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*J Clin Invest.* 1997;100(11):2665-2670. <https://doi.org/10.1172/JCI119811>.

### Research Article

Uncoupling protein-2 and -3 (UCP2 and UCP3) are mitochondrial proteins that show high sequence homology with the brown adipocyte-specific UCP1. UCP1 induces heat production by uncoupling respiration from ATP synthesis. UCP2 is widely expressed in human tissues, whereas UCP3 expression seems restricted to skeletal muscle, an important site of thermogenesis in humans. We have investigated the regulation of UCP2 and UCP3 gene expression in skeletal muscle and adipose tissue from lean and obese humans. UCP2 and -3 mRNA levels were not correlated with body mass index (BMI) in skeletal muscle, but a positive correlation ( $r = 0.55$ ,  $P < 0.01$ ,  $n = 22$ ) was found between UCP2 mRNA level in adipose tissue and BMI. The effect of fasting was investigated in eight lean and six obese subjects maintained on a hypocaloric diet (1,045 kJ/d) for 5 d. Calorie restriction induced a similar 2-2.5-fold increase in UCP2 and -3 mRNA levels in lean and obese subjects. To study the effect of insulin on UCP gene expression, six lean and five obese subjects underwent a 3-h euglycemic hyperinsulinemic clamp. Insulin infusion did not modify UCP2 and -3 mRNA levels. In conclusion, the similar induction of gene expression observed during fasting in lean and obese subjects shows that there is no major alteration of UCP2 and -3 gene regulation in adipose tissue and [...]

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## Increased *Uncoupling Protein-2* and *-3* mRNA Expression during Fasting in Obese and Lean Humans

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### Abstract

Uncoupling protein-2 and -3 (UCP2 and UCP3) are mitochondrial proteins that show high sequence homology with the brown adipocyte-specific UCP1. UCP1 induces heat production by uncoupling respiration from ATP synthesis. UCP2 is widely expressed in human tissues, whereas UCP3 expression seems restricted to skeletal muscle, an important site of thermogenesis in humans. We have investigated the regulation of *UCP2* and *UCP3* gene expression in skeletal muscle and adipose tissue from lean and obese humans. *UCP2* and *-3* mRNA levels were not correlated with body mass index (BMI) in skeletal muscle, but a positive correlation ( $r = 0.55$ ,  $P < 0.01$ ,  $n = 22$ ) was found between *UCP2* mRNA level in adipose tissue and BMI. The effect of fasting was investigated in eight lean and six obese subjects maintained on a hypocaloric diet (1,045 kJ/d) for 5 d. Calorie restriction induced a similar 2–2.5-fold increase in *UCP2* and *-3* mRNA levels in lean and obese subjects. To study the effect of insulin on UCP gene expression, six lean and five obese subjects underwent a 3-h euglycemic hyperinsulinemic clamp. Insulin infusion did not modify *UCP2* and *-3* mRNA levels. In conclusion, the similar induction of gene expression observed during fasting in lean and obese subjects shows that there is no major alteration of *UCP2* and *-3* gene regulation in adipose tissue and skeletal muscle of obese subjects. The increase in *UCP2* and *-3* mRNA levels suggests a role for these proteins in the metabolic adaptation to fasting. (*J. Clin. Invest.* 1997. 100:2665–2670.) **Key words:** obesity • calorie restriction • insulin infusion • adipose tissue • skeletal muscle

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Received for publication 5 August 1997 and accepted in revised form 17 October 1997.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.  
0021-9738/97/12/2665/06 \$2.00  
Volume 100, Number 11, December 1997, 2665–2670  
http://www.jci.org

### Introduction

Obesity results from an imbalance of food intake and energy expenditure. A low rate of energy expenditure may predispose to the development of human obesity (1). The basal metabolic rate, i.e., the obligatory metabolic cost for maintenance of physiological processes and cellular functions, accounts for ~ 60% of total energy expenditure and constitutes a familial trait (2), suggesting that genetic factors controlling energy expenditure might be of importance in the development of obesity (3).

