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Brief Communication

UCP5/BMCP1 transcript isoforms in human skeletal muscle: relationship of the short-insert isoform with lipid oxidation and resting metabolic rates

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Abstract

Uncoupling protein 5 (UCP5) or brain mitochondrial carrier protein-1 (BMCP1) enhances mitochondrial proton leak in vitro and its hepatic and brain expression profiles are modulated by diet and cold exposure in mice. Alternative splicing generates three isoforms: a long form (UCP5L), a short form (UCP5S), and a short form with a 31 amino acid insert (UCP5SI). We investigated the relationship between skeletal muscle UCP5 expression and in vivo energy metabolism in 36 non-diabetic Pima Indians. We determined the expression levels of total UCP5 (UCP5T), and the isoforms UCP5L, UCP5S, and UCP5SI (66.8, 32.5, and 0.8% of UCP5T, respectively). None correlated with body weight or percent body fat. The transcript level of UCP5SI, but not the others, was positively correlated with resting metabolic rate ($r = 0.38$, $P = 0.02$, adjusted for age, sex, fat mass, and fat-free mass) and lipid oxidation rate (adjusted for age, sex, and percent body fat) during a euglycemic clamp with infusion of insulin at a physiologic concentration ($r = 0.42$, $P = 0.01$).

Keywords: Uncoupling protein; UCP5; BMCP1; Isoforms; Skeletal muscle; Energy metabolism; Pima Indians

1. Introduction

Uncoupling proteins (UCPs) are mitochondrial transporters that catalyze proton leaks across the inner mitochondrial membrane, thus uncoupling fuel oxidation from ATP synthesis (for review, see Refs. [1,2]). These transporters are postulated to play a role in regulation of energy balance, basal metabolic rate, and adaptive thermogenesis [2,3]. The first identified mitochondrial uncoupling protein, UCP1, is the most extensively characterized structurally and functionally. UCP1 uncouples mitochondrial respiration in brown adipose tissue [4–6]. Subsequently, UCP2–4 were uncovered by sequence and domain homologies; each protein has six transmembrane regions and three mitochondrial carrier domains [7–11].

Human UCP5 or brain mitochondrial carrier protein-1 (BMCP1), the most recently discovered member of the protein family, is 34–39% identical to UCP1–4 [12,13]. Human UCP5 mRNA is particularly abundant in the brain and testis, and is also widely expressed in other tissues at lower levels. There are three known isoforms of human UCP5: a 325 aa "long" form (UCP5L), a 322 aa "short" form (UCP5S) that lacks amino acids Val–Ser–Gly (VSG) at position 23–25 of UCP5L, and a 353 aa "short-insert" form (UCP5SI) that lacks the VSG amino acids but has a 31-amino acid insertion between transmembrane domains III and IV [13]. UCP5/BMCP1 expression enhances mitochondrial proton leak in an in vitro uncoupling assay using recombinant yeast [12].

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Human kidney 293 cells overexpressing UCP5SI and UCP5L showed decreased mitochondrial membrane potential at a comparable level to those overexpressing UCP3, whereas cells overexpressing UCP5S had a smaller decrease in the mitochondrial membrane potential [13]. Moreover, cold exposure increased brain and liver expression levels of UCP5 in mice, and fasting and a high fat diet increased the hepatic UCP5 expression selectively in obesity-resistant, but not obesity-prone, mice [13]. The precise physiological significance of UCP5 isoforms in human tissues, particularly the possible roles in energy homeostasis and substrate oxidation, has not been investigated up to now.

In this study we measured the expression levels of UCP5 isoforms in human skeletal muscle, a mitochondria-rich tissue that plays an important role in substrate oxidation and energy expenditure [14]. To our knowledge, this is the first study on potential association of UCP5 expression with energy metabolism in humans. Since skeletal muscle UCP3 expression may be linked to the utilization of lipids as fuel substrates [15], and skeletal muscle UCP3 mRNA level correlates with energy expenditure in Pima Indians [16], we analyzed the relationships between the expression levels of the UCP5 isoforms, individually and as a total pool, with in vivo measures of energy metabolism in this population.

