	Acute exercise
UCP-3/18S rRNA					
RG, %		↑ 63*	→		
Control		0.14 ± 0.01			
Treatment		0.23 ± 0.03			
WG, %		↑ 252§	→		
Control		1.3 ± 0.14			
Treatment		3.2 ± 0.14			
MG, %	↑ 331‡			↓ 53‡	↑ 22†
Control	0.04 ± 0.01			0.046 ± 0.002	0.976 ± 0.048
Treatment	0.12 ± 0.01			0.024 ± 0.004	1.188 ± 0.026
UCP-2/18S rRNA					
RG, %		→	→		
WG, %		→	→		
MG, %	↑ 200†			↑ 63†	↑ 55†
Control	0.009 ± 0.001			0.133 ± 0.012	0.017 ± 0.001
Treatment	0.018 ± 0.002			0.217 ± 0.021	0.026 ± 0.001

Northern blot analysis of uncoupling protein 3 (UCP-3) and 2 (UCP-2) mRNA levels from rat and mouse gastrocnemius muscle after acute (2 h of treadmill running) and chronic exercise (9 wk of voluntary wheel running) or denervation. Sedentary rats and mice (exercise studies) or the contralateral limb (denervation studies) served as controls. Results indicate that denervation increased UCP-3 gene expression 331% above control in the rat, but effects were opposite in the mouse (53% decrease in mRNA levels). An acute bout of endurance exercise increased rat UCP-3 gene expression 252 and 63% above sedentary animals in white (WG) and red gastrocnemius (RG), respectively. UCP-3 gene expression was elevated by acute exercise to a lesser extent (22%) above sedentary animals in mouse mixed gastrocnemius (MG). In the rat, there was no effect of acute exercise on UCP-2 gene expression, but UCP-2 mRNA levels in MG were increased 200% above control by denervation. To a lesser extent, denervation (63%) and acute (55%) exercise increased UCP-2 mRNA levels in mouse MG. There was no effect of chronic exercise on UCP gene expression. Original data are normalized to the level of 18S rRNA. Values are means ± SE expressed in arbitrary units. Data as percentages above or below control are comparable only within cells. ↑, Increase; ↓, decrease; →, no change; no arrow, experiment not performed. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$; §$P < 0.0001$.

mouse; *2*) denervation increases UCP-2 mRNA levels in both the rat and mouse MG, although the effect is more pronounced in the rat; *3*) acute, but not chronic, exercise increases UCP-3 mRNA levels in both the rat and the mouse; *4*) the effect of acute exercise is about fourfold greater in rat white vs. red skeletal muscle; and *5*) unlike the rat, UCP-2 levels are increased by acute exercise in mouse skeletal muscle. These results demonstrate that skeletal muscle UCP gene expression is strongly influenced by muscular activity.

Recently, it has been shown that UCP-3 gene expression in rodent muscle is elevated under conditions that increase fatty acid levels, such as those occurring with starvation and endurance exercise. It has also been shown that these UCPs function in the generation of heat by uncoupling oxidative phosphorylation (19). Our findings that UCP-3 mRNA levels are elevated after acute treadmill running are inconsistent with a thermoregulatory role for muscle UCPs during or after acute exercise (i.e., elevated UCP mRNA levels were observed at a time when metabolic heat should be dissipated rather than generated). However, these data do support an association between changes in energy substrate (e.g., fatty acids) availability and UCP gene expression. As an attempt to resolve the paradox that starvation and exercise induce a potentially thermogenic protein to increase at a time when overall energy homeostasis is compromised, Weigle et al. (25) suggest that the induction of UCP-3 may be linked to the use of free fatty acid as a fuel rather than as an increased need of the organism to generate heat. Thus the function of muscle UCPs may be to preserve mitochondrial ionic or osmotic equilibrium under a cellular milieu of greatly increased fatty acid oxidation induced by both fasting and endurance exercise. Although this study did not directly test the dependency of UCP gene expression on cellular fatty acid concentrations, this suggested mechanism is supported by the finding (15) that leptin (known to increase fatty acid oxidation and decrease fatty acid incorporation into triglycerides in skeletal muscle) also increased UCP-3 gene expression in skeletal muscle (10). In contrast, the similar effect of elevating UCPs under conditions of denervation supports the notion that UCPs may also act to facilitate body or tissue-specific temperature homeostasis when presented with this type of metabolic perturbation.

The finding in the present study that rat and mouse UCP-3 is expressed in opposing directions with denervation (and to a lesser extent in the magnitude of increase of UCP-2 and UCP-3 with acute exercise) is perplexing but does suggest that differences in skeletal muscle bioenergetics between species (e.g., metabolic rate) may be reflected by the response of UCP gene expression to perturbations in energy metabolism. The physiological explanation awaits further study, but a potential explanation may lie with differences in species-specific mitochondrial membrane proton leak kinetics, with smaller mammals possessing a "leakier" membrane to protons vs. larger mammals. In general, proton leak kinetics are nonlinear among mammals and differ approximately twofold between the rat and the mouse (20). Thus, in addition to known regulators

(3, 10, 13, 25) of UCP gene expression (e.g., hormonal status, cold exposure, diet/fatty acids, reproductive state, tissue specificity) within a given species, potential differences in the factors that regulate UCP gene expression among mammals may also reflect inherent species differences in mitochondrial bioenergetics.

The results of the present study are dissimilar to those of Boss et al. (2), who report that chronic exercise (8 wk) downregulates UCP-3 and UCP-2 gene expression in tibialis anterior and soleus muscle of pathogen-free (OFA) rats. However, these investigators utilized chronic treadmill running, a forced exercise protocol, which is in contrast to wheel running, which represents volitional exercise. Our laboratory and others have reported that forced treadmill exercise reduces food consumption in male rats, whereas volitional exercise does not (6, 22). Given that food consumption postexercise was not considered in the study by Boss et al., the possibility of differences in feeding behavior accounting for the disparity in the findings between the studies cannot be excluded. To illustrate, male rats rebound from forced running, given a sufficient rest period, by increasing their food intake (22). Thus the potential for effects of refeeding, which has been shown to decrease UCP levels in muscle (10), may in part explain the differences in UCP gene expression between the two studies. The protocol utilized in the present study controlled for feeding behavior by *1*) maintaining animals in the fed state until the start of acute exercise, at which time food was withheld from both control and running groups, and *2*) maintaining animals in the fed state during the chronic exercise protocol and harvesting tissues during their light cycle, at which time animals are in the fed state and markedly reduce activity and food consumption until the next dark cycle period. An alternative explanation for the discrepancy might be that, in the study by Boss et al., UCP-3 mRNA levels were assessed 24–30 h after the last exercise bout. In the present study, muscle samples were obtained at the end of the animal's running (dark) cycle, which would represent only several hours of inactivity. Thus the results by Boss et al. may simply reflect a decline in UCP mRNA levels due to a decline in transcription and/or increased degradation after the lengthy inactive period.

In conclusion, acute exercise and denervation are powerful stimuli for UCP gene expression in mammalian skeletal muscle. The physiological explanation for the finding that perturbations to muscle energy homeostasis and/or thermogenesis elevate mRNA levels is at present unknown. However, because exercise and denervation both alter energy substrate levels (14, 21), for example, fatty acid availability and utilization, the role of alterations in fuel levels on the regulation of UCP gene expression merits further investigation. Furthermore, differences in the effect of denervation on UCP mRNA levels between the rat and mouse indicate that different mechanisms of control for UCP gene expression may exist depending on the species of animal studied and testify to the need for further investigation of the effects of activity on muscle UCP gene expression in the human.

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