Reduced expression of uncoupling proteins-2 and -3 in adipose tissue in post-obese patients submitted to biliopancreatic diversion

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Abstract

Objective: Little is known about the physiological role and the regulation of uncoupling proteins-2 and -3 (UCP-2 and -3) in adipose tissue. We investigated whether the expression of UCP-2 and -3 in adipose tissue was affected by weight loss due to a biliopancreatic diversion (BPD) and related to the daily energy expenditure (24-h EE).

Design: Ten morbidly obese subjects (mean body mass index = 49.80 ± 2.51 kg/m²) were studied before and 18 months after BPD.

Methods: We determined body composition using tritiated water and 24-h EE in a respiratory chamber. Adipose tissue UCP-2 and -3 mRNA, plasma insulin, glucose, free fatty acids (NEFA), free triiodothyronine (FT3), free thyroxine (FT4) and leptin were assayed before and after BPD.

Results: BPD treatment resulted in a marked weight loss (P < 0.001) mainly due to a fat mass reduction. A significant decrease in 24-h EE/fat-free mass (FFM) (P < 0.05) and in UCP-2 (P < 0.05) and UCP-3 (P < 0.05) mRNA was observed. A significant reduction in plasma insulin, glucose, NEFA, FT3, FT4 and leptin was seen after BPD. The decline in plasma leptin and FF A was tightly correlated with the decrease in both UCP-2 and -3. A significant correlation was found between changes in FT3 and variations in 24-h EE (r = 0.64, P < 0.05). In a multiple-regression analysis changes in 24-h EE/FFM after BPD were significantly correlated with changes in UCP-3 expression (P < 0.05).

Conclusion: These findings suggest that UCPs in adipose tissue may play a role in the reduction in 24-h EE observed in post-obese individuals.

Introduction

Changes in the balance between energy intake and energy expenditure may play a role in long-term body weight (BW) regulation and in the development of obesity. A relatively low energy expenditure has been implicated in future weight gain (1) as well as in promoting relapse after short-term intentional weight loss (2–4). Therefore it is essential to understand better the mechanisms involved in the control of energy expenditure in obesity. Resting metabolic rate (RMR), which is the major component of the daily energy expenditure (24-h EE), is the result of complex biochemical reactions leading to a proton leak across the mitochondrial inner membrane, which results in energy dissipation because of uncoupling of oxygen consumption from ATP synthesis (5). Among the mitochondrial carrier proteins playing a major role in this process, the first discovered, uncoupling protein (UCP)-1 (6), is exclusively expressed in brown adipose tissue (BAT), whose presence is quite negligible in adults (7); therefore it is unlikely that this protein may be implicated in regulation of energy expenditure in humans. Two UCP isoforms have been discovered with a high sequence homology to UCP-1. UCP-2 expression is observed in many tissues (8), while UCP-3 is preferentially expressed in skeletal muscle and to a lesser extent in adipose tissue (9). Both these UCPs...
were thought to be implicated in the regulation of EE and may contribute to obesity (10, 11). More recently this role has been questioned after the observations that during fasting, a condition in which there is a clear depression of EE, UCP expression is increased in skeletal muscle (12). This apparent contradiction has been explained by the involvement of these proteins in the mitochondrial free fatty acid (NEFA) oxidation (13). Little information is currently available about factors controlling UCP-2 gene expression in human adipose tissue and the physiological role of this UCP remains still controversial.

Studies in humans have suggested a correlation between UCP-2 gene expression in white adipose tissue (WAT) and body mass index (BMI) (12). However, other authors reported that the obese had reduced UCP-2 mRNA content only in visceral adipose tissue compared with lean subjects, while no difference was observed in s.c. adipose tissue (14). The expression of UCP-2 mRNA in adipose tissue has been reported to be up-regulated by fasting for 5 days (12). However, Barbe et al. (15) reported that weight loss obtained with a modest food limitation did not influence adipose tissue UCP-2 expression. Moreover, in obese women a very-low-calorie diet treatment for a short period of time resulted in a clear decrease in UCP-3 mRNA in adipose tissue tightly correlated with changes in EE, whereas adipose tissue UCP-2 mRNA levels remained unchanged (16). These observations do not allow us to assign to UCP-2 and -3 a precise role in the reduction in EE observed during energy restriction. The reduction in RMR that follows weight loss (17) is mainly due to the decrease in fat-free mass (FFM), which represents the major determinant of EE (18). Nevertheless, the adipose organ is the predominant body compartment to be modified during a high isocaloric diet in obese people. Thus, it is interesting to investigate if changes in adipose tissue UCP mRNA are only due to the reduction of fat mass or are also related to variations in EE.

