

Uncoupling protein gene: quantification of expression levels in adipose tissues of obese and non-obese humans

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Abstract The mitochondrial uncoupling protein (UCP), which is exclusively expressed in brown adipose tissue, regulates energy expenditure in rodents but its importance in the energy homeostasis of adult humans is uncertain. To study associations of UCP gene expression with human obesity, we determined, by a competitive reverse transcription-polymerase chain reaction assay, UCP mRNA expression levels in intra- and extraperitoneal adipose tissues of 79 obese subjects and 17 lean controls. UCP mRNA and internal standard RNA were reverse transcribed and coamplified in one reaction in which the same primers were used. The signal intensities of UCP mRNA products were compared with the signal intensities of standard RNA products to quantify UCP mRNA abundance. UCP mRNA was detected in all intra- and extraperitoneal adipose tissues studied. In both obese and non-obese subjects, UCP mRNA abundance was higher in the intraperitoneal than in the extraperitoneal tissue ($P < 0.001$). Compared to lean controls, morbidly obese subjects showed a significantly lower age- and gender-adjusted UCP mRNA expression level in the intraperitoneal adipose tissue (3.467 ± 2.483 vs. 6.917 ± 4.292 amol/fmol β -actin mRNA; mean \pm SD, $P < 0.002$), while UCP mRNA abundance in extraperitoneal adipose tissue did not differ between obese and non-obese subjects. These data are consistent with reduced energy expenditure in obesity, but it remains to be determined whether the association of decreased intraperitoneal UCP mRNA expression with obesity status reflects a causal contribution of brown adipose tissue function to the pathogenesis of obesity.—Oberkofler, H., G. Dallinger, Y.-M. Liu, E. Hell, F. Krempler, and W. Patsch. Uncoupling protein gene: quantification of expression levels in adipose tissues of obese and non-obese humans. *J. Lipid Res.* 1997. **38**: 2125–2133.

Supplementary key words uncoupling protein • mRNA • obesity • RT-PCR

Obesity is a disorder of energy homeostasis caused by a chronic imbalance of energy intake and expenditure. Energy intake is usually increased in obese subjects, while basal metabolic needs are often reduced. At least in rodents, brown adipose tissue (BAT) plays an impor-

tant role in total-body energy expenditure by burning energy in form of heat, whereas white adipose tissue stores energy in form of triglyceride. Central to the function of BAT is the uncoupling protein (UCP) or thermogenin that is found exclusively in the mitochondrial inner membrane of brown adipocytes (1–3). UCP may directly translocate H^+ across the inner mitochondrial membrane (4) or transport fatty acid anions which can reenter the mitochondria after their protonation (5, 6). In both models, UCP-action causes the H^+ gradient across the mitochondrial inner-membrane to collapse. As a result, substrate oxidation is uncoupled from ATP-generation, and heat is produced instead of chemical energy.

Studies in rodents indicate that BAT regulates total body fat stores. Mice made deficient in BAT through a transgenic approach targeting the UCP gene developed marked obesity. Importantly, obesity was initially not associated with hyperphagia, but was the consequence of reduced energy expenditure. Hyperphagia developed only after the obese phenotype was established. These observations suggest that BAT not only affects energy expenditure, but may also regulate energy intake by yet unknown mechanisms (7). While the importance of BAT in rodents and neonates of larger mammalian species including humans is well documented (8, 9), the functional significance of BAT in adult humans is less clear. The presence of large amounts of UCP has been described in perirenal fat of adult patients with phoe-

Abbreviations: apo, apolipoprotein; BAT, brown adipose tissue; BMI, body mass index; EDTA, ethylenediamine-tetraacetic acid; HDL, high density lipoprotein; RT-PCR, reverse transcription-polymerase chain reaction; SOE, splice overlap extension; UCP, uncoupling protein.

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chromocytoma implying that catecholamines can induce UCP expression (10). Using histological and histochemical techniques, multilocular BAT has been detected around the neck arteries and in the pericardium of outdoor workers (11) and long-term alcohol consumers (12). Studies relying on sensitive molecular techniques to detect UCP mRNA in adult subjects raised the possibility that BAT may also be represented in adipose deposits previously thought to consist only of white adipocytes (13, 14).

