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# Skeletal Muscle Uncoupling Protein 3 Expression Is a Determinant of Energy Expenditure in Pima Indians

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**The recent discovery of uncoupling protein (UCP)-2 and UCP-3, and their high expression in skeletal muscle, has renewed interest in a possible role for these proteins in underlying the variability in energy expenditure and therefore metabolic efficiency. Using reverse transcription–polymerase chain reaction, levels of expression of *UCP-2* and long and short forms of *UCP-3* were measured in skeletal muscle of 19 nondiabetic, male Pima Indians covering a wide range of body weight. Twenty-four–hour energy expenditure was measured in a respiratory chamber in 16 of these individuals. BMI was negatively correlated with the expression levels of the long ($r$ = –0.53, $P$ = 0.025) and short ($r$ = –0.46, $P$ = 0.047) forms of *UCP-3*. BMI was not correlated with *UCP-2* expression. Metabolic rate during sleep, adjusted for fat-free mass and fat mass, was positively correlated with the long form of *UCP-3* ($r$ = 0.69, $P$ = 0.006). These results indicate that UCP-3 may be a determinant of energy expenditure and metabolic efficiency in Pima Indians. *Diabetes* 48:146–149, 1999**

The development of obesity is characterized by an imbalance between energy intake and energy expenditure. Resting metabolic rate (RMR) comprises 50–80% of daily energy expenditure (1) and is quite variable among individuals, even after adjusting for differences in body weight and body composition (2). More importantly, the variability in RMR adjusted for fat-free mass, fat mass, age, and sex aggregates in families, suggesting genetic determinants (3,4). In addition, a low adjusted RMR is a risk factor for weight gain (5). It is therefore important to understand the physiological mechanism(s) underlying the variability in RMR. Some of this variability has been shown to be associated with the variability in skeletal muscle metabolism (6).

In rodents, brown adipose tissue plays an important role in thermogenesis, via the activation of an uncoupling protein gene, *Ucp-1*. This uncoupling protein gene encodes for a mitochondrial protein carrier, which uncouples respiration from ATP production and stimulates heat production (7). *Ucp-1* is only expressed in brown adipose tissue, which is scarce in adult humans and is not thought to play a major role in energy balance. Recently, two new uncoupling proteins—uncoupling protein-2 (UCP-2) (8,9) and uncoupling protein-3 (UCP-3) (10,11)—were discovered. UCP-2 and UCP-3, which have ~55% amino acid identity with uncoupling protein-1, have been shown to have uncoupling activity (9,12). UCP-2 is widely distributed in a variety of tissues, whereas UCP-3 is mainly expressed in skeletal muscle (10,11). These new UCPs are likely candidates to underlie the variability in energy metabolism in humans and may be involved in the development of obesity.

*UCP-2* was mapped to chromosome 11q13 (9) and *UCP-3* is thought to be only 8 kb away (D. Ricquier, personal communication). In the Quebec Family study, RMR was genetically linked to DNA microsatellite markers in the vicinity of 11q13 (13) . *UCP-3* is expressed in a long (*UCP-3L*) and a short form (*UCP-3S*), with the latter lacking exon 7, likely resulting in a truncated protein (14). It is unknown whether this difference is functionally important, although this COOH-terminal region (37 amino acids) is thought to contain a nucleotide binding region.

In the present study, we investigated the relationship between *UCP-2* and *UCP-3* expression in skeletal muscle and obesity/energy metabolism in nondiabetic Pima Indians.

