Acute Endurance Exercise Increases Skeletal Muscle Uncoupling Protein-3 Gene Expression in Untrained But Not Trained Humans

Robert C. Noland, Robert C. Hickner, Mercedes Jimenez-Linan, Antonio Vidal-Puig, Donghai Zheng, G. Lynis Dohm, and Ronald N. Cortright

In rodents, acute exercise increases skeletal muscle uncoupling protein (UCP) gene expression and is associated with elevations in serum nonesterified fatty acids (NEFA). To test whether contractions increase UCP mRNA levels in humans, vastus lateralis biopsies were obtained 1 hour postexercise from untrained and trained subjects and analyzed for UCP-2 and UCP-3 long (UCP-3_L) and short (UCP-3_S) isoforms. The acute exercise bout (graded cycling protocol; 65% to 85% relative $\dot{V}O_2$max) induced significant ($P < .01$) elevations in serum NEFA in both untrained and trained subjects, but the increase in untrained subjects was significantly ($P < .05$) greater (60% *v* 30%). Ribonuclease protection assay demonstrated that basal levels of all UCP isoforms measured were similar between the 2 groups. However, acute exercise induced a significant increase ($P < .02$) in both UCP-3_L and UCP-3_S, but not UCP-2 mRNA levels in untrained, but not trained subjects. Correlation analysis did not show a significant relationship between exercise-induced changes in NEFA and UCP-3 levels. These results demonstrate that acute endurance exercise increases UCP-3 gene expression only in untrained skeletal muscle, but this effect does not seem to be tightly linked to the exercise-induced fluctuations in serum NEFA levels.

THE TRANSFORMATION OF energy from food and stored substrates to adenosine triphosphate (ATP) occurs at the level of the mitochondrial respiratory chain (Mitchell's Chemiosmotic Theory). Uncoupling mitochondrial respiration from ATP synthesis occurs by diminishing the inner mitochondrial proton gradient[1] and results in the release of stored energy in the form of heat. To date, 5 uncoupling proteins (UCPs) have been identified. In rodents, UCP-1 is located exclusively in brown adipose tissue (BAT) and has been shown to serve a thermogenic role. However, because humans express minimal amounts of BAT,[2] emphasis has been placed on the study of UCP-2 and UCP-3 because (1) of their high sequence homology to UCP-1[3]; (2) the finding that they are expressed in skeletal muscle[3-6] (a key tissue for energy homeostasis); and (3) the evidence from several lines of inquiry indicating that like UCP-1, they possess mitochondrial uncoupling activity and hence may play role in human energy metabolism, obesity, and diabetes.[5,7-10]

The biologic stimuli that regulate skeletal muscle UCP gene expression, as well as the physiologic role they may play in mammalian bioenergetics, are not known with certainty. However, it is the recurrent theme in the literature that stimuli that increase skeletal muscle fatty acid oxidation are associated with increased UCP gene expression. Accordingly, a growing number of studies indicate a greater likelihood for a role of skeletal muscle UCPs in the regulation of lipids as fuels rather than thermogenesis.[9,11-13]

It is well known that endurance exercise (as with fasting or starvation) increases fatty acid mobilization and utilization from triacylglycerol storage sites in adipose and skeletal muscle. Accordingly, rodent studies consistently demonstrate elevated UCP gene expression[14,15] and protein content[16] after acute endurance exercise. In contrast, studies examining the effects of acute exercise or training in humans have yielded inconsistent results due, in part, to differences in training status, modes of exercise, and the sampling time postexercise. With respect to the latter, independent laboratories[17,18] have demonstrated that exercise induces a transient increase in skeletal muscle UCP-3 mRNA transcription with expression returning to basal levels by 24 hours after exercise.[15,18] Unfortunately, some studies have measured UCPs 24 to 48 hours after an exercise bout when acute changes in gene expression would be undetectable.

Given that prolonged muscle contractions increase fatty acid mobilization and utilization, that endurance exercise training induces adaptations in skeletal muscle enhancing the mitochondrial capacity to oxidize fatty acids, and the equivocal information regarding the regulation of UCP gene expression that has been obtained from human skeletal muscle thus far, we chose to investigate the effects of acute endurance exercise on expression of UCP-2 and UCP-3 mRNA in trained versus untrained human volunteers. The novelty of the present experimental design was our ability to make direct comparisons between populations distinct in training status, who were biopsied at a time reflective of the effects of acute endurance exercise on UCP gene response. Here we report that acute endurance exercise upregulates skeletal muscle UCP-3 gene expression in untrained, but not trained subjects.

