

# Expression of Peroxisome Proliferator-Activated Receptor- $\gamma_1$ and Peroxisome Proliferator-Activated Receptor- $\gamma_2$ in Visceral and Subcutaneous Adipose Tissue of Obese Women

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Data regarding the expression of peroxisome proliferator-activated receptor (PPAR)- $\gamma_1$  and PPAR- $\gamma_2$  in human visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) are conflicting. To clarify this issue, we studied 50 women who had a BMI  $>35$  kg/m<sup>2</sup> were undergoing gastric reduction surgery. Phenotyping included recording of anthropometric parameters and of a biological profile. Quantification of the expression of PPAR- $\gamma_1$  and PPAR- $\gamma_2$  in samples of VAT and SAT was performed by real-time RT-PCR. In both SAT and VAT, the level of expression of PPAR- $\gamma_2$  were  $>20$ -fold that of PPAR- $\gamma_1$  ( $P < 0.001$  for both). However, only PPAR- $\gamma_1$  was differentially expressed, its levels in SAT being  $216 \pm 34\%$  those in VAT ( $P < 0.001$ ). In a stepwise, multivariate regression analysis, the levels of PPAR- $\gamma_1$  in both SAT and VAT were the major determinants of waist circumference ( $R^2 = 21\%$  for both;  $P < 0.01$ ). Finally, leptin but not PPARs appeared as the single parameter explaining the largest part of the variability of BMI in our cohort of patients ( $R^2 = 22\%$ ,  $P < 0.001$ ). These results are consistent with the putative roles of PPAR- $\gamma_1$  and PPAR- $\gamma_2$  in carbohydrate metabolism and energy homeostasis, respectively. As such, they constitute an important step toward the identification of potential targets for novel therapeutic strategies in the fields of obesity. *Diabetes* 52: 1673–1676, 2003

**A**dipocytes contribute in a major way to the management of energy storage (1–9). In recent years, they have attained the status of full-blown endocrine cells, synthesizing and secreting a variety of peptide and nonpeptide molecules in addition to storing and mobilizing triglycerides (10–13).

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PPAR, peroxisome proliferator-activated receptor, SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

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Because the nuclear hormone receptors peroxisome proliferator-activated receptors (PPARs) (14,15) modulate the expression of a variety of adipocyte-specific genes (16,17), they could be involved in the pathogenesis of obesity and related metabolic disorders (18–20). Consistently, a network of transcription factors promoting the adipocyte differentiation program have recently been identified (21,22). These factors play a crucial role both in the induction of adipose-specific genes and in the phenotypic manifestations of the mature adipose tissue (23,24).

Among the three isoforms of PPARs (25,26), the expression of PPAR- $\alpha$  and PPAR- $\delta$  is not specific to adipose tissue (27–29). In addition, of the three known isoforms of PPAR- $\gamma$ , only PPAR- $\gamma_2$  expression seems specific to adipose tissue. However, their precise pattern of expression in humans remains highly debated. According to Auboeuf et al. (30), PPAR- $\gamma_1$  is expressed at higher levels than PPAR- $\gamma_2$  in subcutaneous adipose tissue (SAT), with no difference between lean and obese subjects. These data have been confirmed by Sewter et al. (31), who found higher levels of PPAR- $\gamma_1$  than PPAR- $\gamma_2$  in both SAT and visceral adipose tissue (VAT) and also in lean subjects compared with obese patients. In contrast, Rieusset et al. (32) reported no difference between PPAR- $\gamma_1$  and PPAR- $\gamma_2$  expression in SAT and no correlation with BMI. Fajas et al. (14) even reported that the expression of PPAR- $\gamma_2$  was higher than that of PPAR- $\gamma_1$  in VAT of three normal subjects. Finally, Vidal-Puig et al. (33) showed that mRNA levels of PPAR- $\gamma_2$  were increased in SAT of obese subjects, whereas no difference was observed in PPAR- $\gamma_1$ .

The small number of patients included in each of these studies, coupled with the considerable variability of the parameters evaluated, probably constitutes a major drawback for the interpretation of these conflicting results (30). To delineate better the possible pathophysiological role of PPAR- $\gamma$ s in human obesity, we assessed the relative expression of PPAR- $\gamma_1$  and PPAR- $\gamma_2$  in visceral and subcutaneous adipocytes obtained from 50 well-characterized obese women who were recruited prospectively.