A substantial part of the basal metabolic rate derives from a leaking of protons across the mitochondrial inner membrane (4, 5). In rodents, an uncoupling protein (UCP)<sup>1</sup> called UCP1, expressed specifically in brown adipose tissue, plays an important role in thermogenesis by promoting the dissipation of the proton electrochemical gradient across the inner mitochondrial membrane (6). The activation of this pathway results in energy dissipation as heat, and has been implicated in the regulation of body temperature and body weight (7–9). In adult humans, UCP1 is not likely to play a major role in the regulation of energy expenditure, since there is little brown adipose tissue present (10). However, the proton leak occurs in mitochondria of cell types other than brown adipocytes (4, 5). Two recently characterized mitochondrial UCPS designated UCP2 and UCP3 are candidates to explain the proton leak (11–14). This assumption is based on several lines of evidence. The amino acid identities between UCP2 and -3, and UCP1, are 59 and 57%, respectively, whereas comparisons with other members of the mitochondrial carrier family show much lower percentages of sequence identity. Like UCP1 and other mitochondrial carriers, UCP2 and -3 contain six predicted transmembrane domains and several conserved protein motifs. UCP2 is active at the mitochondrial level and causes, when expressed in yeast, a decrease in mitochondrial membrane potential. Moreover, the *UCP2* gene maps to a region of human chromosome 11 that has been linked to obesity and hyperinsulinemia.

The tissue distributions of *UCP2* and *-3* in adult humans are markedly different. *UCP2* is expressed in a large number of tissues, including white adipose tissue, skeletal muscle, and tissues rich in macrophages (11, 12, 15). The expression of

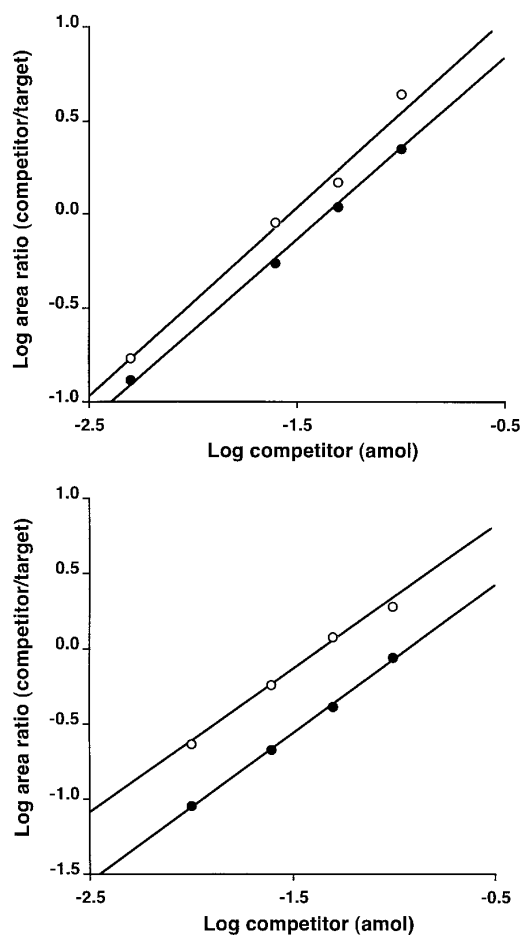
1. Abbreviations used in this paper: BMI, body mass index; RT-competitive PCR, reverse transcription-competitive PCR; UCP, uncoupling protein.

*UCP3* seems restricted in humans to skeletal muscle (13, 14). Skeletal muscle is an important site of energy expenditure, and accounts for much of the variation in metabolic rate between individuals (16).

To investigate whether *UCP2* and *-3* gene expression and regulation are altered in obesity, we measured the mRNA levels of *UCP2* and *-3* in white adipose tissue and skeletal muscle of lean and obese subjects during fasting and insulin infusion.

## Methods

**Subjects.** The subjects comprised 12 lean (3 men and 9 women, mean age  $26 \pm 9$  yr) and 14 nondiabetic obese (4 men and 10 women, mean age  $44 \pm 9$  yr) individuals. All subjects were Caucasian. Percutaneous

