Insulin Acutely Regulates the Expression of the Peroxisome Proliferator-Activated Receptor-γ in Human Adipocytes

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Peroxisome proliferator-activated receptor (PPAR)-γ is one of the key actors of adipocyte differentiation. This study demonstrates *1*) that PPAR-γ mRNA expression is not altered in subcutaneous adipose tissue (n = 44) or in skeletal muscle (n = 19) of subjects spanning a wide range of BMIs (20–53 kg/m^2) and *2*) that insulin acutely increases PPAR-γ mRNA expression in human adipocytes both in vivo and in vitro. The effect of insulin was investigated in abdominal subcutaneous biopsies obtained before and at the end of a 3-h euglycemic-hyperinsulinemic clamp. Insulin significantly increased PPAR-γ mRNA levels in lean subjects (88 ± 17%, n = 6), in type 2 diabetic patients (100 ± 19%, n = 6), and in nondiabetic obese patients (91 ± 20%, n = 6). Both PPAR-γ1 and PPAR-γ2 mRNA variants were increased (P < 0.05) after insulin infusion. In isolated human adipocytes, insulin induced the two PPAR-γ mRNAs in a dose-dependent manner, with half-maximal stimulation at a concentration of ~1–5 nmol/l. However, PPAR-γ2 mRNA was rapidly (2 h) and transiently increased, whereas a slow and more progressive induction of PPAR-γ1 was observed during the 6 h of incubation. In explants of human adipose tissue, PPAR-γ protein levels were significantly increased (42 ± 3%, P < 0.05) after 12 h of incubation with insulin. These data demonstrate that PPAR-γ belongs to the list of the insulin-regulated genes and that obesity and type 2 diabetes are not associated with alteration in the expression of this nuclear receptor in adipose tissue. *Diabetes* 48:699–705, 1999

Peroxisome proliferator-activated receptor (PPAR)-γ is a nuclear receptor belonging to the hormone receptor family that heterodimerizes with a retinoid-X-receptor partner to bind specific *cis*-acting elements in the promoter regions of target genes (1). PPAR-γ is a key actor of adipocyte differentiation (2,3). Since it is expressed at high levels in mature cells, PPAR-γ is also thought to play a critical role in maintaining the metabolic functions of differentiated adipocytes. In agreement, most of its target genes encode proteins involved in lipid storage and metabolism, such as adipose fatty acid–binding protein (2), lipoprotein lipase (4), fatty acid transport protein (5), and leptin (6–8), the cytokine-like hormone produced by adipocytes (9). PPAR-γ exists as two isoforms (γ1 and γ2) generated from the same gene by alternative promoter usage and mRNA splicing, which differ in their NH_2-terminal 30 amino acids (10,11). PPAR-γ1 is always the predominant form in human tissues where PPAR-γ is expressed, and PPAR-γ2 represents only 15% of total PPAR-γ in subcutaneous adipose tissue (12,13).

Alterations in the regulation, activity, or expression of PPAR-γ might participate in the molecular mechanism of pathologies with altered lipid homeostasis and insulin resistance. We have recently shown that the mRNA levels of PPAR-γ are not different in abdominal subcutaneous adipose tissue of lean subjects and obese patients with or without type 2 diabetes (12). In contrast, others have observed a significant correlation between the BMI of subjects and the mRNA level ratio PPAR-γ2/γ1, suggesting that obesity might be associated with an increased expression of PPAR-γ2 in subcutaneous adipose tissue (13). Moreover, a recent report indicated that PPAR-γ expression might be increased in skeletal muscle during obesity, but the authors did not exclude a possible adipose tissue contamination of the obese muscle samples (14). To date, therefore, the relationship between human obesity and possible alterations in the expression of PPAR-γ is not clearly established.

Thiazolidinediones, a new class of antidiabetic drugs, were recently found to bind and activate PPAR-γ (15). Thiazolidinediones enhance insulin sensitivity in vivo in humans as well as in several animal models with insulin resistance (16,17). These results suggest that a cross-talk could exist between insulin action and PPAR-γ functions in mature adipocytes. Insulin is one of the key hormones involved in the control of adipocyte differentiation and fat metabolism in mature cells. Its anabolic effects (antilipolytic action, stimulation of glucose uptake) lead to fat accumulation and adipose tissue development. Insulin also promotes change in the expression of important genes in the metabolic pathways of glucose and fatty acids in adipocytes (18–22). Recent data suggest that insulin might also participate in the control of PPAR-γ expression (13,23). In rodents, PPAR-γ1 and -γ2 expression is decreased by fasting and by insulin deficiency in adipose tissue (23), and treatment of diabetic mice with insulin leads to a partial restoration of PPAR-γ mRNA levels

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DMEM, Dulbecco's modified Eagle's medium; HSL, hormone-sensitive lipase; PBS, phosphate-buffered saline; PPAR, peroxisome proliferator-activated receptor; RT-competitive PCR, reverse transcription–competitive polymerase chain reaction.

in epididymal fat cells (23). In cultured human adipocytes, insulin, in the presence of dexamethasone, has been shown to upregulate PPAR-γ1 and -γ2 mRNA levels (13). Nevertheless, the demonstration that insulin regulates PPAR-γ expression in human adipose tissue in vivo has never been reported.