The present study was designed to examine the levels of UCP-2 and -3 mRNA expression in human adipose tissue in a group of morbidly obese patients before and after biliopancreatic diversion (BPD) and subsequent important weight loss. Another relevant aim of this work was to relate this measure to major changes in adipose tissue and 24-h EE.

**Subjects and methods**

**Subjects**

The study groups consisted of ten (seven male and three female) severely obese subjects (BMI = 49.80 ± 2.51 kg/m² (range 42.46–66.67)) who were studied twice: before and 18 ± 2 months after BPD operation. None had impaired glucose tolerance, diabetes mellitus or any other endocrine or non-endocrine disease. Before inclusion a physical examination was found normal. At the time of the baseline study, all subjects were on a free diet, with the following average composition: 60% carbohydrate, 30% fat, 10% protein (at least 1 g/kg BW). This dietary regimen was maintained for 1 week prior to the study. Their BW was stable for at least 2 months prior to the investigation. After the initial study, patients were submitted to surgery consisting of a partial gastrectomy with a distal Roux-en-Y reconstruction (19, 20). The study protocol was approved by the Institutional Ethical Committee of the Catholic University of Rome; the nature and purpose of the study were carefully explained to all subjects before they provided their written agreement to participate.

**Body composition**

Anthropometric parameters were determined at time 0 and 18 ± 2 months after surgery. BW was measured to the nearest 0.1 kg by a beam scale, and height to the nearest 0.5 cm using a stadiometer (Holatin, Crosswell, UK). Total body water (TBW) was determined using 0.19 Bq of tritiated water in 5 ml saline solution administered as an i.v. bolus injection (21). Blood samples were drawn before and 3 h after the injection. Radioactivity was determined in duplicate on 0.5 ml plasma using a Beta-scintillation counter (Model 1600TR; Canberra-Packard, Meriden, CT, USA). Corrections were made (5%) for non-aqueous hydrogen exchange (22); water density at body temperature was assumed to be 0.99371 kg/l. TBW (kg) was computed as tritiated water dilution space (liters) × 0.95 × 0.99371. The within-subject coefficient of variation (CV) for this method is 1.5% (23). FFM in kg was obtained by dividing the TBW by 0.732 (24).

**Twenty-four hour EE**

The measurement of energy expenditure in a respiratory chamber was performed. Subjects entered the chamber in the morning after an overnight fast and remained there for 24 h. Subjects were fed a standardized diet with the amount of calories calculated according to previously determined equations to achieve energy balance. Meals were provided at 0800, 1200, 1700 and 2000 h. The rate of energy expenditure was measured continuously, calculated for each 15 min interval. Spontaneous physical activity was detected by radar sensors and expressed as the percentage of time over the 24 h period in which activity was detected. Carbon dioxide production (VCO₂) and oxygen consumption (VO₂) were calculated at 15 min intervals, summed for the 24 h in the chamber. The 24 h respiratory quotient (24-RQ) was calculated as the ratio of 24-h VCO₂ and 24-h VO₂ and adjusted for the 24-h energy balance ((24-h energy intake) – (24-h EE)) during the stay in the chamber in a multiple-regression analysis.
as previously described (25). Results are presented as kcal/24 h and after normalization to FFM (kcal/24 h per kg).

**Hyperinsulinemic euglycemic clamp**

Insulin sensitivity was determined in all subjects after an overnight fast by the euglycemic insulin clamp technique with an insulin infusion rate of 7 pmol/min per kg. Whole-body glucose uptake (M-value, in mg/kgBW/min) was determined by averaging the exogenous glucose infusion rates during the last 40 min of the clamp, and correcting them for the glucose space (26).

**Biochemical analysis**

Plasma glucose levels were measured by a glucose oxidase method (Beckman, Fullerton, CA, USA). NEFA levels were determined using a commercial kit (Boehringer–Mannheim KK, Tokyo, Japan).