MATERIALS AND METHODS

Human Subjects

Sixteen subjects (9 trained: 3 women and 6 men; 7 untrained: 2 women and 5 men) between 22 to 40 years of age participated in this project. Individuals were considered trained if they had been bicycling ≥ 100 miles/week for 3 consecutive months, while untrained

From the Departments of Exercise and Sport Science (Human Performance Laboratory) and Physiology, Brody School of Medicine, East Carolina University, Greenville, NC; and the Departments of Histopathology and Clinical Biochemistry, Addenbrookes Hospital, Cambridge, United Kingdom.

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Address reprint requests to Ronald N. Cortright, PhD, Human Performance Laboratory, 371 Ward Sports Medicine Bldg, East Carolina University, Greenville NC 27858-4354; cortrightr@mail.ecu.edu.

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subjects were individuals who engaged in no consistent exercise training. All subjects had not been on any prescribed medications that would affect skeletal muscle energy metabolism (eg, hormone replacement) for at least 1 month before the study. An informed consent explaining the purpose and procedures of the project was obtained before initiation of the test procedures, and the study was approved by the University Medical Center, Institutional Review Board at East Carolina University.

Dietary and Exercise Control

Subjects were advised on procedures for selecting components of their diet and given dietary recall sheets to record their intake for the 3 days preceding the testing date. Subjects were also instructed to refrain from heavy exercise for the 48 hours preceding the test date. Compliance for diet and activity was confirmed by survey. Each participant consumed a standardized meal (1.7 MJ, 400 kcal) between 8 PM to10 PM the night before testing to ensure that an extended fasting period would not confound alterations in UCP gene expression.

Body Composition

Body composition was determined by hydrostatic weighing. Residual volume was determined by the oxygen dilution method using calibrated Ametek (Pittsburg, PA) models S-3A oxygen analyzer and CD-3A carbon dioxide analyzer.

Maximal Oxygen Consumption ($\dot{V}O_2$max) Test

The $\dot{V}O_2$max test was performed on a Lode isokinetic bicycle ergometer (Diversified, Brea, CA). Expired gas analysis was determined by a Model 2900 Sensormedics Metabolic Measurements Cart (Anaheim, CA). Heart rate, rating of perceived exertion, blood pressure, $\dot{V}O_2$, $\dot{V}CO_2$, and respiratory exchange ratio (RER) were monitored during the test. The $\dot{V}O_2$ max obtained was used to establish workloads for the hour-long cycling exercise trial.

Exercise Test Day

Participants reported to the laboratory between the hours of 7 AM to 9 AM in the fasted state (8 to 10 hours; no food intake after midnight). Muscle biopsies from the vastus lateralis were obtained using the percutaneous biopsy procedure, and blood samples were taken from an antecubital vein at rest and 1 hour after completion of the cycling protocol. The 1-hour time point to sample the muscles was chosen based on earlier findings that the mRNA levels for UCP-3 were upregulated at this time postexercise.[14] Furthermore, because the purpose of the study was to determine changes in UCP gene expression in response to an acute exercise bout, additional muscle samples were not obtained for the purpose of determining UCP protein levels, as translation is not thought to occur this rapidly in most systems. In support, Schrauwen et al[13] has recently shown that translation does not accompany UCP-3 mRNA at time points $\leq$ 4 hours postexercise. After the initial biopsy and blood sampling, the participant performed the hour-long endurance cycling bout. The cycling protocol required individuals to exercise at 60% to 65% of his or her respective $\dot{V}O_2$max for 40 minutes, 70% to 75% $\dot{V}O_2$max for the next 10 minutes, and finish the final 10 minutes at 80% to 85% of their $\dot{V}O_2$max. To equalize the relative work performed, subjects were monitored for $\dot{V}O_2$, $\dot{V}CO_2$, RER, and heart rate (Polar Electro, Woodbury, NY) at the end of each 10-minute period, and resistance was adjusted as required.