## RESEARCH DESIGN AND METHODS

**Patients and phenotyping.** Fifty obese white women (BMI  $>35$  kg/m<sup>2</sup>) who were referred consecutively over a period of 24 months for weight reduction surgery were included in the study. The use of oral glucose or lipid-lowering agents, weight reduction therapies, or antihypertensive drugs constituted exclusion criteria. All patients provided informed consent, and the study was

approved by the institutional review board. Gastric banding was applied to 26 patients, with a mean duration of surgery of 63 min. The remaining 24 patients were submitted to gastric bypass surgery, which lasted for a mean duration of 154 min. All surgical procedures were performed via laparoscopy by the same team, and all biopsies were taken by the same operator. Preoperative phenotyping included measurement of weight, height, BMI, and waist and hip circumference (34). In addition, biochemical workup included measurement of fasting blood glucose (Ecoline 100; Merck KGaA, Darmstadt, Germany), total cholesterol (Roche CHOD-PAP; Boehringer Mannheim Systems, Mannheim, Germany), HDL cholesterol (HDL-C plus, 2nd generation; Roche Diagnostic) and triglycerides (TG GPO-PAP; Roche Diagnostic, Boehringer Mannheim Systems) using an automatic Hitachi 917 Roche apparatus. LDL cholesterol was calculated (Friedwald's formula). Insulin (Adaltis Insulin Code 10624, Casalecchio di Reno, BO, Italy) and leptin (Linco, St. Charles, MO) were measured by radioimmunoassay, whereas HbA<sub>1c</sub> (Hemoglobin A<sub>1c</sub> Variant; Bio-Rad Laboratories, Munich, Germany) was measured by HPLC.

**Biopsies and RNA preparation.** Approximately 5 cm<sup>3</sup> of VAT was obtained at the level of the omentum, and another 5 cm<sup>3</sup> of subcutaneous fat was taken at the level of the umbilical fold. Tissue samples were placed on ice in the operating room, and total RNA was extracted the same day following a previously described method (35). Briefly, 1 g of adipose tissue was homogenized in 8 ml of a solution containing guanidinium thiocyanate (4 mol/L, Fluka) and  $\beta$ -mercaptoethanol ( $1.2 \times 10^{-7}$  mol/L) using a Polytron homogenizer. Samples were heated for 2 min at 37°C to liquefy the lipids, shaken vigorously, passed several times through a 21-G needle to disrupt the top layer of cells and to shear genomic DNA, and centrifuged at 10,000 rpm to separate fat from the rest of the solution. The lower aqueous phase was transferred onto a cesium chloride cushion (5.7 mol/L) and submitted to ultracentrifugation at 35,000 rpm overnight. The resulting pellet was resuspended into sodium acetate (0.3 mol/L, pH 6.0) and then ethanol (100%) was precipitated, washed, and finally resuspended into DEPC-treated water and stored at -80°C until use.

Quality of total RNA was assessed using a commercially available kit (RNA 6000 LabChip kit; Agilent Technologies, Meyrin, Switzerland) and an Agilent 2100 bioanalyzer. Quantification was achieved by measuring light absorbency at 260 nm. In cases in which either total RNA quality or quantity was not sufficient to allow further analysis, the extraction procedure was repeated, using frozen tissue samples. The expression of PPAR- $\gamma_1$  and PPAR- $\gamma_2$  was assessed by quantitative RT-PCR, using the LightCycler technology (Roche Diagnostics, Rotkreuz, Switzerland) with SYBR green detection. Reverse transcription was performed with random primers. For PCR, the common antisense primer for PPAR- $\gamma_1$  and PPAR- $\gamma_2$  was CTCCATTACG GAGAGATCC; the PPAR- $\gamma_1$  sense primer was AAAGAAGCCGACACTAAACC; the PPAR- $\gamma_2$  sense primer was GCGATTCTTCACTGATAC. A standard curve was created with serial dilutions of a PCR fragment cloned into pGEM-T (pGEM-T easy Vector system I; Promega, Madison, WI), achieving a sensitivity of 10 copies/tube. Different dilutions of the samples were tested in preliminary experiments to ensure that quantification would be performed within the linear part of this standard curve. After this test, all samples were quantified in at least two different runs. The interassay coefficient of variation (CV) was between 6 and 15%, and a third run was performed for samples with an interassay CV >10%. For quantification purposes, PPAR mRNA levels were always reported to the levels of  $\beta_2$ -microglobulin, a constitutively expressed gene. Primer pairs used for  $\beta_2$ -microglobulin were the following: sense, TGAGTATGCCCTGCCGTGTGA; antisense, GGCATCTTCAAACCTCCATG.

