Expression of mRNAs Encoding Uncoupling Proteins in Human Skeletal Muscle

Effects of Obesity and Diabetes

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To explore the potential role of the uncoupling protein (UCP) family in human obesity and diabetes, we have used the reverse transcription–polymerase chain reaction to quantify UCP mRNA expression in human skeletal muscle. Levels of mRNA for UCP2, and for both short (UCP3S) and long (UCP3L) forms of UCP3, were highly correlated in individuals, indicating that gene transcription of these UCPs may be coordinately regulated by common mechanisms. In normal glucose-tolerant individuals, muscle UCP2 mRNA levels were positively correlated with percentage of body fat and with BMI ($r = 0.6$ and $P < 0.05$ for both). UCP3S mRNA levels were also positively correlated with percentage of body fat ($r = 0.52$, $P < 0.05$), and UCP3L mRNA tended to increase as a function of obesity ($0.05 < P < 0.1$). UCP mRNA levels, however, were not correlated with resting metabolic rate. UCP3S and UCP3L mRNA levels ($P < 0.05$) and the UCP2 mRNA level ($P = 0.09$) were increased by 1.8- to 2.7-fold in type 2 diabetes, an effect that could not be explained by obesity. No significant difference was found for UCP2, UCP3S, or UCP3L mRNA levels between insulin-sensitive and insulin-resistant nondiabetic subgroups. We conclude that *1*) skeletal muscle mRNA levels encoding UCP2 and UCP3 are correlated among individuals and may be coordinately regulated; *2*) UCP3 expression is not regulated by differential effects on UCP3L and UCP3S forms of the mRNA; and *3*) UCP mRNA expression tends to increase in muscle as a function of obesity but not of resting metabolic rate or insulin resistance, and is increased in patients with type 2 diabetes. *Diabetes* 47:1935–1940, 1998

Uncoupling proteins (UCPs) constitute a subgroup of mitochondrial carrier proteins that are localized in the inner mitochondrial membrane (1). By dissipating proton gradients, they act to uncouple respiration from oxidative phosphorylation and convert fuel to heat. So far, three homologous UCP isoforms have been identified. UCP1 was the first UCP to be discovered and is thought to be expressed exclusively in brown adipose tissue. It plays an important role in adaptive thermogenesis and energy expenditure in rodents (2). Cold exposure, food intake, β-adrenergic stimulation, and thyroid hormone can all induce UCP1 gene transcription (3). The importance of UCP1 in adult humans is less clear, because little or no brown adipose tissue is present.

Recently, two other members of the UCP family, which are expressed in adult tissues, have been identified: UCP2 and UCP3. UCP2 is widely expressed in human skeletal muscle, fat, heart, placenta, lung, liver, kidney, and pancreas (4). In white adipose tissue of *ob/ob* and *db/db* mice, the UCP2 transcript is induced approximately fivefold compared with lean littermate controls (4), indicating that the upregulation of UCP2 expression may be a compensatory change to counteract genetically induced obesity. The UCP2 gene has been mapped to human chromosome 11q13 and mouse chromosome 7 in regions that have been linked to obesity and hyperinsulinemia (5). UCP3 exhibits more limited tissue-specific expression confined to skeletal and cardiac muscle and fat tissue (6,7). UCP3 is also distinguished by the existence of two RNA transcripts, differing only by the presence (long form of UCP3 [UCP3L]) and absence (short form of UCP3 [UCP3S]) of the final exonic sequence encoding the COOH-terminal 37 amino acid residues (8). The UCP3 gene has been mapped within 150 kb of UCP2 based on their colocalization on P1 and BAC clones (8).

UCP expression in adult humans indicates that these proteins could be playing an important role in the regulation of energy expenditure and are likely to contribute to obesity and type 2 diabetes. However, little information is available to date on the role of the UCP gene family in human metabolism. In addition to being a major insulin target tissue (9,10), skeletal muscle accounts for a significant proportion of the basal metabolic rate and is a major site for nonshivering thermogenesis in humans (11–14). The relative abundance of UCP2 and UCP3 mRNA in skeletal muscle suggests that UCPs could be important determinants of body weight and insulin sensitivity. Therefore, we have studied the mRNA expression of all UCP isoforms in human skeletal muscle and investigated whether the level of UCP mRNA is altered as a function of obesity and diabetes.