Blood Analysis

Serum leptin, insulin, triglyceride, and nonesterified fatty acid (NEFA) levels were determined to assess whether changes could be associated with skeletal muscle UCP gene expression. The procedures for each were: (1) serum leptin values were determined by radioimmunoassay (Linco, St Louis, MO); (2) serum insulin concentrations were determined using an Abbott IMx analyzer (Abbott Laboratories, Abbott Park, IL); (3) serum triglyceride concentrations were measured colorimetrically (Sigma, St Louis, MO; procedure no. 336); and (4) serum NEFAs were determined using an acyl-CoA oxidase based in vitro enzymatic, colorimetric assay kit (NEFA-C, Wako Pure Chemicals, Osaka, Japan).

Trizol Isolation of mRNA

Muscle samples weighing greater than 50 mg were homogenized on ice in the presence of 1 mL Trizol reagent (Gibco-BRL, Gaithersburg, MD). Samples were transferred to a 1.7-mL microcentrifuge tube, and 200 μL chloroform was added to separate the RNA into an aqueous phase. Samples were vortexed (2 $\times$ 15 seconds) and incubated at room temperature for 3 to 5 minutes. After the incubation period, samples were centrifuged at 12,000 $\times$ *g* for 15 minutes at 4°C. The aqueous phase containing the isolated RNA was transferred to RNase-free microcentrifuge tubes where they were precipitated with 600 μL isopropyl alcohol. After centrifugation (12,000 $\times$ *g*, 10 minutes, 4°C), the supernatant was decanted and the pellet was washed with 100% ethanol. Again the samples were centrifuged (7,500 $\times$ *g*, 5 minutes, 4°C), decanted, and resuspended in 100 μL DEPC-treated water. RNA concentration was determined using 8 μL sample in 992 μL DEPC water and analyzed spectrophotometrically at 260 nm. The values gathered here allowed determination of appropriate sample weight needed for mRNA quantification. A formaldehyde gel was run to verify that the RNA isolation procedure was effective. Samples were aliquoted (8 μg for UCP-3 and 10 to 12 μg for UCP-2) to RNase-free tubes, the volume was brought up to 300 μL with DEPC water, then 12 μL 5 mol/L NaCl, 12 μg tRNA (in 4 μL), and 900 μL 100% ethanol were added to stabilize the RNA. Samples were stored at -80°C until quantification of UCP-2, UCP-3_L, and UCP-3_S mRNA was performed.

Ribonuclease Protection Assay

Partial human UCP-2 and UCP-3 probes were generated by reverse transcriptase-polymerase chain reaction (PCR) using total RNA from human muscle as follows: 2 primers (5′ GCA GTC TTG AAG AAC GGG ACA CC 3′ and 5′ TGG CAG TAG GGG GCA CAT CT 3′) were designed to amplify 210 bp of hUCP-2 and another 2 primers (5′ GGA CTA CCA CCT CAC TG 3′ and 5′ CCC GTA ACA TCT GGA CTT T 3′) were designed to amplify 293 bp of hUCP-3_L and a protected band of 193 bp of hUCP-3_S. Both PCR products were subcloned into PGMT easy TA cloning vector (Promega, Madison, WI). Identity and orientation were confirmed by sequencing. Linearized templates for synthesis of antisense UCP-2 and UCP-3 transcripts were prepared using *Spe* I. Linearized templates for synthesis of reference RNA (ie, sense transcripts) were prepared using *Nco* I. A 103-bp cDNA corresponding to human cyclophilin was used as an internal control (Ambion, Austin, TX) to correct for variations in RNA amount. Antisense probes and reference RNAs were in vitro transcribed from linearized template DNA using T7 and Sp6 (StrataGen Systems, Woodinville, WA), RNA polymerase and labeled (antisense transcripts only) with [^{32}P]-UTP at high-specific activity (1,500 cpm/pg). Transcription reactions were treated with RNase free DNase I, ethanol precipitated in the presence of UCP-2 and UCP-3 mRNA transcripts. Protected bands were visualized by autoradiography and quantitated by phosphorimager analysis using Imagequant software (Molecular Dynamics, Sunnyvale, CA).

