Metabolic and anthropometric factors related to skeletal muscle UCP3 gene expression in healthy human adults

D. J. CALSBEEK,1 T. L. THOMPSON,2 J. A. DAHL,2 N. R. STOB,2 J. T. BROZINICK JR.,5 J. O. HILL,4 AND M. S. HICKEY1–3

Departments of 1Physiology, 2Health and Exercise Science, and 3Food Science and Human Nutrition, Colorado State University, Fort Collins 80523; 4Center for Human Nutrition, University of Colorado Health Science Center, Denver, Colorado 80262; and 5Eli Lilly Corporation, Lilly Corporate Center, Indianapolis, Indiana 46285

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Calsbeek, D. J., T. L. Thompson, J. A. Dahl, N. R. Stob, J. T. Brozinick Jr., J. O. Hill, and M. S. Hickey. Metabolic and anthropometric factors related to skeletal muscle UCP3 gene expression in healthy human adults. Am J Physiol Endocrinol Metab 283: E631–E637, 2002.—This cross-sectional investigation sought to determine the relationship between selected metabolic, endocrine, and anthropometric factors and skeletal muscle UCP3 mRNA in healthy adult humans. Twenty-four healthy adults (13 male and 11 female) across a range of aerobic capacity, age, and body composition were studied. Muscle biopsies were obtained from the vastus lateralis, from which UCP3 mRNA was quantified by Northern blot, and fiber type was determined by use of the myosin ATPase staining procedure. In addition, resting energy expenditure and maximum rate of oxygen consumption were determined by indirect calorimetry, body composition was determined by dual-energy X-ray absorptiometry, and fasting plasma leptin and insulin were determined by ELISA. UCP3 mRNA was correlated positively with the percent type I fibers (r = 0.842, P < 0.001), plasma leptin (r = 0.454, P = 0.026), and plasma insulin (r = 0.615, P < 0.001) and inversely to age (r = −0.411, P = 0.046). Stepwise multiple regression analysis determined that percent type I muscle fibers was the best predictor of vastus lateralis UCP3 mRNA, and no other variable entered the equation (model r² = 0.66). This study suggests that of the variables measured, UCP3 mRNA is primarily related to skeletal muscle fiber type in healthy adults. The factors that contribute to fiber-specific differences in UCP3 mRNA expression will need to be examined in future studies.

uncoupling proteins; leptin; metabolic rate

THE FACTOR(S) THAT CONTRIBUTE to the interindividual variability in resting metabolic rate have been the subject of intense research interest (38, 44, 54). The recent cloning of novel uncoupling protein isoforms (UCP2 and UCP3) has stimulated interest in potential molecular mediators of the variability in metabolic rate and susceptibility to obesity (8, 13, 15). The canonical UCP isoform (UCP1) is well accepted to act as an uncoupler of electron transport and oxidative phos-
UCP3 gene expression in skeletal muscle from healthy adult humans.

SUBJECTS AND METHODS

Subjects. After approval from the Colorado State University Human Subjects Committee, this study was performed on 24 healthy adults (N = 13 males, 11 females) aged 20–48 yr. Exclusion criteria included any disease, condition, or drug that could affect metabolic rate. Smokers and habitual alcohol consumers were not permitted to participate.

Study protocol. Subjects were instructed to report to the Human Performance Clinical Research Laboratory (HPCRL) on four separate occasions within a 2-wk time period. The subjects were urged not to change their lifestyle during their participation in the study with the exception of short-term fasting and limited activity before and after specific protocols.