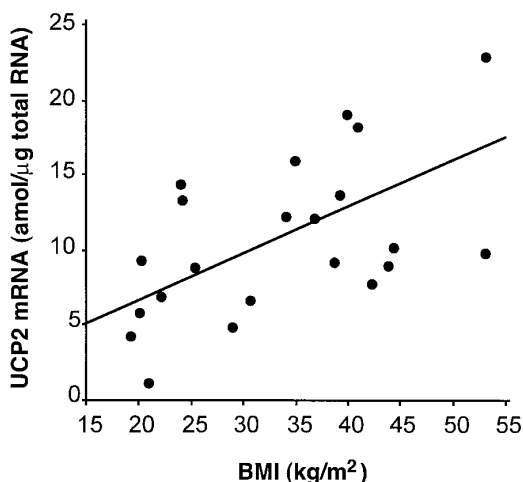


**Figure 1.** Quantification of human *UCP2* and *-3* mRNA levels by RT-competitive PCR. *Top*, Determination of *UCP2* mRNA level in adipose tissue of an obese subject before (6.7 amol/ $\mu$ g total RNA, open symbols) and during (10.6 amol/ $\mu$ g total RNA, filled symbols) calorie restriction. *Bottom*, Determination of *UCP3* mRNA level in skeletal muscle of the same obese subject before (11.0 amol/ $\mu$ g total RNA, open symbols) and during (28.5 amol/mg total RNA, filled symbols) calorie restriction. After the RT step, four PCR reactions were run in parallel with different amounts of competitor, using a dye-labeled sense oligonucleotide. Fluorescent PCR products were separated and analyzed on an automatic DNA sequencer (ALFexpress; Pharmacia Biotech). Ratios of peak areas of competitor to target were plotted against the initial amount of competitor added in the PCR reaction. At the equivalence point (log ratio = 0), initial amount of target cDNA corresponds to initial amount of competitor. mRNA concentrations in the tissue were calculated after correction for dilution factor.

biopsies of the vastus lateralis muscle and abdominal subcutaneous adipose tissue were performed as described previously (17, 18). 14 subjects participated in a 5-d study protocol during which they received a 1,045-kJ/d diet. 3 d before the beginning of the study, the subjects received a standardized diet (104 kJ/kg/d). The first series of biopsies was performed after an overnight fast before the beginning of calorie restriction. The second series of biopsies was performed the morning of the sixth day of calorie restriction. Samples were frozen immediately in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Before and after the diet, the resting metabolic rate of the subjects was measured by indirect calorimetry (Datex monitor; Deltatrac, Anaheim, CA). 11 subjects underwent a 3-h euglycemic hyperinsulinemic clamp (insulin infusion rate of 12 pmol/min/kg) (17, 18). The study was performed after an overnight fast. Biopsies were performed 2 h before and at the end of the clamp. Adipose tissue from the severely obese patients was collected during vertical banded gastroplasty. All subjects had given written consent, and the experimental protocols were approved by the ethics committee of Hospices Civils de Lyon.

**RNA preparation.** Total RNA from skeletal muscle was prepared using guanidinium thiocyanate-phenol-chloroform extraction (19). Total RNA from adipose tissue was obtained using the RNeasy kit (QIAGEN Inc., Chatsworth, CA). The absorption ratios at 260 to 280 nm were between 1.7 and 2.0. RNA integrity was verified on agarose gel electrophoresis. Total RNA was stored at  $-80^{\circ}\text{C}$ .

**Quantification of mRNAs.** Human *UCP2* and *-3* mRNAs were quantified by reverse transcription-competitive PCR (RT-competitive PCR) (20). This technique consists of coamplification of *UCP2* or *-3* cDNA with known amounts of *UCP2* or *-3* competitor DNA added in the same PCR tube. A 290-bp *UCP2* cDNA fragment was obtained by RT-PCR on human adipocyte total RNA using 5'-GACCTATGACCTCATCAAGG-3' as sense primer, 5'-ATAGGTGACGAA-CATCACCACG-3' as antisense primer, and *Taq* DNA polymerase (Perkin-Elmer Corp., Norwalk, CT). *UCP2* competitor DNA was obtained by deletion of 55 bp in the 290-bp sequence using a two-step PCR overlap extension method (21) and high-fidelity *pfu* DNA polymerase (Stratagene, Inc., La Jolla, CA). A 312-bp *UCP3* cDNA fragment was obtained by RT-PCR on human skeletal muscle total RNA with 5'-ATGGACGCCTACAGAACCAT-3' as sense primer and 5'-CTGGGCCACCATCTTTATCA-3' as antisense primer. To construct *UCP3* competitor, 40 bp were deleted from the 312-bp fragment. Identities of the four constructs to published sequences were checked by automatic DNA sequencing (Applied Biosystems, Inc., Foster City, CA). The RT-competitive PCR protocol has been described in detail elsewhere (20). To improve the quantitation of the amplified products, fluorescent dye-labeled sense oligonucleotides



