Uncoupling protein-2 gene: reduced mRNA expression in intraperitoneal adipose tissue of obese humans

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Summary The mitochondrial uncoupling protein-2 (UCP-2) is a recently discovered homologue of the brown adipose tissue-specific uncoupling protein and could be involved in the regulation of energy balance. Since obesity is associated with disturbed energy homeostasis, we tested the hypothesis that UCP-2 gene expression is deficient in this disorder. We determined, by a competitive reverse transcription-polymerase chain reaction assay, UCP-2 mRNA expression in intra- and extraperitoneal adipose tissues of 107 morbidly obese subjects and 31 lean control subjects. In both obese and non-obese subjects, UCP-2 mRNA abundance was higher in the intraperitoneal than in the extraperitoneal tissue ($p < 0.05$), but no association was observed between intra- and extraperitoneal expression in either group. Compared

with lean control subjects, both male and female obese subjects displayed significantly lower average UCP-2 mRNA expression in the intraperitoneal adipose tissue ($p < 0.006$), while UCP-2 mRNA abundance in extraperitoneal adipose tissue was not different between obese and non-obese men and women. Intraperitoneal UCP-2 mRNA remained low in nine obese subjects who lost 23 ± 12 kg of weight over a period of 10 ± 5 months subsequent to weight reducing surgery. These data support the concept that impaired adipose tissue expression of UCP-2 may play a role in the pathophysiology of obesity. [Diabetologia (1998) 41: 940–946]

Keywords Energy homeostasis, gene expression, obesity, RT-PCR, uncoupling protein-2.

Obesity is a highly prevalent disorder in many western societies [1]. It is caused by an imbalance between energy intake and energy expenditure resulting in excess total body fat. Owing to its association with hypertension, hyperlipidaemia, and insulin resistance obesity increases the risk of cardiovascular disease and is an important predictor of morbidity and mor-

talidity [2, 3]. Studies in stable populations, twins and adoptees have clearly established the importance of genetic factors in the aetiology of obesity [4–6]. Some pleiotropic genetic syndromes associated with obesity such as the Prader-Willi syndrome have been mapped to specific chromosomes [7] but the genes contributing to or causing the common forms of obesity have not yet been discovered. The recent discovery of several genes responsible for spontaneous obesity in mice has provided insight into the control of energy homeostasis in mammals. While the *ob*-, *db*-, *tub*-, *A^y*- and *fat* genes [8–12] may all play critical roles in human energy balance, only very few cases of obesity have been directly linked to defects in one of these genes [13, 14].

Small decreases in energy expenditure increase the risk for human obesity [15, 16] and genetic factors determine, at least in part, the resting metabolic rate [17, 18]. Regulated thermogenesis is thought to be

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Abbreviations: Apo, apolipoprotein; BAT, brown adipose tissue; RT-PCR, reverse transcription-polymerase chain reaction; SOE, splice overlap extension; UCP-1, uncoupling protein-1; UCP-2, uncoupling protein-2; UCP-3, uncoupling protein-3; ANOVA, analysis of variance.

an important component of energy expenditure and body weight homeostasis [19, 20]. In rodents, non-shivering thermogenesis is mediated by the brown adipose tissue (BAT)-specific uncoupling protein-1 (UCP-1) which has been implicated in the development of obesity [21–23]. By uncoupling substrate oxidation from ATP generation UCP-1 activity produces heat instead of chemical energy [24, 25]. Significant differences in the intraperitoneal adipose tissue UCP-1 mRNA expression have been observed between obese subjects and lean control subjects [26]. In addition, part of the UCP-1 mRNA abundance variability among obese subjects has been accounted for by common sequence variations at the UCP-1 gene locus [27]. Nevertheless, the pathophysiological significance of UCP-1 for human obesity has not been established. Another candidate for regulating thermogenesis and perhaps body weight in humans is UCP-2 which exhibits structural homology and shares functional properties with UCP-1 [28, 29]. In contrast to UCP-1, UCP-2 is expressed in several human tissues including BAT, white adipose tissue, lung, liver, spleen and macrophages. Very little is known about the role of UCP-2 in humans and its relationship to obesity. We therefore compared UCP-2 mRNA abundance in adipose tissues of morbidly obese subjects and lean control subjects and studied the effect of weight reduction on UCP-2 mRNA expression in obese patients.

