Changes in Gene Expression Profile in Human Subcutaneous Adipose Tissue during Significant Weight Loss

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Key Words
PPARγ · 11βHSD1 · Roux-en-Y gastric bypass · Subcutaneous adipose tissue

Abstract
Objective: To analyze the expression of peroxisome proliferator-activated receptor-γ1 and 2 (PPARγ1 and 2), 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1), and leptin in adipose tissue (AT) of obese women during weight loss following Roux-en-Y gastric bypass (RYGB) and to compare these levels with those obtained in AT of nonobese subjects. Methods: Gene expression was determined by real-time RT-PCR prior to surgery and at 3, 6, and 12 months after RYGB. Results: All obese patients lost weight, reaching a mean BMI of 29.3 ± 1.0 kg/m² at 1 year after surgery (~33.9 ± 1.5% of their initial body weight). In obese subjects leptin and 11βHSD1 were over-expressed, whereas PPARγ1 was expressed at lower levels compared to controls. After surgery, leptin and 11βHSD1 gene expression decreased, whereas PPARγ1 expression increased. At 12 months after RYGB, these 3 genes had reached levels similar to the controls. In contrast, PPARγ2 gene expression was not different between groups and types of tissue and remained unchanged during weight loss. We found a positive correlation between BMI and levels of gene expression of leptin and 11βHSD1. Conclusion: Gene expression of leptin, PPARγ1, and 11βHSD1 in AT is modified in human obesity. This default is completely corrected by RYGB.

Introduction

Surgical treatment of obesity (reviewed in [1, 2]) and its positive consequences on the overall body composition, the inflammation profile, the insulin resistance, the cardiac status, the endothelial function, and the bone status are well documented (reviewed in...
Gene network analysis applied to obesity and caloric restriction, e.g. following bariatric surgery, highlighted the importance of pathways for glycerolipid metabolism (reviewed in [6, 7]). Peroxisome proliferator-activated receptor-γ 1 and 2 (PPARγ1 and 2) and 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) are among the glycerolipid metabolism-related genes. Both PPARγ agonism and inhibition of 11βHSD1 improve lipid metabolism and lipemia. Moreover, therapeutic inhibition of 11βHSD1 will be beneficial in most cellular contexts, with clinical trials supportive of this concept. Although the key role of PPARγ and 11βHSD1 in adipose tissue (AT) development and differentiation are not debated, their mRNA levels in human AT are still controversial.

PPARγ1 and PPARγ2 isoforms are highly expressed in human subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) [8–10]. However, the literature does not give a clear view about PPARγ mRNA expression in obesity. Some authors found no difference between obese and nonobese subjects [8, 11, 12]. Some others reported an increase of PPARγ expression in obesity. According to Bortolotto et al. [13], PPARγ expression increase in SAT of obese subjects, decrease in retroperitoneal AT, and is not changed in VAT. For Lefebvre et al. [14], PPARγ in VAT is increased in obesity, but not in SAT. Finally, Vidal-Puig et al. [10] showed that PPARγ1 and PPARγ2 were differently regulated and that only PPARγ2 expression increased in obese versus lean subjects. Consistently, Viguerie et al. [15] reported a decrease of PPARγ expression following 10-week hypocaloric diet program. In contrast, Pou lain-Godefroy et al. [16] published recently results demonstrating a significant decrease of PPARγ expression in SAT and a trend toward a decrease in VAT of obese compared to lean patients.

11βHSD1 mRNA expression in AT in human obesity is also highly debated in the literature. Several papers reported an overexpression or an overactivity of 11βHSD1 in AT of obese women and men [17–20]. Polycystic ovary syndrome and obesity are independently associated with increased expression of 11βHSD1 [21]. Consistently, Mericq et al. [22] demonstrated a positive correlation between mRNA expression of 11βHSD1 in VAT and BMI in children. However, others studies have failed to show this increase and this relationship [23, 24]. Tomlinson et al. [25] even showed that weight loss increases 11βHSD1 expression in human isolated adipocytes. Moreover, data regarding gene expression of 11βHSD1 in VAT and SAT are also conflicting [22–24, 26, 27].