The aims of the present work were *1*) to characterize the mRNA expression of PPAR-γ1 and PPAR-γ2 in subcutaneous adipose tissue and in skeletal muscle of a large number of subjects spanning a wide range of BMIs and *2*) to investigate the in vivo regulation by insulin of PPAR-γ mRNA expression in human subcutaneous adipose tissue. The effects of insulin on PPAR-γ expression were further characterized in more detail in human isolated mature adipocytes.

RESEARCH DESIGN AND METHODS

Subjects and protocols. Forty-four subjects were involved in the study: 14 healthy lean volunteers (5 men and 9 women, age 28 ± 9 years, BMI 23 ± 2 kg/m^2), 11 patients with type 2 diabetes (4 men and 7 women, age 51 ± 7 years, BMI 32 ± 2 kg/m^2), and 19 nondiabetic obese subjects (6 men and 13 women, age 43 ± 8 years, BMI 38 ± 7 kg/m^2). Among these subjects, 18 participated in the investigation of the effect of insulin (6 control subjects: 1 man and 5 women, basal fasting glycemia 4.4 ± 0.1 mmol/l, basal fasting insulinemia 30 ± 1 pmol/l; 6 type 2 diabetic patients: 4 men and 2 women, basal fasting glycemia 10.7 ± 0.8 mmol/l, basal fasting insulinemia 56 ± 13 pmol/l, duration of diabetes 4 ± 1 years; and 6 obese subjects: 3 men and 3 women, basal fasting glycemia 5.0 ± 0.4 mmol/l, basal fasting insulinemia 113 ± 26 pmol/l). After an overnight fast, these 18 subjects underwent a 3-h euglycemic-hyperinsulinemic clamp (insulin infusion rate of 12 pmol · kg^{-1} · min^{-1}) done exactly as previously reported (21,24). Similar levels of hyperinsulinemia were achieved in the three groups of subjects during the last hour of the clamp (1,145 ± 163 pmol/l, n = 18). Biopsies of abdominal subcutaneous adipose tissue were taken by needle aspiration 2 h before and at the end of the euglycemic clamp, as described previously (21). Samples (330 ± 180 mg wet wt, n = 36, with no significant difference between groups or between samples obtained before and after clamp) were immediately frozen in liquid nitrogen and stored at –80°C until analysis.

Subcutaneous adipose tissue biopsies were obtained, in the basal state after an overnight fast, from the 26 other subjects that did not undergo the hyperinsulinemic clamp. Skeletal muscle (vastus lateralis) biopsies were also taken from 19 of these subjects (9 control, 5 type 2 diabetic, and 5 nondiabetic obese subjects) using a Weil Blakesley plier as previously described (24). All subjects gave their written consent after being informed of the nature, purpose, and possible risks of the study. The experimental protocol was approved by the ethics committee of Hospices Civils de Lyon and performed according to French legislation.

Preparation of human isolated adipocytes and tissue explants. Abdominal subcutaneous adipose tissue (5.3 ± 0.4 g wet wt) was obtained during surgery from 15 additional subjects (BMI 26.5 ± 1.5 kg/m^2), with the consent of the patients. The tissue was immediately immersed in Hanks' medium kept at 4°C and brought to the laboratory within 10 min after tissue sampling. Adipocytes (n = 10) were isolated according to the method of Rodbell (25) with minor modifications. Briefly, the digestion of the tissue with collagenase (1 mg/ml) (type II; Sigma, La Verpillère, France) was done at 37°C during 20 min, without agitation, in 10 ml Hanks' medium buffered with 20 mmol/l HEPES and supplemented with 200 nmol/l adenosine (Sigma) and 4% (wt/vol) free fatty acid bovine serum albumin (Boehringer, Meylan, France). Cells were filtered through a 500-µm mesh nylon filter and washed three times in Dulbecco's modified Eagle's medium (DMEM) medium containing 4% free fatty acid bovine serum albumin. Isolated adipocytes were incubated at 37°C in a final volume of 1 ml in the same medium. The average cell number in the incubation medium varied from 50,000 to 150,000 cells per ml. Insulin (Sigma) was added in the incubation medium after 30 min of preincubation at 37°C. At the end of the incubation periods, infranatant was removed by aspiration and cells were immediately frozen in liquid nitrogen.

For preparation of adipose tissue explants (n = 5), fat samples were cut into small pieces under sterile conditions, rinsed once in DMEM, weighed, and incubated (400 mg tissue [wet wt] in 2 ml medium) in DMEM supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin, but without bovine serum albumin and fetal calf serum, at 37°C under 5% CO_2, 95% O_2 atmosphere. At the end of the incubation period, tissue pieces were frozen in liquid nitrogen and stored at –80°C until quantification of PPAR-γ protein levels.

Total RNA preparation. Total RNA from adipose tissue samples and from isolated adipocytes was prepared using the RNeasy total RNA kit from Quiagen (Courtaboeuf, France) as previously indicated (8,26). Total RNA concentration was determined by absorbance measurement at 260 nm. The 260/280 nm absorption ratio of all preparations was between 1.8 and 2.0. The average yield of total RNA was 1.2 ± 0.5 µg/100 mg of adipose tissue (n = 62), with no significant difference between groups, and about 1 µg/100,000 isolated cells. For skeletal muscle, frozen tissue samples (53 ± 3 mg, n = 19) were pulverized in liquid nitrogen, and total RNA was prepared as described (14). The average yield was 26 ± 2 µg/100 mg of tissue (n = 19), with no difference between groups.