**Data analysis.** First, the ratio of either PPAR- $\gamma_1$  or PPAR- $\gamma_2$  over  $\beta_2$ -microglobulin was calculated for each sample. For the comparison of the relative expression of PPAR- $\gamma_1$  and PPAR- $\gamma_2$ , a value of 100% was arbitrarily attributed to PPAR- $\gamma_1$ , and PPAR- $\gamma_2$  was expressed as a percentage of PPAR- $\gamma_1$ . For comparisons between VAT and SAT expression of PPARs, a value of 100% was attributed to the levels measured in VAT, and SAT levels were expressed as a percentage of VAT levels. All results were reported as means  $\pm$  SE. Total PPARs were calculated as the sum of PPAR- $\gamma_1$  and PPAR- $\gamma_2$ . Differences between levels of expression of PPARs in VAT and SAT, as well as between levels of expression of PPAR- $\gamma_1$  and PPAR- $\gamma_2$ , were assessed by Student's *t* test.

Potential univariate relationships between the levels of PPAR expression and the biological or anthropometric parameters recorded were assessed by Spearman's correlation analyses. Differences were considered significant at *P* < 0.05. Then, stepwise regression analysis was performed to identify potential links between the level of expression of PPARs and the obese syndrome. PPAR mRNA levels, together with the various biological parameters, were therefore introduced in the model as the independent variables, whereas BMI and hip and waist circumference, which represent reliable indexes of obesity, were used as the dependent variables. All analyses were performed using the Jump 4 statistical package (SAS Institute, Cary, NC).

TABLE 1  
Anthropometric and biological parameters of the 50 patients

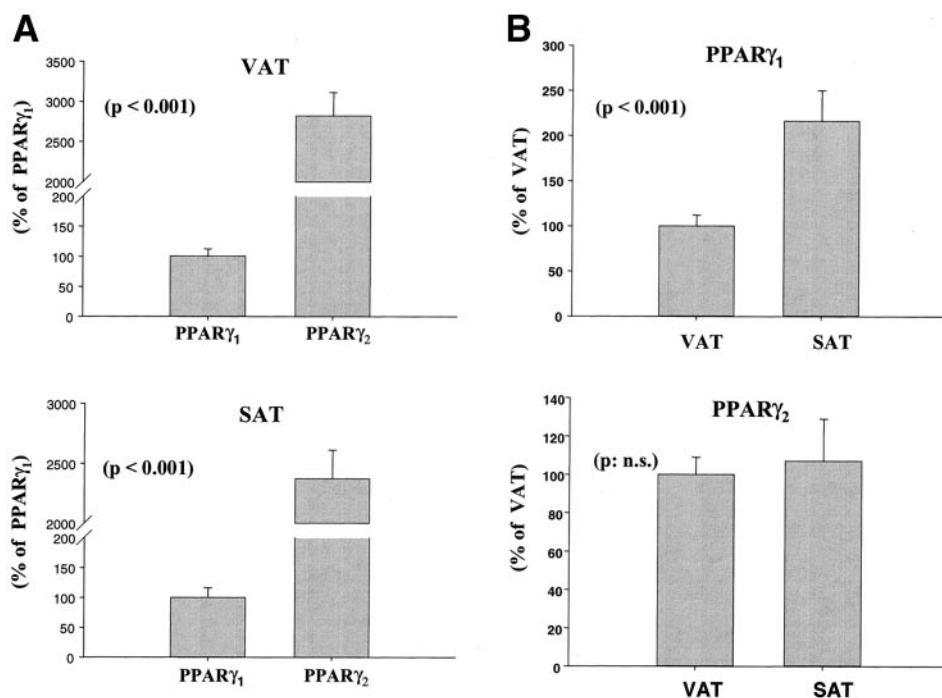
	Mean	SE	Range
Age (years)	38	1.2	(21–55)
Weight (kg)	121.4	2.9	(86.7–161.6)
Height (cm)	162	1.1	(143–180)
BMI (kg/m <sup>2</sup> )	46	0.9	(36.8–64.9)
Waist (cm)	117	2.1	(83–163)
Hip (cm)	140	2	(113–180)
Glycemia (mmol/L)	5.7	0.2	(4.6–10.4)
Total cholesterol (mmol/L)	5.1	0.1	(2.9–7.7)
HDL cholesterol (mmol/L)	1.2	0.04	(0.5–1.7)
Total/HDL	4.2	0.1	(2.6–6.1)
LDL cholesterol (mmol/L)	3.2	0.1	(1.9–4.9)
Triglycerides (mmol/L)	1.4	0.1	(0.5–3.3)
Insulin (UI/L)	30.8	3.9	(9.9–193)
Leptin ( $\mu$ g/L)	46.9	2.3	(17–81)
HbA <sub>1c</sub> (%)	5.8	0.2	(4.1–10)