2. Materials and methods

2.1. Subjects

Thirty-six unrelated non-diabetic Pima Indians volunteered to undergo muscle biopsies for this study. The characteristics of this group are summarized in Table 1. All studies were approved by the Tribal Council of the Gila River Indian Community and the Institutional Review Board of the National Institutes of Diabetes and Digestive and Kidney Diseases. All subjects provided written informed consent prior to participation. All were in good health as determined by medical history, physical examination, and routine blood and urine tests, and were not taking medication during the study.

2.2. Clinical measurements

Volunteers were admitted to the Clinical Research Unit for 8–10 days, where they were fed a weight-maintaining diet for 2–3 days prior to metabolic testing. Body composition was measured by dual energy X-ray absorptiometry (DEXA) using a total body scanner (DPX-L, Lunar Radiation, Madison, WI) [17]. Oral glucose tolerance tests using 75 g glucose were conducted after a 12-h overnight fast to exclude diabetic subjects as defined by WHO [18].

Insulin action was measured at physiologic and maximally stimulating insulin concentrations during a two-step hyperinsulinemic–euglycemic glucose clamp as described previously [19]. Following an overnight fast, a primed continuous intravenous insulin infusion was administered for 100 min at a constant rate of 40 mU/m^2 body surface area/minute with a steady-state plasma insulin concentration of 140 ± 42 μU/ml (mean ± S.D.). The second insulin infusion was then administered for 100 min at 400 mU/m^2/min with steady-state plasma insulin concentrations of 2220 ± 580 μU/ml. Plasma glucose concentrations were maintained at ~100 mg/dl with a variable infusion of a 20% glucose solution. Rates of insulin-stimulated glucose disposal for physiologic and maximally stimulating insulin infusion dosages were calculated for the last 40 min of each phase and corrected for endogenous glucose output as previously described [19–21]. Using a ventilated hood system, indirect calorimetry was performed to measure resting metabolic rate (RMR) for 40 min prior to the clamp [22], and to determine rates of glucose and lipid oxidation throughout the clamp [23].

2.3. RNA extraction from muscle biopsies and cDNA synthesis

In the morning after a 12-h overnight fast, percutaneous skeletal muscle biopsies of the vastus lateralis muscle were performed using Bergstrom needles (Depuy) under local anesthesia with 1% lidocaine. The biopsy was cleaned of any visible fat, rinsed in sterile 0.9% NaCl solution, snap-frozen in liquid nitrogen and stored at −70 °C. Total RNA was isolated from the frozen tissues using Trizol Reagent (Life Technologies, Gaithersburg, MD). A portion of the total RNA was treated with DNA-free reagent (Ambion, Austin, TX) to minimize potentially contaminating genomic DNA. The quality of the RNA before and after DNAse treatment was assessed by agarose gel electrophoresis. For a few samples, mRNA was extracted from the total RNA using Oligotex direct mRNA Midi/Maxi kit (Qiagen, Santa Clarita, CA). Oligo dT-primed cDNA was subsequently synthesized using the Advantage RT-for-PCR kit (Clontech, Palo Alto, CA) from 1 μg of RNA or 0.2 μg of mRNA samples. PCR amplification of GAPDH transcript using GAPDH primers provided in the Clontech kit was carried out to confirm successful cDNA synthesis.

2.4. Quantitative Real Time PCR

Transcript level quantification for total UCP5 (UCP5T) that include all isoforms, UCP5L, and UCP5SI was performed using previously described primer–probe sets for Real Time PCR [13] using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster

Table 1
Anthropometric and metabolic characteristics of the subjects, and the P values of their Pearson correlations with UCP5SI transcript level

