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Decreased Uncoupling Protein Expression and Intramyocytic Triglyceride Depletion in Formerly Obese Subjects

Geltrude Mingrone, Giuseppina Rosa,* Aldo V. Greco,* Melania Manco,* Nathalie Vega,† Matthijs K. Hesselink,‡ Marco Castagneto,‡ Patrick Schrauwen,§ and Hubert Vidal†*

Abstract

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Objective: To examine the muscular uncoupling protein expression 2 (UCP2) and UCP3 gene expression in morbid obese subjects before and after bariatric surgery [biliopancreatic diversion (BPD)].

Research Methods and Procedures: Eleven obese subjects (BMI = 49 ± 2 kg/m^2) were studied before BPD and 24 months after BPD. Skeletal muscle UCP2 and UCP3 mRNA was measured using reverse transcriptase-competitive polymerase chain reaction and UCP3 protein by Western blotting. Intramyocytic triglycerides were quantified by high-performance liquid chromatography. Twenty-four-hour energy expenditure and respiratory quotient (RQ) were measured in a respiratory chamber.

Results: After BPD, the average weight loss was ~38%. Nonprotein RQ was increased in the postobese subjects (0.73 ± 0.00 vs. 0.83 ± 0.02, $p < 0.001$). The intramyocytic triglyceride level dropped (3.66 ± 0.16 to 1.60 ± 0.29 mg/100 mg of fresh tissue, $p < 0.0001$) after BPD. Expression of UCP2 and UCP3 mRNA was significantly reduced (from 35.9 ± 6.1% to 18.6 ± 4.5% of cyclophilin, $p = 0.02$; from 60.2 ± 14.0% to 33.4 ± 8.5%, $p = 0.03$; respectively). UCP3 protein content was also significantly reduced (272.19 ± 84.13 vs. 175.78 ± 60.31, AU, $p = 0.04$). A multiple regression analysis ($R^2 = 0.90$) showed that IMTG levels ($p = 0.007$) represented the most powerful independent variable for predicting UCP3 variation.

Discussion: The strong correlation of UCP expression and decrease in IMTG levels suggests that triglyceride content plays an even more important role in the regulation of UCP gene expression than the circulating levels of free fatty acids or the achieved degree of weight loss.

Key words: uncoupling proteins, muscle biopsies, calorimetric chamber, morbid obesity

Introduction

Uncoupling proteins (UCPs)[1] are mitochondrial proton carriers that can uncouple respiration from adenosine triphosphate synthesis. There are at least three UCPs expressed in a variety of tissues in mammals (1–5). UCP1 is almost exclusively expressed in brown adipose tissue (BAT) and has been demonstrated to play a major role in the control of body temperature (6). Because adult humans lack a significant amount of BAT, other uncoupling proteins, namely UCP2 and UCP3, have been postulated to participate in the regulation of energy metabolism (1–5). In addition, the genomic location (11q13) of human *UCP2* and *UCP3* genes is coincident with quantitative trait loci for obesity in whites (7), suggesting a possible role of these

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*Istituto di Clinica Medica and ‡Clinica Chirurgica and Centro CNR Fisiopatologia Shock, Università Cattolica S. Cuore, Rome, Italy; †Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 449 and Centre de Recherche et Nutrition Humaine, Lyon, France; and §Departments of Human Biology and Movement Sciences, NUTRIM, Maastricht University, Maastricht, The Netherlands.
Address correspondence to Geltrude Mingrone, Istituto di Medicina Interna, Università Cattolica S. Cuore, Largo A. Gemelli, 8, 00168 Roma, Italy.
E-mail: gmingrone@rm.unicatt.it

[1] Nonstandard abbreviations: UCP, uncoupling protein; BAT, brown adipose tissue; BPD, bilio-pancreatic diversion; 24-hour EE, 24 hour energy expenditure; REE, resting energy expenditure; RQ, respiratory quotient; FFA, free fatty acid; TG, triglyceride; EI, energy intake; RT-cPCR, reverse transcription competitive polymerase chain reaction; IMTG, intramyocytic triglyceride; FFM, fat-free mass; FM, fat mass; DAG, diacylglycerol.

proteins in the control of body weight. On the basis of a large number of experiments in both rodents and humans, it has also been hypothesized that UCP2 and UCP3 may have a role as modulators of glucose/lipid use as fuel substrates (1,2,8).