## RESULTS

Results of the preoperative phenotyping are summarized in Table 1. All patients were markedly obese (mean BMI,  $46 \pm 0.9$  kg/m<sup>2</sup>) with high circulating leptin levels ( $46.9 \pm 2.3$  ng/l). Of these 50 patients, 5 had a diagnosis of diabetes and were treated with insulin. In addition, 15 patients had dyslipidemia according to the criteria of the Swiss Society for Cardiology, but they were on no medication. As a group, however, the mean glucose and lipid levels of the 50 patients were in the normal range.

Figure 1A demonstrates the relative expression of PPAR- $\gamma_1$  and PPAR- $\gamma_2$  in the VAT (top) and SAT (bottom). In both types of tissue, PPAR- $\gamma_2$  was expressed at higher levels than PPAR- $\gamma_1$  ( $2,820 \pm 290\%$  those of PPAR- $\gamma_1$  in VAT, and  $2,370 \pm 240\%$  those of PPAR- $\gamma_1$  in SAT; *P* < 0.001 for both). Figure 1B displays the relative expression of the two PPARs between the SAT and VAT in the same patients: PPAR- $\gamma_1$  was differentially regulated, with a significantly higher expression in SAT ( $216 \pm 34\%$  of VAT; *P* < 0.001). Of note, these comparisons performed in adipose tissue samples obtained from the subgroup of women without diabetes or dyslipidemia (*n* = 30) yielded exactly the same results (data not shown). Therefore, all patients were included in the final analysis. Finally, there was no difference between patients who were subjected to gastric banding or gastric bypass surgery (data not shown).

A Spearman correlation (Table 2) showed that subcutaneous PPAR- $\gamma_2$  was inversely correlated with BMI and weight (*P* < 0.05 for both parameters). In addition, the subcutaneous levels of both PPAR- $\gamma_1$  and PPAR- $\gamma_2$  were inversely correlated with circulating leptin levels (*P* < 0.05). Table 3 summarizes the results of the stepwise regression analysis, demonstrating that the visceral and subcutaneous levels of PPAR- $\gamma_1$  mRNA together accounted for 21% of the variability of waist circumference in this model (*P* < 0.01). Leptin was the parameter explaining the largest part of the variability of BMI (22%; *P* < 0.001). Similarly, leptin was also strongly linked to hip circumference, its levels explaining 13% of its variability (*P* < 0.02). Finally, in a further stepwise regression analysis, 22% of the variability of insulin levels could be attributed to triglycerides and subcutaneous levels of PPAR- $\gamma_1$  (*P* < 0.01, data not shown).



**FIG. 1.** A: PPAR- $\gamma_1$  and PPAR- $\gamma_2$  mRNA levels in human VAT (top) and SAT (bottom) of obese women, demonstrating the higher expression of PPAR- $\gamma_2$  in both tissues. Results are expressed as percentage of PPAR- $\gamma_1$  (means  $\pm$  SE). B: PPAR- $\gamma_1$  and PPAR- $\gamma_2$  mRNA levels in SAT and VAT of the same obese patients, illustrating the differential expression of PPAR- $\gamma_1$ . Results are expressed as percentage of VAT levels (means  $\pm$  SE).

