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

Patrick Schrauwen, James Xia, Clifton Bogardus, Richard E. Pratley, and Eric Ravussin

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 (UCP)-1, which has ~55% amino acid identity with UCP-2, 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 informed consent before participation. Subjects were admitted to the Clinical Research Unit for 7–10 days and were provided a standard weight-maintenance 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., with all 15-min periods during which spontaneous physical activity was detected <15% 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 vast...
TABLE 1
Subject characteristics, energy expenditure, and UCP-2/3 expression in 19 nondiabetic Pima Indians

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (Range)</th>
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<tr>
<td>Age (year)</td>
<td>33 ± 8 (19–50)</td>
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<tr>
<td>Height (m)</td>
<td>1.71 ± 0.05 (1.63–1.81)</td>
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<tr>
<td>Weight (kg)</td>
<td>94.0 ± 20.8 (54.1–140.7)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>33 ± 7 (18–44)</td>
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<tr>
<td>Body fat (%)</td>
<td>33 ± 8 (10–44)</td>
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<tr>
<td>Fasting insulin (pmol/l)</td>
<td>113 ± 66 (24–258)</td>
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<tr>
<td>Thyroid-stimulating hormone (mU/l)</td>
<td>2.1 ± 1.2 (0.8–4.8)</td>
</tr>
<tr>
<td>24-h energy expenditure (kJ/day)*</td>
<td>9,802 ± 1,504 (7,440–13,223)</td>
</tr>
<tr>
<td>SMR (kJ/day)*</td>
<td>7.079 ± 896 (5.384–9.123)</td>
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<tr>
<td>UCP-2 mRNA expression (ratio with β-actin)</td>
<td>0.21 ± 0.10 (0.12–0.49)</td>
</tr>
<tr>
<td>UCP-3L mRNA expression (ratio with β-actin)†</td>
<td>0.47 ± 0.16 (0.13–0.72)</td>
</tr>
<tr>
<td>UCP-3S mRNA expression (ratio with β-actin)†</td>
<td>0.46 ± 0.15 (0.18–0.78)</td>
</tr>
<tr>
<td>Total UCP-3 mRNA expression (ratio with β-actin)†</td>
<td>0.93 ± 0.29 (0.31–1.50)</td>
</tr>
<tr>
<td>UCP-3S/UCP3L†</td>
<td>0.99 ± 0.22 (0.67–1.49)</td>
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Data are means ± SD (range). *n = 16; †n = 18.

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 UCPs 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.](image-url)
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 lower 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), p3 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.

**ACKNOWLEDGMENTS**

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**REFERENCES**

Author Queries (please see Q in margin and underlined text)

Q1: Please check that author affiliations are correct.
Q2: To distinguish between the $UCP$ genes and the UCP proteins, please check carefully that when referring to the gene, the abbreviations are italicized. When referring to the protein, the abbreviations should not be italicized. Also, please check capitalization of the abbreviations, as a few are in the format $Ucp$. Are these different from those formatted $UCP$?
Q3: Please provide all author names (last name and first initial[s]) for personal communication.
    Please define “kb.”

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