The explanation for these discrepancies is not clear. The large variability observed might be related to the degree of sensitivity of the methods or to the criteria of recruitment for the cohorts. Furthermore, discrepancies could be due to circadian pattern in 11βHSD1 and PPARγ gene expression [8]. Indeed, clock genes have been recently implicated in the human metabolic syndrome [9–31].

What we propose is to follow the levels of expression of PPARγ1, PPARγ2, and 11βHSD1 genes in SAT and VAT during substantial weight reduction, to compare these levels with those obtained in AT of nonobese subjects, and to determine if the expression of one of these genes is predictive of weight loss. AT were obtained from obese women during Roux-en-Y gastric bypass (RYGB). Additional SAT biopsies were taken by liposuction at the level of the umbilical fold:

a) at 3 months after surgery, during rapid weight loss associated with significant food restriction and hypercatabolism;

b) at 6 months, at a time of moderate weight loss and modest food restriction;

c) 12 months, in steady state conditions.
Participants and Methods

Subjects
The study group included 30 obese women and 5 healthy nonobese female volunteers (table 1), matched to the patients by age (39 ± 2 [range 28–62] years versus 39 ± 4 [range 29–48] years), who underwent cholecystectomy and who served as the control group. All selected subjects are Caucasian. They provided informed consent, and the institutional review board approved the study.

Analytical Procedures
Fasting blood glucose (Ecoline 100 Merck, KgaA, Darmstadt, Germany), total cholesterol (Roche CHOD-PAP; Roche Molecular Biochemicals Systems, GmbH, Mannheim, Germany), high-density lipoprotein cholesterol (HDL-C) (HDL-C plus, second generation; Roche Diagnostic GmbH, Mannheim, Germany), and triglycerides (TG GPO-PAP; Roche Diagnostic) were measured using an automatic Hitachi 917 Roche apparatus. Low-density lipoprotein cholesterol (LDL-C) was then calculated by the Friedwald’s formula. Plasma insulin was assayed by specific radioimmunoassay (Aldatis Insulin, code 10624, Casalvecchio di Reno, BO, Italy).

Table 1. Anthropometric characteristics of obese and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Age, years</th>
<th>Weight, kg</th>
<th>Height, cm</th>
<th>BMI, kg/m²</th>
<th>Glycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese</td>
<td>39 ± 9 (28–62)</td>
<td>117 ± 16 (81–157)</td>
<td>162 ± 7 (146–179)</td>
<td>44 ± 5 (38–56)</td>
<td>6.0 ± 2.1 (4.7–16)</td>
</tr>
<tr>
<td>Ctrl</td>
<td>39 ± 8 (29–48)</td>
<td>69 ± 8 (62–78)</td>
<td>166 ± 5 (160–172)</td>
<td>25 ± 2 (23–26)</td>
<td>5.5 ± 0.3 (5.2–5.8)</td>
</tr>
</tbody>
</table>

$a$ Data are presented as: mean ± SD (range). The control (ctrl) group included 5 healthy volunteers, glycemia was known for three of them.

Table 2. Sequences of PCR primers

<table>
<thead>
<tr>
<th>cDNA probe</th>
<th>Direction</th>
<th>Sequence 5′–3′</th>
<th>Product length, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARγ1</td>
<td>for</td>
<td>AAAGAAGCCAACACTAAACC</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>CTTCATTACGGAGAGATCC</td>
<td></td>
</tr>
<tr>
<td>PPARγ2</td>
<td>for</td>
<td>GCGATTCCTCTACTGATAAC</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>CTTCATTACGGAGAGATCC</td>
<td></td>
</tr>
<tr>
<td>11βHSD1</td>
<td>for</td>
<td>GTTTCTGGGATAGTCATGCAA</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>CCATCAGAAAGGAATATTCAGTG</td>
<td></td>
</tr>
<tr>
<td>β2-Microglobulin</td>
<td>for</td>
<td>TGAGTATGCTGGCTCTGTA</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>GGCATCTTTGAAACCTCCATG</td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>for</td>
<td>TCTATGTCGAAGCTGTGCCCAT</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>ATAAGTCGAGATGGGTGGAG</td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>for</td>
<td>GGGCATGCCGGCCATA</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>GTTTCACCGATGTCCTCCCTAGG</td>
<td></td>
</tr>
<tr>
<td>Glut4</td>
<td>for</td>
<td>GCTACCTCCTACATCACAGATCTC</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>rev</td>
<td>CCAGAAACATCGGCCCA</td>
<td></td>
</tr>
</tbody>
</table>
Biopsies, RNA Preparation, and Gene Expression Analysis