Quantification of PPAR-γ mRNA. The mRNA levels of PPAR-γ1 and PPAR-γ2 were determined by reverse transcription reaction followed by competitive polymerase chain reaction (RT-competitive PCR), which is the coamplification of PPAR-γ cDNAs with a known amount of a specific competitor DNA in the same tube (26). The construction of the competitor DNA and the validation of the RT-competitive PCR for PPAR-γ have been described in detail (11,12). The RT-competitive PCR reactions and the analysis of the PCR products were performed in the conditions previously indicated (11,12). Hormone-sensitive lipase (HSL) mRNA levels were also determined by RT-competitive PCR using the multispecific competitor and the conditions previously described (14).

Quantification of PPAR-γ protein. Explants of adipose tissue were homogenized in a phosphate-buffered saline (PBS) lysis buffer containing 1% Nodinet P-40 (Sigma), 0.5% sodium desoxycholate (Sigma), and 0.1% SDS (Sigma) and supplemented with a freshly prepared cocktail of protease inhibitors (400 µg/ml 4-(2-aminoethyl)-benzenesulfonyl fluoride, 5 mg/ml EDTA, 1 µg/ml leupeptin, and 1 µg/ml pepstatin) (ICN Pharmaceuticals, Orsay, France). After centrifugation of the lysate at 4°C, protein concentration was measured with the Bio-Rad protein assay system. Proteins (0.5 µg) were separated by SDS-PAGE, transferred to polyvinylidine difluoride (PVDF) membrane (Millipore, Guyancourt, France) and blocked overnight at 4°C in blocking buffer (PBS, 0.1% Tween-20, 5% skim milk). PPAR-γ was identified using a rabbit polyclonal antibody that recognizes human, mouse, and rat PPAR-γ 1 and 2 (BIOMOL Research Laboratory, Plymouth Meeting, PA). Membranes were incubated for 1 h at room temperature with the antibody (1/2,000) and next developed with an anti-rabbit IgG peroxidase conjugate (Sigma) diluted at 1:10,000. The complex was visualized using a chemiluminescence kit (Specichrom, Lyon, France). Films (Biomax ML, Kodak, Cretell, France) were analyzed with a Vernon photometer-integrator.

Lipolysis assay in isolated adipocytes. To test the antilipolytic effect of insulin in human isolated adipocytes, about 50,000 cells were preincubated for 30 min at 37°C in 1 ml DMEM containing 4% free fatty acid bovine serum albumin. After the preincubation period, isolated adipocytes were treated with isoproterenol (10^{-6} mol/l) alone or in combination with various concentrations of insulin. After 30 min at 37°C, glycerol released was determined spectrophotometrically after perchloric acid/K_2CO_3 deproteinization of the whole incubation medium (27).

Presentation of the results. The results of PPAR-γ mRNA levels are presented in attomoles per microgram of total RNA and are expressed as means ± SE. When required, statistical analyses were performed using Wilcoxon (paired values) or Mann-Whitney (unpaired values) nonparametric tests. Significance of the correlations was examined using the nonparametric Spearman's rank correlation coefficient. The threshold for significance was set at $P < 0.05$.

RESULTS

Characterization of PPAR-γ mRNA expression in adipose tissue and skeletal muscle. PPAR-γ1 and PPAR-γ2 mRNA levels were determined in subcutaneous adipose tissue biopsies from 44 subjects spanning a wide range of BMIs. Figure 1*A* clearly shows that there was no correlation between BMI and total PPAR-γ (γ1 + γ2) mRNA levels (r = 0.02, P = 0.91). There was no correlation when men (r = 0.004, P = 0.066, n = 15) or women (r = 0.016, P = 0.125, n = 29) were analyzed separately or when the diabetic patients were excluded (r = 0.005, P = 0.074, n = 32). PPAR-γ mRNA levels were not associated with the age of the subjects (r = 0.03, P = 0.052, n = 42). Moreover, when the results were presented as the mRNA ratio PPAR-γ2/γ1, there was also no relationship with the BMI of the subjects (Fig. 1*B*) (r = –0.12, P = 0.42). Therefore, in lean as well as in largely obese subjects, PPAR-γ2 mRNA represented about 15% of PPAR-γ1 mRNA level in human abdominal subcutaneous adipose tissue.

PPAR-γ mRNA could be quantified in human skeletal muscle, but, as shown in Fig. 2, the expression levels were very low (at least 100 times lower than those found in adipose tissue). PPAR-γ2 mRNA was detectable, but was under the limit of the assay by RT-competitive PCR (<0.05 amol/µg total RNA) (26). Although there were important variations in indi-