Serum immunoreactive insulin was assayed by using microparticle enzyme immunoassay (Abbott, Pasadena, CA, USA). Thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were measured using microparticle enzyme immunoassay (Abbott, Pasadena, CA, USA). Thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were measured using microparticle enzyme immunoassay (Abbott, Pasadena, CA, USA). Thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were measured using microparticle enzyme immunoassay (Abbott, Pasadena, CA, USA). Thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were measured using microparticle enzyme immunoassay (Abbott, Pasadena, CA, USA). Thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were measured using microparticle enzyme immunoassay (Abbott, Pasadena, CA, USA). Thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were measured using microparticle enzyme immunoassay (Abbott, Pasadena, CA, USA). Thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were measured using microparticle enzyme immunoassay (Abbott, Pasadena, CA, USA).

**Adipose tissue biopsies**

Biopsies were obtained under local anesthesia with lidocaine from the s.c. adipose tissue obtained by needle aspiration in the vastus lateralis muscle region. Tissue samples were washed to remove blood, immediately frozen in liquid nitrogen and stored at −80°C for further analysis of UCP-2 and -3 mRNA levels. Biopsies were obtained before and after BPD surgical treatment.

**Isolation of RNA and RT-PCR determination of UCP-2 and -3 mRNA**

Total RNA was isolated from adipose tissue using the RNeaZol method (TEL-TEST, Inc., Friendswood, TX, USA). The concentration and purity of RNA were determined by absorbance at 260 and 280 nm. All the samples had a 260 to 280 absorbance ratio of about 2.0.

RNA was treated for 1 h at 37°C with 1U RNase-free DNase I/μg RNA in 10 μl buffer containing 40mmol/l Tris–HCl, pH 8.0 and 10 mmol/l MgSO4 and 1 mmol/l CaCl2. Two micrograms of total RNA were reverse-transcribed with 200U Moloney murine leukemia virus reverse transcriptase (Promega Corporation, Madison, WI, USA) and 20U RNAsin ribonuclease inhibitor in 50 μl buffer containing dNTP mix (0.5 mmol/l each) and 100 ng oligo(dT)15 primer (Promega) in 50 mmol/l Tris–HCl pH 8.3, 75 mmol/l KCl, 3 mmol/l MgCl2 and 10 mmol/l dithiothreitol. PCR was performed using Hot Star Taq DNA polymerase (Qiagen GmbH, Hilden, Germany) in 25 μl standard buffer with 1.5 mmol/l MgCl2, 200μmol/l each dNTP and 40 pmol of each sense and antisense specific oligonucleotide primer. The primer sequences were chosen using OMIGA 2.0 and were 5′-TAAAGACCTCTTATGGCAACAGT-3′ and 5′-CACGATGGAGGCGCCTACTCATC-3′ for β-actin, 5′-CTACGTGCACGTGAAGTCTTC-3′ and 5′-TCGGCGCATGTTTTTGAGG-3′ for UCP-2, 5′-ACAGATGTGGTGAAGTTTC-3′ and 5′-TACGAACATCACCAGTTCC-3′ for UCP-3. Amplification products were of 241, 472 and 468 bp respectively.

The primers for β-actin were added at the tenth cycle of each PCR amplification to avoid a plateau situation. All the genes were amplified using 30 cycles at 94°C for 30s, 60°C for 30s and 72°C for 30s, followed by 5min final extension at 72°C.

After amplification, 10μl reaction mixture were separated by electrophoresis (1% agarose gel in Tris–borate–EDTA buffer), visualized using ethidium bromide staining, revealed with Image Master VDS (Amersham Pharmacia Biotech Europe GmbH, Freiburg, Germany) and densitometrically analyzed with Image Master Total Lab 1.00 software (Amersham Pharmacia).

The number of cycles for the semiquantitative RT-PCR analysis and the conditions of the reaction temperature were estimated to be optimal for a linear relationship between the amount of input template and the amounts of PCR product generated over a significant concentration range: 20–100 ng from total RNA. In particular the linearity of the RT-PCR amplifications for all the tested genes was measured at 15, 30 and 40 cycles (data not shown).

Levels of mRNA were expressed as the ratio of signal intensity for the target genes relative to that for β-actin. The RT-PCR analyses were performed three times on the same sample: the intra-assay CV was <5%.