RESEARCH DESIGN AND METHODS

Clinical characterization of the human study groups. We studied eight insulin-sensitive (IS) and eight insulin-resistant (IR) subjects with normal oral glucose tolerance (15), as well as eight overtly hyperglycemic patients with type 2 diabetes All subjects were chemically euthyroid and without renal, hepatic,

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IR, insulin-resistant; IS, insulin-sensitive; PCR, polymerase chain reaction; UCP, uncoupling protein; UCP3L, long form of UCP3; UCP3S, short form of UCP3.

or cardiac disease. The protocols were approved by the Institutional Review Board, and informed consent was obtained from every subject. As shown in Table 1, individuals were categorized as IS or IR based on maximally insulin-stimulated glucose uptake rates >5 or <12 mg · kg^{-1} lean body mass · min^{-1}, respectively, during hyperinsulinemic-euglycemic clamp studies as previously described (16–18). The clamp studies assessed insulin responsiveness at a maximally effective insulin concentration (insulin infusion rate: 200 mU · m^{-2} · min^{-1}). Type 2 diabetes was diagnosed according to the criteria of the National Diabetes Data Group (15). Before the study, all type 2 diabetic patients were withdrawn from therapy for at least 3 weeks and were followed on an outpatient basis. All subjects from the IS, IR, and type 2 diabetes subgroups were allowed to equilibrate on a weight-maintenance diet (28–32 kcal · kg^{-1} · day^{-1}) consisting of 50% carbohydrate, 35% fat, and 15% protein for 2 days. On the next morning, after an overnight fast, percutaneous needle biopsy of the vastus lateralis skeletal muscle was performed as previously described (16–19). The muscle tissue retrieved was placed immediately into liquid N_2 and stored at –80°C.

Percentage of body fat and lean body mass were measured by dual-energy X-ray absorptiometry (DEXA) (Lunar, Madison, WI) as previously described (16–18). Plasma glucose was measured by the glucose oxidase method with a glucose analyzer (YSI 2300; YSI, Yellow Springs, OH). Serum insulin levels were measured using a microparticle enzyme immunoassay kit (Abbott, Chicago). Energy expenditure rates were assessed using an open-circuit indirect respiratory calorimeter (Deltratrac II; SensorMedics, Yorba Linda, CA) as previously described (18). Measures were taken before (resting metabolic rate) and at the end of the hyperinsulinemic clamps, and the difference in energy expenditure represents insulin-induced thermogenesis. Metabolic rates were normalized per kilogram of metabolically active body mass according to the method of Ravussin et al. (20).

Measurement of UCP2, UCP3S, and UCP3L mRNA levels by quantitative polymerase chain reaction. Total RNA was isolated from vastus lateralis muscle biopsies by acid guanidinium isothiocyanate extraction (21). Oligo(dT)$_{12–18}$ primers and the Super Script Preamplification System (GibcoBRL, Gaithersburg, MD) were used to convert 2 µg total RNA to first-strand cDNA in a 20-µl reaction. For quantitative polymerase chain reaction (PCR), the target sequences (UCPs) and the endogenous standard sequence (β-actin) were simultaneously amplified from 1 µl of first-strand cDNA in separate 20-µl reactions containing 10 mmol/l Tris-HCl (pH 8.3), 1.5 mmol/l $MgCl_2$, 50 mmol/l KCl, 200 µmol/l each dNTP, 2 µCi [α-^{32}P]dCTP, 1 U Taq DNA polymerase (GibcoBRL), and 1 µmol/l of each specific primer (Table 2). The reaction was conducted in a GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT) using the following program: 94°C for 1 min, 53°C for 1 min, and 72°C for 2 min. The cycle numbers for UCP2, UCP3S, UCP3L, and β-actin were 20, 19, 19, and 18, respectively, which were determined from amplification curves (number of cycles versus logarithm of the amounts of amplified products) to assure that the reactions were always stopped within the linear range (22). PCR products were resolved by 5% nondenaturing PAGE and were visualized on autoradiograms of the dried gels. Quantification of PCR products was accomplished by aligning corresponding autoradiograms and gels, cutting the specific bands from the gel, and counting radioactivity of the gel slices. The ratios of the amount of PCR products generated by UCP and β-actin in different samples were then determined and compared.