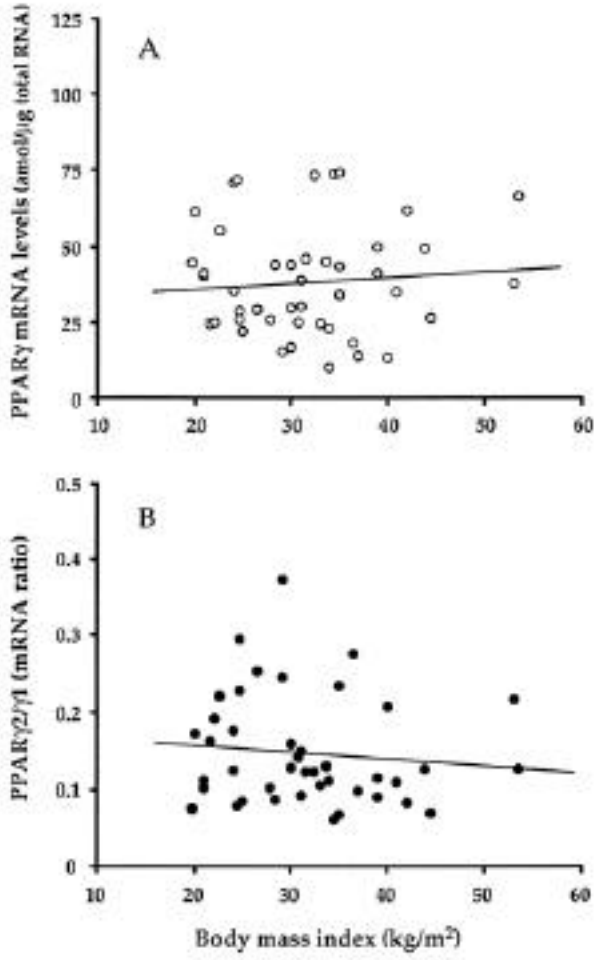

FIG. 1. Lack of correlation between PPAR-γ mRNA levels (*A*) or the mRNA level ratio of PPAR-γ2/γ1 (*B*) and BMI in human adipose tissue. The levels of the two PPAR-γ mRNA isoforms were measured by RT-competitive PCR in abdominal subcutaneous adipose tissue biopsies of 44 subjects spanning a wide range of BMIs.

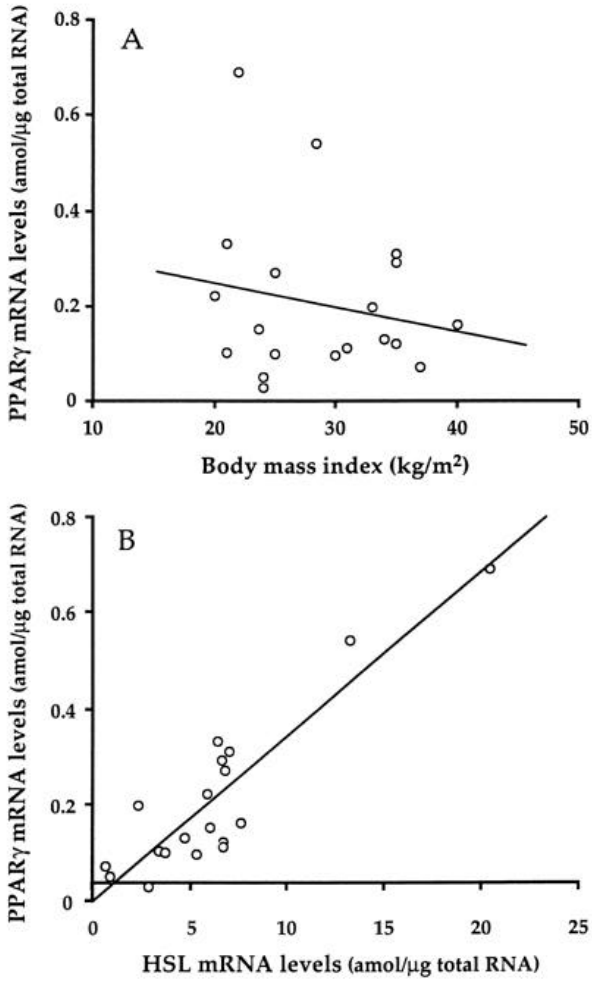

FIG. 2. PPAR-γ mRNA expression levels in human skeletal muscle. *A*: Lack of correlation between PPAR-γ mRNA levels and the BMI of the subjects (P = 0.75, n = 19) *B*: Relationship between HSL and PPAR-γ mRNA levels in skeletal muscle samples (P = 0.0015, n = 19).

vidual values (from 0.05 to 0.69 amol/μg total RNA), the levels of PPAR-γ mRNA in skeletal muscle did not correlate with the BMI of the subjects (Fig. 2*A*) (r = –0.08, P = 0.75). Since PPAR-γ is abundantly expressed in adipose tissue, it was important to verify whether PPAR-γ mRNA levels in skeletal muscle reflected a contamination of the tissue samples by adipocytes. To this end, we determined the mRNA levels of HSL, which is expressed at high levels in adipose tissue (402 ± 41 amol/μg total RNA, n = 25) but at very low levels, if any, in skeletal muscle. Figure 2*B* shows a highly significant positive correlation (r = 0.75, P = 0.0015, n = 19) between HSL and PPAR-γ mRNA levels in the muscle samples. In contrast, there was no correlation between PPAR-γ and HSL mRNA levels in adipose tissue samples (r = –0.03, P = 0.84, n = 25) (data not shown). Thus, the observed variation in PPAR-γ mRNA levels in muscle likely reflected different adipose content of the samples.