To investigate a possible role of BAT and/or an imbalance between BAT and white adipose tissue in human obesity, a method for the quantification of functional BAT is essential. As UCP mRNA is expressed solely in BAT, we developed a quantitative assay for UCP mRNA that is based on reverse transcription of total adipocyte RNA in the presence of an *in vitro* synthesized competitor RNA and subsequent amplification of cDNA. Using this assay, UCP mRNA was detectable in all intra- and extraperitoneal adipose tissues studied. In intraperitoneal adipose tissue, morbidly obese subjects expressed significantly lower levels of UCP mRNA than lean controls.

MATERIALS AND METHODS

Human adipose tissue samples, isolation of total adipose tissue RNA and DNA, and biochemical measurements

Tissue samples were obtained from morbidly obese subjects who underwent weight reduction surgical treatment through a gastric banding procedure. Control subjects underwent elective surgical procedures such as cholecystectomy or repair of hernias. Study subjects provided informed consent and the study was approved by the institutional review board. After an overnight fast, general anesthesia was induced between 8:00 A.M. and 10:00 A.M. by a short-acting barbiturate and maintained by alfentanil-hydrochloride. Fat biopsies were taken from comparable sites of the abdominal subcutaneous fat (referred to as extraperitoneal fat) and the omentum (referred to as intraperitoneal fat) at the beginning of the surgical procedure. Adipose tissue specimens were submerged in ice-cold saline and transported to the laboratory where they were divided into aliquots and frozen at -70°C .

Total RNA was isolated from 2 g of adipose tissue according to the method of Chomczynski and Sacchi (15). The integrity of the RNA was ascertained by the electrophoretic patterns of rRNA in formaldehyde gels. RNA

concentrations were determined by absorbance measurements at 260 nm. Genomic DNA was isolated from adipose tissue specimens using the QIAamp Tissue Kit (Qiagen Inc., Hilden, Germany) according to the manufacturers' instructions. DNA concentrations were determined by absorbance measurements at 260 nm.

Body mass index (BMI, kilograms per meters squared) was calculated from measurements of weight and height. After an overnight fast, venous blood was collected at 8:00 A.M. into tubes containing EDTA. Plasma glucose was measured by a hexokinase/glucose-6-phosphate dehydrogenase method. Plasma insulin was measured by immunoassay (MEIA, Abbott Laboratories, Abbot Park, IL). Cholesterol and triglyceride were measured by enzymatic procedures using a Hitachi 717 analyzer (Boehringer Mannheim Diagnostics, Mannheim, Germany) and the respective enzymatic kits (catalogue Nos. 1489437 and 1058550, Boehringer Mannheim Diagnostics). HDL cholesterol was determined in supernates after precipitation of plasma with phosphotungstic acid/magnesium chloride. ApoB and apoA-I levels were determined using nephelometric procedures (Array 360, Beckman, Palo Alto, CA). Plasma leptin levels were measured with a RIA kit (Linco Inc., St. Charles, MO) using an antibody raised against highly purified human leptin and recombinant human leptin as tracer and standard. The inter- and intra-assay coefficients of variation were 8% and 7%, respectively.

UCP and β -actin ribonuclease (RNase) protection assays

A 202-bp fragment of human β -actin mRNA and a 312-bp fragment of human UCP mRNA were amplified from human adipose tissue total RNA using RT-PCR. The sequences of the β -actin primers were 5'-ACTCTTCCAGCCTTCCTT-3' (+821, +840) and 5'-GATCTTCATTGTGCTGGG-3' (+1335, +1316), respectively. Sequences of the UCP primer were 5'-ACCTCGCTACACGGGGAC-3' (+450, +467) and 5'-GCACAGTTGGGCA CACTTTTGT-3' (+740, +761), respectively. The numbers in parentheses designate the 5' and 3' ends in the cDNA relative to the translation start site (Genbank accession numbers U 28480 and M 10277 for UCP and β -actin, respectively). Primers were synthesized by using a Beckman Oligo 1000 DNA Synthesizer (Beckman Instruments Inc., Fullerton, CA). Adipose tissue RNA (0.5 μg /assay) was reverse transcribed using 200 units of Moloney murine leukemia virus reverse transcriptase (Gibco BRL Life Technologies, Grand Island, NY), 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 5 mM MgCl_2 , 2.5 μM random hexamers, 1 mM dNTP, and 20 units of RNasin (Promega Corp., Madison, WI) in a volume of 20 μL .