## RESEARCH DESIGN AND METHODS

**Subjects.** A total of 19 male nondiabetic Pima Indians were studied. Sixteen of them also had 24-h energy expenditure measured in a respiratory chamber (1). The characteristics of this group are given in Table 1. All subjects were in good health as determined by physical examination and routine blood and urine tests. All subjects were clinically euthyroid, and their concentrations of serum thyroid-stimulating hormone were within the normal range (Table 1). None took prescribed or over-the-counter medications. This study was approved by the ethics committee of the National Institute of Diabetes and Digestive and Kidney Diseases and by the Tribal Council of the Gila River Indian Community, and all subjects gave written informed consent before participation. Subjects were admitted to the Clinical Research Unit for 7–10 days and were provided a standard weight-maintaining diet containing 50% carbohydrates, 30% fat, and 20% protein for at least 3 days before metabolic testing. Glucose tolerance was assessed by an oral glucose tolerance test according to World Health Organization criteria (15), and insulin concentrations were also measured (Concept 4; ICN, Costa Mesa, CA).

**Body composition and energy metabolism.** Percent total body fat was measured by dual-energy X-ray absorptiometry using a total body scanner (DPX-L; Lunar Radiation Corp, Madison, WI), as previously described (16). After at least 3 days on a weight-maintenance diet, subjects entered the respiratory chamber at 7:30 A.M. for a 23-h stay (1). Sleeping metabolic rate (SMR) was calculated between 11:00 P.M. and 5:00 A.M., using all 15-min periods during which spontaneous physical activity was detected <1.5% of the time by 2 microwave motion detectors (1).

**Muscle biopsy and RNA analysis.** After subjects had at least 7 days on a weight-maintenance diet, a percutaneous muscle biopsy was taken from the vas-

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bp, base pair; RMR, resting metabolic rate; RT-PCR, reverse transcription–polymerase chain reaction; SMR, sleeping metabolic rate; UCP, uncoupling protein; UCP-3L, uncoupling protein 3, long form; UCP-3S, uncoupling protein 3, short form.

TABLE 1
Subject characteristics, energy expenditure, and UCP-2/3 expression in 19 nondiabetic Pima Indians

| | |
|---|---|
| Age (year) | 33 ± 8 (19–50) |
| Height (m) | 1.71 ± 0.05 (1.63–1.81) |
| Weight (kg) | 94.0 ± 20.8 (54.1–140.7) |
| BMI (kg/m$^2$) | 33 ± 7 (18–44) |
| Body fat (%) | 33 ± 8 (10–44) |
| Fasting insulin (pmol/l) | 113 ± 66 (24–258) |
| Thyroid-stimulating hormone (mU/l) | 2.1 ± 1.2 (0.8–4.8) |
| 24-h energy expenditure (kJ/day)* | 9,802 ± 1,504 (7,440–13,223) |
| SMR (kJ/day)* | 7,079 ± 896 (5,384–9,123) |
| *UCP-2* mRNA expression (ratio with β-actin) | 0.21 ± 0.10 (0.12–0.49) |
| *UCP-3L* mRNA expression (ratio with β-actin)† | 0.47 ± 0.16 (0.13–0.72) |
| *UCP-3S* mRNA expression (ratio with β-actin) | 0.46 ± 0.15 (0.18–0.78) |
| Total *UCP-3* mRNA expression (ratio with β-actin)† | 0.93 ± 0.29 (0.31–1.50) |
| *UCP-3S*/*UCP3L*† | 0.99 ± 0.22 (0.67–1.49) |

Data are means ± SD (range). *$n$ = 16; †$n$ = 18.