**Statistical analysis**

Data are shown as means±S.E.M. and one-way ANOVA was used to compare before and after values (P<0.05 was considered significant). Single-linear and multiple-regression analyses were performed. All analyses were performed with STATISTICA for Windows package (StatSoft, Inc., Tulsa, OK, USA).

**Results**

**Effects of BPD on body composition and 24-h EE**

After BPD the subjects had lost around 32% of their initial BW (mean weight loss 44.89±5.94 kg,
range 22–80 kg). Changes in body composition are reported in Table 1. EE expressed in absolute terms (−721.56 ± 135.35 kcal/24 h) or normalized by FFM (−6.51 ± 1.27 kcal/24 h per kg) was significantly reduced after BPD, as reported in Table 2.

**Insulin sensitivity**

A significant reduction in plasma insulin, glucose, NEFA and triglycerides was seen after BPD (Table 3). On the clamp, insulin-mediated whole-body glucose uptake that appeared markedly impaired before treatment was significantly improved after weight loss in the BPD group (Table 3).

**Leptin and thyroid hormones**

After BPD (18 ± 2 months) we observed a significant fall in leptin plasma levels (−61%) that exceeded the percent of decrease in fat stores (−42%) (Table 3). Table 3 shows the changes in thyroid hormones before and after weight reduction. Both FT3 and FT4 declined significantly, while TSH remained unchanged.

**Effects of BPD on adipose tissue UCP-2 and -3 mRNA levels**

UCP-2 mRNA levels in adipose tissue were significantly reduced after weight loss by 70% (82.80 ± 27.48 vs 25.13 ± 7.41 arbitrary units (a.u.), P < 0.05) The adipose tissue level of UCP3 mRNA was reduced by 50% (96.11 ± 15.45 vs 47.51 ± 12.47 a.u., P < 0.05) in the biopsies obtained after BPD as compared with initial values (Fig. 1).

**Table 1** Anthropometric characteristics of obese subjects (n = 10) at the beginning of the study and after BPD; means ± S.E.M. Statistics were performed by one-way ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>Before BPD</th>
<th>After BPD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>135.80 ± 6.46</td>
<td>92.30 ± 5.89</td>
<td>0.00002</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>49.80 ± 2.51</td>
<td>34.15 ± 2.90</td>
<td>0.00002</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>62.50 ± 3.24</td>
<td>26.60 ± 3.59</td>
<td>0.00001</td>
</tr>
<tr>
<td>FM (%)</td>
<td>45.98 ± 0.72</td>
<td>26.08 ± 5.21</td>
<td>0.00257</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>73.30 ± 3.49</td>
<td>65.70 ± 3.68</td>
<td>0.02196</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>54.02 ± 0.72</td>
<td>71.87 ± 2.42</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

FM, fat mass.

**Table 2** Effects of BPD on changes in the energy metabolism profile as assessed by a calorimetric chamber; means ± S.E.M. of 10 subjects. Statistics were performed by one-way ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>Before BPD</th>
<th>After BPD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂ (ml/min)</td>
<td>359.33 ± 18.94</td>
<td>263.69 ± 7.69</td>
<td>0.00037</td>
</tr>
<tr>
<td>VCO₂ (ml/min)</td>
<td>284.67 ± 15.58</td>
<td>225.66 ± 5.84</td>
<td>0.00198</td>
</tr>
<tr>
<td>RQ</td>
<td>0.79 ± 0.00</td>
<td>0.86 ± 0.01</td>
<td>0.00001</td>
</tr>
<tr>
<td>EE (kcal/24h)</td>
<td>2563.10 ± 174.27</td>
<td>1878.50 ± 92.48</td>
<td>0.00273</td>
</tr>
<tr>
<td>EE/FFM (kcal/24h/kg)</td>
<td>34.95 ± 1.52</td>
<td>28.81 ± 0.81</td>
<td>0.00062</td>
</tr>
<tr>
<td>N₂ (g/24h)</td>
<td>10.39 ± 0.46</td>
<td>13.70 ± 0.40</td>
<td>0.00012</td>
</tr>
</tbody>
</table>