Effect of insulin infusion on PPAR-γ mRNA levels in vivo. To investigate the acute effect of insulin on PPAR-γ expression, 18 subjects underwent a 3-h euglycemic-hyperinsulinemic clamp. Normoglycemia (4.3 ± 0.3, 4.9 ± 0.2, and 4.5 ± 0.2 mmol/l in control, type 2 diabetic, and obese subjects, respectively) was achieved during hyperinsulinemia and maintained by adapted glucose infusions. The rates of glucose infusion required to maintain euglycemia during the last hour of the clamp were 57.7 ± 2.7, 31.9 ± 4.4, and 30.2 ± 2.7 μmol · kg^{-1} · min^{-1} in control, type 2 diabetic, and obese subjects, respectively (P < 0.05, type 2 diabetic and obese subjects versus control), indicating a marked state of insulin resistance of whole-body glucose disposal rate in type 2 diabetic and obese patients. Plasma free fatty acid concentrations markedly decreased (P < 0.05) during insulin infusion in the three groups (from 462 ± 72 to 35 ± 11, from 652 ± 13 to 83 ± 12, and from 654 ± 77 to 51 ± 8 μmol/l in control, type 2 diabetic, and obese subjects, respectively; P < 0.05 in type 2 diabetic and obese subjects versus control in the basal state).

Figure 3 shows PPAR-γ1 mRNA levels in abdominal subcutaneous adipose tissue biopsies taken before and at the end of insulin infusion in the three groups of subjects. After 3 h of hyperinsulinemia, the levels of PPAR-γ1 mRNA were significantly increased in all the subjects (P < 0.02). Although in this study, the obese group seemed to express lower basal levels of PPAR-γ1 than control and type 2 diabetic subjects, the difference was not significant (P = 0.416). The magnitude of insulin effect was similar in control (87 ± 16%), type 2 diabetic (95 ± 18%), and obese (94 ± 12%) groups. Insulin also induced about a twofold increase in mRNA levels of PPAR-γ2 in the three groups of subjects (92, 98, and 146% in control, type 2

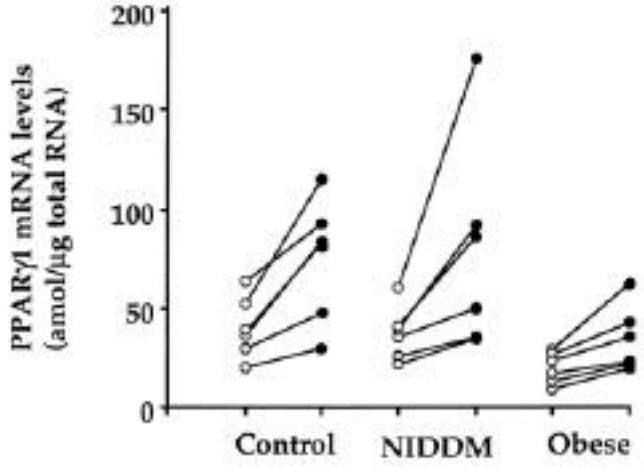

FIG. 3. Effect of insulin on PPAR-γ1 mRNA levels in abdominal subcutaneous adipose tissue. The amount of PPAR-γ1 mRNAs was measured by RT-competitive PCR in adipose tissue biopsies of six lean, six type 2 diabetic, and six nondiabetic obese subjects before (○) and at the end of (●) a 3-h euglycemic-hyperinsulinemic clamp, as described in METHODS. P = 0.019 for the lean subjects, P = 0.014 for the type 2 diabetic patients, and P = 0.009 for the obese subjects.

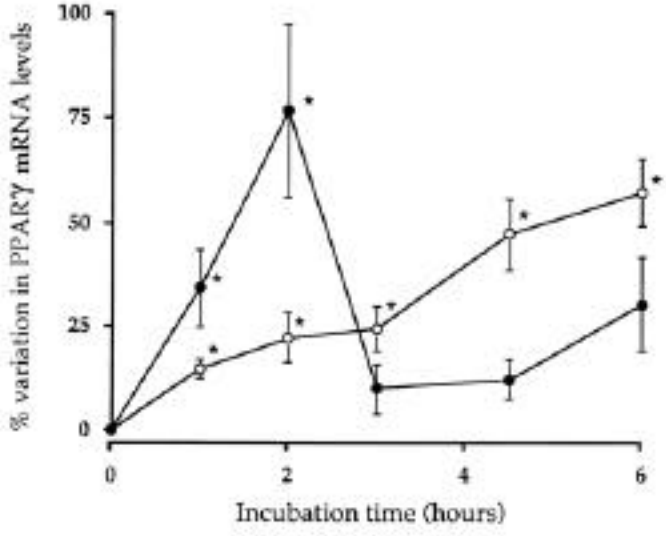

FIG. 4. Time-course of insulin effect on PPAR-γ mRNA levels in isolated human adipocytes. Adipocytes were isolated from subcutaneous abdominal adipose tissue from lean subjects and incubated with 10^{-7} mol/l insulin (n = 5 different preparations). ○, insulin-induced variations in PPAR-γ1 mRNA levels; ●, data concerning PPAR-γ2 mRNA. The basal levels were 28 ± 2 amol/µg total RNA for PPAR-γ1 and 4.9 ± 0.7 amol/µg total RNA for PPAR-γ2. *P < 0.05 in the presence vs. the absence of insulin.

diabetic, and obese subjects, respectively; $P < 0.05$ in each group) (data not shown). Before insulin infusion, PPAR-γ2 mRNA levels represented about 15% of PPAR-γ1 mRNA in the three groups as shown in Fig. 1 (PPAR-γ2, 12.8 ± 1.4, 13.6 ± 1.9, and 15 ± 2.9% of PPAR-γ1 in the control, type 2 diabetic, and obese subjects that participated in the clamp study; no significant differences). Insulin did not modify this ratio (PPAR-γ2 = 13.7 ± 2.1, 16.9 ± 4.1, and 16.7 ± 4.5% of PPAR-γ1 in control, type 2 diabetic, and obese subjects after the hyperglycemic clamp; no significant differences).