Statistical analysis. Data are means ± SE. Statistical significance was determined using analysis of variance together with Tukey's honestly significant difference to assess post hoc intergroup differences. Simple correlation analysis was used to evaluate the relationships among the UCP isoforms as well as between clinical characteristics and UCP mRNA levels. All statistical analyses were performed using Statistica for Windows, version 5.1 (1997 edition) (StatSoft, Tulsa, OK). Statistically significant differences were indicated by $P < 0.05$.

RESULTS

Coordinate expression of UCP isoforms. By using primers specific for various UCP isoforms, we were able to amplify UCP2, UCP3S, and UCP3L cDNA fragments from human skeletal muscle after a single-round PCR. UCP1 could also be amplified from human muscle, although a double-round PCR was necessary (data not shown). This difference may result from either the low level of UCP1 mRNA that is also expressed in human muscle, or the derivation of UCP1 mRNA from small numbers of fat cells present in muscle tissue. PCR products for all UCPs have been verified for sequence correctness. Our study was designed to assess whether UCP mRNA levels were altered as a function of insulin resistance and diabetes. Therefore, we have measured the relative levels of UCP2, UCP3S, and UCP3L mRNA in skeletal muscle from healthy IS individuals as well as from IR and type 2 diabetic subjects (see below). The relationships between mRNA levels of different UCP isoforms were also examined. Figure 1 shows the strong positive correlations among UCP isoforms in individual subjects. A remarkably high correlation was observed between the two UCP3 isoforms ($r = 0.83$, $P < 0.001$). UCP2 was also found to be positively correlated with UCP3S ($r = 0.64$, $P = 0.001$) and UCP3L ($r = 0.44$, $P = 0.031$). The positive correlations between the two UCP3 isoforms and between UCP2 and UCP3 suggest that gene transcription of these UCPs may be controlled by common regulators.

Muscle UCP mRNA expression and diabetes. Quantitative PCR was used to quantify UCP mRNA expression in human skeletal muscle. Figure 2*A* is an autoradiogram showing the relative amount of UCP2, UCP3S, UCP3L, and β-actin PCR products in the IS, IR, and type 2 diabetes subgroups. UCP mRNA levels were normalized for the amount of β-actin, which provided an internal control because its mRNA levels were not significantly different among the IS, IR, and type 2 diabetes subgroups (NS, data not shown). As shown in Fig. 2*B*, UCP2, UCP3S, and UCP3L mRNA levels in the IR subgroup were not significantly different from those in the IS subgroup (NS). However, when diabetic and nondiabetic subjects were compared, UCP3S and UCP3L mRNA levels in the type 2 diabetes subgroup were 84–170% higher than those in the IS and IR subgroups ($P < 0.05$). The UCP2

TABLE 1
Clinical characteristics of the study groups

	IS	IR	Type 2 Diabetes
n	8	8	8
Sex (F/M)	7/1	2/6	5/3
Race (white/black)	7/1	7/1	5/3
Age (years)	35 ± 4	36 ± 4	45 ± 3
% Body fat	31.2 ± 2.9	28.5 ± 2.6*	26.2 ± 2.9
BMI (kg/m^2)	26.0 ± 1.2	26.6 ± 1.5	27.4 ± 2.0
Fasting insulin (pmol/l)	28.4 ± 6.0	49.9 ± 6.2	51.5 ± 15.0
Fasting glucose (mmol/l)	5.0 ± 0.1	5.1 ± 0.1	11.9 ± 0.8
Glucose uptake rate (mg · kg^{-1} lean body mass · min^{-1})	16.9 ± 0.5	11.4 ± 0.4	8.6 ± 1.1

Data are means ± SE. IS and IR subjects were defined on the basis of maximally insulin-stimulated glucose uptake rates >15 or <12 mg · kg^{-1} lean body mass · min^{-1}, respectively. *One IR subject's percentage of body fat was unavailable ($n = 7$).