AT samples were obtained by biopsy during laparoscopic surgery as previously described [9, 32]. Obese and nonobese patients were operated by the same surgical team for RYGB and removal of the gallbladder, respectively. Moreover, needle biopsies of SAT of obese patients were sampled 3, 6, and 12 months following RYGB. RNAs were extracted using the RNA-STAT 60 Reagent method (AMS biotechnology Europe Ltd., Bioggio-Lugano, Switzerland). Reverse transcription was performed on 500 ng of total RNA using the SuperScript™ II Reverse Transcriptase (Invitrogen, Basel, Switzerland) and random primers (Promega, Catalys, Wallissellen, Switzerland). Relative expression of genes was assessed by real time PCR using the LightCycler technology (Roche Diagnostics, Rotkreuz, Switzerland) with the Quantitect Sybr® Green PCR Kit (Qiagen, Hilden, Germany). cDNAs of interest were amplified using specific primers synthesized by Microsynth (Windish, Switzerland) which are listed in table 2. All samples were quantified in at least two runs, to obtain an inter-assay coefficient variation <10%. β2-microglobulin was used as the reference gene. Relative expression was then determined using crossing point values and amplification efficiencies of the target gene and the reference gene.

Statistical Analysis

All analyses were performed using the Jump 5.1 statistical package (SAS Institute, Cary, NC, USA). All data are given as mean ± SEM, and potential differences between groups were assessed by Student’s t-test. Potential univariate relationship between the levels of gene expression and BMI were done by Spearman’s correlation analyses. Stepwise analysis of independent variables was used to determine possible predictors of weight loss. P ≤ 0.05 was considered statistically significant.

Results

Anthropometric and Biological Measures after Surgery

We studied a cohort of 30 women undergoing RYGB, and we recorded anthropometric and biological parameters before and after 3, 6, and 12 months following surgery. All bariatric patients significantly lost weight after surgery (fig. 1), starting with a BMI of 44.2 ± 0.8 (range 38.1–55.9) kg/m² and reaching 29.3 ± 1.0 (range 22.5–36.9) kg/m². At 6 months after surgery, they had lost 25.6 ± 1.3% of their initial body weight and at 12 months 33.9 ± 1.5%. Finally at 12 months, their BMI was still increased compared to nonobese controls but not significantly different (BMI of 24.9 ± 0.7 (range 22.7–26.4) kg/m²). Their mean fasting insulin levels decreased from 23.6 ± 2.5 UI/l to 16.4 ± 1.9 (p = 0.03), 14.0 ± 1.4 (p = 0.001) and 12.2 ± 1.4 (p = 0.0002) after 3, 6 and 12 months after surgery, respectively. As their mean glucose and lipid levels were more or less in the normal range at baseline, they were not markedly modified with major weight loss (fig. 2 A–F). However, at 12 months after gastric bypass, HDL-C, LDL-C, and triglycerides were still significantly lower compared to baseline (HDL-C 2.8 ± 0.2 vs. 3.6 ± 0.2 mmol/l (p = 0.007); LDL-C 2.3 ± 0.2 vs. 2.9 ± 0.2 mmol/l (p = 0.01); triglycerides 1.2 ± 0.1 vs. 1.7 ± 0.2 mmol/l (p = 0.01)).