tus lateralis muscle after an overnight fast. After local anesthesia, a 5-mm diameter side-cutting needle was passed through a 7-mm skin incision. Muscle specimens were immediately homogenized in denaturation solution, and total RNA was isolated (Ambion, Austin, TX). Oligo-dT-primered cDNAs were synthesized from 2 µg of total RNA in a 20 µl volume using SuperScript cDNA synthesis kit from Gibco BRL (Gaithersburg, MD). The integrity of each RNA sample was verified by gel electrophoresis. For quantitative comparisons, reverse transcription–polymerase chain reaction (RT-PCR) was performed. All primer combinations were designed to span at least one intron to avoid co-amplification of genomic DNA, which may contaminate the RNA preparation. For *UCP-2*, a 964–base pair (bp) cDNA fragment was obtained using 5′-catctcctgggacgtag-3′ as a sense and 5′-atcaggtcagcagcaggagag-3′ as an antisense primer. A 993-bp *UCP-3L* cDNA fragment was obtained using 5′-aggactatggttggactgaa-3′ as a sense primer and 5′-cattcttaactggtttcggacac-3′ as an antisense primer. These primers are in exon 2 and exon 7, respectively. For *UCP-3S*, a 868-bp cDNA fragment was obtained using the same sense primer as in *UCP-3L*, and an antisense 5′-gttctctgggagggagtgc-3′ primer, which is in the untranslated region of exon 6. A 535-bp fragment of the β-*actin* gene was co-amplified as an internal control. Aliquots (5 µl) were taken from each tube every four cycles after 22 cycles to determine whether the amplification was in the exponential phase for each product. The products were resolved on a 1% agarose gel containing 1 µl ethidium bromide, which was photographed using Polaroid 665 film (Cambridge, MA), and the relative concentration of PCR products was measured by scanning densitometry (BioImage version 3.3; Sun SparcStation 5, Ann Arbor, MI). Each experiment was performed in triplicate, and the mean value was calculated for analysis. Levels of mRNA were expressed as the ratio of signal intensity for the target genes relative to β-*actin* and were corrected for the size of the product. Total *UCP-3* expression was calculated by summing the corrected ratios for *UCP-3L* and *UCP-3S*. For determination of the ratio of *UCP-3S*:*UCP-3L*, the target genes were amplified together without the control gene β-*actin*.

**Statistical analysis.** All data were analyzed using the procedures of the SAS Institute (Cary, NC). Metabolic rate (sleeping or over 24 h) was adjusted by linear regression model for the independent effects of fat-free mass and fat mass. Pearson correlation coefficients were calculated to determine the relationship between selected variables. Because of the small number of subjects, Spearman correlation coefficients were also calculated to confirm the significance of correlations. Multiple regression models also assessed the effect of UCP-2 and UCP-3 expression, independent of fat-free mass and fat mass, on energy metabolism. Data are expressed as means ± SD, and $P$ values <0.05 are considered significant.

## RESULTS

The mean levels of expression of *UCP-2*, *UCP-3L*, *UCP-3S*, total *UCP-3*, and ratio of *UCP-3S*/*UCP-3L* are given in Table 1. The expression of *UCP-3L* correlated with the expression of *UCP-3S* ($r$ = 0.60, $P$ < 0.01). The ratio of *UCP-3S* to *UCP-3L* mRNA expression did not correlate with any of the measured variables.

BMI was negatively correlated with *UCP-3L* ($r$ = –0.53, $P$ = 0.025, Fig. 1), *UCP-3S* ($r$ = –0.46, $P$ = 0.047), and total *UCP-3* ($r$ = –0.56, $P$ = 0.017). BMI was not correlated with *UCP-2*. Percent body fat tended to correlate negatively with *UCP-3L* ($r$ = –0.42, $P$ = 0.09), *UCP-3S* ($r$ = –0.40, $P$ = 0.09), and total *UCP-3* ($r$ = –0.46, $P$ = 0.06), but not with *UCP-2* . Fasting plasma insulin concentration was correlated negatively with *UCP-3L* ($r$ = –0.53, $P$ = 0.04) and only tended to correlate with total *UCP-3* ($P$ = 0.09). None of the *UCP*s were correlated with thyroid-stimulating hormone concentration.