UCP2 mRNA is found in a variety of tissues including white adipose tissue, BAT, skeletal muscle, and cells from the immune system (5). In contrast, UCP3 is expressed mainly in skeletal muscle and, to a lesser extent, in cardiac muscle (3,4). In some situations, the expression of UCP2 and UCP3 has been shown to be modulated by diet, and particularly by dietary fat consumption (5,9,10). In humans, for example, it has been demonstrated in a group of endurance athletes that skeletal muscle UCP2 and UCP3 mRNA expression was enhanced by a high-fat diet (10). This upregulation appeared more pronounced in subjects with high proportions of type IIA muscle fibers, which are known to have a high capacity to shift from carbohydrate to fat oxidation, suggesting a role for UCPs in lipid use (10,11).

To further explore the possible role of muscle UCP2 and UCP3 as modulators of glucose/lipid use as fuel substrates or as mediators of thermogenesis, we investigated their transcriptional expression in vastus lateralis muscle biopsies of morbid obese subjects before and after malabsorptive bariatric surgery. Peculiar to these subjects is the fact that they have a very high energy intake with a fat-rich diet but a low daily fat availability because of lipid malabsorption (12).

Research Methods and Procedures

Subjects

Eleven obese subjects (two men and nine women; age, 42 ± 2 years; BMI, 49 ± 2 kg/m^2) were enrolled in the study. Subjects had maintained a stable body weight for at least 2 months before the beginning of the protocol. The subjects were not on regular medication and did not show known complications of obesity, such as established hypertension, diabetes, or dyslipidemia. All subjects were white. The subjects went through a metabolic investigation before surgery (basal situation), after which they underwent biliopancreatic diversion (BPD). Postoperatively, they underwent a second metabolic investigation (24 months later) for the follow-up study. Biopsies of the vastus lateralis muscle, as well as all the metabolic measurements, were performed after an overnight fast during the 2 investigation days, before and after surgery. All subjects had given written informed consent, and the experimental protocols were approved by the Institutional Review Board.

BPD

This essentially malabsorptive surgical procedure (13) consists of ~60% distal gastric resection with stapled closure of the duodenal stump. The residue volume of the stomach is ~300 mL. The small bowel is divided at 2.5 m from the ileo-cecal valve, and its distal end is anastomosed to the remaining stomach. The proximal end of the ileum, comprised of the remaining small bowel that carries the bilio-pancreatic juice and excluded from food transit, is anastomosed in an end-to-side fashion to the bowel 50 cm proximal to the ileo-cecal valve. Consequently, the total length of absorbing bowel is brought to 250 cm, whose final 50 cm, the so-called common channel, represents the site where ingested food and bilio-pancreatic juices mix up.

Restrictive bariatric surgery, such as the long gastroplasty, allows 20- to 30-mL pouch limit capacity.

Body Composition

Before and after the surgical procedure, body weight was measured to the nearest 0.1 kg, using a beam scale, and height was measured to the nearest 0.5 cm using a stadiometer (Holatin, Crosswell, Wales, UK). Total body water was determined using 0.19 Bq of tritiated water in 5 mL of saline solution administered as an intravenous bolus injection (14). Blood samples were drawn before and 3 hours after the injected dose. The dpm were counted in duplicate on 0.5 mL of plasma using a β-scintillation counter (Model 1600TR; Canberra-Packard, Meriden, CT). Corrections were made (5%) for nonaqueous hydrogen exchange (15), and water density at body temperature was assumed to be 0.99371 kg/L. Total body water (kilograms) was computed as ^3H_2O dilution space (liters) × 0.95 × 0.99371. The within-person day-by-day CV reported for this method is 1.5% (16).