**Table 3** Effects of BPD on changes in the endocrine and metabolic parameters; means ± S.E.M. of 10 subjects. Statistics were performed by one-way ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>Before BPD</th>
<th>After BPD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>90.90 ± 1.73</td>
<td>72.20 ± 0.76</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Tryglycerides (mg/dl)</td>
<td>181.90 ± 6.25</td>
<td>88.90 ± 4.33</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>NEFA (µmol/l)</td>
<td>948.80 ± 66.17</td>
<td>480.80 ± 34.94</td>
<td>0.00001</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>30.40 ± 2.14</td>
<td>9.10 ± 1.23</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>M-value (mg/kg BW/min)</td>
<td>2.34 ± 0.18</td>
<td>6.28 ± 0.48</td>
<td>0.00004</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>46.10 ± 3.03</td>
<td>17.67 ± 1.71</td>
<td>0.00003</td>
</tr>
<tr>
<td>TSH (µlU/I)</td>
<td>1.76 ± 0.22</td>
<td>2.00 ± 0.21</td>
<td>ns</td>
</tr>
<tr>
<td>FT3 (pmol/l)</td>
<td>2.85 ± 0.07</td>
<td>2.91 ± 0.23</td>
<td>0.04003</td>
</tr>
<tr>
<td>FT4 (pmol/l)</td>
<td>13.35 ± 0.58</td>
<td>10.99 ± 0.55</td>
<td>0.00035</td>
</tr>
</tbody>
</table>

**Correlations between UCP mRNA, body composition, and 24-h EE**

Before surgery 24-h EE (kcal/24 h) was significantly correlated with FFM in kg (r = 0.76, P < 0.01), FM in kg (r = 0.69, P < 0.05) and total BW (r = 0.75, P < 0.01). Only UCP-3 mRNA was significantly correlated with BMI (r = 0.86, P < 0.001), FFM (r = 0.95, P < 0.001), FM (r = 0.57, P < 0.05), 24-h EE (r = 0.98, P < 0.001) and 24-h EE/FFM (r = 0.69, P < 0.05).

There was a positive correlation between the fall in EE and changes in BW (r = 0.93, P < 0.001), FFM in kg (r = 0.59, P < 0.05) and FM in kg (r = 0.92, P < 0.001). Also 24-h EE/FFM positively correlated with changes in FM in kg (r = 0.72, P < 0.01).

There was also a positive correlation between the changes in adipose tissue UCP-3 mRNA and the fall in 24-h EE (r = 0.72, P < 0.01) and 24-h EE/FFM (r = 0.81, P < 0.01), but not with adipose tissue UCP-2 mRNA (Table 4).

A significant correlation was found between changes in FT3 and variations in 24-h EE (r = 0.64, P < 0.05); on the contrary no evidence existed for an influence of FT4 on calorimetric parameters. However, a significant negative correlation was observed between changes in FT4 and both UCP-2 and -3 modifications before and after weight reduction (Table 4).

Moreover, a significant correlation between changes in serum leptin levels after weight loss and the magnitude of the reduction of both 24-h EE (r = 0.84, P < 0.01) and 24-h EE/FFM (r = 0.69, P < 0.05), and either adipose tissue UCP-2 (r = 0.99, P < 0.001) and UCP-3 (r = 0.72, P < 0.01) mRNA levels was detected (Table 4).

The decline in plasma NEFA was significantly correlated with the decrease in both UCP-2 (r = 0.70, P < 0.05) and UCP-3 (r = 0.61, P < 0.05) mRNA levels in adipose tissue (Table 4).

Multiple-regression analysis, using changes in 24-h EE/FFM as the dependent variable and changes in UCP-3 as the independent variable, revealed that only percentage change in adipose tissue UCP-3 mRNA was independently correlated with 24-h EE/FFM when the
other variables (changes in BMI, insulin, NEFA and M-value) were taken into account in a standard regression model (adjusted $R^2 = 0.89$, $F = 17.73$, $P < 0.05$).