Twenty-four–hour energy expenditure and SMR were adjusted for their two major determinants, fat-free mass and fat mass. Adjusted SMR was positively correlated with *UCP-3L* ($r$ = 0.69, $P$ = 0.006, Fig. 1) and total *UCP-3* ($r$ = 0.60, $P$ = 0.02), but not with *UCP-2* expression. Twenty-four–hour

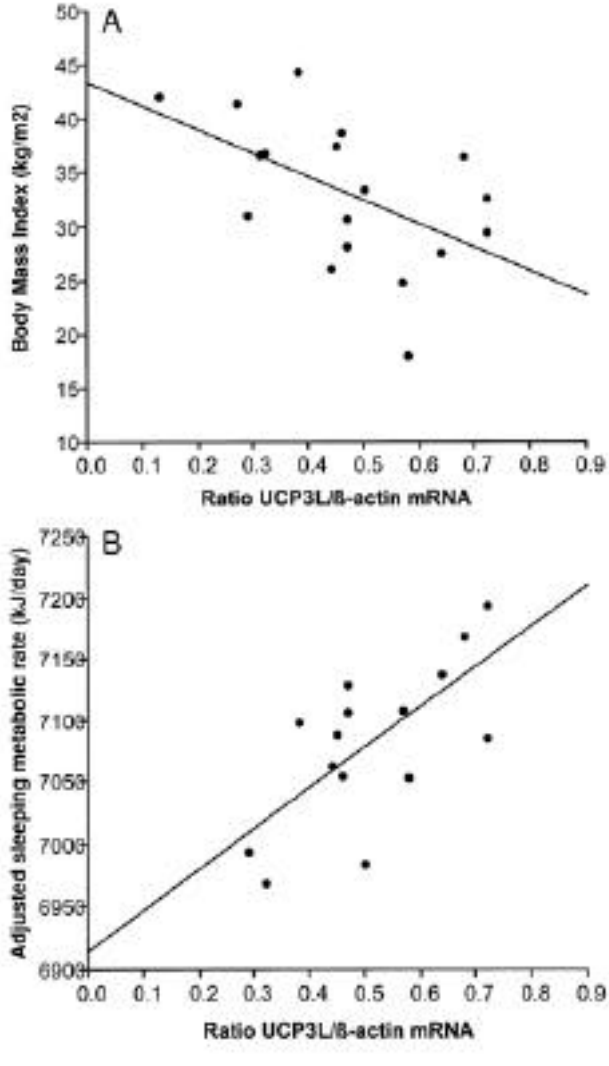


**FIG. 1. Relationship between BMI (*A*) and SMR (*B*) and relative *UCP-3L* mRNA expression (*UCP-3L*/β-*actin*) in skeletal muscle determined by RT-PCR. The mRNA expression is the mean of triplicate measurements. *A:* BMI ($r$ = –0.53, $P$ = 0.025). *B:* SMR ($r$ = 0.69, $P$ = 0.006). SMR is adjusted for fat-free mass and fat mass.**

energy expenditure only tended to correlate with total *UCP-3* ($P = 0.07$).

## DISCUSSION

RMR is an important determinant of 24-hour energy expenditure, accounting for ~50–80% of daily energy expenditure (1). The major determinants of RMR are fat-free mass, fat mass, and sex, but even after adjustment for these factors there is still considerable variability between individuals. It is important to understand the determinants of this variability, because a low "relative" metabolic rate is a predictor of weight gain (5). Part of the remaining variability in RMR can be accounted for by differences in skeletal muscle metabolism (6). The recently discovered mitochondrial proteins UCP-2 (8,9) and UCP-3 (10,11) are both expressed in skeletal muscle and have been shown to have uncoupling activity, thereby dissipating energy as heat. Therefore, these UCPs are likely candidates to underlie the physiological variability in resting energy expenditure in humans. In this study, we found positive correlations between SMR, adjusted for fat-free mass and fat mass, and the expression of the *UCP-3* gene, which indicates that *UCP-3* may be a determinant of energy expenditure in humans.