Subjects and methods

Study subjects and adipose tissue samples. This study included 157 unrelated patients, 107 morbidly obese subjects, 9 post-obese subjects and 31 non-obese control subjects. Tissue samples were obtained from morbidly obese subjects who underwent weight reduction surgical treatment through a gastric banding procedure. Control subjects and post-obese subjects underwent elective surgical procedures such as cholecystectomy, repair of hernias, adjustment or removal of the gastric tape. Study subjects gave informed consent and the study was approved by the institutional review board. After an overnight fast general anaesthesia was induced and fat biopsies were taken from the abdominal subcutaneous fat, referred to as extraperitoneal adipose tissue, or the omental fat, referred to as intraperitoneal fat or both, at the beginning of the surgical procedure. Adipose tissue specimens were processed as described [26]. Body mass index (BMI; kilograms per meters squared) was calculated from measurements of weight and height.

Laboratory methods. After an overnight fast, blood was collected 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, Abbott Park, Ill., USA). Cholesterol and triglyceride were measured by enzymatic procedures (catalogue Nos. 1489437 and 1058550, Boehringer Mannheim Diagnostics, Mannheim, Germany). Plasma free fatty acids were determined by an enzymatic colourimetric method (Boehringer Mannheim Diagnostics, catalogue No. 1383175). High-density lipoprotein (HDL) cholesterol was de-

termined in supernates after precipitation of plasma with phosphotungstic acid/magnesium chloride. Apolipoprotein (apo) B and apoA-I levels were determined using nephelometric procedures (Array 360, Beckman, Palo Alto, Calif., USA). Plasma leptin was measured by RIA (Linco Inc., St. Charles, Mo., USA).

Isolation of adipose tissue total RNA and DNA. Total RNA was isolated from 2 g of adipose tissue according to the method of Chomczynski and Sacchi [30]. The integrity of RNA was ascertained by electrophoresis in formaldehyde gels. DNA from adipose tissue was isolated using the QIAamp Tissue Kit (Qiagen Inc., Hilden, Germany). DNA and RNA concentrations were determined by absorbance measurements at 260 nm.

UCP-2 mRNA quantification by competitive reverse transcription-polymerase chain reaction (RT-PCR). Adipose tissue total RNA was reverse transcribed using 200 units of Moloney murine leukaemia virus reverse transcriptase (Gibco BRL Life Technologies, Grand Island, N. Y., USA), 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 6 mmol/l MgCl₂, 2.5 µmol/l random hexamers, 1 mmol/l dNTP, and 20 units of RNasin (Promega Corp., Madison, Wis., USA) in a volume of 20 µl. The primer pairs for PCR amplification were 5'-GTAAAGGTCC-GATTCCAAGC-3' (UCP-2 upper primer; +418, +437) and 5'-GCATGGCCCCGGCTAGAGACAAAGC-3' (UCP-2 lower primer; +985, +962). The numbers in parentheses designate the 5' and 3' ends in the cDNA relative to the translation start site [28, 29]. Primers were synthesized using a Beckman Oligo 1000 DNA Synthesizer (Beckman Instruments Inc., Fullerton, Calif., USA). The PCR reactions contained 0.2 µmol/l of each upstream and downstream primer, 200 µmol/l of dNTP, 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 2.5 mmol/l MgCl₂ and 2.5 units of Pfu-DNA polymerase (Stratagene, La Jolla, Calif., USA) 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 59°C (cycles 4–6) and at 57°C (cycles 7–28), 1 min extension at 72°C, 1 min denaturation at 95°C and a final extension at 72°C for 5 min. With this procedure, a single 568 bp PCR product spanning nucleotides +418 to +985 relative to the translation start site was obtained (not shown). PCR reactions using the same primers and human genomic DNA as template produced a DNA fragment of more than 2 kb. Dye terminator cycle-sequencing of RT-PCR products using an ABI PRISM 310 Genetic Analyser (Perkin Elmer-Applied Biosystems, Foster City, Calif., USA) confirmed the published sequence. Sequencing of the genomic PCR product indicated the presence of at least two introns between nucleotides +533 and +812 relative to the translation start site (Fig. 1A). Thus, the possibility that DNA contamination of our RNA samples contributed to signal intensities of RT-PCR products was excluded.