Investigation of insulin action in human isolated adipocytes. Using RT-competitive PCR, PPAR-γ mRNAs could be quantified in total . b RNA prepared from as few as 50,000 isolated cells (data not shown). After isolation, adipocytes were first preincubated for 30 min at 37°C to obtain stable levels of PPAR-γ mRNA (PPAR-γ1 mRNA levels 31.2 ± 3.3 amol/µg total RNA, n = 10). Similarly to what was found in the whole tissue, PPAR-γ1 was the predominant form expressed in adipocytes (84.5 ± 1.4% of PPAR-γ total mRNAs, n = 10). We have first verified the viability of the cells and the stability of PPAR-γ mRNA levels during incubation at 37°C. After 6 h, the activity of lactate dehydrogenase released in the medium never exceeded 30% of total cell activity (data not shown). During the same incubation periods, the levels of PPAR-γ mRNAs remained unchanged (PPAR-γ1 mRNA levels after 6 h 30.0 ± 3.5 amol/µg total RNA, n = 10). Before investigating the effect of insulin on PPAR-γ expression, we verified whether the human isolated adipocytes were responsive to insulin with respect to its antilipolytic effect. In five independent cell preparations, isoproterenol (10^{-6} mol/l) produced a robust 10-fold increase in the amount of glycerol released by the cells (98 ± 33 vs. 9 ± 5 nmol glycerol/ml, 30 min). Insulin inhibited the effect of isoproterenol in a dose-dependent manner (half-maximal effect at about 5 nmol/l, n = 2) with a maximal inhibitory effect of 45 ± 5% (n = 5) with 10^{-6} mol/l of insulin.

Figure 4 shows the time courses of insulin action on both PPAR-γ1 and γ2 mRNAs. Insulin (0.1 µmol/l) induced a rapid and transient increase in PPAR-γ2 mRNA levels, with a maximal effect after 2 h (77 ± 21%, n = 5). After 3 h, PPAR-γ2 mRNA returned to basal level. In contrast, PPAR-γ1 mRNAs increased slowly and constantly during the 6 h of incubation with insulin, to reach values 57 ± 8% (n = 5) higher than basal levels at the end of the incubation. During the same incubation period, insulin did not affect the mRNA expression of the reference gene β-actin measured by RT-competitive PCR (123 ± 28 vs. 138 ± 32 amol/µg total RNA after 6 h of incubation with versus without 10^{-7} mol/l insulin, n = 3). To further verify whether the effect of insulin was selective for PPAR-γ, the mRNA levels of the metabolic enzymes lipoprotein lipase, HSL, and the glucose transporter GLUT4 were measured by RT-competitive PCR (24) in four of the five experiments presented in Fig. 4. After 6 h of incubation, insulin did not modify the mRNA levels of lipoprotein lipase (3 ± 21%, NS) and HSL (–16 ± 7%, NS) but significantly increased GLUT4 mRNA expression (69 ± 21%, $P < 0.05$).

To further characterize the action of insulin on PPAR-γ1 and -γ2 mRNA abundance, we investigated the dependence of the effects on the hormone concentration. Insulin action on PPAR-γ2 mRNA was studied after 2 h, while the effect on PPAR-γ1 mRNA was examined after 6 h of incubation. The dose-response curves obtained (Fig. 5) clearly demonstrated that the induction of PPAR-γ1 and -γ2 mRNAs was dependent on insulin concentration. The half-maximal effect occurred with insulin concentrations ~1–5 nmol/l for both PPAR-γ1 and PPAR-γ2 mRNAs.

We next investigated whether the insulin induction of PPAR-γ mRNA levels has a consequence on the nuclear receptor protein amounts. Using a commercially available antibody that recognizes both isoforms of PPAR-γ, we first did not observe any change in the protein levels after 6 h of incubation in isolated human adipocytes (data not shown). Because longer incubation periods resulted in a dramatic