Gene Expression at Baseline

Figure 3 shows the mRNA expression of PPARγ1, PPARγ2, 11βHSD1, and leptin in the VAT (black columns) and SAT (white columns) in both obese and nonobese women. At baseline, PPARγ1 was expressed at lower levels in obese patients compared to controls in both VAT and SAT (−39% (p = 0.006) and −34% (p = 0.02), respectively), whereas PPARγ2 expression was not significantly different between groups (+10% (p = 0.68) and −1% (p = 0.99), respectively) (fig. 3A–B). Consistently, target genes of PPARγ pathway, such as the glucose transporter Glut4, were decreased in obesity (VAT −53% (p = 0.007) and SAT −62% (p = 0.0008)) compared to nonobese women. In contrast, 11βHSD1 was expressed at higher levels in obese compared to control subjects in both VAT and SAT (2.9 × (p = 0.006) and 2.2 × (p = 0.003), respectively) (fig. 3C). Leptin levels were markedly enhanced in obese women.
Leptin and 11βHSD1 gene expression were decreased after surgery, whereas PPARγ1 expression was increased (fig. 4A, C, D). At 12 months post RYGB, these 3 genes had reached levels similar to controls. In contrast, PPARγ2 gene expression levels remained unchanged during weight loss following RYGB (fig. 4B). Moreover, we found a positive correlation between levels of gene expression of leptin as well as 11βHSD1 and BMI (or weight) in both VAT and SAT (table 3). We saw no correlation between levels of PPARγ1 as well as PPARγ2 expression and weight or BMI.

In order to evaluate the predictive value of gene expression on weight loss, we performed a stepwise analysis. By comparing weight loss 0–6 months and 0–12 months with baseline gene expression levels, the result was significant for 11βHSD1 (p < 0.05). However, we found no significant correlation between the changes of 11βHSD1 expression levels and the decrease of weight, thus confirming the results of the stepwise analysis.
Discussion

This study is the first detailed description of PPARγ1, PPARγ2, 11βHSD1, and leptin gene expression in adipose tissue taking during and after RYGB surgery. The present results demonstrate that our candidate genes cannot provide conclusive predictive information and do not have immediate clinical utility. However, our data suggest that analyzing the expression of a set of genes in adipose tissue biopsied in a time-dependent manner should give preponderant information to predict weight loss.

Leptin

As expected and consistently with the literature, we found that leptin gene levels are enhanced in obesity in both VAT and SAT [33]. Although bariatric procedures are often prescribed to obese subjects, little information is available on the changes that occur in gene expression in AT during weight loss. Here, we showed that the rapid and massive weight loss following RYGB surgery resulted in the drop of leptin gene levels at 3 months of surgery, reaching levels similar to control. This result is in agreement with the work of Bastard et al. [34] who showed a 33% decrease of leptin mRNA during a very-low-calorie diet. Although the decline of leptin gene expression was rapid, it was not due to surgery itself. Indeed, Schoff
et al. [35] established that leptin gene expression in VAT and SAT was not changed during surgical stress. Our present observation perfectly reflected the well-known decrease of plasmatic leptin as soon as 1 month after RYGB [33, 36–39]. It is consonant with the effect of a 3-week very-low-calorie diet (weight loss of about 3 kg), resulting in a decrease of leptin at the protein level in SAT [40]. In contrast, it is interesting to note that 12-week training (−5.9% of initial body weight) leads to a decrease of plasmatic leptin concentrations, but had no effect on its gene expression in SAT [41]. Therefore, it would be interesting to look at leptin gene 2 or 3 years post RYGB, when a subset of patients regains some weight. Indeed, authors performing RYGB in rats have shown that 75% of rats that sustained weight loss had less leptin secretion compared to those unable to sustain weight loss [42, 43]. Here, we observed that, in a subset of patients, leptin gene expression slightly enhanced between 6 and 12 months of surgery, although the weight of these patients was still decreasing.