The template for in vitro synthesis of competitor RNA was constructed by splice overlap extension (SOE) PCR [31]. The primer pairs for the first round of PCR amplifications were i) UCP-2 upper primer and 5'-AAGGGCACAG-GAGGTTGGCTTTCAGGAGGG-3' (SOE primer 1; +774, +765 and +627, +608) and; ii) UCP-2 lower primer and 5'-AGCCAACCTCCTGTGCCCTTACCATGCTCC-3' (SOE primer 2; +618, +627 and +765, +784). PCR conditions were as described above. Equimolar amounts of overlapping gel-purified PCR products were 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 ampli-

Fig. 1A, B. Construction of Δ UCP-2 cDNA by splice overlap extension PCR. **A** representation of the portion of the UCP-2 gene encompassed by the primers used to prepare cDNA from total adipose tissue RNA by RT-PCR. Exon and intron sequences are written in large and small letters, respectively. **B** generation of overlapping PCR products from the 568 bp UCP-2 cDNA fragment using UCP-2 sense primer and splice overlap extension primer SOE-1 or UCP-2 antisense primer and splice overlap extension primer SOE-2. The resulting deletion construct Δ UCP-2 was cloned into pGEM 3Zf. Numbers in **A** and **B** refer to the nucleotide positions relative to the translation start site [28, 29]

fied using the upper and lower UCP-2 primer. The resulting Δ UCP-2 fragment that contained a central 137 bp deletion was cloned into Sma I digested pGEM-3Zf (Fig. 1B). Sequences of both the wild-type UCP-2 and the UCP-2 deletion construct were verified by sequencing.

For assay standardization, sense RNA of wild-type and competitor cDNA was transcribed using the Riboprobe-Systems in-vitro transcription kit (Promega Corp.) in the presence of $[5\text{-}^3\text{H}]\text{UTP}$ (specific activity 10 Ci/mmol; Amersham Life Science, Buckinghamshire, UK). The amount of gel-purified RNA was calculated from the nucleotide composition and the radioactivity incorporated. Increasing doses of competitor RNA were analysed with decreasing doses of in vitro synthesized wild-type RNA (Fig. 2). Comparison of molar input ratios of competitor and wild-type RNA with corrected signal intensity ratios revealed a linear relationship ($R = 0.997$) and the slope of the regression line was 0.87. Thus, the concentration of

Fig. 2A, B. Validation of RT-PCR with synthetic wild-type and competitor Δ UCP-2 $[^3\text{H}]\text{RNA}$. **A** autoradiograph of RT-PCR products separated by denaturing electrophoresis in 4% polyacrylamide gels. Lanes 1–5 represent products obtained by RT-PCR of increasing amounts of wild-type $[^3\text{H}]\text{RNA}$ (1.6, 3.2, 6.4, 12.8 and 25.6 amol/assay) and decreasing amounts of Δ UCP-2 $[^3\text{H}]\text{RNA}$ (21.4, 10.7, 5.4, 2.7 and 1.3 amol/assay). **B** relation of molar input ratios with corrected signal intensity ratios of PCR products determined by scanning autoradiographs

primers and substrates was not limiting over a wide range of RNA doses. Possible influences of plateau effects on the quantification procedure were excluded, as the signal intensity ratio for wild-type and competitor RNA remained constant after 20, 24 and 28 amplification cycles.