**Figure 2.** Linear relationship between BMI and *UCP2* mRNA levels in adipose tissue.

were used. The PCR products were separated and analyzed on an ALFexpress DNA sequencer (Pharmacia Biotech, Piscataway, NJ) using Fragment Manager software. To validate the RT-competitive PCR assay, *UCP2* and *-3* RNAs were synthesized by in vitro transcription (Promega Corp., Madison, WI) and quantified over a wide range of concentrations (from 0.1 to 25 amol) (20). The absence of contamination by genomic DNA was checked by omitting reverse transcriptase in the reactions.

**Statistical analysis.** Values are given as means  $\pm$  SEM except body mass index (BMI) and age (mean  $\pm$  SD). The Wilcoxon nonparametric test for paired values was used for comparisons before and during fasting, and before and after the hyperinsulinemic clamp. The nonparametric U Mann-Whitney test for unpaired values was used for

comparisons between groups of subjects. In some cases, simple regression analysis was performed. Statistical calculations were performed with a software statistical package (Statview; Abacus Concepts, Inc., Berkeley, CA).  $P < 0.05$  was the threshold of significance.

## Results and Discussion

RT-competitive PCR was used to measure *UCP2* and *-3* mRNA levels in skeletal muscle and abdominal subcutaneous adipose tissue (Fig. 1). *UCP3*, unlike the ubiquitously expressed *UCP2*, has been detected in rodents only in skeletal muscle and brown adipose tissue (13, 14). Since there is little