TABLE 2
Primers used in quantitative PCR analysis

Gene	GenBank accession no.	Primers used	Product size	Base pair no. amplified
UCP1	U28480	sense TCTCTCAGGATCGGCCTCTA antisense GTGGGTTGCCCAATGAATAC	134	268–401
UCP2	U82819	sense CCTTACCATGCTCCAGAAGG antisense TCAGCTGCTCATAGGTGACG	113	859–971
UCP3S	U82818	sense CTCCAGGCCAGTACTTCAGC antisense TGTACTCTTCACCGCTACATCC	185	899–1,083
UCP3L	U84763	sense CTCCAGGCCAGTACTTCAGC antisense GATGCACCGTTTTCTTCCAT	263	899–1,161
β-Actin	X00351	sense AAGAGAGGCATCCTCACCCT antisense TACATGGCTGGGGTGTTGAA	218	222–439

mRNA level in the type 2 diabetes subgroup also tended to be ~80% higher than in the IS and IR subgroups, although these differences did not achieve statistical significance (NS). Therefore, diabetes, but not insulin resistance, was associated with increased UCP3 mRNA expression in human skeletal muscle.

Before concluding that muscle UCP mRNA expression was not related to insulin sensitivity, we had to consider the caveat that there were mostly women in the IS group and mostly men in the IR group (Table 1), with the possibility that differences in gender composition could have obscured such a relationship. When the nine nondiabetic women were compared with the seven nondiabetic men, the mean values for muscle UCP2, UCP3S, and UCP3L mRNA levels were comparable (NS), as were mean values for age, fasting glucose, BMI, and resting metabolic rate. No gender-based differences in muscle UCP mRNA levels were apparent. We then studied the relationship between UCP mRNA levels and maximally stimulated glucose uptake rates in men separately from females. In men, neither UCP2 mRNA (r = –0.49, P = 0.26), UCP3S mRNA (r = –0.02, P = 0.96), nor UCP3L mRNA (r = –0.07, P = 0.88) were correlated with the maximal glucose uptake rates. Similarly, in women, neither UCP2 nor UCP3L mRNA levels were correlated with glucose uptake rates; however, UCP3S mRNA levels did show a significantly negative correlative correlation (r = –0.79, P = 0.011). Therefore, with the exception of UCP3S mRNA in women, no evidence of a relationship between muscle UCP mRNA and insulin sensitivity was found in either sex.

Correlation analyses in Table 3 further support a relationship between UCP mRNA expression and type 2 diabetes. Significant positive correlations of quantities of UCP2, UCP3S, and UCP3L mRNA with fasting glucose levels were observed (r = 0.61, 0.83, and 0.65, respectively; P < 0.01 for all). We also found significant negative correlations of UCP2, UCP3S, and UCP3L mRNA levels with maximally stimulated glucose uptake rates (r = –0.46, –0.60, and –0.53, respectively; P = 0.03, P < 0.01, and P = 0.01, respectively). However, when type 2 diabetic patients were removed from the analysis, the significant correlations with fasting glucose levels and glucose uptake rates disappeared (Table 3), indicating that the observed correlations are the results of significant differences that existed between type 2 diabetic and nondiabetic subjects. Furthermore, no significant correlation was found between UCP2, UCP3S, or UCP3L mRNA levels and fasting plasma insulin levels (Table 3). Because both IR and type 2 diabetes share the characteristics of increased fasting insulin levels and decreased glucose uptake rates, the combined data indicate that hyperglycemia (or some other aspect of the diabetic state), but not insulin resistance, is associated with increased expression of UCP mRNAs.

Muscle UCP mRNA expression and obesity. Relationships between UCP mRNA expression and two independent measures of obesity, percentage of body fat and BMI, were also examined. As shown in Table 3, UCP2, UCP3S, and UCP3L mRNA were not correlated with either percentage of body fat or BMI when type 2 diabetic patients were included in the sample. Considering that diabetes may primarily affect UCP expression independent of obesity, these analyses were repeated, this time excluding type 2 diabetic patients, and significant positive correlations between UCP2 mRNA levels and percentage of body fat and BMI were detected (Fig. 3). We also observed a significant positive correlation between UCP3S and percentage of body fat, and we noted trends for positive correlations between UCP3L mRNA levels and BMI as well as percentage of body fat (Table 3). Thus, obesity is associated with increased expression in muscle of UCP2 and UCP3 mRNA in subjects with normal glucose tolerance.