The PCR reactions contained 0.2 μM of each β -actin or UCP upstream and downstream primer, 200 μM of each dNTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, and 2.5 units of Pfu-DNA polymerase (Stratagene, La Jolla, CA) in a 100 μL reaction volume that was overlaid with mineral oil. Samples were processed through initial denaturation for 5 min at 95°C; 35 cycles of amplification each consisting of 1 min annealing at 57°C (cycles 1–5), at 55°C (cycles 6–10), and at 53°C (cycles 11–35), 1 min extension at 72°C, 1 min denaturation at 95°C, and a final extension at 72°C for 10 min. PCR products were cloned into Sma I digested pGEM-3Z (Promega). The sequence of inserts exhibited perfect homology to the respective published sequence.

After linearization of plasmid DNA, labeled β -actin and UCP antisense RNA was transcribed from the T7-promoter using Riboprobe Systems in vitro Transcription kit (Promega) and [α -³²P]UTP (specific activity 3000 Ci/mmol; Amersham Life Science, Buckinghamshire, UK) according to the instructions of the manufacturer. After denaturation at 95°C for 5 min, aliquots of 8×10^4 cpm of gel-purified ³²P-labeled RNA were hybridized with 5 or 5 to 100 μg adipose tissue total RNA for quantitation of β -actin or UCP mRNA, respectively, at 43°C overnight. Samples were incubated with 0.5 units of RNase A and 20 units of RNase T1 (Ambion RPAII Kit; Ambion Inc., Austin, TX) at 37°C for 30 min in a total volume of 220 μL to digest unhybridized RNA. RNase inactivation/precipitation mixture supplied by the manufacturer was added to precipitate ³²P-labeled RNA-RNA hybrids. Precipitates were washed with 70% ethanol and subjected to electrophoresis in 4% polyacrylamide-urea gels. Protected fragments were quantified by scanning of autoradiographs with a Model GS-700 Imaging densitometer using the Molecular Analyst software (Bio-Rad, Hercules, CA).

UCP mRNA quantification by competitive reverse transcription-polymerase chain reaction (RT-PCR)

For synthesis of a competitor fragment that contained a 75 bp deletion at its center, splice overlap extension (SOE)-PCR was used (16, 17). The primer pairs for the first round of PCR amplifications were 1) 5'-ACCTCGC TACACGGGAC-3' (UCP upper primer, +450, +467) and 5'-CCTCGAATAAGCTTTGA AATTGATGATGACA CTTCT-3' (SOE primer 1; +562, +542); and 2) 5'-GCA CAGTTGGGCACACTTTTGT-3' (UCP lower primer, +761, +740) and 5'-TCAAAGCTTATTCTCGAGGCACT TGGTGTGGCTCTT-3' (SOE primer 2; +655, +638). The complementary sequence of the SOE primers is written in italics. Reverse transcription of adipose tissue total RNA and PCR reactions were performed as above

except for the annealing temperatures during cycling (61°C, cycles 1–5; 57°C, cycles 6–10; and 53°C, cycles 11–35). Overlapping PCR products were gel purified, mixed in equimolar amounts, and allowed to anneal. Extension of single strands was performed with 2.5 units Pfu-DNA polymerase at 72°C for 4 min. Extended strands were amplified by using the upper and lower UCP primer as described above. The resulting UCP-fragment that contained a foreign sequence of 19 bp instead of a central 75 UCP sequence was cloned into Sma I digested pGEM-3Z. Sequences of both the wild-type UCP and the UCP-deletion construct were verified by the dideoxy chain-termination sequencing method (18).

For assay standardization, sense RNA of wild-type and competitor cDNA was transcribed in vitro in the presence of [^3H]UTP (sp act 10 Ci/mmol; Amersham). Plasmids containing the respective inserts were linearized with Hind III or EcoR I and transcribed from the T7 or SP6 promoter using the Riboprobe-Systems in vitro Transcription kit. In vitro transcribed RNA was gel purified and the amount of ³H-labeled RNA synthesized was calculated from its nucleotide composition and the amount of radioactivity incorporated. Radioactivity was determined by liquid scintillation counting (Wallac 1450 Microbeta PLUS, EG&G Berthold, Bad Wildbach, Germany).