Resting energy expenditure. Resting energy expenditure (REE) was measured after a 12-h fast and 48 h without regular exercise. Subjects were instructed to report to the HPCRL in the morning after a normal night of sleep. Energy expenditure was then assessed using the SensorMedics VMAX ventilated hood indirect calorimeter (SensorMedics, Yorba Linda, CA). The subjects were tested in the supine position in a darkened, thermoneutral room under a clear plastic hood. Subjects were also supplied with foam earplugs to reduce any auditory stimuli. The subjects were instructed to refrain from performing any limb movements through the duration of the assessment. Measurements were taken for 60–90 min, depending on the time required to reach and maintain a metabolic steady state. Carbon dioxide and oxygen volumes of the last 30 min of each assessment were averaged, converted to kilocalories using the Weir equation (55), and expressed on a per minute basis. Estimated fat and carbohydrate oxidation rates were calculated on the basis of the average respiratory exchange ratio of the final 30 min of the measurement period using the caloric equivalents of Livesey and Elia (28).

Plasma leptin and insulin concentrations. Immediately after the measurement of REE, blood samples were taken from an antecubital vein. Samples were stored at −80°C until processing. Plasma leptin and insulin levels were determined in duplicate by ELISA (Diagnostic Systems Labs, Dallas, TX).

Aerobic fitness. Maximal oxygen consumption (VO₂ max) was determined using the Modified Balke Multistage Progressive treadmill protocol, in which the slope of the treadmill (Quinton model no. 24-72, Bothell, WA) was increased by 1% per minute while the speed remained constant (1). Oxygen consumption and carbon dioxide production were measured using the open-circuit indirect calorimeter system of the SensorMedics 2900 metabolic measurement chart (SensorMedics, Yorba Linda, CA) at rest, during exercise, and during recovery from exercise. An individual’s VO₂ max was calculated by averaging the highest three values of oxygen consumption during the test.

To ensure that VO₂ max had been achieved, the following criteria were met: 1) a plateau in VO₂ max was observed with increasing workload, 2) the heart rate of the subject neared the maximum age-predicted heart rate (220 – age), 3) the subject’s rate of perceived exertion (RPE; Ref. 6) exceeded 17, and 4) the respiratory exchange ratio (RER) at peak exercise intensity exceeded 1.1. Subjects were instructed not to eat for 4 h before this test and not to have exercised that day. Before testing, subjects were familiarized with the test protocol and equipment so as to reduce test anxiety.

Muscle biopsy. A muscle biopsy was taken from the vastus lateralis under local anesthesia. Subjects were instructed not to exercise for 48 h before the biopsy. Fifty to one hundred milligrams of tissue were harvested and separated for assay and slide preparation. The aliquot saved for assay was immediately frozen in liquid nitrogen and stored at −80°C until analysis. The remainder of the sample was suspended and frozen in a gum tragacanth medium and stored at −80°C for histochemical analysis.

UCP3 mRNA and histochemical analysis. The mRNA for UCP3 was isolated from the biopsy samples via the methods of Chomczynski and Sacchi (12). RNA from human muscle were isolated using TRIZOL (Life Technologies, Rockville, MD) as described in the manufacturer’s instructions. Fifteen micrograms of sample RNA were size fractionated on a 1.5% agarose gel containing 0.66 M formaldehyde, and the integrity of the RNA was verified by observation of the 16 and 18S ribosomal bands under ultraviolet light. The RNA was transferred to Nytran membranes by use of the Turboblotter system (Schleicher and Schuell, Keene, NH), following the manufacturer’s instructions. The blots were prehybridized for 1 h at 42°C in UltraHyb (Ambion, Austin, TX). The blots were then hybridized overnight at 42°C in fresh hybridization solution containing 1 × 10⁶ counts-min⁻¹ (cpm)–mL⁻¹ of a [γ-³²P]CTP (3,000 Ci/mmol; New England Nuclear, Boston, MA). A random primed labeled full-length UCP3 cDNA. The CDNA probe was generated from cloned human UCP3 by PCR and was labeled using the random primers labeling system (Life Technologies, Rockville, MD). The filters were washed 2 × 5 min in 1 × sodium chloride-sodium citrate (SSC)-0.5% SDS at 42°C and for 2 × 15 min in 0.1 × SSC-0.5% SDS at 42°C. Blots were visualized by phoshorimaging and quantified with the use of Imagequant software (Molecular Dynamics, Sunnyvale, CA). After quantification for UCP3 mRNA, blots were stripped and reprobed for actin mRNA using the full-length actin CDNA that was labeled with [γ-³²P]CTP using the random primers labeling system (Life Technologies). All UCP3 signals were normalized to the actin signal to account for unequal loading of RNA.