To quantify UCP-2 mRNA in adipose tissue samples, 0.6 μg of adipose tissue total RNA was reverse transcribed with three increasing doses of competitor RNA using 100 units of Moloney murine leukaemia virus reverse transcriptase, 2.5 mmol/l UCP-2 lower primer, 50 mmol/l Tris-HCl, pH 8.3, 40 mmol/l KCl, 6 mmol/l MgCl_2 , and 2 mmol/l of dNTP in a 20 μl reaction volume for 30 min at 42°C. PCR amplification was performed as described, but using 2.5 units of Amplitaq (Perkin-Elmer Corp.) and including 2 μCi $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ (specific activity 3000 Ci/mmol; Amersham Life Science). PCR products were separated on 4% denaturing polyacrylamide gels. After removal of urea, gels were dried and exposed to x-ray film. Intensities of bands were quantified by scanning autoradiographs with a Model GS-700 Imaging densitometer using the Molecular Analyst software (Bio-rad Laboratories, Hercules, Calif., USA) and the linear range of films. Signal intensity ratios of wild-type to competitor cDNA were corrected for their molar G/C content and plotted as a function of the known amount of competitor RNA to determine the point of equivalence (i.e. where the molar ratio was 1.0). UCP-2 mRNA abundance in adipose tissue total RNA was normalized for β -actin mRNA abundance determined by RNase protection assay [26]. The possibility that transcripts of UCP-1 and UCP-3, other members of the UCP gene family [24, 32, 33], confounded UCP-2 quantification was excluded, as up to 10 fg of near full-length UCP-1 or UCP-3 cDNA used as a template failed to produce an amplification product in the UCP-2-specific PCR. UCP-1 mRNA expression levels were determined by a competitive RT-PCR method [26].

Table 1. Characteristics of study subjects

Variable	Female		Male	
	Obese	Non-obese	Obese	Non-obese
no. of subjects	87	16	20	15
BMI (kg/m ²)	43.0 ± 5.7	23.3 ± 3.6	48.5 ± 11.9	24.4 ± 2.6
Age (years)	36 ± 11	39 ± 23	33 ± 10	53 ± 18 ^a
Glucose (mmol/l)	4.9 ± 1.7	4.4 ± 0.8	4.9 ± 1.2	4.6 ± 0.6
Insulin (pmol/l)	74 ± 55	44 ± 21 ^c	83 ± 51	31 ± 17 ^a
Leptin (ng/ml)	40.3 ± 18.2	9.0 ± 6.1 ^a	31.3 ± 23.6	3.0 ± 1.9 ^a
Cholesterol (mmol/l)	5.26 ± 1.14	4.43 ± 1.06 ^b	5.12 ± 1.40	5.00 ± 1.01
Triglyceride (mmol/l)	3.55 ± 4.08	1.62 ± 0.69 ^b	3.94 ± 2.99	2.50 ± 1.32
HDL cholesterol (mmol/l)	0.88 ± 0.43	1.09 ± 0.26 ^c	0.70 ± 0.38	0.91 ± 0.34
ApoA-I (mg/dl)	122 ± 26	129 ± 18	98 ± 30	112 ± 12
ApoB (mg/dl)	105 ± 27	74 ± 23 ^a	99 ± 34	92 ± 26

Results represent proportions or means ± SD. ^a $p < 0.001$, ^b $p < 0.01$, ^c $p < 0.05$, analysis of variance within the respective gender group

Table 2. UCP-2 mRNA abundance levels, by gender, in intra- and extraperitoneal adipose tissues of obese and non-obese subjects

Adipose Tissue	UCP-2 mRNA expression level (amol/fmol β -actin)					
	Female			Male		
	Obese	Non-obese	p^a	Obese	Non-obese	p^a
Intraperitoneal	53.1 ± 19.1	70.1 ± 25.8	0.0061	57.8 ± 14.5	89.2 ± 31.4	0.0009
no. of subjects	78	13		17	15	
Extraperitoneal	46.9 ± 16.9	48.9 ± 14.0	0.7260	43.8 ± 10.0	52.2 ± 12.4	0.1192
no. of subjects	68	9		14	10	

Results are expressed as means ± SD. ^a Analysis of variance.