## DISCUSSION

The aims of this study were to investigate the relative distribution of PPAR- $\gamma$ s in SAT and VAT of a large population of obese women and to evaluate their possible pathophysiological implication in human obesity. The two major observations of our study are that 1) PPAR- $\gamma_2$  is more abundant than PPAR- $\gamma_1$  in SAT and VAT and 2) PPAR- $\gamma_1$  is differentially regulated, being expressed at higher levels in SAT than VAT. In addition, both VAT and SAT levels of PPAR- $\gamma_1$  are major determinants of the variability of waist circumference, and stepwise regression analysis indicates that PPAR- $\gamma_1$  in SAT may be implicated in the determination of plasma insulin levels. Given the number of patients included as well as their wide range of anthropometric and biochemical characteristics, these results can probably be extrapolated to individual obese women. However, whether they can also be extrapolated to male patients remains an open question.

The chain of events, from biopsy to measurement of PPAR expression, was also carefully standardized. These precautions probably account at least partially for the relatively low variability of our results, thus allowing firmer conclusions to be drawn. It has also been suggested that the large variability observed in previous data might be related to short-term regulations (30), such as those induced by the degree of intraoperative stress preceding the biopsy procedure. Therefore, 24 patients who underwent gastric bypass surgery, a heavy operation lasting

**TABLE 2**

Nonparametric correlation according to Spearman between BMI, weight, leptin, and subcutaneous PPAR- $\gamma_1$  and PPAR- $\gamma_2$

Variable	By variable	Spearman RHO	Prob > RHO
PPAR- $\gamma_2$	BMI	-0.2976	0.036
PPAR- $\gamma_2$	Weight	-0.3019	0.033
PPAR- $\gamma_1$	Leptin	-0.2988	0.039
PPAR- $\gamma_2$	Leptin	-0.2859	0.049

~150 min, were compared with 26 patients who benefited from gastric banding, an intervention lasting ~60 min. We found no difference in PPAR expression between these two groups, suggesting that the type and duration of anesthesia are unlikely to play any role.

Previous studies investigating the expression of PPAR- $\gamma_1$  and PPAR- $\gamma_2$  in human adipose tissue have generated conflicting results (31–33) that are also somewhat inconsistent with animal data (27). Our data now demonstrate in the largest available cohort of obese women that PPAR- $\gamma_2$  is the most abundant isoform. In addition, our results suggest for the first time that PPAR- $\gamma_1$  may be differentially regulated between VAT and SAT. This observation, together with the significant correlation existing between SAT PPAR- $\gamma_1$  levels and circulating insulin concentrations, may underlie the role of PPAR- $\gamma_1$  in the insulin signaling pathway and its possible pathophysiological importance in the development of complications of obesity. Finally, the higher expression of PPAR- $\gamma_1$  in SAT of obese patients may be consistent with the hypothesis

**TABLE 3**

Results of a stepwise regression analysis, illustrating the parameters significantly linked to the dependant variables

Dependant variable	Independent variable	P	R <sup>2</sup>
Waist	VAT PPAR- $\gamma_1$	0.062	0.07
	SAT PPAR- $\gamma_1$	0.007	0.21
	HDL	0.075	0.27
Hip	Leptin	0.014	0.13
	HDL	0.06	0.19
	Triglycerides	0.033	0.28
BMI	Leptin	0.001	0.22
	Glucose	0.036	0.29
	Insulin	0.205	0.32

R<sup>2</sup> gives an estimate of the variability of the dependant parameter that can be explained by the various biological parameters measured.

that the tissue-specific effects of thiazolidinediones, which stimulate a higher increase in the body content of SAT than VAT, may be linked to differences in the regional expression of PPAR- $\gamma$  (31).

In our model, leptin appears as the single parameter explaining the largest portion of BMI variability, whereas the expression of PPAR- $\gamma$ s was linked with neither BMI nor hip circumference. Although such correlations can never prove causality, these results still suggest that PPAR- $\gamma$ s do not play a key role in the regulation of absolute fat mass storage. If this hypothesis is correct, then it may implicate other known transcription factors, coactivators, or modulators (e.g., ADD/SREBP1, C/EBP, tumor necrosis factor, IGF) in the cascade of events that eventually induce and maintain the expression of PPARs and hence the proliferation and differentiation of adipocytes. In contrast, PPAR- $\gamma_1$  was significantly linked to waist circumference in this cohort of patients, suggesting that it is somehow related to the development of metabolic and cardiovascular complications of obesity. However, further work will be necessary to address these hypotheses.

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