Statistical Analysis

A 1-way analysis of variance (ANOVA) using SigmaStat PC software (SPSS, Chicago, IL) was used to test for significant differences in subject demographic and dietary data. A group (untrained *v* trained) by

Table 1. Subject Characteristics

	Untrained (N = 7)	Trained (N = 9)	*P* Value
Age (yr)	28.4 ± 2.4	30.0 ± 2.3	.647
Height (m)	1.75 ± 0.03	1.74 ± .03	.939
Weight (kg)	84.5 ± 8.4	73.8 ± 3.8	.229
BMI (kg/m^2)	27.5 ± 2.1	24.3 ± 1.0	.160
Percent fat (hydrostatic)	26.1 ± 2.1	13.0* ± 2.2	.001
Fat-free mass (kg)	61.4 ± 4.1	64.1 ± 3.5	.618
Fat mass (kg)	23.2 ± 4.5	9.6* ± 1.8	.009
$\dot{V}O_2$max (mL/kg/min)	35.4 ± 3.5	54.0* ± 2.8	.001
$\dot{V}O_2$max (L/min)	2.65 ± 0.26	3.95* ± .27	.005

NOTE. Data are presented as mean ± SEM.
*Significant difference between trained and untrained.

time (pre- and postexercise), 2-way repeated measures ANOVA was used to determine whether significant differences in UCP levels and serum variables existed based on training status and in response to an acute exercise bout. A Tukey's hsd (main effects) or simple effects (interaction effects) analysis was used where appropriate. Pearson product moment correlation analysis was also performed to examine whether uncoupling protein levels had significant relationships with either demographic or serum variables. Statistical significance was set a priori at $P < .05$ for all analyses performed. All data are expressed as mean ± SEM.

RESULTS

Human Subjects

No significant differences due to training status were detected with regard to subject age, height, weight, or body mass index (BMI) (Table 1). However, significant differences ($P < .05$) were observed when comparing body fat percentage of the 2 groups. Interestingly, the subjects exhibited similar values for lean body mass, but had markedly different fat mass levels ($P < .05$). The average daily energy consumption of the trained (169.5 ± 3.5 kJ/kg fat-free mass/d) and the untrained group (157.2 ± 4.9) were similar, although on average, the trained subjects consumed more energy (~1.3 MJ/d; 305 kcal; not significant).

Although not elite athletes, the trained subjects were competitive cyclists training for local competitions. Comparison between the 2 groups on activity log data indicated that subjects cycled > 100 miles per week, and as expected, the trained subjects had a higher mean $\dot{V}O_2$max ($P < .01$; Table 1) than the untrained group. No significant changes in activity level 2 weeks before the study were noted for the sedentary subjects. Therefore, we accomplished our original goal of selecting a distinct subject sample in that these individuals were significantly more fit and had incurred a training adaptation versus their sedentary counterparts.

All subjects maintained similar cycling workloads relative to fitness level (as demonstrated by a similarly achieved percent of $\dot{V}O_2$max at each exercise stage) over the hour-long exercise bout. Also, no significant differences were found between the 2 groups with regard to RER values during each stage of the experimental protocol, thus indicating no differences in substrate selection during exercise (Table 2). Furthermore, due to a greater absolute work (Watts) requirement to achieve the same relative $\dot{V}O_2$max versus the sedentary counterparts, the trained group utilized ~1.2 MJ (280 kcal) more over the course of the cycling protocol, which was offset by their matched increase in daily energy intake (~1.3 MJ/d, 305 kcal/d) indicating that both groups had similar energy reserves after the exercise session.

Table 2. Mean RER Values for Trained and Untrained Subjects at Each Stage/Intensity of a 1-Hour Exercise Cycling Protocol

	Untrained RER Value	Trained RER Value	*P* Value
60% to 65% $\dot{V}O_2$max	0.88 ± 0.01	0.86 ± 0.01	.571
70% to 75% $\dot{V}O_2$max	0.94 ± 0.03	0.94 ± 0.03	.885
80% to 85% $\dot{V}O_2$max	1.00 ± 0.03	1.01 ± 0.02	.645

NOTE. Data are presented as mean ± SEM.

Blood Analysis

Previous studies have demonstrated an association between serum lipid levels, leptin, and UCP gene expression. Figure 1 depicts the response of serum hormone levels to an acute bout of exercise. ANOVA with post hoc analysis indicated that insulin values were lower ($P < .03$) after exercise in only the untrained subjects. In contrast, leptin concentration was re-