The accuracy of the UCP-2 quantification in adipose tissue total RNA was ascertained by adding a constant amount of synthetic wild-type UCP-2 RNA to several RNA preparations from adipose tissues exhibiting differences in UCP-2 mRNA abundance. Recoveries determined in the presence of competitor RNA averaged 96.5%. The reliability of measurements was determined in 3 aliquots each of 6 adipose tissue total RNA preparations. The mean intra-assay coefficient of variation was 10% (range 5–14%).

Statistical analyses. Analysis of variance (ANOVA; [34]) was used to test the equality of continuous variables such as age and biochemical measurements between obese and non-obese male or obese and non-obese female subjects. A transformation was made on the original variable, if the equal variance and normality assumptions of the one-way ANOVA were rejected. For some analyses, UCP-2 mRNA expression levels were adjusted, by multiple regression, for gender or age or both. To compare categorical variables, a contingency chi-square test was used. The paired t -test was used to analyse UCP-2 mRNA levels before and after weight reduction surgery.

Results

Table 1 shows characteristics, stratified by gender and obesity status, of study subjects in whom UCP-2 mRNA expression levels were determined. The BMI of obese patients was almost twice that of control subjects in both genders. The average age of obese and non-obese women was similar, but was lower

in obese men than in non-obese men. In both genders obese subjects had significantly higher average values than control subjects for plasma concentrations of insulin and leptin, while average levels for triglyceride and apoB differed only in female subjects. Average values of HDL cholesterol were significantly lower in obese females in comparison with control females.

In both obese and lean subjects, average UCP-2 mRNA expression in the intraperitoneal tissue was higher than in extraperitoneal fat deposits ($p < 0.05$). Neither obese nor non-obese subjects displayed a significant association between intra- and extraperitoneal UCP-2 mRNA abundance. Compared with lean controls, the gender-specific average intraperitoneal UCP-2 mRNA level, normalized for β -actin mRNA abundance, was significantly lower in obese subjects (Table 2). Since obese males were younger than non-obese males, the analysis was repeated with UCP-2 mRNA values adjusted for age, but the significant difference between obese and lean males was maintained ($p < 0.0047$). Average values of UCP-2 mRNA concentrations in extraperitoneal adipose tissue tended to be lower in obese than in control subjects, but the difference was not statistically significant in either gender (Table 2). The average DNA content in lean subjects was about 50 per cent higher than in obese subjects in both intra- (32 vs 48 μ g/100 mg tissue, $p < 0.001$) and extraperitoneal fat (24 vs 35 μ g/100 mg tissue, $p < 0.001$), indicating similar

Table 3. Characteristics of nine obese subjects before and after weight reducing surgery

	Surgery I ^a	Surgery II ^a
no. of subjects (female/male)	6/3	
BMI (kg/m ²)	41.9 ± 8.8	32.8 ± 6.4 ^b
Glucose (mmol/l)	5.1 ± 0.5	4.1 ± 0.8 ^c
Insulin (pmol/l)	81 ± 74	45 ± 40
Leptin (ng/ml)	35.4 ± 33.4	12.7 ± 14.8 ^c
Fatty acids (μmol/l)	0.728 ± 0.262	0.684 ± 0.207
intra-peritoneal UCP-2 mRNA (amol/fmol β-actin)	54.6 ± 18.8	49.1 ± 17.8

Results are means ± SD; ^a Surgery I refers to the initial weight reduction surgery and surgery II refers to an elective surgical procedure performed 10 ± 5 months later. ^b $p < 0.001$, ^c $p < 0.02$, paired *t*-test.

fractional differences in cell size between lean and obese subjects in both tissue locations. Thus, differences in cell size between the two tissue locations were unlikely to explain the selective reduction of UCP-2 mRNA levels in the intra-peritoneal fat deposits of obese subjects.