Respiratory Chamber

On different days and according to a random design, subjects spent a day in the respiratory chamber. At 8:00 AM of the respiratory chamber experimental day, subjects entered the respiratory chamber (volume 23.6 m^3) in the Metabolism Unit, Catholic University, School of Medicine in Rome (Italy) and spent the following 24 hours in it. Twenty-four-hour energy expenditure (24-hour EE) and resting energy expenditure (REE) were measured as previously described (17,18). Briefly, the carbon dioxide concentration was measured by a 2% full scale (0–2%) infrared absorption analyzer (URAS 3G; Hartmann & Braun, Frankfurt, Germany), and the oxygen concentration was assessed by a 2% full scale (19–21%) paramagnetic analyzer (Magnos 4G; Hartmann & Braun, Frankfurt, Germany). Both gas analyzers operated with a precision of 0.02 vol%. The zero values of both analyzers were calibrated by allowing fresh air to flow through the sample and the reference lines simultaneously, whereas the span values were calibrated using commercially available gas mixtures (Rivoira, Torino, Italy). The composition of the gas mixture used to calibrate the O_2 analyzer was 19.48% O_2 and 80.52% N_2. The com-

position of the gas mixture used to calibrate the CO_2 analyzer was 1.5% CO_2 and 98.5% N_2. The calibration procedure was conducted every day at the beginning of each experimental session. The algorithm used for computing oxygen consumption (VO_2 mL/min) and carbon dioxide production (VCO_2 mL/min) approximated the gas consumption/production during a time interval by adding two independent quantities, dynamic (open) and static (closed) gas production (19); consumption was negative production. Values were corrected for temperature, barometric pressure, and humidity. EE was calculated according to Ferrannini (20). Protein oxidation was determined from 24-hour urinary nitrogen excretion. Calibration procedures, precision, and variability of the respiratory chamber were previously published (17,21).

Physical activity was monitored by means of two orthogonal ultrasound sensors; the sensitivity of the receivers was set so that respiratory movements were not detected. Twenty-four-hour urinary urea nitrogen was measured by BUN Analyzer (Beckman Instruments, Fullerton, CA) to correct respiratory quotient (RQ).

Fat, starch, and nitrogen were measured in 24-hour stools by near infrared reflectance analysis according to Benini and Caliari (22).

Seven-day food diaries were analyzed by a dietitian using computerized food tables (Food processor II; Esha Research, Salem, OR; modified according to the food tables of the Istituto Nazionale di Nutrizione, Roma, Italy).

Euglycemic-Hyperinsulinemic Clamp Procedure

Peripheral insulin sensitivity was evaluated by the euglycemic-hyperinsulinemic clamp procedure (23). After inserting a cannula in a dorsal hand vein for sampling arterialized venous blood and another one in the antecubital fossa of the contralateral arm for infusions, the subjects rested in a supine position for at least 1 hour. They were placed with one hand warmed in a heated air box set at 60 °C to obtain arterialized blood samples. Whole-body glucose uptake (M value) in μmoles/kg_{FFM}/min was determined during a primed-constant infusion of insulin (at the rate of 7 pmol/min/kg). The fasting plasma glucose concentration was maintained throughout the insulin infusion by means of a variable glucose infusion and blood glucose determinations every 5 minutes. Whole-body peripheral glucose use was calculated during the last 40-minute period of the steady-state insulin infusion.

Blood Chemistry

Plasma glucose levels were measured by a glucose-oxidase method (Beckman). Serum immunoreactive insulin was assayed by using microparticle enzyme immunoassay (Abbott, Pasadena, CA). Serum free fatty acids (FFA), cholesterol, and triglycerides (TGs) were measured by enzymatic colorimetric methods.

Calculations

The metabolizable energy was calculated by subtracting the energy loss from the energy intake (EI). The energy balance represents the difference between the EE and the EI.

Muscle Biopsies

Muscle biopsy specimens were obtained under local anesthesia from the vastus lateralis portion of the quadriceps femoris muscle, immediately placed in liquid nitrogen, and stored at −80 °C for further analysis.

Total RNA Extraction and Quantification of mRNA

Frozen muscle biopsy specimens (20–30 mg) were crushed in liquid nitrogen, and total RNA was prepared using guanidinium thiocyanate-phenol-chloroform extraction (24). Concentration of each sample was assessed by absorbance measurement and its purity by the 260:280 ratio. The average yield of total RNA was 0.27 ± 0.02 μg/mg of tissue (wet weight). Total RNA samples were stored at −80 °C until quantification of the target mRNAs. Human uncoupling protein-2 (UCP2) and -3 (UCP3) mRNAs were quantified by reverse transcription followed by competitive polymerase chain reaction (RT-cPCR), during which known amounts of competitor DNA were coamplified with the target cDNA in the same tube (25). The competitor was designed to be amplified with the same primers as the target but to yield a PCR product of slightly different size so that they could be separated and quantified by gel electrophoresis. The RT reaction was performed from 0.1 μg of skeletal muscle total RNA in the presence of a thermostable RT enzyme and a specific antisense primer to warrant optimal synthesis of the first strand cDNA (25). A Cy-5 5′-end-labeled sense primer was used in the cPCR to generate fluorescent PCR products that were analyzed with an automated fluorescence DNA sequencer (ALFexpress; Pharmacia, Uppsala, Sweden) in a 4% denaturing polyacrilamide gel. The initial concentration of the target mRNA was determined at the competition equivalent point, as described in detail (25). The RT-cPCR assays for UCP2 and UCP3 mRNAs have been previously described and validated (26). Cyclophilin mRNA was also measured by RT-cPCR, as a reference gene (27), and the levels of UCP2 and UCP3 mRNAs were expressed as percentage ratio referred to the expression of cyclophilin.