Two findings support the argument that higher UCP mRNA levels in type 2 diabetes are specifically a characteristic of diabetes and cannot be explained by obesity. First, percentage of body fat and BMI in the type 2 diabetes subgroup were similar to those in the IS and IR subgroups (NS). Second, the relationships between percentage of body fat and muscle UCP mRNA levels were statistically significant among nondiabetic individuals, whereas significance was lost when type 2 diabetic subjects with higher UCP mRNA levels were included in these analyses.

Muscle UCP mRNA expression and energy expenditure. Given the putative role of UCP2 and UCP3 in thermogenesis, it was interesting to examine mRNA levels in relationship to energy expenditure. UCP mRNA levels were not correlated with resting metabolic rates (Table 3). However, insulin-induced thermogenesis was negatively correlated with both UCP3S (r = –0.62, P < 0.01) and UCP3L (r = –0.49, P < 0.05) mRNA levels when type 2 diabetic and nondiabetic subjects were considered together. Because type 2 diabetic patients exhibit decreased insulin-induced thermogenesis by virtue of insulin resistance (23), we considered that the negative correlations might represent a byproduct of high UCP mRNA levels in the type 2 diabetes subgroup. Analyses were then performed with only the type 2 diabetic individuals excluded,

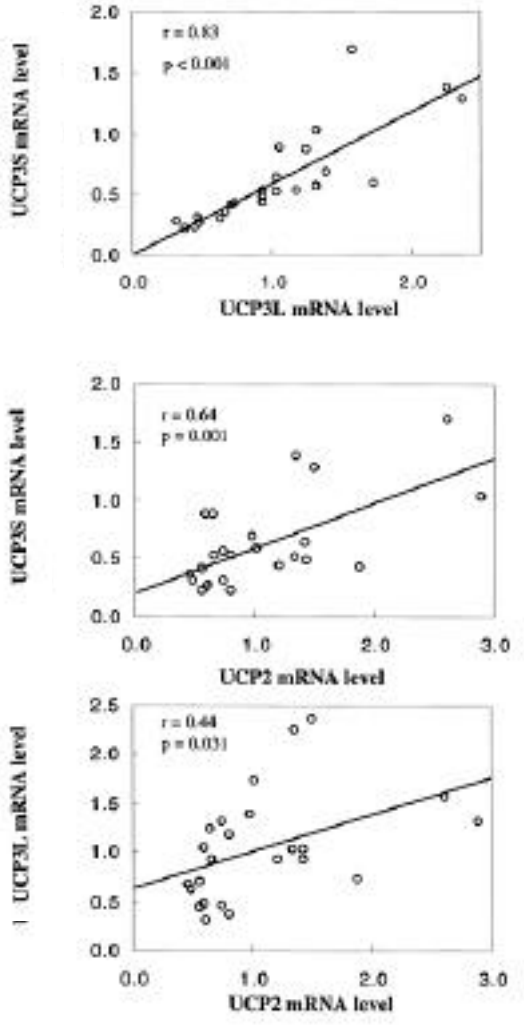

FIG. 1. Interrelationships between mRNA levels and different UCP isoforms. Relative levels of UCP2, UCP3S, and UCP3L mRNA were measured by quantitative PCR in human skeletal muscle of eight subjects each from the IS, IR, and type 2 diabetes subgroups. Simple correlation analysis was used to examine the relationships between mRNA levels of different UCP isoforms. The data represent ratios of UCP mRNA to β-actin mRNA expressed in relative units.