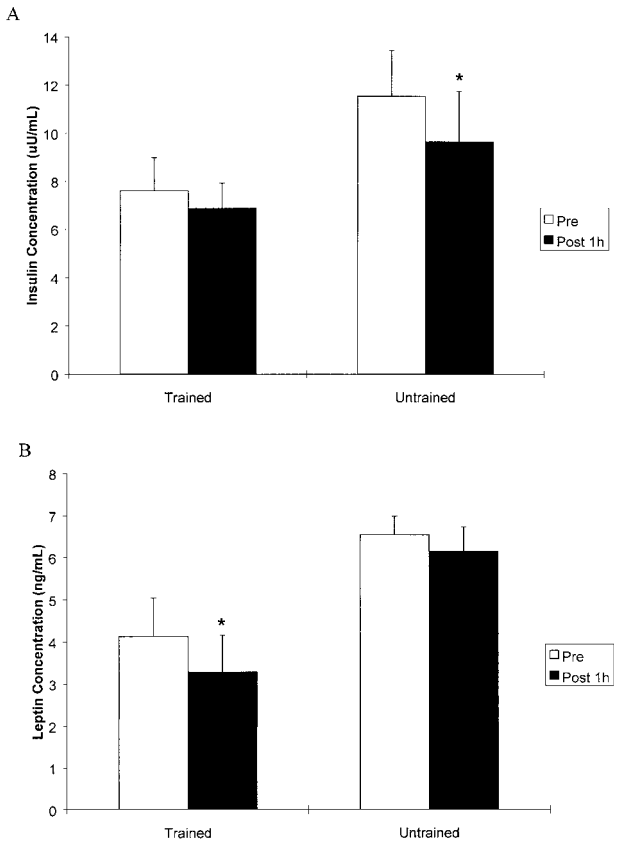

Fig 1. Effects of acute cycling exercise on serum insulin and leptin levels in untrained (N = 7) and trained (N = 9) human subjects. Values for (A) insulin and (B) leptin are shown. Data are presented as mean ± SEM. *Significant difference ($P < .03$ for insulin and $P < .01$ for leptin) in response to acute exercise.

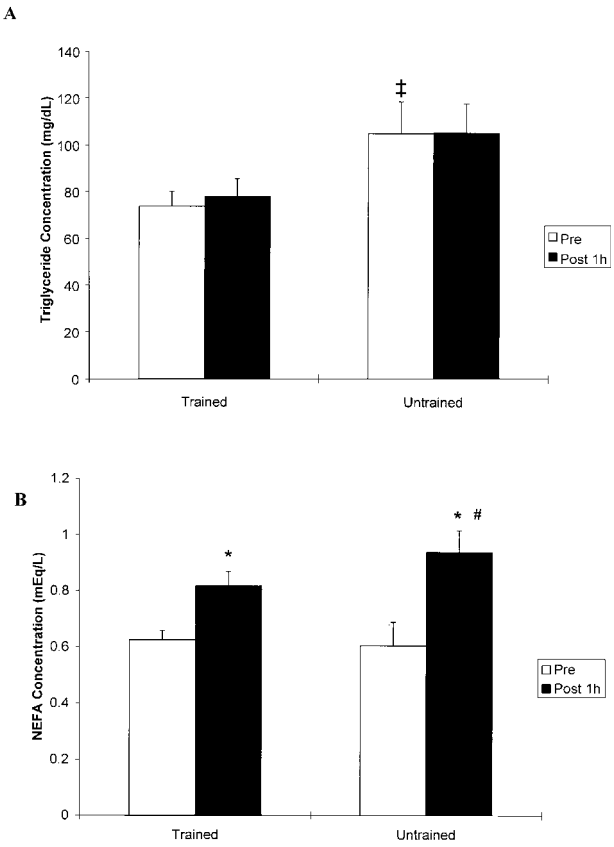

Fig 2. Effect of acute cycling exercise on (A) serum triglycerides and (B) NEFAs pre- and 1 hour post acute endurance exercise in untrained (N = 7) and trained (N = 9) human subjects. Data are presented as mean ± SEM. ‡Significant difference ($P < .05$) due to training status. *Significant difference ($P < .001$) in response to acute exercise. #Significant difference ($P < .05$) pre- and postexercise due to training status.

duced ($P < .01$) in response to exercise, but only in the trained subjects.

Figure 2 depicts the effect of the acute exercise bout on selected serum lipid levels. Resting triglyceride concentrations were higher ($P < .05$) in untrained subjects, but did not change after the acute exercise bout for either group. Basal NEFA levels were similar between groups, and as expected, were elevated in both groups postexercise ($P < .001$). Further analysis indicated an interaction effect between training status and time point, indicating that the untrained group had a significantly ($P < .05$) greater increase in NEFA levels in response to the acute exercise protocol versus their trained counterparts (60% *v* 30%).