UCP3 Protein Assay

Muscle biopsies from five subjects were homogenized in ice-cold Tris-EDTA buffer in phosphate-buffered saline at pH 7.4. The homogenates were subsequently sonicated for 3 × 15 s. After sonication, two volumes of each skeletal muscle homogenate and one volume sodium dodecyl sulphate-sample buffer were boiled for 4 minutes (28). All samples contained equal amounts of proteins, as determined with BioRad Protein Assay, and were loaded simulta-

neously on a 13% polyacrylamide gel containing 0.1% sodium dodecycl sulphate. Electrophoresis was performed using a Mini-Protean 3 Electrophoresis Cell (Bio-Rad Laboratories, Hercules, CA) followed by Western blotting using a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad Laboratories). The separated polypeptides were transferred to a nitrocellulose membrane (0,45 μm; Bio-Rad Laboratories) by Western blotting (29) for 1 hour at 100 V in a cold (4 °C) buffer containing 25 mM Tris, 192 mM glycine, and 20% methanol.

For detection of UCP3, an affinity purified rabbit polyclonal antibody (code 1331, kindly provided from LJ Slieker, Eli Lilly and Company, Indianapolis, IN) prepared against a 20 amino-acid peptide (human sequence amino acids 147–166) was used. We recently validated this UCP3 antibody and showed that it recognizes UCP3 and passed all specificity checks (30,31).

After protein transfer, a nitrocellulose sheet containing all samples was blocked with 5% nonfat dry milk in 0.05% Tween-20/phosphate-buffered saline and subsequently incubated with the UCP3 antiserum, diluted 1:5000 in 5% nonfat dry milk in 0.05% Tween-20/phosphate-buffered saline. Incubation was performed and shaken gently overnight at room temperature. Thereafter, the blot was incubated for 60 minutes with horseradish peroxidase–conjugated swine anti-rabbit Ig (DAKO, Glostrup, Denmark) at a dilution of 1:10,000. The blot, containing all muscle samples, was washed for 2 hours in 0.05% Tween20/phosphate-buffered saline and treated for 1 minute with chemiluminescence substrate according a standardized method (Super Signal West Dura Extended Duration Substrate; Pierce, Rockford, IL). Finally, a clear blue X-ray film (CL-Xposure Film; Pierce) was exposed to the nitrocellulose sheet for 1 minute. The reaction product of each sample was analyzed by densitometry using Imagemaster (Pharmacia Biotech, Roosendaal, The Netherlands).