To quantify UCP mRNA in adipose tissue samples, 0.6 μg of adipose tissue total RNA was reverse transcribed along with four increasing doses of competitor RNA using 2 units of *Tth* DNA polymerase (Perkin Elmer-Applied Biosystems, Foster City, CA), 2.5 μM UCP-lower primer, 10 mM Tris-HCl, pH 8.3, 90 mM KCl, 10 mM MgCl₂, and 2 mM of each dNTP in a 20 μL reaction volume for 30 min at 70°C. PCR amplification was performed using 0.2 μM of each upper and lower UCP primer, 200 μM of each dNTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 2 mCi [α -³²P]dCTP (Amersham), and 2.5 units of Amplitaq (Perkin-Elmer Corp.) in a 100 μL reaction volume that was overlaid with mineral oil. Samples were processed through initial denaturation for 5 min at 95°C; 28 cycles of amplification each consisting of 1 min annealing at 61°C (cycles 1–3), at 57°C (cycles 4–6), and at 53°C (cycles 7–28), 1 min extension at 72°C, 1 min denaturation at 95°C and a final extension at 72° for 10 min. PCR products were separated on 4% denaturing polyacrylamide gels containing urea. After removal of urea, gels were dried and exposed to X-ray film. Intensities of bands were quantified by scanning autoradiographs with a densitometer using the linear range of films. Signal intensity ratios of wild-type to competitor cDNA were corrected for their molar C/G content and plotted as a function of the

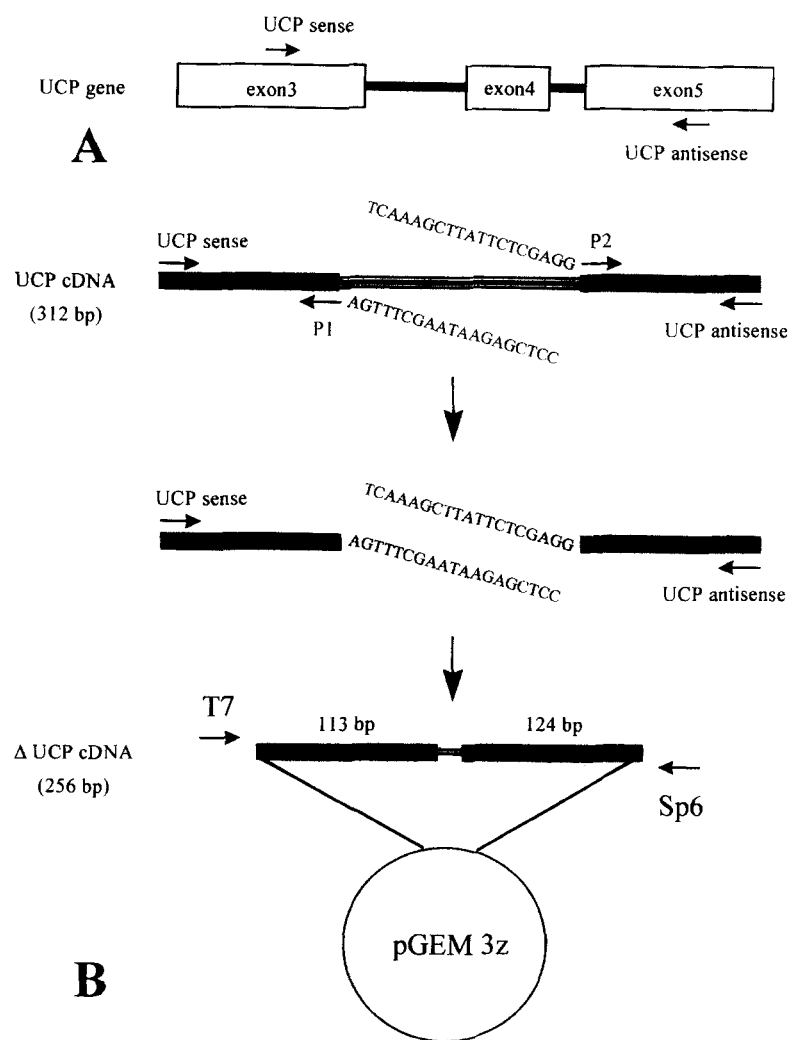


Fig. 1. Construction of Δ UCP cDNA by splice overlap extension PCR. **A:** diagram of the portion of the UCP gene encompassed by the primers used to prepare cDNA from total adipose tissue RNA by RT-PCR. **B:** generation of overlapping PCR products from the UCP cDNA fragment by using UCP sense primer and splice overlap extension primer P1 or UCP antisense primer and splice overlap extension primer P2. The complementary sequence of P1 and P2 shown in the upper and middle diagram is indicated in the lower diagram by an open box that replaces the central portion of the UCP wild-type cDNA in the Δ UCP construct which was cloned into pGEM 3z.

known amount of competitor RNA to determine the point of equivalence (i.e., where the molar ratio was 1.0). UCP mRNA abundance in adipose tissue total RNA was normalized for β -actin mRNA abundance determined by RNase protection assay.