**Discussion**

Metabolic predisposition to obesity might depend not only on initial rates of EE, but also on how this parameter and the underlying control mechanisms change after weight loss. The marked weight loss produced by BPD in morbidly obese subjects significantly reduced FM, FFM and 24-h EE. Metabolic adaptation in post-obese individuals who have returned to a normal BW or have decreased their BW considerably and maintained the weight loss over months is commonly accompanied by a small reduction in EE. This phenomenon might be amplified after a more rapid decrease in BW (27), as in our experimental conditions. However, even small differences in EE can have an important influence on BW over the long-term (1) thus explaining, at least in part, the high rate of weight relapse after dieting in obese people. The decline in total EE strongly depends on changes in RMR that can be explained by a reduction in FFM (4). This is the case also in our experiments in which a significant correlation is present between changes in FFM and 24-h EE. It is widely accepted that skeletal muscle plays the major role in the regulation of RMR in humans (18), but also FM has been considered as a significant, independent predictor of this parameter (28), therefore we cannot exclude the possibility that relevant modifications in fat mass and in molecular factors controlling fat cell energy metabolism (i.e. UCP-2 and -3) might influence whole-body EE. Before surgery, 24-h EE was closely correlated with BW, BMI, FM and FFM and with the mRNA levels of UCP-3. After weight loss, a positive correlation was also found between the fall in 24-h EE and changes

![Figure 1](https://www.eje.org)

**Figure 1** Changes in UCP-2 and -3 expression in adipose tissue before and after BPD. (A) RT-PCR products of representative samples of one subject before (b) and after (a) BPD were resolved on agarose gel together with the 100 bp molecular weight ladder (Mw), the 500 bp band of which is present at triple the intensity of the other fragments. (B) UCP mRNA levels means $\pm$ S.E.M. of ten subjects before and after BPD were compared. Statistics was performed by one-way ANOVA. *$P < 0.05$.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\Delta$ UCP-2 (a.u.)</th>
<th>$\Delta$ UCP-3 (a.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>$-0.29$ ns</td>
<td>$0.72 &lt; 0.01$</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>$-0.54$ ns</td>
<td>$0.52$ ns</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>$-0.75 &lt; 0.01$</td>
<td>$0.23$ ns</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>$0.17$ ns</td>
<td>$0.91 &lt; 0.001$</td>
</tr>
<tr>
<td>EE (kcal/24 h)</td>
<td>$-0.16$ ns</td>
<td>$0.72 &lt; 0.01$</td>
</tr>
<tr>
<td>EE/FFM (kcal/24 h/kg)</td>
<td>$0.52$ ns</td>
<td>$0.81 &lt; 0.01$</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>$-0.80 &lt; 0.01$</td>
<td>$-0.75 &lt; 0.01$</td>
</tr>
<tr>
<td>NEFA (μmol/l)</td>
<td>$0.70 &lt; 0.05$</td>
<td>$0.61 &lt; 0.05$</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>$-0.52$ ns</td>
<td>$0.43$ ns</td>
</tr>
<tr>
<td>M-value (mg/kg BW/min)</td>
<td>$0.40$ ns</td>
<td>$-0.62 &lt; 0.05$</td>
</tr>
<tr>
<td>FT3 (pmol/l)</td>
<td>$-0.50$ ns</td>
<td>$0.43$ ns</td>
</tr>
<tr>
<td>FT4 (pmol/l)</td>
<td>$-0.64 &lt; 0.05$</td>
<td>$-0.94 &lt; 0.001$</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>$0.08$ ns</td>
<td>$0.73 &lt; 0.01$</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>$0.99 &lt; 0.001$</td>
<td>$0.72 &lt; 0.01$</td>
</tr>
</tbody>
</table>
in FM and FFM; moreover, a clear reduction in both adipose tissue UCP-2 and -3 mRNA levels was observed, but only the change in adipose tissue UCP-3 mRNA was independently correlated with the decrease in 24-h EE/FFM. However, divergent results have previously been described concerning the correlation between UCP gene expression, BMI and changes in BW (10, 12, 15, 16, 29–31).

It has been reported that the reduction of thermogenesis that occurs during weight loss is somewhat determined by the degree of depletion of the fat stores and that it persists during subsequent weight recovery, with the energy thus conserved being directed at accelerating the refill of the fat stores (32). Lipogenesis and lipolysis in adipose tissue are tightly dependent on ATP levels (33) and the respiratory uncoupling may therefore contribute to the control of fat cell energy metabolism (34–37). As a matter of fact, pharmacological treatments that induce the expression of UCPs in WAT are able to reduce adiposity.

The correlation between changes in the expression of UCP-2 and -3 in WAT and the modifications in fat mass we found are in keeping with this view, even if WAT is not a major thermogenic tissue in humans. Moreover, since UCP-2 is expressed at a much higher level than UCP-3 in adipose tissue it seems questionable if its variations in this organ may play a major role in EE. Nevertheless, in our experimental conditions only changes produced by BPD in adipose tissue UCP-3 mRNA seem to contribute independently to the variation in EE.

