Original Article

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

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Key Words

PPAR γ · 11 β HSD1 · Roux-en-Y gastric bypass · Subcutaneous adipose tissue

Abstract

Objective: To analyze the expression of peroxisome proliferator-activated receptor- γ 1 and 2 (PPAR γ 1 and 2), 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD1), 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 ($-33.9 \pm 1.5\%$ of their initial body weight). In obese subjects leptin and 11 β HSD1 were over-expressed, whereas PPAR γ 1 was expressed at lower levels compared to controls. After surgery, leptin and 11 β HSD1 gene expression decreased, whereas PPAR γ 1 expression increased. At 12 months after RYGB, these 3 genes had reached levels similar to the controls. In contrast, PPAR γ 2 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 11 β HSD1. **Conclusion:** Gene expression of *leptin*, PPAR γ 1, and 11 β HSD1 in AT is modified in human obesity. This default is completely corrected by RYGB.

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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

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[3–5]). 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 (*PPAR γ 1* and 2) and 11 β -hydroxysteroid dehydrogenase type 1 (*11 β HSD1*) are among the glycerolipid metabolism-related genes. Both *PPAR γ* agonism and inhibition of 11 β HSD1 improve lipid metabolism and lipemia. Moreover, therapeutic inhibition of 11 β HSD1 will be beneficial in most cellular contexts, with clinical trials supportive of this concept. Although the key role of *PPAR γ* and 11 β HSD1 in adipose tissue (AT) development and differentiation are not debated, their mRNA levels in human AT are still controversial.

PPAR γ 1 and *PPAR γ 2* 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 *PPAR γ* mRNA expression in obesity. Some authors found no difference between obese and nonobese subjects [8, 11, 12]. Some others reported an increase of *PPAR γ* expression in obesity. According to Bortolotto et al. [13], *PPAR γ* expression increase in SAT of obese subjects, decrease in retroperitoneal AT, and is not changed in VAT. For Lefebvre et al. [14], *PPAR γ* in VAT is increased in obesity, but not in SAT. Finally, Vidal-Puig et al. [10] showed that *PPAR γ 1* and *PPAR γ 2* were differently regulated and that only *PPAR γ 2* expression increased in obese versus lean subjects. Consistently, Viguerie et al. [15] reported a decrease of *PPAR γ* expression following 10-week hypocaloric diet program. In contrast, Poulain-Godefroy et al. [16] published recently results demonstrating a significant decrease of *PPAR γ* expression in SAT and a trend toward a decrease in VAT of obese compared to lean patients.

11 β HSD1 mRNA expression in AT in human obesity is also highly debated in the literature. Several papers reported an overexpression or an overactivity of 11 β HSD1 in AT of obese women and men [17–20]. Polycystic ovary syndrome and obesity are independently associated with increased expression of 11 β HSD1 [21]. Consistently, Mericq et al. [22] demonstrated a positive correlation between mRNA expression of 11 β HSD1 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 11 β HSD1 expression in human isolated adipocytes. Moreover, data regarding gene expression of *11 β HSD1* 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 *11 β HSD1* and *PPAR γ* 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 *PPAR γ 1*, *PPAR γ 2*, and *11 β HSD1* 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 gene 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.

Table 1. Anthropometric characteristics of obese and control subjects

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

^aData 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

cDNA probe	Direction	Sequence 5'–3'	Product length, bp
PPAR γ 1	for rev	AAAGAAGCCAACACTAAACC CTTCCATTACGGAGAGATCC	150
PPAR γ 2	for rev	GCGATTCTTCACTGATAC CTTCCATTACGGAGAGATCC	123
11 β HSD1	for rev	GTTTCTGGGATAGTCCATATGCAA CCATCAGAAAGGAATATTCAGTG	164
β 2-Microglobulin	for rev	TGAGTATGCCTGCCGTGTGA GGCATCTTCAAACCTCCATG	100
Leptin	for rev	TCTATGTCCAAGCTGTGCCCAT ATAAGGTCAGGATGGGGTGGAG	168
Adiponectin	for rev	GGGCATCCGGGCCATA GTT TCACCGATGTCTCCCTTAGG	116
Glut4	for rev	GCTACCTCTACATCATCCAGAATCTC CCAGAAACATCGGCCCA	78

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, Casa-
lecchio di Reno, BO, Italy).