Skeletal Muscle Lipid Analysis

To measure intramyocytic lipids (32) a specimen of ~100 mg was taken and immediately placed into a calcium-free Hanks solution combined with EDTA and bubbled with O_2 95% and CO_2 5%. The sample was washed and then immersed in a fresh Hanks solution combined with collagenase type IV 50 mg and calcium ions and agitated in a Dubnoff water-bath maintained at 37 °C until the tissue appeared soft. At this point, the specimen was gently removed, and cells were brushed with a blunted spatula, filtered, suspended in phosphate-buffered saline, and centrifuged twice at 50*g* for 2 minutes. The supernatant was discarded, and the muscle cells were dried under nitrogen stream. After protein precipitation with 5–10 mg of trichloroacetic acid, lipids were extracted twice with 8 volumes of chloroform-methanol (2:1, vol/vol), with the solutions stirred at 60 °C for 15 minutes. The combined extracts were dried in a GyroVap apparatus (GV1; Gio. DeVita, Rome, Italy) operating at 60 °C, coupled with a vacuum pump and a gas trap (FTS System, Stone Ridge, NY). The dry weight of lipid extracts was obtained by weighing the sample tube before and after drying the extracts. The above extracts were redissolved in chloroform-methanol (2:1, vol/vol) and fractionated into their various components by thin-layer chromatography on standard thin layer plates (Stratocrom SI AP; Carlo Erba, Milan, Italy) coated with a 0.25-mm-thick layer of silica gel and activated by heating at 120 °C for 20 minutes. The plates were developed into successive solvent system as described by Passi et al. (33). The area of silica gel corresponding to the R_f of a triolein and tripalmitin standard mixture was scraped off and extracted with peroxide-free diethyl ether. The intramyocytic triglyceride (IMTG) fraction eluted from the thin-layer chromatography plates was saponified by a treatment with 2 N KOH in methanol and successive acidification to pH 2–3 with 2 N HCl. FFAs were thus obtained and finally separated and measured according to a previously described method (34). Recovery of glycerol—measured enzymatically with a fuorimeter (35) in triplicate—from tripalmitoyl-glycerol standard (Sigma Chemical Co. Ltd., Milan, Italy) was 98 ± 3% (mean± SD) and that from a tripalmitoylglycerol standard added to skeletal muscle cell samples was 95 ± 5%.

Data Analysis

Values are given as means ± SE. The nonparametric Wilcoxon signed-rank test was used for comparisons before and after BPD. Linear regression analysis was used to examine associations. Multiple linear regression analysis was performed to identify the best predictors of the changes in UCP2 and UCP3 mRNA levels using the independent variables, IMTGs, plasma levels of FFA, fat-free mass (FFM), and fat mass (FM). $p < 0.05$ was the threshold of significance.

Results

After BPD operation, the average weight loss was ~38%, mainly depending on a massive decrease of fat mass (~55%), with some contribution also of FFM, which was reduced by ~19% (Table 1).

Circulating TGs dropped from 1.48 ± 0.14 to 0.98 ± 0.10 g/L ($p < 0.001$) after BPD, and a similar observation was made for plasma FFAs (875.7 ± 118.0 to 447.6 ± 94.3 mM; $p < 0.0001$), plasma cholesterol (4.84 ± 0.17 to 2.38 ± 0.33 mM; $p < 0.01$), fasting plasma insulin (133.80 ± 6.32 to 60.40 ± 8.12 pM; $p < 0.0001$), and fasting plasma glucose (5.54 ± 0.23 to 4.52 ± 0.16 mM, $p < 0.05$).

In all patients, the plasma glucose concentration variation in the last 40 minutes of the 2-hour clamp was lower than 10%, and a steady-state glucose infusion rate was always

Table 1. Subject characteristics

	Before BPD	After BPD	*p*
Weight (kg)	135.0 ± 3.89	82.9 ± 5.58	<0.0001
BMI (kg/m^2)	49.6 ± 2.35	30.0 ± 1.63	<0.0001
FM (kg)	68.7 ± 3.59	30.7 ± 3.68	<0.0001
FFM (kg)	67.5 ± 3.71	52.2 ± 2.89	<0.01

Values are expressed as mean ± SD.

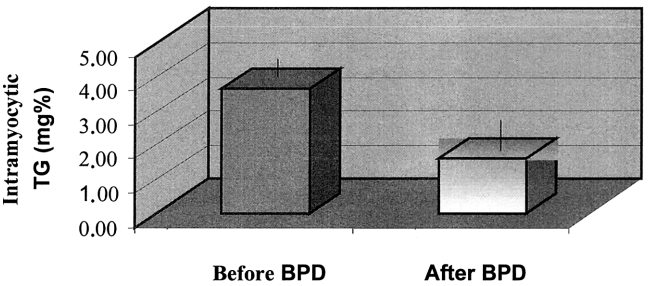

Figure 1: Concentration of intramyocytic triglycerides (mg/100 of fresh tissue) before and after BPD. Data are expressed as mean ± SE.