The fiber type percentage was determined after myosin ATPase staining, as described previously (9). An average of 868 (±185) muscle fibers were counted for each subject.

Body composition. Total body mass was measured to 0.1 kg and body height to the nearest centimeter on a physician’s balance scale (Detecto, Webb City, MO). Body mass index (BMI) was calculated from body weight and height (kg/m²). A DPX-IQ full-size densitometer was used to determine body density (Lunar, Madison, WI). Subjects were asked to not eat for 4 h before the dual-energy X-ray absorptiometry (DEXA) scan. The assessment required subjects to remove all metal articles and lie flat in a supine position on the DEXA table. The speed of the scan was determined by the thickness of the subject’s chest. The variables determined by each scan include percent body fat, fat-free mass (in kg), and lean body mass (in kg).

Statistics. All statistical procedures were performed with Statistical Package for the Social Sciences (SPSS) 10.0. Means and standard deviations were calculated and are reported in Table 1. Student’s t-tests were performed to compare means between genders; this is also shown in Table 1. Pearson correlations were performed for all variables. The level of statistical significance was set at P ≤ 0.05. Partial correlations were performed to control for the variables of body mass and fat mass. Stepwise regression analysis was employed to determine the best prediction model of UCP3 mRNA expression.

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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males (n = 13)</th>
<th>Females (n = 11)</th>
<th>Total (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>31.4 ± 9.4</td>
<td>27.2 ± 6.6</td>
<td>29.5 ± 9.1</td>
</tr>
<tr>
<td>Body Mass, kg</td>
<td>78.7 ± 11.4</td>
<td>64.9 ± 5.9</td>
<td>72.4 ± 11.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>(21–48)</td>
<td>(20–48)</td>
<td>(20–48)</td>
</tr>
<tr>
<td>Fat Mass, kg</td>
<td>24.3 ± 9.4</td>
<td>23.6 ± 6.6</td>
<td>23.9 ± 6.6</td>
</tr>
<tr>
<td>Fat-Free Mass, kg</td>
<td>11.3 ± 5.5</td>
<td>18.4 ± 5.4</td>
<td>14.5 ± 6.5</td>
</tr>
<tr>
<td>%Body Fat</td>
<td>62.9 ± 6.8</td>
<td>43.0 ± 4.2</td>
<td>53.7 ± 11.6</td>
</tr>
<tr>
<td>%Type I Fiber</td>
<td>14.7 ± 5.8</td>
<td>29.6 ± 7.0</td>
<td>21.5 ± 9.8</td>
</tr>
<tr>
<td>Insulin, pm/ml</td>
<td>48.9 ± 9.5</td>
<td>51.3 ± 12.8</td>
<td>50.8 ± 11.1</td>
</tr>
<tr>
<td>REE, kJ/min</td>
<td>34.6 ± 12.4</td>
<td>47.9 ± 20.0</td>
<td>40.7 ± 17.3</td>
</tr>
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</table>