UCP mRNA Expression

The major UCP found in the vastus lateralis of humans appears to be the UCP-3_L isoform. On average, UCP composition of the subjects participating in this study was approximately 60% UCP-3_L, 21% UCP-3_S, and 19% UCP-2. These values are representative of skeletal muscle in both trained and untrained subjects.

No significant main effects were found for training status, time point, nor was there an interaction between these variables with regard to UCP-2 mRNA levels (data not shown). ANOVA results for UCP-3_L showed a significant main effect for time ($P < .02$) indicating exercise resulted in elevations in this isoform regardless of training status (Fig 3B). Post hoc analysis showed that only the increase seen in UCP-3_L levels of untrained individuals (63%) reached statistical significance ($P < .01$). Similarly, UCP-3_S (Fig 3A) mRNA levels were significantly elevated 1 hour postexercise. Again, post hoc analysis showed that only the 25% increase observed in the untrained subjects reached statistical significance ($P < .02$).

In an effort to determine the potential factors involved in the acute exercise response of human skeletal muscle UCPs, a correlation analysis was performed. A strong positive relationship was found between changes in UCP-3_L levels in response to acute exercise with BMI ($r = .586$; $P < .02$), percent fat ($r = .596$; $P < .02$), and fat mass ($r = .890$; $P < .01$). In contrast, blood analysis for changes in insulin, leptin, and triglyceride

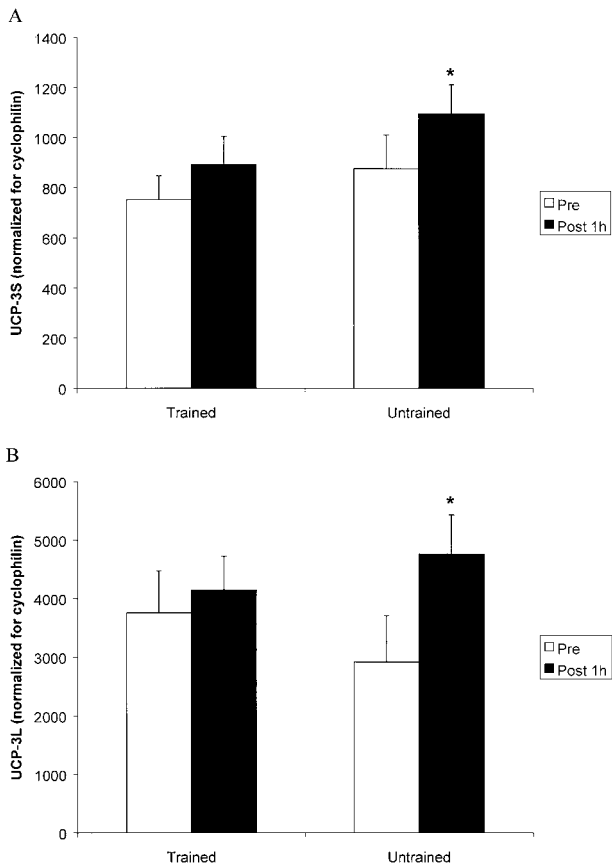

Fig 3. Effect of acute endurance exercise on skeletal muscle UCP-3 mRNA expression in untrained (N = 7) and trained (N = 9) human subjects. Muscle biopsies were obtained from the vastus lateralis, and mRNA was isolated by Trizol extraction and assessed for (A) UCP-3_S and (B) UCP-3_L by RPA. Values are presented as arbitrary units normalized for cyclophilin (a mitochondrial marker) for the (A) short and (B) long isoforms of UCP-3. Data are presented as mean ± SEM. *Significant difference ($P < .01$ for UCP-3_L and $P < .02$ for UCP-3_S) in response to acute exercise.

levels were not correlated with changes in UCP gene expression pre- to postexercise. Unexpectedly, serum NEFA values were also not correlated with changes in either skeletal muscle UCP-$3_{L,S}$ or UCP-2 mRNA pre- to postexercise.