The other AT-secreted proteins did not seem to have similar preponderant implication as leptin. In particular, adiponectin is one AT-secreted protein, and, although adiponectin plays an important role in glucose and lipid metabolism, it did not seem to be associated with obesity. Indeed, according to previous findings, we observed that the levels of adiponectin

![Diagram of relative mRNA expression of PPARγ1, PPARγ2, 11βHSD1, and leptin in VAT and SAT obtained from control (ctrl) and obese subjects. Results are expressed relatively to values of the ctrl (AU: arbitrary unit; mean ± SEM; *p < 0.05).]
mRNA were not affected in obese women compared to controls and by weight loss occurring after RYGB [15, 44]. However and in contrast, adiponectin gene expression in SAT could be differently regulated in obese men undergoing RYGB [45]. Nevertheless, this should further confirmed in a larger number of male patients.

**PPARγ**

PPARγs are nuclear receptors that function as transcriptional regulators. Although PPARγ is found in a variety of human tissues, it is most highly expressed in AT [10]. More specifically, both PPARγ1 and PPARγ2 isoforms, which are derived from the same gene by alternative promoter usage and splicing, are expressed at high levels in adipocytes and macrophages [10, 46–48]. Previous studies investigating the gene expression of PPARγ in human AT in obesity have generated conflicting results, which were possibly due to the distinction between isoform 1 and 2 or not, to the localization of the AT, and to the mean BMI of the cohorts of patients [10, 11, 13, 16, 49]. Here, we showed that PPARγ1 and PPARγ2 were differently regulated in obesity. In both VAT and SAT, only PPARγ1 was decreased in obese women. This is consistent with a paper from Poulain-Godefroy et al. [16] who reported a
significant decrease of PPARγ in SAT and a trend toward a decrease in VAT in obese normoglycemic versus lean subjects. However, although the levels of PPARγ1 mRNA were decreased in obesity, they were not associated with obesity, BMI, leptin gene expression in SAT and insulin [8]. Furthermore, we observed that PPARγ1 increased further with weight loss to reach levels similar to the controls at 12 months of surgery. For PPARγ2, we observed a slight decrease at 3 months post surgery, but this change was not significant. These results differed from previous studies which reported a decrease of PPARγ following a hypocaloric diet program [34, 44, 50]. Nevertheless, they are not contradictory since Vidal-Puig et al. [10] showed that the decrease of PPARγ2 attributed to weight loss returns to initial levels after 4 weeks of weight maintenance. Adipose PPARγ appears to be an essential mediator for maintenance of whole body insulin sensitivity. It protects nonadipose tissue against lipid overload and guarantees appropriate production of adipokines such as adiponectin and leptin from adipocytes [51, 52] (reviewed in [53]). Among obese people, this balance is disturbed. PPARγ1 is decreased in AT, and other organs are thus not protected from excess fat. Therefore, there will be a development of metabolic comorbidities as well as leptin and insulin resistance. It is thus consistent that deletion of PPARγ in adipose tissues of mice protects against HFD-induced obesity and insulin resistance [54, 55].

11βHSD1

In AT of both origins, we found elevated levels of 11βHSD1 in obesity consistently with previous studies [17–20, 56]. Polycystic ovary syndrome and obesity are independently associated with increased expression of 11βHSD1 [21]. Moreover, these levels were positively correlated with BMI as it was already shown in children [22]. Consistently, the levels changed to go to the normal range at 6 months of bariatric surgery.

Conclusion

Finally, is the expression of one gene sufficient to predict weight loss success? When initiating this study, we thought that one of our candidate genes could be detected as a predictive factor of the weight loss occurring after RYGB. Here, we showed that it was not the case. Indeed, neither the changes of PPARγ1 and 2 gene expression nor the changes of 11βHSD1 gene expression can predict the evolution of the weight. Nevertheless, a stepwise analysis (weight loss 0–6 and 0–12 months vs. initial gene expression levels) was significant for 11βHSD1 (p < 0.05). However, we found no significant correlation between the changes of 11βHSD1 expression levels and the decrease of weight allowing confirming the pertinence of the stepwise result.

Further studies are needed to increase our understanding of the role of specific genes in weight maintenance and obesity and to define the network of genes that could be used as a biological predictor of the likelihood of success in weight loss and therefore in RYGB.

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Disclosure Statement

There were no conflicts of interest to declare.
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