Table 2. Pearson R correlations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Leptin (ng/ml)</th>
<th>UCP3 mRNA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>-0.411*</td>
<td>-0.272</td>
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<tr>
<td>Body mass, kg</td>
<td>-0.071</td>
<td>-0.251</td>
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<tr>
<td>BMI, kg/m²</td>
<td>0.114</td>
<td>0.056</td>
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<tr>
<td>Fat mass, kg</td>
<td>0.241</td>
<td>0.317</td>
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<tr>
<td>Fat-free mass, kg</td>
<td>-0.191</td>
<td>-0.405*</td>
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<tr>
<td>%Body fat</td>
<td>0.317</td>
<td>0.427*</td>
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<tr>
<td>%Type I fiber type</td>
<td>0.842†</td>
<td>0.356</td>
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<tr>
<td>%Type II fiber type</td>
<td>-0.856†</td>
<td>-0.338</td>
</tr>
<tr>
<td>Insulin, pm/ml</td>
<td>0.015</td>
<td>0.788†</td>
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<tr>
<td>Leptin, ng/ml</td>
<td>0.454*</td>
<td>1.000</td>
</tr>
<tr>
<td>VO2max, ml·kg⁻¹·min⁻¹</td>
<td>-0.281</td>
<td>-0.175</td>
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<tr>
<td>REE, kJ/min</td>
<td>-0.175</td>
<td>-0.339</td>
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<td>RER</td>
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<td>VO2, ml/min</td>
<td>0.093</td>
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<td>Fat oxidation, g/min</td>
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<td>-0.333</td>
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<td>CHO oxidation, g/min</td>
<td>-0.065</td>
<td>0.208</td>
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<tr>
<td>UCP3 mRNA</td>
<td>1.000</td>
<td>0.454*</td>
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</table>

*P ≤ 0.05, †P ≤ 0.01.

RESULTS

Table 1 presents selected anthropometric and metabolic characteristics of the population sample. The data presented include the mean and standard deviation and minimum and maximum data points for each variable. Table 2 presents selected Pearson R correlations of interest.

UCP3 mRNA. UCP3 mRNA was not related to resting rate of oxygen consumption (VO2; r = -0.091, P = 0.672) or REE (r = -0.175, P = 0.415) but was related to plasma leptin (r = 0.454, P = 0.026; Fig. 1A), percentage of type I fiber (%type I fiber; r = 0.842, P < 0.001; Fig. 1B), age (r = -0.411, P = 0.046; Fig. 1C), and plasma insulin (r = 0.615, P = 0.001; Fig. 1D). Only two of the correlations remained significant when male and female subjects were analyzed separately. The relationship between UCP3 mRNA and %type I fiber remained significant for both genders [male (M): r = 0.893, P < 0.001; female (F): r = 0.805, P = 0.003], and the relationship between UCP3 mRNA and plasma insulin remained significant for females (M: r = 0.181, P = 0.554; F: r = 0.774, P = 0.005). For males, UCP3 mRNA was not related to resting VO2 (r = 0.130, P = 0.671), REE (r = 0.021, P = 0.947), leptin (r = 0.219, P = 0.473), or age (r = -0.295, P = 0.328). For females, UCP3 mRNA was also not related to resting VO2 (r = 0.414, P = 0.205), REE (r = 0.371, P = 0.262), leptin (r = 0.449, P = 0.166), and age (r = -0.443, P = 0.172). Step-wise multiple regression was performed to determine the best prediction model of UCP3 mRNA. The %type I fibers was the best predictor of UCP3 mRNA (r = 0.809, P < 0.01) and was the only variable to fit into the model.

REE and resting VO2. In this data set, REE (kJ/min) was related to lean body mass (r = 0.871, P < 0.001), body mass (r = 0.807, P < 0.001), body fat percentage (r = -0.419, P = 0.042), and body surface area (r = 0.807, P < 0.001). Resting VO2 was also related to lean body mass (r = 0.744, P < 0.001), body mass (r = 0.578, P = 0.003), and body surface area (r = 0.585, P = 0.003).

DISCUSSION

The primary finding from the present study is that there are significant relationships between UCP3 mRNA and plasma leptin concentration, plasma insulin concentration, %type I fiber, and age. Also of importance is that no relationship was found between REE or VO2max and UCP3 mRNA.

Fiber type-UCP3 mRNA relationship. The first account regarding UCP3, by Boss et al. (8), reported mRNA to be highest in rodent skeletal muscle. More specifically, UCP3 mRNA was highest in tensor fascia latae (fast-twitch glycolytic), tibialis anterior (fast-twitch oxidative-glycolytic), and gastrocnemius (mixed) muscles and lower in soleus muscle (slow-twitch oxidative). More recently Hesselink et al. (20) developed and utilized a UCP3-specific antibody in
an immunohistochemical procedure to show that UCP3 protein levels are expressed most abundantly in type IIb, second in type IIa, and lowest in type I human skeletal muscle fibers. Thus work both in rodents and the single report in humans suggests that UCP3 mRNA (rodents) or protein (human) expression is higher in fast twitch fibers.