Characteristics	Mean ± S.D.	Range	P value of UCP5SI correlations
Number of subjects (M/F)	23/13		
Age (year)	29 ± 8	18–44	0.3
Height (m)	1.68 ± 0.08	150–182	0.2
Weight (kg)	93.3 ± 22.8	45.5–147.0	0.9
Body fat (%)	31 ± 7	15–47	0.5
Fasting glucose (mg/dl)	87 ± 6	71–99	0.006
2 h glucose (mg/dl)	116 ± 29	67–187	0.03
Fasting insulin (μU/ml)	41 ± 16	24–101	0.09
2 h insulin (μU/ml)	156 ± 110	28–426	0.001
M-low (mg/min/kg-EMBS)[a]	2.78 ± 0.93	1.80–5.71	0.2
M-high (mg/min/kg-EMBS)[b]	8.83 ± 2.25	3.65–13.49	0.4
Resting metabolic rate (kJ/min)	5.64 ± 0.88	3.91–7.13	0.6
Basal lipid oxidation (mg/kg-FFM/min)[c]	0.74 ± 0.22	0.24–1.21	0.11
Lipid oxidation during physiologic insulin infusion (mg/kg-FFM/min)	0.51 ± 0.29	−0.32–1.26	0.06

[a] M-low = glucose disposal rate at physiological insulin concentration during a two-step hyperinsulinemic–euglycemic clamp; EMBS = estimated metabolic body size.
[b] M-high = glucose disposal rate at maximally stimulating insulin concentration during the clamp.
[c] FFM = fat-free mass.

City, CA). Reaction mixture for each sample contains 2.5% of the above synthesized cDNA sample. The relative abundance of UCP5S was calculated by subtracting UCP5L and UCP5SI from UCP5T levels. Human β-actin was used to normalize the UCP5 isoform transcript levels (see below), since its abundance did not significantly correlate with any clinical parameters of the samples and the primers did not amplify any detectable product from RNA alone (data not shown). A standard curve for each primer–probe set was generated by serial dilution of cDNA from a healthy subject done in triplicate. Each sample was run in duplicate and the mean values of the duplicates were used to calculate transcript level.

2.5. Statistical analysis

Statistical analyses were performed using the Statistical Analysis System of the SAS Institute (Cary, NC). For continuous variables, general linear regression models were used to adjust for the effects of age, sex, and body composition (percent body fat for lipid oxidation rate; fat mass and fat-free mass for RMR). Plasma insulin concentration, rates of glucose disappearance during the low-dose insulin infusion, and gene expression levels were log transformed to approximate a normal distribution. The log-transformed levels of UCP5 isoform transcripts were normalized to log-transformed β-actin levels. Results were expressed as mean ± S.D., and the P values < 0.05 (unadjusted for multiple testing) were considered significant.

3. Results

Using quantitative Real Time PCR, we determined the relative abundance of skeletal muscle UCP5 isoforms in 36 unrelated non-diabetic Pima Indians whose anthropometric and metabolic characteristics are summarized in Table 1. All samples expressed quantifiable amounts of each isoform. The expression ranges of the measured UCP5T, UCP5L, and UCP5SI were 0.31–1.50, 0.21–0.82, and 0.002–0.008 arbitrary units, respectively. The total UCP5 trancript pool was composed of UCP5L as the most abundant isoform (66.8 ± 2.6% of UCP5T), followed by UCP5S (32.5 ± 2.6% of UCP5T), and UCP5SI (0.8 ± 0.05% of UCP5T). None of the individual isoforms or the total transcript pool correlated with body weight or percent body fat. Pearson correlation analysis of UCP5T and the different isoform levels revealed significant correlations between UCP5SI expression and several in vivo metabolic measures (Table 1). Further analysis using general linear models indicated that people with higher UCP5SI transcript level had a higher RMR ($r = 0.38$, $P = 0.02$) and lipid oxidation rate during the euglycemic clamp with a physiologic dose of insulin infusion ($r = 0.42$, $P = 0.01$).