Statistical analyses

One-way ANOVA was used to test the distributions of the continuous variables such as age and biochemical measurements between obese subjects and lean controls. The Kruskal-Wallis test was used, or a transformation was made on the original variable, if the equal variance and normality assumptions of the one-way ANOVA were rejected. Analysis of covariance was used to adjust UCP-mRNA expression levels for age and gender. To compare categorical variables, a contingency χ^2 test was used.

RESULTS

Our initial efforts were directed towards establishing an RNase protection assay for the quantification of UCP mRNA abundance in human adipose tissue samples. While an RNA fragment of the expected size of 312 nucleotides was protected in some tissue samples, no such fragment was detected in the majority of specimens despite the use of 100 μ g total RNA for hybridization (not shown). The abundance level of UCP mRNA in the majority of specimens was thus below the detection level of our RNase protection assay. We therefore turned to the more sensitive method of RT-PCR.

Using a primer complementary to exon 5 for reverse transcription and the same primer along with an exon 3 primer for amplification of cDNA (**Fig. 1A**), a single PCR product spanning nucleotides +450 to +761 rela-

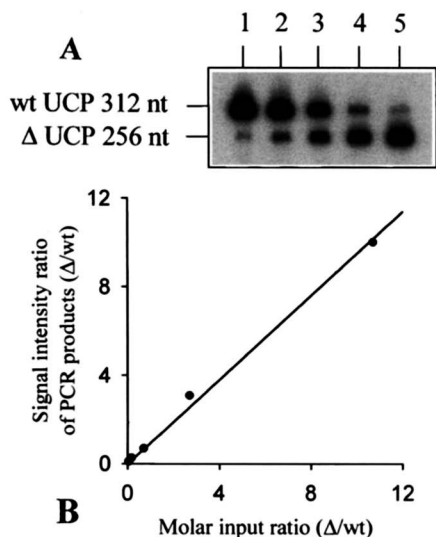


Fig. 2. Validation of RT-PCR with synthetic wild-type and competitor Δ UCP [^3H]RNA. A: autoradiograph of RT-PCR products separated by denaturing electrophoresis in 4% polyacrylamide gels. Lanes 1–5 represent products obtained by RT-PCR of decreasing amounts of wild-type [^3H]RNA (0.6, 0.3, 0.15, 0.075, and 0.034 amol/assay) and increasing amounts of Δ UCP [^3H]RNA (0.024, 0.048, 0.096, 0.19, and 0.38 amol/assay). B: relation of molar input ratios with corrected signal intensity ratios of PCR products determined by scanning autoradiographs.

tive to the translation start site was obtained (not shown). To control for variability during reverse transcription and PCR amplification, a template for in vitro synthesis of competitor RNA was constructed by splice overlap extension PCR. The resulting PCR product contained UCP cDNA extending from position +450 to +562, a 19 bp sequence representing the complementary region of the SOE primers, and UCP cDNA extending from position +638 to +761 relative to the translation start site (Fig. 1B). Both wild-type and deletion constructs were cloned into p-GEM-3Z vectors for in vitro synthesis of the respective RNA species.

To establish and validate reaction conditions, increasing doses of competitor RNA were analyzed along with decreasing doses of in vitro synthesized wild-type RNA (Fig. 2). As both RNA molecules were transcribed in the presence of [^3H]UTP, the doses of the two synthetic RNA species could be calculated precisely. Comparison of molar input ratios of competitor and wild-type RNA with corrected signal intensity ratios revealed a linear relationship ($r = 0.995$) and the slope of the regression line was 0.95. Thus, the concentration of primers and substrates was not limiting for the RNA doses used and the signal intensity ratios were proportional to the molar input ratios over a wide range. To exclude possible influences of plateau effects on the quantification procedure, aliquots of PCR mixtures were removed after

20, 22, 24, 26, and 28 cycles and analyzed as described. Over this full range, the signal intensity ratio for wild-type and competitor RNA remained constant.

To quantify UCP mRNA levels in human adipose tissue samples, 0.6 μg total RNA was reverse transcribed along with decreasing concentrations of competitor RNA and cDNA products were amplified by PCR. The autoradiographs shown in Fig. 3A demonstrate clear differences in signal intensity ratios of competitor and target RNA that were isolated from the intraperitoneal adipose tissue of two obese subjects. These differences are substantiated in plots of the molar amount of competitor RNA added per assay versus the corrected molar signal intensity ratios (Fig. 3B).