Comparison, in intra- and extra-peritoneal tissues, of UCP-2 mRNA levels with UCP-1 mRNA levels determined in the same subjects [26, 27] revealed no association in any of the four groups studied. Moreover, no significant association was observed between intra- or extra-peritoneal adipose tissue UCP-2 mRNA levels and plasma insulin or leptin concentrations in our study groups.

In 14 female and 4 male post-obese patients intra-peritoneal adipose tissue was obtained during an elective surgical procedure that was performed 4 to 103 months after the weight reduction surgery. The BMI in these patients had decreased by 12.9 ± 7.1 kg/m² (mean ± SD) and their intra-peritoneal UCP-2 mRNA expression level, adjusted for age and gender, was 56.6 ± 20.4 amol/fmol β-actin mRNA. This value differed significantly from the age and gender adjusted UCP-2 mRNA expression of non-obese subjects shown in Table 2 ($p < 0.002$). In nine of these subjects, intra-peritoneal adipose tissue had also been collected during the initial gastric banding procedure. The time between the two surgical procedures was 10 ± 5 months in these patients. Table 3 shows patient characteristics referring to the time of the respective surgical procedure. Plasma concentrations of glucose and leptin were significantly decreased after weight loss, while the decrease in insulin was not significant. Average plasma non-esterified fatty acid concentrations and UCP-2 mRNA expression in intra-peritoneal adipose tissue were similar before and after weight reduction and no relationship between changes in UCP-2 mRNA and changes in BMI or levels of glucose, insulin, leptin, and non-esterified fatty acids was observed in these patients.

Discussion

Consistent with the putative role of UCP-2 as a regulator of energy efficiency [28, 29], morbidly obese patients exhibited significantly lower mean and median UCP-2 expression in the intra-peritoneal adipose tissue in comparison with lean control subjects. Like in many previous studies on adipocyte gene expression [35, 36, 37], UCP-2 mRNA data were normalized for β-actin mRNA abundance in our study. β-actin is also expressed in stromovascular cells and the content of these cells could vary in adipose tissues of lean and obese subjects. However, a greater degree of differentiation would be expected in tissue samples of obese subjects. Thus, the possibility that normalization for β-actin accounted for the main finding of our study is unlikely, but can not be excluded entirely.

Since the UCP-2 mRNA expression in adipose tissue was higher than in any other human tissue studied [29], the difference in adipose tissue expression between obese and lean subjects is likely to have pathophysiological relevance. Previous studies have linked the UCP-2 gene with quantitative trait loci for obesity in rodents [38] and the insulin dependent diabetes locus-4 in humans [39]. In addition, UCP-2 expression studies in adipose tissues of two mouse strains reflected feed efficiency in that UCP-2 mRNA abundance was greater in the obesity-resistant A/J mice than in the obesity-prone B6 mice [28]. Even though the difference in UCP-2 mRNA abundance between obese and lean subjects is biologically plausible, our findings await confirmation in other study populations and, if confirmed, their possible causal role in the pathophysiology of obesity remains to be proven.

Comparison of UCP-2 with UCP-1 mRNA abundance measured in the same population [26, 27] showed more than tenfold higher mRNA expression of UCP-2. This result was not unexpected since the UCP-2 gene is expressed not only in BAT, but also in white adipose tissue. In contrast to UCP-1 mRNA expression which was tenfold higher in the intra- than in the extra-peritoneal tissue [26, 27], UCP-2 mRNA expression was only marginally different between intra- and extra-peritoneal adipose deposits. These site-specific differences in UCP-1 and UCP-2 expression could relate to differences in BAT content or the expression level of β3-adrenergic receptor mRNA or both [40]. Studies in mice showed that β3-adrenergic receptor agonists had no effect on UCP-2 expression [28], but enhanced UCP-1 expression [41, 42]. No significant association was found between UCP-2 mRNA abundance and insulin, supporting the argument against a specific effect of high ambient insulin on UCP-2 expression in intra-peritoneal adipocytes. No association between plasma insulin concentrations and UCP-2 mRNA expression in subcutaneous adipose tissue was observed in hyperinsulinaemic clamp studies [43]. Nevertheless, various degrees of

insulin resistance in our obese subjects could have masked such an association.