achieved in the last 40 minutes of the euglycemic-hyperinsulinemic clamp. The M value almost doubled after surgery (35.1 ± 9.7 to 64.2 ± 10.9 μmol/kg$_{FFM}$/min; $p < 0.001$), becoming similar to the values currently reported in the literature for healthy subjects (36). Similar results were obtained by using the ratio M/I, which is obtained by dividing the average value of M, calculated over the last 40 minutes of the clamp, by the value of plasma insulin averaged on the same period (0.038 ± 0.004 to 0.063 ± 0.009 μmol/kg$_{FFM}$/min/pmol). The data regarding energy metabolism are summarized in Table 2. Energy intake was significantly higher after BPD; however, because of a marked fecal lipid loss, metabolizable energy was reduced after surgery. REE and 24-hour EE were not different before and after BPD (Table 2). However, nonprotein RQ was significantly increased in the postobese subjects (0.73 ± 0.00 vs. 0.83 ± 0.02, $p < 0.001$), indicating a marked reduction of lipid use as energy fuel after BPD. Under these conditions of large weight loss accompanied by massive fecal lipid loss, we observed a significant drop (of ~54%) in the IMTG concentration (3.66 ± 0.16 to 1.60 ± 0.29 mg/100 mg of fresh muscle, $p < 0.0001$; Figure 1).

Figure 2 shows the mRNA levels of UCP2 and UCP3 in skeletal muscle of the obese subjects before and after BPD. The mRNA levels of UCP2 were significantly ($p = 0.02$) decreased by ~35% (from 35.9 ± 6.1% to 18.6 ± 4.5% of cyclophilin mRNA) after BPD. UCP3 mRNA also dropped ~28% (from 60.2 ± 14.0% to 33.4 ± 8.5% of cyclophilin mRNA, $p = 0.03$). No significant difference was found in the average concentration of cyclophilin mRNA before (20.7 ± 2.4 amol/mg total RNA) and after BPD (19.7 ± 3.5 amol/mg total RNA, $p = 0.84$). On average, UCP3 protein was reduced by 35% (272.19 ± 84.13 vs. 175.78 ± 60.31 AU, $p = 0.04$).

A multiple regression analysis (Table 3) was used to evaluate the joint effect of changes in IMTG levels and circulating levels of FFAs, FFM, and FM on the variation of UCP2 and UCP3 skeletal muscle expression; IMTG levels ($p = 0.007$) were the most powerful independent variable for predicting UCP variation.

A significant positive correlation (Figures 3, A and B) was found between the change in IMTG concentration and the change in UCP2 mRNA levels ($y = 0.59x - 33.33$; $R^2 = 0.80$, $p < 0.001$), as well as the change in UCP3 mRNA levels ($y = 0.53x - 36.92$; $R^2 = 0.90$, $p < 0.0001$). Moreover, a weaker but still significant negative correlation was observed between changes in nonprotein RQ and UCP mRNA level modification (UCP2: $y = -4.55x + 27.71$; $R^2 = 0.61$; $p < 0.01$; UCP3: $y = -0.86x - 45.35$; $R^2 = 0.51$; $p < 0.01$). The change in nonprotein RQ was also correlated with the reduction in IMTGs ($y = 0.59x - 35.17$; $R^2 = 0.65$; $p < 0.01$). Furthermore, it should be indicated that no significant correlation was observed with any of the measured parameters using the actual values of UCP2 and UCP3 mRNA levels instead of their changes. Finally, the

Table 2. Metabolic variables (mean ± SD)

	REE (kcal/ 24 h/kg$_{FFM}$)	24-hour EE (kcal/24 h/ kg$_{FFM}$)	24-hour EI (kcal/24 h/ kg$_{FFM}$)	Fecal lipid loss (g)	Metabolizable energy (kcal/ 24 h/kg$_{FFM}$)	Energy balance (kcal/24 h)
Pre-BPD	35.19 ± 1.84	45.74 ± 2.40	47.48 ± 2.19	—	46.00 ± 1.89	118.16 ± 37.52
Post-BPD	34.86 ± 2.54	43.45 ± 2.54	64.85 ± 3.23*	114 ± 6.63	43.92 ± 2.40*	244.67 ± 110.31

* $p < 0.01$. Energy Balance = Metabolizable Energy − 24-hour EE.