To determine the accuracy of UCP quantification, in vitro transcribed UCP RNA was added to RNA preparations from adipose tissues exhibiting differences in UCP mRNA abundance (Table 1). Recoveries determined in the presence of competitor RNA were 90 and 103% in the RNA preparation with low and high UCP mRNA abundance level, respectively. To ascertain the reliability of measurements, UCP mRNA was quantified in three aliquots each of six adipose tissue total RNA preparations. The mean intra-assay coefficient of variation was 12% (range 7–19%). To determine the variability of UCP mRNA expression levels in individual adipose tissue samples, RNA was extracted from three separate aliquots of adipose tissue samples and UCP mRNA abundance was quantified in triplicate. The mean intra-tissue coefficient of variation in four adipose tissues from different subjects was 17.5% (range 13.8–20.3%).

UCP gene expression was measured in intra- and/or extraperitoneal adipose tissue samples of 79 morbidly obese subjects and 17 lean controls. The BMI of morbidly obese subjects was nearly twice as high as in lean controls (44.4 ± 7.9 vs. 23.4 ± 3.7 kg/m^2 , mean \pm SD). No significant difference was found in the gender distribution between groups (Table 2). Non-obese controls were significantly older than obese patients ($P < 0.01$). Obese subjects had significantly higher average values than controls for plasma concentrations of insulin (9.8 vs. 5.6 mU/ml), leptin (38 vs. 8 ng/ml), triglyceride (350 vs. 151 mg/dl), and apoB (102 vs. 83 mg/dl). Average values for plasma concentrations of HDL cholesterol were significantly lower in obese cases than in lean controls (33 vs. 41 mg/dl).

Among both obese and lean subjects, UCP mRNA expression levels exhibited considerable inter-individual variability and UCP-mRNA levels in intraperitoneal tissues were severalfold higher than in extraperitoneal tissues in both groups ($P < 0.001$) (Table 3 and Fig. 4). Compared with lean controls, the average intraperitoneal UCP mRNA level, normalized for β -actin mRNA abundance and adjusted for age and gender, was sig-

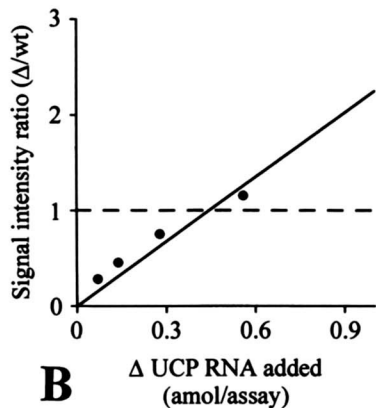
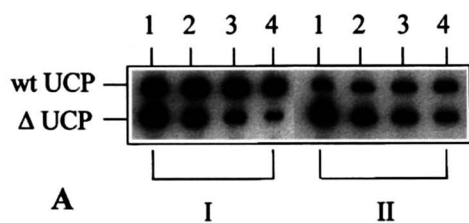
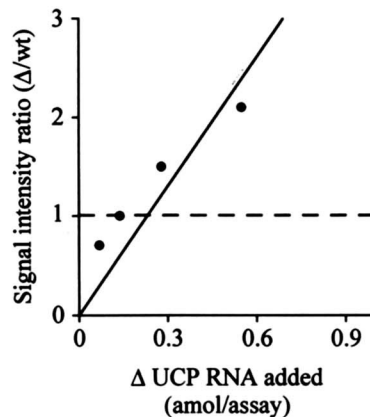


Fig. 3. Quantification of UCP mRNA abundance in human intra-peritoneal adipose tissue. A: autoradiograph of gel-separated PCR products of target (wt UCP) and competitor (Δ UCP) RNA in two subjects (I, II). Decreasing amounts of Δ UCP [3 H]RNA (0.56, 0.28, 0.14, and 0.07 amol in lanes 1, 2, 3, and 4, respectively) and 0.6 μ g of total adipose tissue RNA were used for RT-PCR. B: Relation of Δ UCP [3 H]RNA added as competitor with corrected signal intensity ratio of products representing competitor and target RNA. The line of equivalence is indicated by dashes.



nificantly lower in obese subjects ($P = 0.0011$). Median UCP mRNA levels were also significantly lower in obese subjects than in controls (2.900 vs. 6.583 amol/fmol β -actin mRNA, $P < 0.003$). No difference between obese and non-obese individuals was observed in mean and average extraperitoneal UCP mRNA expression levels. Neither obese subjects ($n = 22$) nor non-obese individuals ($n = 7$) displayed a significant association between intra- and extraperitoneal UCP mRNA abundance. Similarly, no correlation was observed in lean or obese subjects between the intra- or extraperitoneal UCP mRNA expression level and plasma lipid or lipoprotein levels or plasma concentrations of leptin or glucose.