DISCUSSION

It has previously been demonstrated in rodent models[7,14-16] that a single bout of endurance exercise will result in significant elevations in skeletal muscle UCP mRNA. Unfortunately, the current literature in humans is not definitive on this topic due to inconsistencies in experimental design among investigations (eg, time of biopsy sampling postexercise, training status, UCP isoform studied, and mode of muscle contractions). Therefore, the present study used an experimental design enabling direct comparisons between populations distinct in training status and who were biopsied at a time reflective of the effects of acute exercise on the response of the skeletal muscle UCPs. The major findings of this study are: (1) basal skeletal muscle UCP gene expression is similar between untrained and trained individuals; (2) both UCP-3_L and UCP-3_S, but not UCP-2 mRNA levels, are significantly elevated in untrained, but not trained subjects, after acute endurance exercise; and (3) this response was not tightly linked to changes in serum NEFA levels or whole body substrate metabolism.

To differentiate the effects of acute exercise versus training, it is important to understand the time course of gene expression for skeletal muscle UCPs. Accordingly, in human subjects Pilegaard et al[18] found transcription rates and UCP-3 mRNA levels peaked 1 to 4 hours postexercise, but had returned to basal levels within 22 to 24 hours. These results were corroborated in the rodent by Tsuboyama-Kasaoka et al,[15] who further determined that UCP mRNA decreased below basal levels 44 hours into recovery. Therefore, muscle biopsies taken after 24 hours postexercise should be considered to reflect the basal or recovered state, whereas skeletal muscle sampled 1 to 4 hours into recovery are reflective of the effects of acute exercise.[13,14,17] With this in mind, the existing literature regarding the effect of training status is equivocal. The present study found no differences in basal UCP mRNA levels between trained and untrained human subjects; a finding that is supported by other studies showing UCP mRNA[18,19] and protein[10] levels are not altered in trained (aerobic exercise or electrical stimulation) muscle. Alternatively, other studies[12,20] demonstrated basal UCP mRNA levels are lower in skeletal muscle posttraining. The probable explanation for these discrepancies is due to methodologic inconsistencies among the existing investigations, including the duration of exercise training (5 days to 3 years), the mode of training (eg, electrical stimulation, 1-legged knee extensions, regimented *v* self-reported cycling), subjects recruited (eg, recreational *v* elite athletes *v* tetraplegics), and the method chosen to normalize gene expression. Including the present study, the consensus is that UCP gene expression is not altered in the basal state after endurance exercise training.

The results of the present investigation indicate that only untrained human subjects responded to an acute exercise bout by increasing skeletal muscle UCP mRNA, and that this effect was isolated to the UCP-3 isoforms. Outside of the current investigation, only 3 studies have reported results in humans analyzing skeletal muscle UCP gene expression within the known time course to be considered a response to acute exercise. Similar to the current findings, Schrauwen et al[12] reported UCP-3 mRNA levels were not altered after acute exercise in trained subjects, whereas a more recent report by the same investigators showed UCP-3_L mRNA in untrained subjects was significantly increased 4 hours postexercise.[13] In addition, Pilegaard et al[18] found both untrained (4 hours cycling) and trained (5 days of 1-legged endurance training) human skeletal muscle had significantly elevated UCP-3 transcription rates by 1 to 2 hours postexercise, yet gene expression increased only in the untrained group, possibly indicating differential posttranscriptional modification/stabilization between these 2 populations. Thus, it appears to be a unifying theme that only untrained subjects will induce UCP mRNA in response to acute exercise, and that this may be specific to UCP-3 isoforms.

Because upregulation of the UCP-3 isoforms was unique to the untrained group, we attempted to address the variables that may have contributed to this finding. A common observation in the literature is that UCPs are associated with physiologic stimuli leading to enhanced fatty acid utilization (e.g., fasting/starvation), suggesting they may function in the metabolism of lipids as fuels.[9,11,21,22] To illustrate, Yoshitomi et al[23] have demonstrated that in mouse skeletal muscle, UCP-2 and UCP-3 mRNA expression was positively correlated with changes in blood fatty acids. Similarly, UCP mRNA levels have been shown to be dramatically increased in rodent skeletal muscle after intralipid infusion[24] and in C_2C_{12} myotubes incubated in the presence of fatty acids.[25] Likewise, in humans, Khalfallah et al[26] demonstrated increased plasma NEFA levels (by lipid infusion) increased UCP-3 mRNA.