Recently, Walder et al. (17) reported an association between polymorphisms in *UCP-2* and SMR in Pima Indians. *UCP-3* is located in the same BAC and P1 clones as *UCP-2* (14), indicating that the two genes are physically near each other. Therefore, it is possible that the association between *UCP-2* polymorphisms and SMR might be due to variants in *UCP-3*. In this study, we found a positive correlation between SMR and *UCP-3* mRNA levels. Assuming that mRNA levels reflect UCP-3 protein concentrations, these data indicate that reduced skeletal muscle *UCP-3* results in a low SMR. Interestingly, Barbe et al. (18) recently reported a positive correlation between RMR and *UCP-2* expression in adipose tissue of obese women after 25 days on a very-low-calorie diet. Because a low relative RMR is a predisposing factor for weight gain (5), it is expected that individuals with low *UCP-2* and/or *UCP-3* gene expression would eventually have higher body weight. Only prospective studies in humans will tease out the cause-and-effect relationship between *UCP-3* gene expression and body weight gain. Alternatively, transgenic animal models in which *UCP-3* expression is absent or upregulated will provide information on the role of *UCP-3* as a determinant of metabolic rate and obesity.

The negative correlation between skeletal muscle *UCP-3* expression and BMI in Pima Indians is in contrast with the results of Millet et al. (19), who found no difference in *UCP-3* mRNA expression in skeletal muscle between obese and lean Caucasians. In Pima Indians, the prevalence of obesity is higher than in Caucasians, probably because of a stronger genetic susceptibility. The lack of correlation between BMI and *UCP-3L* in Caucasians might be explained by a lower susceptibility to obesity in this population but does not rule out a role for *UCP-3* in energy expenditure and obesity in Caucasians.

*UCP-3* is expressed as a long and a short form. *UCP-3S* lacks exon 7, which encodes for a domain that is highly homologous to COOH-terminal residues found in *UCP-1* and *UCP-2* (14). In UCP-1, this terminal region is believed to participate in purine nucleotide-mediated inhibition of uncoupling activity (20). This suggests that UCP-3S might have altered uncoupling activity. In the present study, *UCP-3L* and *UCP-3S* were equally expressed in skeletal muscle, confirming previous results (14). There was, however, some variability in the ratio between *UCP-3S* and *UCP-3L* expression among individuals, but this ratio was not related to any of the measured variables.

Fasting insulin concentration was negatively correlated with *UCP-3L* expression. This relation is unlikely to be due to a direct effect of insulin, as acute hyperinsulinemia does not appear to alter the expression of *UCP-3* in skeletal muscle (19). Because fasting insulin concentrations are related to BMI and percent body fat, it is possible that *UCP-3L* expression and insulin concentrations were related through their common association with obesity. However, it is also possible that *UCP-3* or a closely linked gene directly affects insulin. In support of this, *Ucp-2* was linked to hyperinsulinemia in mice (9), and the region containing *UCP-2/UCP-3* showed some evidence of linkage to 2-h insulin concentrations during an oral glucose tolerance test in nondiabetic Pima Indians (21).

The cause(s) of the two- to threefold variation in skeletal muscle *UCP-3* mRNA expression in Pima Indians remains to be determined. *Ucp-3* mRNA concentrations are increased by thyroid hormones (12), leptin (12), β3 agonists (12), glucocorticoids (12), and free fatty acids (22). Whether these factors or DNA polymorphisms in, or near, the *UCP-3* gene result in differential expression of *UCP-3* mRNA remains to be determined.

In conclusion, our results indicate that *UCP-3* mRNA expression in skeletal muscle varies two- to threefold and may be a determinant of the variability in rates of energy expenditure and, thereby, in the degree of obesity. Additional studies are needed to demonstrate that UCP-3 protein concentrations are reflected by *UCP-3* mRNA concentrations and that UCP-3 protein concentrations are also correlated with rates of energy expenditure. Also, the genetic and/or other hormonal and metabolic determinants of variations in skeletal muscle *UCP-3* mRNA and protein concentrations in Pima Indians need to be identified.

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