DISCUSSION

Previous studies have detected UCP mRNA, albeit in small amounts, in several adipose depots of adult hu-

man subjects (14). As UCP is only expressed in brown adipocytes, these findings suggested that brown adipocytes are present in adipose tissue deposits previously thought to contain white adipocytes only. Our results confirm and extend these studies in that *i*) UCP mRNA was reproducibly and accurately measured in virtually all intra- and extraperitoneal tissue samples studied; *ii*) UCP mRNA expression levels were severalfold higher in intraperitoneal than in extraperitoneal tissues; and *iii*) morbidly obese subjects displayed significantly lower UCP mRNA levels in intraperitoneal fat deposits than non-obese controls.

The competitive RT-PCR assay used in our study was carefully validated. Primers encompassing introns were selected to avoid coamplification of DNA that could have contaminated RNA preparations. A synthetic competitor RNA was included in the assay to control for vari-

TABLE 1. Analytical recovery of wild-type UCP [3 H]RNA in total adipose tissue RNA

	UCP-mRNA Measured		Recovery
	No UCP [3 H]RNA Added	UCP [3 H]RNA Added (0.2 amol/assay)	
	<i>amol/assay</i>		
Sample A	0.070 \pm 0.008	0.250 \pm 0.032	90
Sample B	0.256 \pm 0.021	0.467 \pm 0.031	103

UCP-RNA was determined in 0.6 μ g total adipose tissue RNA using RT-PCR in the presence of competitor RNA. Results are means \pm SD of triplicate determinations.

TABLE 2. Characteristics of study subjects

Variable	Obese Subjects	Lean Controls	<i>P</i>
Gender (f/m)	14/65	5/12	0.2747
Age (years)	36 \pm 11	47 \pm 26	0.0062
Glucose (mg/dl)	87 \pm 32	82 \pm 15	0.5634
Insulin (mU/ml)	9.8 \pm 6.7	5.6 \pm 3.4	0.0395
Leptin (ng/ml)	38 \pm 19	8 \pm 6	0.0001
Cholesterol (mg/dl)	197 \pm 44	185 \pm 68	0.4881
Triglyceride (mg/dl)	350 \pm 397	151 \pm 79	0.0001
HDL-cholesterol (mg/dl)	33 \pm 14	41 \pm 12	0.0429
ApoA-I (mg/dl)	119 \pm 30	120 \pm 27	0.9256
ApoB (mg/dl)	102 \pm 30	83 \pm 36	0.0403

Results represent proportions or age- and gender-adjusted means \pm SD.

TABLE 3. UCP-mRNA abundance levels in intra- and extraperitoneal adipose tissues of morbidly obese subjects and lean controls

Tissue	UCP mRNA				P ^a
	Obese Subjects	n	Lean Subjects	n	
		<i>amol/fmol β-actin mRNA</i>			
Intraperitoneal adipose	3.467 ± 2.483	78	6.917 ± 4.292	14	0.0011
Extraperitoneal adipose	0.633 ± 0.425	23	0.517 ± 0.508	10	0.1868

Results are expressed as age- and gender-adjusted means ± SD.

^aAnalysis of variance of log-transformed values with age and gender as covariates.

ability during reverse transcription and PCR amplification. The UCP target mRNA and the internal standard RNA amplified with comparable efficiency. The accuracy and precision of the assay was established in recovery experiments and measurements of replicates. Considering variability in RNA extraction, the intra-tissue variability of UCP-mRNA expression was shown to be of similar magnitude as the analytical variability of UCP mRNA quantification. Thus, our data are consistent with the notion that brown adipocytes are evenly interspersed among white adipocytes in both intraperitoneal and extraperitoneal fat deposits.