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 gall-bladder, 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, Wallisellen, 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 (Windisch, Switzerland) which are listed in table 2. All samples were quantified in at least two runs, to obtain an inter-assay coefficient variation <10%. β_2 -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 $\pm$ 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. $P \leq 0.05$ 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/m^2 and reaching 29.3 ± 1.0 (range 22.5–36.9) kg/m^2 . At 6 months after surgery, they had lost $25.6 \pm 1.3\%$ of their initial body weight and at 12 months $33.9 \pm 1.5\%$. 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/m^2). 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. 2A–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 PPAR γ 1, PPAR γ 2, 11 β HSD1, and leptin in the VAT (black columns) and SAT (white columns) in both obese and nonobese women. At baseline, PPAR γ 1 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 PPAR γ 2 expression was not significantly different between groups ($+10\%$ ($p = 0.68$) and -1% ($p = 0.99$), respectively) (fig. 3A–B). Consistently, target genes of PPAR γ 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, 11 β HSD1 was expressed at higher levels in obese compared to control subjects in both VAT and SAT ($2.9\times$ ($p = 0.006$) and $2.2\times$ ($p = 0.003$), respectively) (fig. 3C). Leptin levels were markedly enhanced in obese women

Fig. 1. Mean weight of obese patients at the time of RYGB and at 3, 6 and 12 months after surgery and compared to the mean weight of control subjects (mean ± SEM; *p < 0.05).

Table 3. Nonparametric correlation according to Spearman between BMI and gene expression of leptin and 11βHSD1

Variable	By-variable	Spearman RHO	Prob > RHO
<i>Leptin</i> SAT	BMI	0.4746	0.0165
<i>Leptin</i> VAT	BMI	0.5985	0.0016
<i>11βHSD1</i> SAT	BMI	0.4146	0.0393
<i>11βHSD1</i> VAT	BMI	0.4954	0.0118

(7.1× (p = 0.009) and 1.9× (p = 0.03), respectively) (fig. 3D). In contrast, levels of adiponectin mRNA were not affected in obese women (VAT −4% (p = 0.89) and SAT −16% (p = 0.51) compared to nonobese women).

Gene Expression after Surgery

We next determined the effect of weight loss on the expression of the selected genes. *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.

Fig. 2. Measurements of **A** glycemia, **B** urate, **C** cholesterol (Chol), **D** triglycerides, **E** LDL, and **F** Chol/HDL of obese patients at the time of RYGB and at 3, 6 and 12 months after (mean ± SEM; *p < 0.05).

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

Fig. 3. Relative mRNA expression of **A** PPAR γ 1, **B** PPAR γ 2, **C** 11 β HSD1, and **D** leptin in VAT (white columns) and SAT (black columns) obtained from control (ctrl) and obese subjects. Results are expressed relatively to values of the ctrl (AU: arbitrary unit; mean $\pm$ SEM; * p < 0.05).

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

Fig. 4. Relative mRNA expression of **A** PPAR γ 1, **B** PPAR γ 2, **C** 11 β HSD1, and **D** leptin in SAT obtained from control (ctrl) and obese subjects. SAT obtained from obese women was taken during RYGB and at 3 months, 6 months, and 12 months of surgery. Results are expressed relatively to values of the ctrl (AU: arbitrary unit; mean $\pm$ 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.

PPAR γ

PPAR γ s are nuclear receptors that function as transcriptional regulators. Although PPAR γ is found in a variety of human tissues, it is most highly expressed in AT [10]. More specifically, both PPAR γ 1 and PPAR γ 2 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 PPAR γ 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 PPAR γ 1 and PPAR γ 2 were differently regulated in obesity. In both VAT and SAT, only PPAR γ 1 was decreased in obese women. This is consistent with a paper from Poulain-Godefroy et al. [16] who reported a

significant decrease of PPAR γ in SAT and a trend toward a decrease in VAT in obese normoglycemic versus lean subjects. However, although the levels of PPAR γ 1 mRNA were decreased in obesity, they were not associated with obesity, BMI, *leptin* gene expression in SAT and insulin [8]. Furthermore, we observed that PPAR γ 1 increased further with weight loss to reach levels similar to the controls at 12 months of surgery. For PPAR γ 2, 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 PPAR γ following a hypocaloric diet program [34, 44, 50]. Nevertheless, they are not contradictory since Vidal-Puig et al. [10] showed that the decrease of PPAR γ 2 attributed to weight loss returns to initial levels after 4 weeks of weight maintenance. Adipose PPAR γ 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. PPAR γ 1 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 PPAR γ in adipose tissues of mice protects against HFD-induced obesity and insulin resistance [54, 55].

11 β HSD1

In AT of both origins, we found elevated levels of 11 β HSD1 in obesity consistently with previous studies [17–20, 56]. Polycystic ovary syndrome and obesity are independently associated with increased expression of 11 β HSD1 [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 PPAR γ 1 and 2 gene expression nor the changes of 11 β HSD1 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 11 β HSD1 ($p < 0.05$). However, we found no significant correlation between the changes of 11 β HSD1 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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