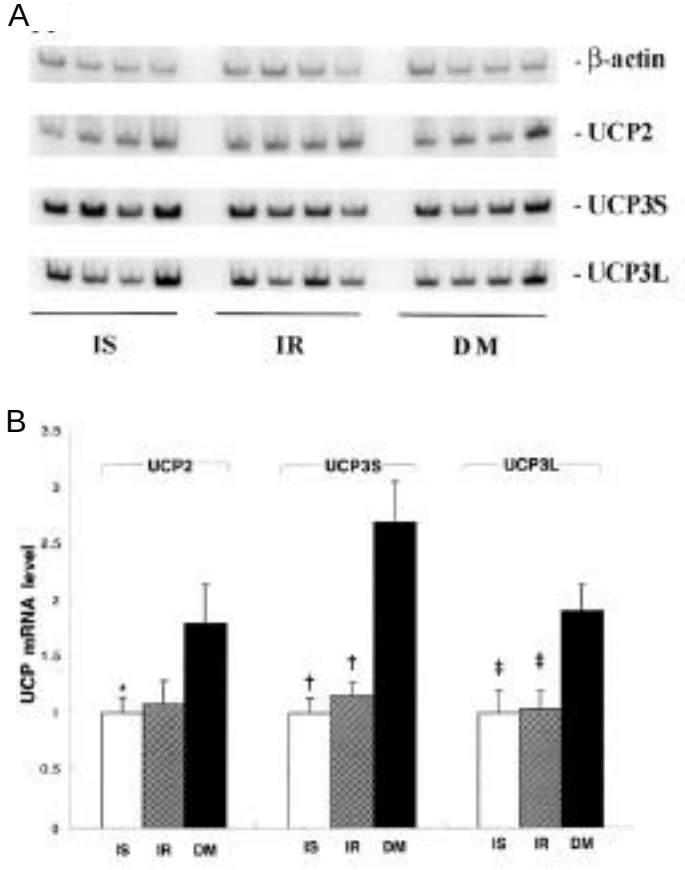

FIG. 2. Measurement of UCP2, UCP3S, and UCP3L mRNA in human skeletal muscle by quantitative PCR. Eight subjects each from the IS, IR, and type 2 diabetes (diabetes mellitus [DM]) subgroups were studied. *A*: Representative autoradiogram involving four subjects in each group. *B*: Plot of the ratios of UCP to β-actin PCR products. Data are means ± SE of eight subjects from each group, with the mean value in the IS group assigned a value of 1. *0.05 < *P* < 0.1; †*P* < 0.01; ‡0.01 < *P* < 0.05.

and the correlations between UCP mRNA levels and insulin-induced thermogenesis were no longer significant (Table 3).

DISCUSSION

UCPs have been implicated in thermogenesis and body weight regulation in animal studies (1,2). To date, however, little information is available on the role of the UCP gene family in human metabolism. To address this issue, we have studied the mRNA expression of all UCP isoforms in human skeletal muscle and have investigated the relationships between UCP mRNA expression and metabolic parameters relevant to obesity and diabetes, such as insulin sensitivity and energy expenditure. We assessed UCP mRNA in skeletal muscle because this tissue (not fat) accommodates the bulk of insulin-stimulated glucose uptake and is a major contributor to nonshivering thermogenesis in adult humans (11–14). All currently identified members of the UCP family, UCP1, UCP2, UCP3S, and UCP3L, were detected in human skeletal muscle, although the level of UCP1 mRNA was very low. The positive interrelationships of UCP2, UCP3S, and UCP3L mRNA levels in skeletal muscle were important, indicating that expression of these mRNA species could be coordinately regulated. In particular, a remarkably high degree of correlation was observed between long and short forms of UCP3 mRNA. With respect to the potential metabolic significance of muscle UCP expression, obesity was associated with increases in UCP2 and UCP3 mRNA levels, and type 2 diabetes exerted an independent effect that augmented UCP2 and UCP3 mRNA expression. UCP isoform mRNA expression had no relationship to the degree of insulin sensitivity or resistance, assessed as maximally stimulated glucose uptake rates during euglycemic clamp studies, with one exception: in nondiabetic females, UCP3S mRNA levels were negatively correlated with glucose uptake rates. Rates of resting energy expenditure (in all subjects) or insulin-induced thermogenesis (in nondiabetic subjects only) were not correlated with UCP mRNA expression.