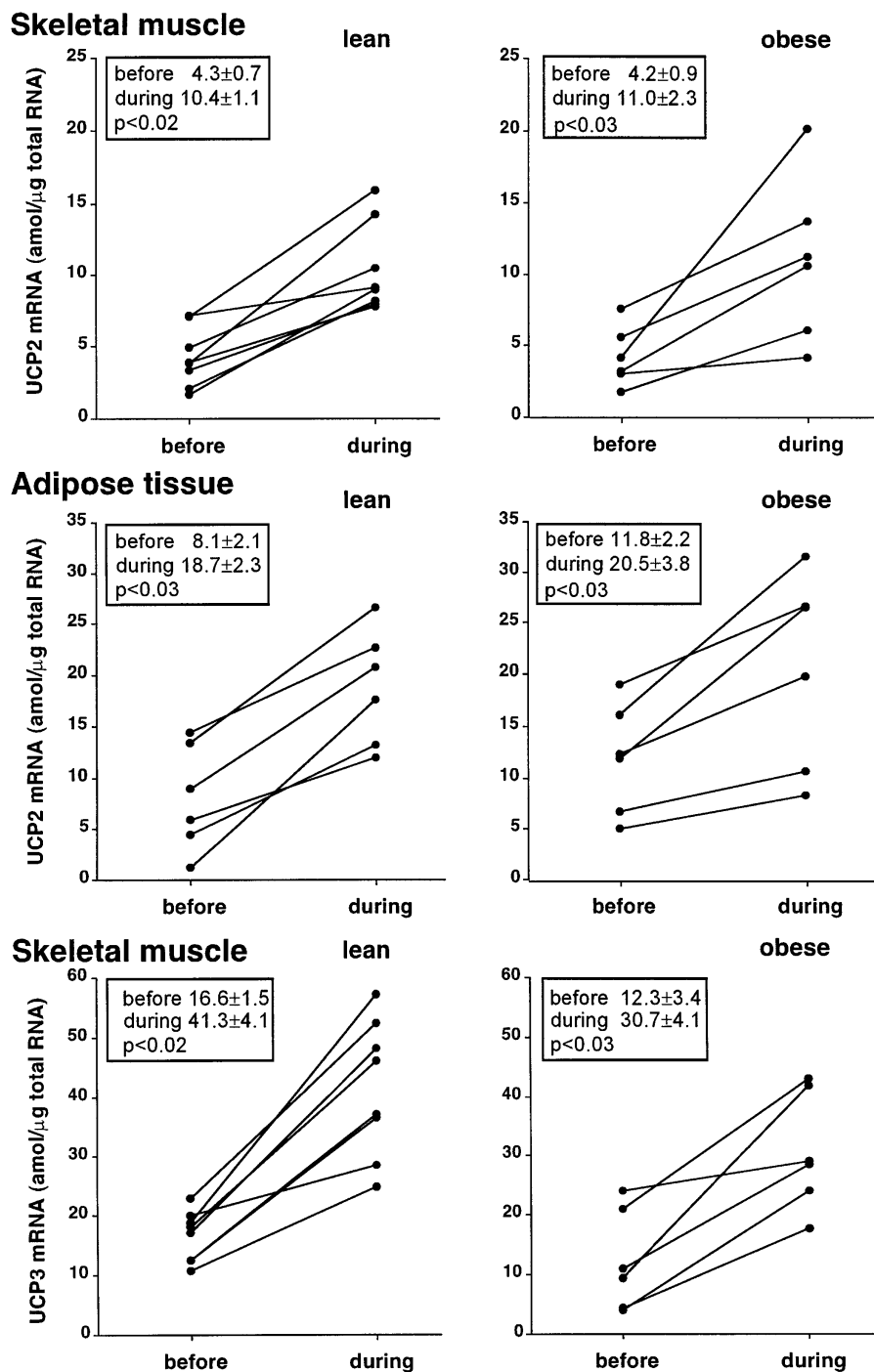


Figure 3. Effect of 5 d of calorie restriction on *UCP2* and *-3* mRNA levels. Amounts of *UCP2* and *-3* mRNA were determined using RT-competitive PCR in skeletal muscle and adipose tissue of lean (two men and six women) and obese (two men and four women) subjects. Insets, Means  $\pm$  SEM before and during calorie restriction, and  $P$  value.

brown adipose tissue present in adult humans, the main site of expression is thought to be skeletal muscle. No *UCP3* mRNA was detected in subcutaneous adipose tissue from five individuals. As shown in rodents (13, 14), *UCP3* mRNA ( $15.0 \pm 1.5$  amol/ $\mu$ g total RNA,  $n = 18$ ) is more abundant in skeletal muscle than *UCP2* mRNA ( $4.4 \pm 0.5$  amol/ $\mu$ g total RNA,  $n = 18$ ). In skeletal muscle, no correlation was found between BMI (range 19–40 kg/m<sup>2</sup>), and *UCP2* ( $P = 0.8$ ,  $n = 18$ ) and *-3* ( $P = 0.6$ ,  $n = 18$ ) mRNA levels. Over the same range of BMI, a positive correlation was found between BMI and *UCP2* mRNA levels in adipose tissue ( $r = 0.65$ ,  $P < 0.02$ ,  $n = 14$ ). Including

determinations performed on adipose tissues of eight severely obese subjects, *UCP2* mRNA level remained positively correlated to BMI ( $r = 0.55$ ,  $P < 0.01$ ) (Fig. 2). These data are consistent with the higher *UCP2* mRNA level found in white adipose tissue of *ob/ob* and *db/db* obese mice compared with lean littermates (12). An increase in *UCP2* mRNA expression was reported in lipid-accumulating brown adipocytes of *UCP1*-deficient mice and of transgenic mice overexpressing glycerol-3-phosphate dehydrogenase (22). These data suggest that the level of *UCP2* mRNA is associated with the lipid content of adipocytes. Moreover, our results show that there is no