Our results, however, show a significant positive relationship between skeletal muscle UCP3 mRNA and the percentage of type I skeletal muscle fibers ($r = 0.842, P < 0.001$). Although our data are in agreement with expected fiber type differences in mitochondrial density, i.e., highest mitochondrial density in type I fibers (41), it is clear from the available work in this area that neither UCP3 mRNA nor the corresponding protein content is necessarily a simple marker for mitochondrial density (8, 20). Given the prior observations that UCP3 protein content is higher in type II muscle in humans (20), it must be argued that transcriptional and translational regulation of UCP3 is complex. Considerable additional work must be done to characterize those factors that contribute to changes in both UCP3 mRNA and the expression of the mature protein.

A major advantage in the work of Hesselink et al. (20) is that these authors utilized an immunohistochemical technique to measure UCP3 protein in cross sections that were serially stained to determine fiber type. In contrast, the present study measured gene expression (steady-state UCP3 mRNA) from whole muscle homogenates and determined fiber type on cross sections from the same tissue sample. Thus differences in methodology make straightforward comparisons to the work of Hesselink et al. difficult. It is possible that both transcription and translation rates for UCP3 are not uniform between fiber types (i.e., despite lower mRNA levels in type II skeletal muscle, this tissue translates more efficiently than type I skeletal muscle). Giacobino (14) described a series of studies in which the amplitude of UCP3 mRNA changes in response to fasting was less robust than the corresponding change in UCP3 protein. Sivitz et al. (47) demonstrated UCP3 mRNA and protein changing concomitantly in rodent skeletal muscle but strongly suggested that both transcriptional and translational regulation of UCPs may be isoform, tissue, species, and perturbation specific.

In addition to differences in the methodological approach utilized, it must be noted that Hesselink et al. (20) studied only 3 healthy and 10 type II diabetic men, whereas the present study included both genders, all of good health. We performed a partial correlation controlling for gender and found that UCP3 mRNA and percentage of type I fibers remained statistically significant ($r = 0.84$). The correlations between UCP3 mRNA and percentage of type I fibers for males and females were $r = 0.893 (P = 0.001)$ and $r = 0.805 (P = 0.003)$, respectively. As noted earlier, there was no statistical difference between men and women regarding UCP3 mRNA or percentage of type I fibers.

Several putative roles for UCP3, in addition to decreasing the mitochondrial protonmotive force, have been proposed (18, 29). One proposed function of UCP3 is the reduction of reactive oxygen species (ROS) (19, 21, 50). Intracellular ROS are generated as byproducts of cellular metabolism. Imbalanced cellular redox status is a potent pathogenic factor that leads to various degenerative diseases (52). Fiber type differences in

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**Fig. 1. Relationship between uncoupling protein (UCP)-3 mRNA and plasma leptin concentration (A), %type I fibers (B), age (C), and plasma insulin (D).**
ROS formation are not well understood, although recent work (27) has provided some evidence of increased accumulation of markers of oxidative stress in type I muscle. More research on this specific topic must be carried out before any formal conclusions can be made. In addition to the potential function in defense of ROS formation, both Himms-Hagen and Harper (22) and Schrauwen et al. (42) have recently suggested that UCP3 may act to protect the mitochondria against accumulation of nonesterified fatty acids (NEFA) by translocating fatty acids outward from the mitochondrial matrix. Prior research has shown that red muscle has a higher capacity for protein-mediated fatty acid transport into the mitochondria (5, 24), suggesting that type I or red muscle fibers may have a greater need for removing excess NEFA. In the context of the proposed function of UCP3 in the outward transport of NEFA, the higher expression of UCP3 observed in type I fibers in the present study might be an adaptive response. A recent report by Moore et al. (31) revealed that mitochondrial thioesterase (MTE)-1 mRNA expression is increased in skeletal muscle of transgenic mice over-expressing UCP3, which also supports the hypothesis of UCP3 as a fatty acid exporter. Nevertheless, more research is needed to explicate the role of UCP3 in skeletal muscle.