As noted above, we observed strong positive correlations between UCP2, UCP3L, and UCP3S mRNA levels in

TABLE 3
Simple correlation analyses

	Pearson's correlation coefficient					
	All subjects (n = 24)*			Subjects with normal glucose tolerance (n = 16)*		
	UCP2	UCP3S	UCP3L	UCP2	UCP3S	UCP3L
% Body fat	0.08	–0.10	–0.11	0.64†	0.52†	0.46‡
BMI	0.25	0.22	0.29	0.59†	0.45‡	0.43‡
Resting metabolic rate	0.07	0.19	–0.04	0.03	–0.12	–0.19
Fasting glucose	0.61§	0.83§	0.65§	0.20	0.32	0.33
Fasting insulin	–0.02	–0.02	–0.01	–0.02	0.01	–0.03
Glucose uptake rate	–0.46†	–0.60§	–0.53§	–0.17	–0.29	–0.17
Insulin-induced thermogenesis	–0.38	–0.62§	–0.49†	–0.43	–0.11	–0.16

*One IR subject's percentage of body fat was unavailable. †0.01 < P < 0.05; ‡0.05 < P < 0.10; §P < 0.01.

skeletal muscle, including a high degree of correlation between the two isoforms UCP3L and UCP3S. Differential expression of UCP3S and UCP3L could be an important site of regulation because UCP3S deletes the last exon and transmembrane region of full-length UCP3 encoded by UCP3L mRNA (8) and thus could result in the translation of a dysfunctional protein. However, we find no evidence of this process in human muscle. UCP2 and UCP3 mRNA exhibit differences in tissue-specific expression (6,7) and are differentially regulated in rodent skeletal muscle by thyroid hormone, by fasting and refeeding, and by leptin treatment (24). In human muscle, however, UCP2 and UCP3 mRNA levels were positively correlated, raising the interesting possibility that UCP2 and UCP3 mRNA species could be coordinately regulated in human muscle. UCP2 and UCP3 genes are localized within 150 kb of each other on human chromosome 11q13 and could be regulated by common metabolic and hormonal factors, and by common *trans*- or *cis*-elements.

Increased expression of UCP2 and UCP3 mRNA was associated with obesity in nondiabetic individuals. This association did not appear to be a function of insulin resistance because except for UCP3S, which was negatively correlated with glucose uptake rates in female subjects only, muscle UCP2 and UCP3 mRNA levels were not correlated with maximally stimulated glucose uptake rates. Elevated UCP mRNA levels were also observed in type 2 diabetes, and this effect was independent of obesity because there were no differences in BMI or percentage of body fat between nondiabetic and diabetic subgroups. Upregulation of UCP gene transcription could be a compensatory change to counteract obesity and diabetes, as was observed in *ob/ob* and *db/db* mice (4).

We also observed that UCP2 and UCP3 mRNA levels were not correlated with the resting metabolic rate, which is inconsistent with the concept of a thermogenic role for the corresponding proteins. Rates of insulin-induced thermogenesis were negatively correlated with UCP3 mRNAs when diabetic subjects were included in the analysis, primarily because insulin resistance (23) and enhanced expression of UCP mRNAs in these individuals result in a low incidence of insulin-induced thermogenesis. Among nondiabetic individuals, we found no correlation between muscle UCP mRNAs and insulin-induced thermogenesis. Thus, we could find no definitive relationship between energy expenditure and UCP mRNA expression in skeletal muscle in this cross-sectional analysis. Millet et al. (25) have recently reported that UCP2 and UCP3 mRNA levels in human skeletal muscle were increased during food deprivation. Fasting is associated with reduced thermogenesis and increased lipid utilization. Therefore, UCP2 and UCP3 may participate in the regulation of lipids as fuel substrates rather than mediators of regulatory ther-