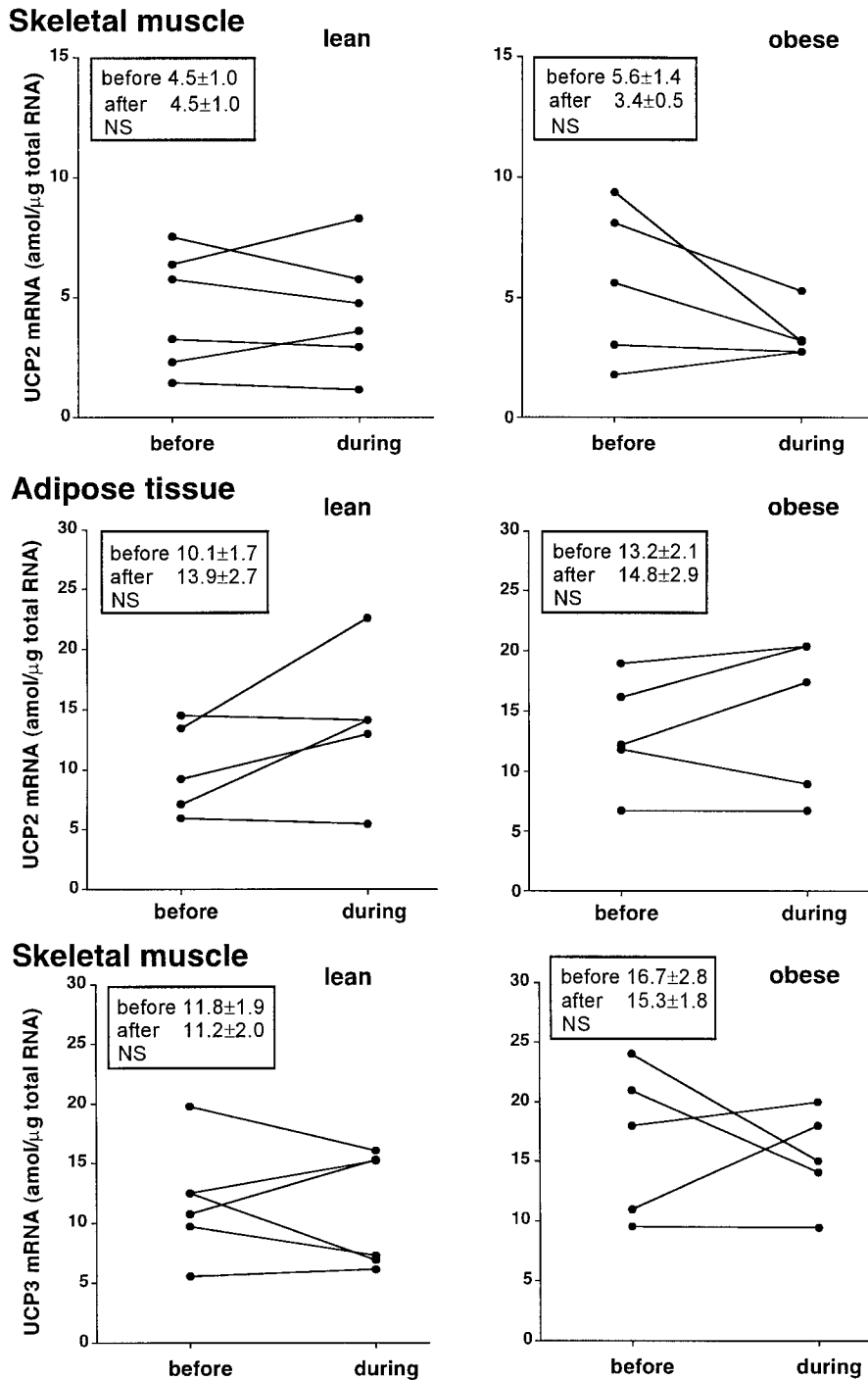


Figure 4. Effect of insulin infusion on *UCP2* and *-3* mRNA levels in lean and obese subjects. Amounts of *UCP2* and *-3* mRNA were determined using RT-competitive PCR in skeletal muscle and adipose tissue of lean (one man and five women) and obese (three men and two women) subjects. Insets, Means  $\pm$  SEM before and during calorie restriction.

marked defect of *UCP2* and *-3* mRNA expression associated with obesity in skeletal muscle, which represents an important determinant of whole-body energy expenditure in humans.

In this study, there was no selection of the subjects on the basis of gender or age. No correlation was found between *UCP2* and *-3* mRNA levels and age, and we did not find difference in gene expression between men and women (data not shown). However, the limited number of subjects studied does not allow excluding an effect of age and/or sex on UCP gene expression.