However, the putative thermogenetic role of these UCPs has been recently questioned and several findings indicate that NEFA might be involved in the control of UCP expression (13). As a matter of fact, the increased gene expression of UCP-3 in adipose tissue and skeletal muscle in rodents (38, 39) and humans (12) produced by a prolonged fast is probably due to the accompanying increase in circulating NEFA release from adipocytes and their oxidation in muscle.

In our experimental model we observed an important reduction in FM and a concomitant decline in plasma NEFA, thus leading to a decrease in both UCP-2 and -3 adipose tissue expression. In agreement with this view, we recently observed that the increase in circulating NEFA levels during Intralipid plus heparin infusion in healthy subjects increased both UCP-2 and -3 in subcutaneous fat (40). The BPD treatment and the Intralipid infusion represent two different physiological conditions: the former is characterized by an increased release of NEFA from adipocytes to supply muscle energy requirements, while the latter is characterized by an enhanced flux of NEFA preferentially channeled to adipose tissue. Since it has been recently described that, in the fasting state, adipose tissue UCP-2 expression correlated inversely with epigastric venous fatty acids concentration (31), the reduced UCP expression may be considered therefore as the consequence of an increased lipid fuel flux leaving adipose tissue.

Different hormones are known to regulate the mRNA expression of UCPs: leptin, thyroid hormones and β-3-adrenergic agonists all appear able to stimulate UCP mRNA expression in adipose tissue (34, 41).

FT3 produces an organ-specific enhancement of UCP-2 and -3 mRNA in rats, namely in the heart, BAT, WAT and skeletal muscle (42, 43). Muscle UCP-3 levels were found to be decreased in hypothyroid rats (34). Recently it has been shown that, in humans, a doubling of plasma FT3 levels leads to an up-regulation of UCP-2 and -3 mRNA levels in adipose tissue (44). These data obtained in animals and in humans suggest that UCP-2 and -3 could mediate, at least to some extent, the effects of T3 on energy metabolism (45). We confirmed that changes in FT3 and variations in 24-h EE may be linked together. However, we did not observe any significant correlations between changes in serum concentrations of FT3 and the adipose tissue UCP-2 and -3 mRNA expression, while a significant negative correlation was observed between changes in FT4 and both UCP-2 and -3 modifications. These apparent contradictory results may be explained by the fact that data we collected either before surgery or after weight loss are consistent with an euthyroid status of the patients we analyzed, and we cannot exclude the possibility that a more marked fall in thyroid hormone concentrations may have a different impact on UCP gene expression.

The significant correlation we observed between changes in serum leptin levels and differences in 24-h EE also confirms previous data, even if in this field conflicting results have been obtained. A link between leptin levels and EE was also recently suggested in a group of overweight and obese women in whom a negative correlation between RMR and leptin levels was found after controlling for FM and FFM. Moreover, in a multiple-regression analysis, leptin contributed significantly to RMR, independently of body composition (46). A direct thermogenic effect of leptin was recently demonstrated in skeletal muscle (47), but so far no clear evidence for a direct effect of leptin on adipose energy metabolism has been reported.

In our study we were able to detect a significant correlation between the decrease in serum leptin concentrations after weight loss and either adipose tissue UCP-2 and -3 mRNA levels, thus supporting the idea of a role for leptin in controlling UCP expression also during a remarkable weight loss. As a matter of fact, it has been shown that leptin increases UCP-2 mRNA in pancreatic islets and in WAT (48). Moreover adipose tissue UCP-2 and skeletal muscle UCP-3 mRNA levels were increased upon a central nervous system leptin infusion in rats (49, 50).

In conclusion, the change in 24-h EE observed after BPD was closely correlated with the change in FM and FFM. Prolonged energy restriction caused by BPD
and the subsequent weight loss affects both UCP-2 and -3 mRNA in adipose tissue. Changes in UCP-3 expression may contribute to the variation in 24-h EE. However, we cannot exclude the possibility that the decrease in the UCP expression may be simply the result of changes in the fat cell lipid metabolism. Our data further suggest a role for leptin in the control of energy metabolism either directly or through its effects on UCP-2 and -3, whose impact on the development of obesity and whose link with the frequent relapse after dieting still remain to be elucidated.

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