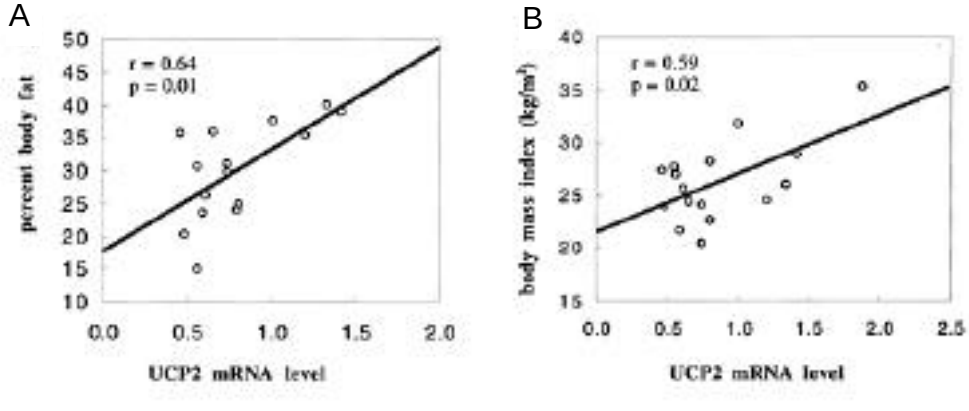

FIG. 3. The relationships between muscle UCP2 mRNA expression and obesity in nondiabetic humans. Relative UCP2 mRNA levels in muscle biopsy specimens were correlated with percentage of body fat (*A*) and BMI (*B*).

mogenesis. In fact, Boss et al. (26) have detected a significant positive correlation between UCP3 (but not UCP2) mRNA and circulating free fatty acids in human muscle from obese patients. However, in the current study, we could not detect any significant correlation between UCP2 or UCP3 mRNA and free fatty acids, regardless of whether the type 2 diabetic patients were included or excluded (NS, data not shown).

The functional significance of these observations involving mRNA levels should be interpreted cautiously when data on UCP levels are unavailable. It is important to consider that UCP expression may be regulated differentially at the level of mRNA and protein and that the protein may subserve functional effects other than thermoregulation. Nevertheless, skeletal muscle UCP mRNA upregulation in obesity and diabetes is consistent with UCP participation in these disease states. Accordingly, we have recently detected association of a UCP2 gene polymorphism with obesity in African-Americans (A. Brown, L. Robinson, B.W., G. Argyropoulos, W.T.G., unpublished observations). Additional experiments assessing UCP mRNA and protein expression in metabolically characterized subjects are necessary before conclusions can be drawn regarding the physiological role of UCP in humans.

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

Q1: Please indicate which coauthors are affiliated with the Department of Medicine, Medical University of South Carolina, and which are affiliated with the Ralph H. Johnson Veteran Affairs Medical Center. Thanks.
Q2: Should “literate” be “littermate”?
Q3: “and are likely to contribute to obesity and type 2 diabetes” as meant?
Q4: Please note that “diabetes mellitus (DM)” has been changed throughout to “type 2 diabetes,” in accordance with journal style.
Q5: Should “DEXA” be “DPX-L DEXA” or “DPX-g DEXA”? The abbreviation DEXA appears incomplete for a product name.
Q6: OK to change “group-by-group” to “intergroup”?
Q7: Is sentence “This difference may be due to…” OK as edited?
Q8: Is “tended to be ~80% higher” as meant?
Q9: Fig 1—Is “data represent ratios of UCP mRNA to β-actin mRNA” OK as edited (as meant)?
Q10: Figure 2—Please note that symbols a, b, and c have been changed to dagger, double dagger, and asterisk, respectively, per journal style.
Q11: Figure 2—OK to add “β-actin” to “Measurement of UCP2, UCP3S, and UCP3L mRNA”?
Q12: Table 3—Please note that a, b, and c have been changed to section mark, dagger, and double dagger, respectively, per journal style.
Q13: OK to add “isoforms” after “two” in “between the two UCP3L and UCP3S”?
Q14: OK to add “process” after “this” in the sentence “However, we find no evidence of this in human muscle”? If not, do you wish to supply another word for clarification?
Q15: Is “by thyroid hormone, by fasting and refeeding, and by leptin treatment” as meant?
Q16: Is the sentence “Rates of insulin-induced thermogenesis were…” OK as edited (as meant)?
Q17: By “without UCP protein levels, do you mean “when data on UCP levels are unavailable”? If so, OK to change?
Ref. 27—Has this paper been accepted yet for publication? If so, please provide details (journal name, volume no, page range); if not, the reference will be deleted from the list and cited in text as “unpublished observations.”