Alterations in the regulation of *UCP2* and *-3* gene expression could occur in obesity. Calorie restriction and insulin infusion, two conditions known to modify intracellular metabolism through modulation of gene expression (23), were investigated. Six obese patients (BMI [mean±SD] 34.3±4.0 kg/m<sup>2</sup>) and eight lean subjects (BMI 22.6±2.5 kg/m<sup>2</sup>) were maintained on a restricted diet of 1,045 kJ/d for 5 d. The diet induced an average weight loss of 2.8 kg (range 1–5 kg) in the two groups. Fasting glycemia decreased from 4.4±0.2 to 3.8±0.2 mM ( $P < 0.02$ ) in lean subjects, and from 5.0±0.3 to 4.3±0.2 mM ( $P < 0.05$ ) in obese subjects. Fasting plasma insulin concentrations decreased from 35±3 to 30±1 pM ( $P < 0.05$ ) in lean subjects, and from 91±23 to 48±5 pM ( $P < 0.04$ ) in obese subjects. Plasma FFA levels increased during the hypocaloric diet in the lean (from 500±89 to 951±98 μM,  $P < 0.05$ ) and obese (from 646±53 to 830±44 μM,  $P < 0.05$ ) groups. Daily urinary free cortisol levels did not change during the 5-d calorie restriction (data not shown). Plasma free triiodothyronine levels were not modified in the lean (before fasting, 4.4±0.3 pM; during fasting, 4.6±0.2 pM) and obese (before fasting, 5.2±0.4 pM; during fasting, 4.7±0.5 pM) groups. The resting metabolic rate estimated by indirect calorimetry decreased from 3,766±132 to 3,556±117 kJ/m<sup>2</sup>/24 h ( $P < 0.01$ ) in lean subjects, and from 3,535±149 to 3,358±141 kJ/m<sup>2</sup>/24 h ( $P < 0.05$ ) in obese subjects. A 2.4-fold increase in *UCP2* mRNA levels was observed in adipose tissue and skeletal muscle of lean subjects (Fig. 3). In obese patients, 2.6- and 1.7-fold increases were seen in adipose tissue and skeletal muscle, respectively. *UCP3* mRNA expression in skeletal muscle was increased 2.5-fold in lean and obese subjects. No correlation was found between *UCP2* and *-3* mRNA levels, and resting metabolic rates (data not shown). Therefore, *UCP2* and *-3* are upregulated during fasting, and the induction of gene expression is not different between obese and lean subjects.

To test the acute effect of insulin on the UCP gene expression, six lean and five obese subjects underwent a 3-h euglycemic hyperinsulinemic clamp. Insulin concentrations rose to 1,195±142 pM in the 11 subjects, whereas glycemia remained constant at 4.5±0.1 mM. No variation in *UCP2* and *-3* mRNA levels was observed during the clamp (Fig. 4). The data suggest that insulin per se does not acutely regulate *UCP2* and *-3* gene expression in humans.

Calorie restriction provokes a complex metabolic adaptation involving numerous hormonal and metabolic changes. Leptin, the product of the *obese* (*ob*) gene, was shown recently to regulate positively *UCP2* gene expression (24). A 5-d calorie restriction does not modify *ob* mRNA levels in adipose tissue (17), and longer food restriction is associated with a decrease in plasma leptin levels (25). Therefore, leptin is not a likely candidate to underlie the upregulation of *UCP2* and *-3* during fasting. Calorie restriction induces an increase in adipose tissue lipolysis (26), resulting in an important fatty acid

release from body fat stores. Fatty acids and other ligands of the peroxisome proliferator-activated receptors stimulate *UCP2* gene expression (27), and adipose tissue *UCP2* mRNA levels are increased by high-fat feeding in mice (11). Therefore, fatty acids might contribute to the increased *UCP2* and *-3* gene expression occurring during fasting.

Body weight reduction obtained by underfeeding is associated with a decrease in resting and nonresting energy expenditure (28). This adaptive mechanism may account for the poor long-term efficacy of weight-reducing programs. In that context, an increase in *UCP2* and *-3* mRNA expression during calorie restriction is unexpected, suggesting that the putative defect is not at the level of gene transcription. Regulation of mitochondrial uncoupling activity could be altered. Therefore, the increased *UCP2* and *-3* gene expression could represent a compensatory mechanism. However, the existence of other UCPs involved in the control of energy balance cannot be ruled out.

To conclude, *UCP2* mRNA level was correlated to BMI in adipose tissue but not in skeletal muscle. An association between obesity and *UCP2* mRNA levels in other tissues cannot be excluded. The similar induction of *UCP2* and *-3* observed during fasting in lean and obese subjects shows that there is no major alteration of gene regulation in adipose tissue and skeletal muscle of obese subjects. The increase in *UCP2* and *-3* mRNA levels suggests that these proteins may play a role in metabolic adaptation to fasting.

## Acknowledgments

We thank Nathalie Vega for expert technical assistance.

This work was supported in part by Groupe Danone and Fondation de France.

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