Calculations based on DNA measurements in adipose tissue samples and on the assumptions of 8 pg DNA (3×10^9 base pairs per haploid human genome) and 100

molecules of an individual mRNA per cell, suggest that in intraperitoneal adipose tissue approximately one brown adipocyte would be found per 100–200 white adipocytes. UCP mRNA abundance in the extraperitoneal fat tissues was much lower than in intraperitoneal adipose deposits. Similar results were reported in another study showing higher UCP mRNA levels in omental than in subcutaneous fat (14). In these deposits, UCP mRNA levels paralleled those of β_3 -adrenergic receptor. Such an association is supported by several studies indicating that signalling via the β_3 -adrenergic receptor plays an important role in UCP gene expression and thermogenesis (19, 20).

Compared to lean controls, obese patients exhibited significantly lower UCP mRNA abundance levels in the intraperitoneal adipose tissue. These results are consistent with reduced metabolic expenditure known to be a risk factor for obesity (21, 22), but await confirmation in another population. Furthermore, the abundance of UCP-containing brown adipocytes in adult humans is limited, and it remains to be determined whether reduced expression of UCP in obese subjects represents an association of major importance contributing to the pathogenesis of obesity in some subjects. The difference of intraperitoneal UCP mRNA abundance between obese and non-obese subjects as well as the large inter-individual variability of UCP mRNA expression in both groups may reflect differences in the proportion of brown and white adipocytes and/or differences in the UCP expression level in a constant number of brown adipocytes. Our measurements do not allow us to distinguish between these possibilities.

Unlike in rodents, the importance of brown adipose tissue in the regulation of body fat stores in adult humans has not been studied in detail. Nevertheless, a contribution of brown adipose tissue to the pathogenesis of obesity is suggested by some studies. Pima Indians with a Trp64Arg mutation in the β_3 -adrenergic receptor gene tended to have a lower metabolic resting rate and exhibited an earlier onset of non-insulin-dependent diabetes mellitus (23). In other populations, the same mutation in the β_3 -adrenergic receptor gene was found to be associated with abdominal obesity and resistance to insulin (24) or an increased capacity to gain weight

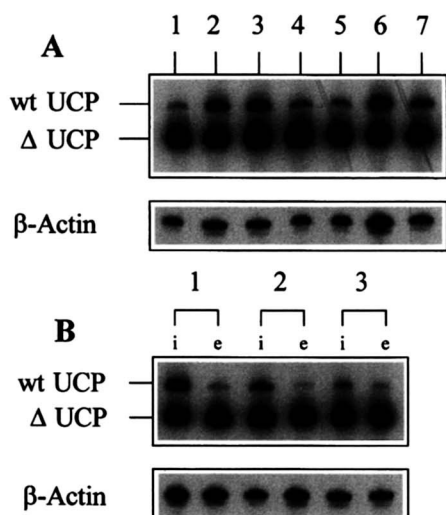


Fig. 4. Interindividual variability of UCP mRNA expression levels in intraperitoneal and extraperitoneal adipose tissues of obese subjects. A: autoradiograph of gel-separated cDNA products obtained by RT-PCR of 0.6 μg total intraperitoneal adipose tissue RNA from seven obese subjects (1–7) in the presence of 2 amol competitor RNA. B: autoradiograph of gel-separated cDNA products obtained by RT-PCR of 0.6 μg total intraperitoneal (i) or extraperitoneal (e) adipose tissue RNA from three obese subjects (1–3) in the presence of 2 amol competitor RNA; wt UCP and Δ UCP refer to PCR products of target and competitor RNA, respectively. RNase protection assays for quantification of β-actin mRNA levels are shown below the UCP PCR products of the respective RNA samples.

(25). It has been proposed that this mutation may lead to decreased lipolysis and thermogenesis thereby participating in the pathogenesis of obesity and/or accelerating its morbid complications. This hypothesis is supported by a recent study showing an additive effect of a genetic variant of the UCP gene with the Trp64Arg mutation in the β_3 -adrenergic receptor gene on weight gain in obese subjects (26). Moreover, the same genetic variant of the UCP gene was associated with a reduced body weight loss during a low calorie diet (27). The results presented here lend further credit to an association of BAT function with obesity and the RT-PCR method described for the quantification of UCP gene expression should be useful to gain more insight into associations among obesity indices, thermogenesis, and sequence variations in the UCP and β_3 -adrenergic receptor gene. ■

This study was supported by a grant of the Medizinische Forschungsgesellschaft Salzburg, by the Jubilaeumfondsprojekt Nr. 5175 of the Oesterreichische Nationalbank, and a grant from Dr. Karl Thomae GmbH, Biberach, Germany.

Manuscript received 11 April 1997 and in revised form 1 July 1997.

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