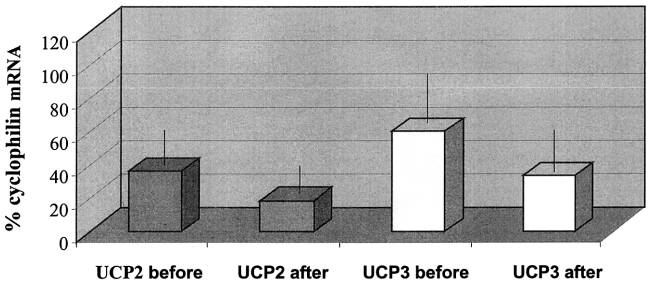

Figure 2: UCP2 and UCP3 mRNA levels (as percentage of cyclophilin mRNA) in skeletal muscle biopsies before and after BPD. Data are expressed as mean ± SE.

duration of follow-up did not show any significant impact on the change in UCP mRNA as demonstrated by including the duration of follow-up as a covariate in a statistical model for repeated measures ANOVA.

Discussion

This study shows that, in obese subjects with postbariatric lipid malabsorption, there is a reduction in the mRNA expression of uncoupling proteins (UCP2 and UCP3) in skeletal muscle; this result is supported by a concomitant decrease of the UCP3 protein content, which is significantly associated with a decrease in IMTG concentration. Furthermore, although the subjects lost weight and FM, the change in UCP expression was not associated with the degree of FM loss.

There is increasing evidence that fatty acids and their metabolites can modulate *UCP* gene expression in skeletal muscle (2,25) and also in other tissues like adipose tissue (5,6) and hepatocytes (37). In rodents, it has been shown that UCP mRNA expression in rat gastrocnemius muscle is dependent on dietary manipulations associated with changes

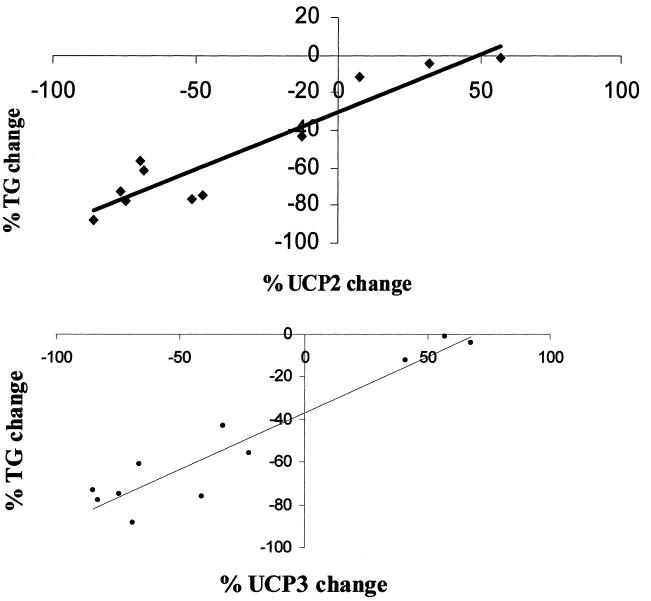

Figure 3: (A) Correlation ($y = 0.59x - 33.33$; $R^2 = 0.80$; $p < 0.001$) between changes in intramyocytic triglyceride concentration and changes in UCP2 mRNA levels. (B) Correlation ($y = 0.53x - 36.92$; $R^2 = 0.90$; $p < 0.0001$) between changes in intramyocytic triglyceride concentration and changes in UCP3 mRNA levels.

in the plasma levels of fatty acids. During starvation, a situation associated with elevated concentrations of nonesterified fatty acids, there is a rise in the expression of the UCP2 and UCP3 in skeletal muscle (9). In contrast, during re-feeding on a low-fat diet, an adaptive suppression of UCP2 and UCP3 expression was observed (9). Acute exercise, a condition known to be associated with elevated fatty acid levels, also results in an increase of skeletal muscle UCP2 and UCP3 mRNA (38,39). In humans, an association

Table 3. General linear regression model with percent changes in UCP2 ($R^2 = 0.81$, $p = 0.03$) mRNA levels and UCP3 ($R^2 = 0.84$, $p = 0.02$) as dependent variables

Dependent variables	Parameters	Coefficient	SE	*p*
UCP2 percent variation	Intercept	52.54	62.65	0.449
	FFA percent change	0.560	0.845	0.544
	IMTG percent change	1.404	0.280	0.007
	FFM percent change	−0.282	1.241	0.832
	FM percent change	−0.221	1.193	0.862
UCP3 percent variation	Intercept	127.1	76.81	0.173
	FFA percent change	0.143	1.036	0.897
	IMTG percent change	1.734	0.343	0.007
	FFM percent change	0.810	1.521	0.622
	FM percent change	1.347	1.462	0.409

between plasma FFA levels and the expression of the UCPs in muscle has also been documented in a number of studies (25,40–43). Recently, direct transcriptional effects of fatty acids on *UCP2* gene expression have been characterized in vitro in muscle cells from both rodents (44) and humans (45).