4. Discussion

Uncoupling protein 5 (UCP5/BMCP1) is a recently identified member in the family of mitochondrial transporters, whose potential role in the control of thermogenesis and/or substrate oxidation remains to be clarified. In this report, we determined the expression levels of UCP5 isoforms in human skeletal muscle, and showed that the relative abundance of the isoforms was UCP5L > UCP5S ≫ UCP5SI. Despite the low abundance of the UCP5SI isoform, we were able to detect it in all samples, in contrast to a previous report indicating that UCP5SI was undetectable in human skeletal muscle

[13]. The latter result may be a particular characteristic of the single human skeletal muscle sample used in that study.

The uncoupling activity of mitochondrial transporter proteins may contribute to regulation of energy expenditure. We found a positive correlation between UCP5SI mRNA concentration and resting metabolic rate (RMR), adjusted for age, sex, fat-free mass, and fat mass. This result is reminiscent of the positive correlation between sleeping metabolic rate of Pima Indians (adjusted for fat mass and fat-free mass) with UCP3L transcript level [16]. Since RMR contributes ~50–80% of daily energy expenditure [24], the expression of UCP5SI appears to be associated with energy expenditure in the Pima Indian subjects.

In addition to the correlation with RMR, we also observed a positive correlation between the transcript level of UCP5SI and lipid oxidation during the euglycemic clamp with a low-dose insulin infusion. The correlation between UCP5SI and lipid utilization is intriguing, since increased circulating fatty acid level stimulated muscle UCP3 expression [25]. In the fasting condition, which is associated with increased free fatty acid level, there were higher levels of human skeletal muscle UCP2 and UCP3 expression [26,27], leading to the proposal that these proteins regulate lipid utilization as fuel substrates [28,29]. Thus, our finding indicates that skeletal muscle UCP5SI may play a role in lipid metabolism.

The cross-sectional design of this study did not allow determination of whether changes in skeletal muscle UCP5SI expression cause variations in RMR and lipid oxidation rates. Furthermore, the reasons for these metabolic measures being correlated with UCP5SI only and not the other UCP5 isoforms are not clear. Thus, this first report on correlation of skeletal muscle UCP5SI expression with RMR and lipid oxidation rate lays the foundation for further prospective studies in humans that include measurements of skeletal muscle UCP5 protein isoform concentrations and activity levels to elucidate the potential mechanistic role of UCP5SI in metabolic rate and lipid utilization.

The expression levels of skeletal muscle uncoupling proteins UCP3L and UCP3S have been previously shown to correlate negatively with body mass index (BMI) in non-diabetic Pima subjects [16]. In contrast, UCP2 mRNA level does not have significant correlations with adiposity measures in these subjects [16]. In Caucasian subjects, neither skeletal muscle UCP2 nor UCP3 expression correlated with BMI [26]. Our results showed that neither the total UCP5T nor the UCP5 isoforms in skeletal muscle of the non-diabetic Pimas in this study had expression levels that correlate with body weight or percent body fat, indicating that skeletal muscle UCP5 may not play a major role in obesity.

Pearson correlation analyses indicated no significant relationship between other in vivo metabolic measurements of the donors with skeletal muscle UCP5T, UCP5S or UCP5L. In contrast, the expression level of UCP5SI, the lowest abundant isoform, positively correlated with fasting plasma glucose and insulin, and 2 h plasma insulin, even after adjustments for the clinical determinants (age, sex, and percent body fat; data not shown). Nevertheless, UCP5SI mRNA concentration was not correlated with insulin action (two-step hyperinsulinemic glucose clamp). The physiological significance of this observation needs further investigation.

In this exploratory study, the results were not adjusted for the potential number of multiple testing, and, thus, should be considered preliminary. Nevertheless, the present study supports the postulated role for UCP5 and its isoforms in energy and lipid metabolisms and serves as a foundation for further functional studies.

In summary, the mitochondrial uncoupling protein UCP5 is expressed in human skeletal muscle as three different isoforms, and the most abundant isoform is UCP5L, followed by UCP5S and UCP5SI, respectively. The expression level of UCP5SI positively correlated with resting metabolic and lipid oxidation rates during the euglycemic clamp with a low-dose insulin infusion. The results indicate a potential association of skeletal muscle UCP5SI expression with energy expenditure and lipid oxidation.

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