Exercise is known to alter skeletal muscle metabolism and substrate flux, and the energy required for submaximal endurance exercise (40% to 60% $\dot{V}O_2max$) is primarily derived from fatty acid oxidation, suggesting they may provide a link between exercise and the regulation of UCP gene expression. Recently, Schrauwen et al[13] found that acute exercise increased UCP-3_L mRNA in untrained human skeletal muscle. This effect, however, was abolished when plasma fatty acid levels were prevented from increasing during exercise and recovery by administration of an oral glucose load. It was concluded that the exercise-induced elevation in skeletal muscle UCP gene expression is the result of alterations in fat metabolism rather than energy metabolism. Our data support the contention that the UCP response to acute exercise is not linked to the metabolic energy state per se, as the trained group consumed an average of 1.3 MJ/d (305 kcal/d) more than controls; an extra energy intake sufficient to offset the additional energy demand of the exercise bout (1.2 MJ, 280 kcal).

The untrained group in the present study had larger ($P < .05$) increases in serum NEFA levels 1 hour postexercise (Fig 2). Additionally, leptin (shown to increase fatty acid oxidation[27] and skeletal muscle UCP mRNA[28]) did not change from pre- to postexercise in untrained subjects, whereas a significant reduction was noted in trained subjects (Fig 1). These findings led us to suspect these serum factors were involved in the differential regulation of skeletal muscle UCP-3 mRNA. Unexpectedly however, further analysis of the data indicated that neither

changes in serum NEFA nor leptin levels were correlated with changes in skeletal muscle UCP-3 gene expression. Furthermore, no differences were observed between groups with respect to whole body substrate metabolism (by indirect calorimetry). These findings do not completely dissociate a role for serum fatty acids in the regulation of skeletal muscle UCP-3 gene expression, but do suggest that other (intracellular) variables may be more directly linked with their induction. In support, Schrauwen et al[13] speculated that UCP mRNA expression might be more a function of the lipid environment at the level of the mitochondria outer membrane and/or the concentration of matrix fatty acids. Collectively, these findings direct future studies toward identifying the intracellular factors that act directly to increase gene transcription of skeletal muscle UCPs.

In the context of the present experiment, it could be argued that the more pronounced effect of acute exercise on UCP-3 mRNA expression in untrained subjects might be due to differences in body composition rather than training status. However, this line of reasoning is less probable, as partial correlational analysis indicated that only 30% of the variance could be accounted for by differences in the percent body fat between groups. In support, other investigations in humans[18] and rodents[15] have yielded results indicating a more dramatic response in untrained subjects with similar body morphometry. Collectively, our interpretation of the existing data indicates that although body composition may play a role in UCP gene expression, enough evidence exists to suggest an independent effect of training status on UCP gene induction after acute exercise.

With regard to the possible physiologic role(s) for skeletal muscle UCPs during or postexercise, Vidal-Puig et al[29] have demonstrated excess reactive oxygen species (ROS) production in UCP-3 knockout mice and have proposed UCPs may be a physiologic compensation against excess ROS formation. Indeed, reducing the membrane potential by adding electron transport chain uncouplers decreases ROS generation.[30] The formation of ROS increases in the face of high-fatty acid flux as occurs with prolonged exercise.[31] Endurance training provides protective effects against ROS development by increasing antioxidant capabilities,[32,33] and subsequently, endurance-trained individuals have been reported to incur less oxidative stress during an exhaustive exercise bout than their untrained counterparts.[34] The present study showed that at similar relative exercise intensities and RER values, only the untrained group had significantly elevated UCP-3 mRNA levels. A potential explanation for this finding may be that UCP gene expression is upregulated as a compensatory mechanism against excess ROS production brought on by exercise due to a combination of increased fatty acid flux and a low antioxidant capacity in untrained versus trained people.[35] However, the present study did not assess ROS production and clearly, further research providing more direct evidence for a role of human UCPs in ROS formation is necessary.

In conclusion, our results support previous studies indicating UCP-3 mRNA levels are elevated after acute endurance exercise in untrained, but not chronically trained, humans. The explanation for the differential response due to training status remains unclear, although our data suggests that this effect is not closely linked to changes in serum NEFA levels or whole body fatty acid oxidation after acute exercise. Whereas the present report is limited to speculation as to the role skeletal muscle UCPs may have in human bioenergetics, our results emphasize the importance of sampling time in recovery when assessing the effects of exercise on UCP gene expression and thus serve to clarify some of the inconsistencies in the literature. Given the present findings, it is clear that future investigation must now be directed toward identifying the intracellular factors that may more directly regulate UCP gene expression.

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