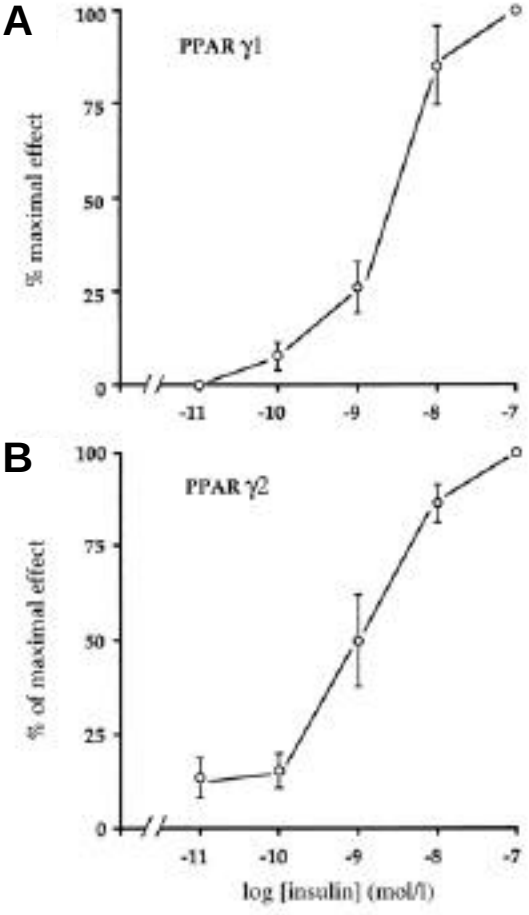

FIG. 5. Dependence of PPAR-γ mRNA induction on insulin concentrations. Isolated adipocytes from lean subjects were incubated with various concentrations of insulin during 6 h for the assay of PPAR-γ1 (*A*) and 2 h for the assay of PPAR-γ2 (*B*). The absolute mRNA levels obtained in the presence of 10^{-7} mol/l insulin (100%) were 54 ± 6 amol/μg total RNA for PPAR-γ1 and 8.0 ± 0.8 amol/μg total RNA for PPAR-γ2. Data show the means ± SE of three different preparations of isolated adipocytes.

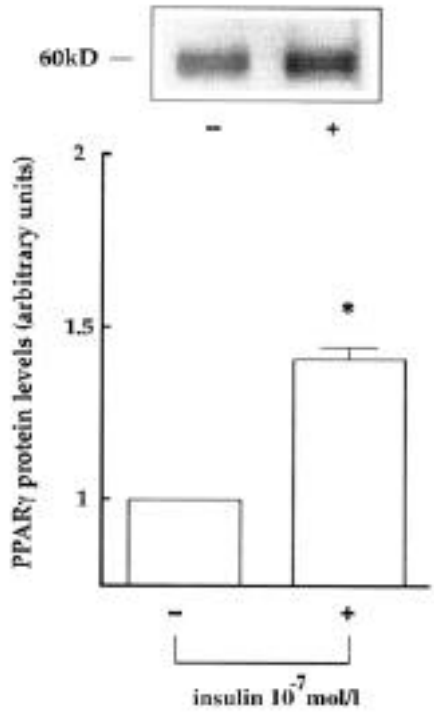

FIG. 6. Effect of insulin on PPAR-γ protein levels in subcutaneous adipose tissue. Western blots of PPAR-γ were performed in human adipose tissue explants (400 mg/2 ml culture medium) incubated for 12 h with or without 10^{-7} mol/l insulin. The bars represent the effect of insulin (mean ± SE) in five separate experiments (P = 0.04).

loss of adipocyte viability, we used adipose tissue explants. Figure 6 clearly shows that insulin (10^{-7} mol/l) produced a significant increase in PPAR-γ protein levels after 12 h of incubation (141 ± 3%, P = 0.04, n = 5).

DISCUSSION

PPAR-γ is the adipocyte-specific isoform of the PPAR- subfamily of nuclear receptors (2,3). Several lines of evidence strongly suggest that PPAR-γ plays a crucial role in the initiation and the maintenance of the adipocyte phenotype: first, PPAR-γ is rapidly expressed during adipocyte differentiation (2); second, forced expression of PPAR-γ into fibroblasts leads to the transformation of those cells into adipocytes (28); third, high levels of PPAR-γ expression are maintained in mature adipocytes (3,11–13,23); and finally, most of the known PPAR-γ target genes encode a key protein involved in the regulation of adipocyte functions and metabolism (3,4–8,29–31). In addition to natural ligands that are thought to be lipid metabolites (like the prostaglandin J2 derivatives) (32,33), PPAR-γ also binds the thiazolidinediones (15), leading to improved insulin sensitivity in vivo and in vitro (16). Since insulin is one of the main controlling hormones of adipocyte development and function, a cross-talk between insulin action and PPAR-γ function is expected but is not yet clearly established. Recently, effects of insulin on PPAR-γ transcriptional activity were reported, but depending on the experimental cellular model used, insulin either stimulates (34,35) or inhibits (36) PPAR-γ activity. However, it seems indubitable that insulin can lead to phosphorylation of PPAR-γ (34,36).

In this study, we demonstrate that insulin acutely upregulates PPAR-γ mRNA and protein levels in human adipocytes. In subcutaneous abdominal adipose tissue, the mRNA concentrations of both PPAR-γ1 and PPAR-γ2 were twofold increased in vivo after a 3-h insulin infusion. The stimulating effect of insulin was similar in lean subjects, type 2 diabetic patients, and nondiabetic obese subjects. In isolated human adipocytes, insulin induced PPAR-γ mRNAs in a dose-dependent manner, with half-maximal stimulation for a concentration of about 1–5 nmol/l, suggesting that the observed in vivo effect was due to a direct action of insulin on adipocytes and was probably mediated by insulin receptor activation. The observed changes in mRNA levels could appear small (less than twofold increases), but they were significant and undoubtedly reliable when using a highly sensitive and reproducible RT-competitive PCR assay (26). In addition, β-actin, HSL, and lipoprotein lipase mRNA levels, measured with the same methodology, did not change when isolated adipocytes were incubated with insulin. Interestingly, the mRNA levels of the glucose transporter GLUT4 were significantly increased by insulin in isolated adipocytes. As for PPAR-γ, in vivo clamp studies have already shown that GLUT4 mRNA expression is upregulated by insulin in human subcutaneous adipose tissue (21). There-

fore, our results confirm and extend to humans recent data obtained in rodents that suggested a positive effect of insulin on PPAR-γ mRNA expression in epididymal adipose tissue (23). Moreover, we demonstrate that this effect results in a significant increase in PPAR-γ protein levels. Further studies are required to determine the physiologic consequences of this effect of insulin in adipose tissue. Nevertheless, these findings reinforce the concept that insulin and PPAR-γ functions can be closely linked in adipocytes.