Targeted disruption of the UCP-1 gene in transgenic mice resulted in defective thermoregulation, but not in obesity or hyperphagia [44]. UCP-2 was selectively upregulated in the brown adipose tissue of transgenic animals suggesting that loss of UCP-1 was compensated for by induction of UCP-2 expression. In our study groups no relationship between UCP-2 and UCP-1 mRNA abundance was observed in intra- or extraperitoneal adipose tissue samples. This result is not surprising as BAT abundance in adipose tissues of adult humans is very low in comparison with rodents. Therefore, a possible relationship between UCP-1 and UCP-2 expression levels in BAT could have escaped our detection, even if the backup-system described in UCP-1 deficient mice had existed.

A proton leak in mitochondria has been related to the basal metabolic rate and shown to vary inversely with body mass in various species [45, 46]. The fact that intraperitoneal UCP-2 mRNA remained low in several subjects who experienced a substantial weight reduction after the gastric banding surgery is consistent with an inherent defect in energy expenditure. Such a proposal is supported by some studies showing a lower basal metabolic rate in post-obese subjects in comparison with never-obese controls [47 and references therein]. Nevertheless, this conclusion must be qualified, as weight loss has been associated with a reduction of energy expenditure [48] and the physiological response of UCP-2 gene expression to pronounced weight losses is not known. The possibility that the subnormal UCP-2 mRNA levels in our obese subjects resulted from increased food consumption or metabolic changes associated with obesity seems unlikely, since *ob/ob* and *db/db* mice harbouring a genetic defect in leptin signalling exhibited an upregulation of UCP-2 mRNA expression in comparison with lean control animals [29].

The lack of an association of leptin with UCP-2 mRNA abundance may appear at variance with studies in lean rats in whom adenovirus-mediated leptin overexpression increased adipose tissue UCP-2 mRNA abundance [49]. Leptin in our control group was much lower than in the animal model used. Nevertheless, a larger sample size of lean subjects may be required to detect such an association, should it indeed exist in humans. The absence of an association between UCP-2 expression and leptin in the larger sample of obese subjects with high plasma leptin possibly relates to leptin resistance. In addition, the mechanism(s) underlying a possible relationship of leptin and UCP-2 expression may be defective in morbidly obese subjects.

Albeit that the reduced UCP-2 mRNA in both obese and post-obese subjects supports the argument for functional consequences in energy homeostasis, a reduced mRNA abundance does not necessarily pre-

dict a diminished amount or functional activity of the protein. Linkage studies in pedigrees from the Quebec Family Study showed associations of markers in the vicinity of the UCP-2 and UCP-3 gene locus with resting metabolic rate and regional fat mass [50]. UCP-3 mRNA expression in muscle tissue did not differ between obese and lean subjects [43]. Sequence substitutions at the UCP-3 gene locus that affect mRNA expression in muscle and play a role in obesity may therefore be uncommon. However, the regulation of UCP-3 in muscle and adipose tissue differed in rodents [51], but data on UCP-3 expression in human adipose tissue are not yet available. Notwithstanding the possible role of UCP-3 in human obesity, the differences in UCP-2 mRNA expression between obese and lean subjects reported here are consistent with the notion that the UCP-2 gene locus possibly contributes to human obesity. Studies in a Danish population failed to identify obesity-associated mutations in the coding region of the UCP-2 gene [52]. Hence, mutations in regulatory regions of the UCP-2 gene could explain the differences in UCP-2 mRNA expression between lean and obese subjects as well as the linkage data reported in the Quebec Family Study.

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