Plasma leptin and insulin concentrations. The hormone leptin is produced primarily in adipose tissue and is the protein product of the ob gene. Leptin has been shown to act on several peripheral target tissues (3, 32, 45), but the majority of leptin action appears to occur in the hypothalamus, where it acts to decrease food consumption and presumably increase energy dissipation (23). However, research has shown that leptin treatment to ob/ob mice (lack of functional leptin) causes an increase in muscular UCP3 mRNA expression (16, 17), indicating that leptin can regulate UCP3 gene expression. The results of this study are in agreement with the previously observed relationship between plasma leptin levels and UCP3 mRNA levels in skeletal muscle. Note that we did not find a relationship between UCP3 mRNA expression and body fat percentage in this study; however, Bao et al. (2) have shown a positive correlation of skeletal muscle UCP3 mRNA with body fat percentage and BMI in nondiabetic individuals. A potential explanation for the absence of this relationship within the current data is that this sample is relatively lean. The average body fat percentages of subjects in the study conducted by Bao et al. were considerably higher (26.2 ± 2.9, 28.5 ± 2.6, and 31.2 ± 2.9%) than the percentage of the present study (21.5 ± 9.8%). It is possible that the relationship between UCP3 mRNA and body fat percentage observed by Bao et al. is only observed at higher body fat levels. Although the females studied had a comparable body fat percentage (29.6 ± 7.0%), this relationship was not significant. Other confounding factors not examined in this study may have influenced this relationship.

Bovin et al. (4) found no evidence of a significant relationship between skeletal muscle expression of UCP3 and fasting plasma insulin concentrations in 18 healthy males. In contrast, the present data present a robust relationship between these parameters (r = 0.615, P = 0.001). When separated for the effect of gender, the UCP3 mRNA and plasma insulin relationship remained significant only in the females (r = 0.774, P = 0.005). Thus our data agree with those of Bovin et al. regarding the UCP3 mRNA-insulin relationships in healthy males but suggest that this relationship is gender dependent.

Age. Older individuals have often been characterized by lower REE (10, 37, 46, 47, 49). The age-related decline in REE is also associated with increased body weight and fatness, decreased physical activity (37, 47, 49), decreased energy intake (30), and decreased lean body mass (35, 36). In the context of proposed functions of UCP3, the age-related decline in REE may, in part, be a function of a concomitant reduction in UCP3-mediated uncoupling. Interestingly, while the present study does suggest that UCP3 decreases with age, UCP3 mRNA was unrelated to REE. Thus functions for UCP3 other than thermogenesis must be considered. The decline of UCP3 mRNA with age may be important in explaining the increased prevalence of other degenerative diseases with age. As stated previously, UCP3 may function to reduce the accumulation of ROS resulting from fatty acid oxidation. If UCP3 levels are declining with age, this may explain the means by which ROS levels are elevated and subsequently cause degenerative diseases (26, 51, 52). Clearly, additional work must be done to clarify what role, if any, UCP3 might play in skeletal muscle ROS accumulation. Furthermore, our data only include men and women between the ages of 18 and 50 yr, a relatively young sample. Additional research must be performed to investigate the relationships between UCP3, ROS, and aging in an older population sample.

In conclusion, we analyzed metabolic and anthropometric variables of 24 men and women, aged 20–48 yr, to identify the relationship between these variables and UCP3 gene expression in skeletal muscle. Our results indicated that skeletal muscle UCP3 mRNA is directly associated with plasma leptin concentration, plasma insulin concentration, and percentage of type I skeletal muscle fibers and inversely related to age. Additional research must be done to further elucidate the relationship between these factors and the role of UCP3 in skeletal muscle fatty acid metabolism and ROS accumulation.

REFERENCES