In the obese subjects after BPD, we found an increased nonprotein RQ, suggesting a shift from lipids to carbohydrates as fuel substrate. This was also supported by the observed increase in insulin sensitivity as demonstrated by the values of whole-body glucose uptake during euglycemic hyperinsulinemic clamp. Under such conditions, UCP mRNA expression was significantly reduced after BPD. There are contrasting results in the literature regarding the relationship between *UCP* gene expression, protein content, and weight loss. In fact, some authors have reported that body weight loss, induced by calorie restriction, does not cause any change in skeletal muscle UCP2 protein content (17). Other authors have shown that, in the skeletal muscle of obese subjects, UCP2 mRNA is significantly increased after weight loss, whereas UCP3 mRNA is reduced (46). Finally, some other authors have found a significant muscular reduction of both UCP2 and UCP3 mRNA expression associated with weight loss (31). Our patients represent a peculiar experimental model in that their weight loss occurs at the time of a very-high-energy intake (47). Therefore, they are very different from the obese subjects generally described in the literature, where a strict diet is required to obtain a certain degree of weight loss. In post-BPD subjects, the carbohydrate absorption rate is conserved, with a subsequent normal availability of carbohydrate as an energy source for skeletal muscle. In contrast, in subjects under a strict diet, the extent to which carbohydrates contribute to the energy source depends on diet composition. If there is little carbohydrate in the diet, there will not be enough carbohydrates for the skeletal muscle, and the myocytes will accumulate a large amount of lipids as an energy source; thus, the IMTGs of the strict diet subjects probably will be higher than that of the malabsorption subjects. Actually, obese subjects who undergo BPD lose the major part of dietary lipids with feces; therefore, their real dietary fat availability is poor (46,48). Under this particular situation, these patients show significantly lower IMTG concentrations. The original finding of our work was the demonstration that the higher the reduction in IMTG concentrations the higher the decrease in muscle UCP mRNA; furthermore, the best predictor of UCP mRNA reduction was the IMTG level, whereas circulating FFAs did not play a significant role. This result suggests, therefore, that an important regulator of *UCP* gene expression might be the quantity of lipids immediately available in the muscle compartment, whereas in a dynamic status, such as that observed during weight loss, the plasma FFA compartment may play a pivotal role in the control of *UCP* gene expression. It should be also noted that reduction in IMTGs in post-BPD patients is a very rapid event, occurring before massive weight loss (49). Accordingly, we did not find significant correlation between the degree of weight loss and the reduction in UCP mRNA expression.

The exact role of UCPs in cell physiology is still unclear. The fact that a high fatty acid supply induces its expression led to the suggestion that UCPs may protect the cells from a high fatty acid oxidation rate, with subsequent increase of reactive oxygen species (10). The high levels of reactive oxygen species in the macrophages of UCP2-knockout mice also support this hypothesis (50).

Another mechanism through which postobese subjects, after malabsorptive bariatric surgery, greatly improve their insulin sensitivity at the level of skeletal muscle might be related to the reduction of other intramyocellular lipid moieties, such as ceramide and diacylglycerol (DAG). In fact, it has been shown that DAG activates isozymes of the protein kinase C family and that ceramide has several effectors, including protein kinase C and protein phosphatase (51). Thus, both of these lipid molecules inhibit insulin signaling and promote cellular insulin resistance. Unfortunately, we do not have data about the skeletal muscle levels of these lipids. Furthermore, no information is available in the literature on a possible action of ceramide and DAG on *UCP* gene expression. This interesting topic might represent the starting point of a new study in the human model of tissue lipid pauperization, which is realized through intestinal lipid malabsorption in post-BPD subjects.

In conclusion, our data show that *UCP2* and *UCP3* gene expression was reduced in skeletal muscle of obese subjects who lost weight after a malabsorptive bariatric operation. Moreover, the change in UCP mRNA expression was strongly correlated with a decrease in IMTG levels, suggesting that the content of IMTGs plays a relevant important role in the regulation of *UCP* gene expression.

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