In human isolated adipocytes, PPAR-γ1 mRNA levels were progressively increased during the whole incubation period, whereas a rapid, but transient, rise in PPAR-γ2 mRNA was observed during the first hours of incubation. PPAR-γ1 and -γ2 are derived from the same gene by differential promoter usage and differential splicing (10,11). In the human gene, evidence for a functional specific promoter for each isoform was provided by transfection experiments with promoter constructs (11) and supported by the tissue-specific expression of PPAR-γ1 and PPAR-γ2 (11,12). The different effects of insulin on the two mRNA isoforms may argue for a different type of action of insulin on each promoter. Nevertheless, different effects of the hormone on the stability of the two PPAR-γ mRNAs could not be excluded at this step of the study.

Insulin can change the transcriptional activities of PPAR-γ1, PPAR-γ2, or both that are initially present in the cells (34–36), and this effect might be involved in the observed induction of PPAR-γ mRNAs by the hormone. The possibility that PPAR-γ directly participates in the regulation of its own gene is supported by the recent finding that thiazolidinediones, which bind and activate PPAR-γ, also induce PPAR-γ gene expression in rat adipose tissue (30). Supporting this hypothesis, sequence consensus for PPAR-response element have been found in the proximal promoter of PPAR-γ1 (L. Fajas, J.A., unpublished observations). However, the mechanism of action of insulin will be difficult to ascertain as long as insulin effects on PPAR-γ transcriptional activity are not clearly defined in normal cells.

In vivo, a 3-h hyperinsulinemia resulted in about a twofold increase in PPAR-γ1 and -γ2 mRNA levels in abdominal subcutaneous adipose tissue. The same effect was observed in lean and in obese subjects with or without type 2 diabetes, strongly suggesting that these pathologies are not associated with altered regulation by insulin of PPAR-γ gene expression. Furthermore, although the patients exhibited a marked state of insulin resistance of whole-body glucose uptake, our results indicated that the pathway involved in the regulation of PPAR-γ gene expression is not resistant to the action of insulin. This suggests distinct insulin signaling mechanisms for the control of glucose metabolism and of PPAR-γ mRNA expression.

In addition to the demonstration of a positive regulation by insulin of PPAR-γ expression in human fat tissue, this study confirmed our previous observation that basal expression levels of PPAR-γ are not altered in adipose tissue of obese and type 2 diabetic patients (12). Recently, Vidal-Puig et al. (13) claimed that obesity might be associated with a slight increase in PPAR-γ2 mRNA levels in subcutaneous adipose tissue. Because those authors did not observe any change in PPAR-γ1 mRNA abundance, a positive correlation was found between PPAR-γ2/γ1 relative expression levels and the BMI of the subjects (13). This was not observed in this study, which included 44 subjects with a wide range of BMIs. The reasons for the discrepancy between our data and those of Vidal-Puig et al. (13) are unknown but could be related to methodology differences in the quantification of PPAR-γ mRNAs. Alternatively, differences in the population studied cannot be excluded. In addition to the lack of correlation with obesity, we also did not observe correlation between basal fasting insulinemia and basal PPAR-γ mRNA levels, although insulin acutely regulates PPAR-γ expression. These results may suggest that differences in plasma insulin concentrations between control and obese subjects (30 vs. 113 pmol/l) were not large enough to promote changes in PPAR-γ mRNA expression.

PPAR-γ mRNA could be quantified in human skeletal muscle, but the levels of expression were very low compared with those found in adipocytes. As in adipose tissue, we did not observe any relationship between PPAR-γ mRNA levels in muscle biopsies and the BMI of the subjects. Moreover, the correlation between HSL and PPAR-γ mRNA strongly suggested that the variations in the observed PPAR-γ mRNA levels reflected different adipose content of the muscle samples. Although minute expression of PPAR-γ could exist in the muscle cells, its role in human skeletal muscle functions and in thiazolidinedione effects in vivo is probably very low.

In overweight patients, however, the lack of alteration in basal PPAR-γ expression and in its regulation by insulin do not exclude the fact that defects in PPAR-γ function occur and participate in the development of body fatness. The regulation of the transcriptional activity of the nuclear receptors is very complex, involving several partners and also frequently phosphorylation/dephosphorylation processes (37). To date, the existence of potential alterations in these different regulatory steps is unknown in human pathologies.

In conclusion, we provide evidence for an acute stimulation by insulin of PPAR-γ mRNA and protein expression in human adipocytes, both in vivo and in mature isolated cells. Although the physiologic role of this effect is not yet established, these results reinforce the concept that a cross-talk occurs in adipocytes between PPAR-γ and insulin, two fundamental actors of fat differentiation and metabolism.

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