

Peroxisome Proliferator-activated Receptor Gene Expression in Human Tissues

Effects of Obesity, Weight Loss, and Regulation by Insulin and Glucocorticoids

Antonio J. Vidal-Puig,* Robert V. Considine,† Mercedes Jimenez-Liñan,* Ariel Werman,* Walter J. Pories,§ Jose F. Caro,‡ and Jeffrey S. Flier*

*Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215; †Department of Medicine, Thomas Jefferson Medical School, Philadelphia, Pennsylvania 19107-5083; and §Department of Surgery, School of Medicine, East Carolina University, Greenville, North Carolina 27858-4354

Abstract

The peroxisome proliferator activated receptor (PPAR γ) plays a key role in adipogenesis and adipocyte gene expression and is the receptor for the thiazolidinedione class of insulin-sensitizing drugs. The tissue expression and potential for regulation of human PPAR γ gene expression *in vivo* are unknown. We have cloned a partial human PPAR γ cDNA, and established an RNase protection assay that permits simultaneous measurements of both PPAR γ 1 and PPAR γ 2 splice variants. Both γ 1 and γ 2 mRNAs were abundantly expressed in adipose tissue. PPAR γ 1 was detected at lower levels in liver and heart, whereas both γ 1 and γ 2 mRNAs were expressed at low levels in skeletal muscle. To examine the hypothesis that obesity is associated with abnormal adipose tissue expression of PPAR γ , we quantitated PPAR γ mRNA splice variants in subcutaneous adipose tissue of 14 lean and 24 obese subjects. Adipose expression of PPAR γ 2 mRNA was increased in human obesity (14.25 attomol PPAR γ 2/18S in obese females vs 9.9 in lean, $P = 0.003$). This increase was observed in both male and females. In contrast, no differences were observed in PPAR γ 1/18S mRNA expression. There was a strong positive correlation ($r = 0.70$, $P < 0.001$) between the ratio of PPAR γ 2/ γ 1 and the body mass index of these patients. We also observed sexually dimorphic expression with increased expression of both PPAR γ 1 and PPAR γ 2 mRNAs in the subcutaneous adipose tissue of women compared with men. To determine the effect of weight loss on PPAR γ mRNA expression, seven additional obese subjects were fed a low calorie diet (800 Kcal) until 10% weight loss was achieved. Mean expression of adipose PPAR γ 2 mRNA fell 25% ($P = 0.0250$ after a 10% reduction in body weight), but then increased to pretreatment levels after 4 wk of weight maintenance. Nutritional regulation of PPAR γ 1 was not seen. *In vitro* experiments revealed a synergistic effect of insulin and corticosteroids to induce PPAR γ expression in isolated human adipocytes in culture. We conclude that: (a) human PPAR γ mRNA ex-

pression is most abundant in adipose tissue, but lower level expression of both splice variants is seen in skeletal muscle; to an extent that is unlikely to be due to adipose contamination. (b) RNA derived from adipose tissue of obese humans has increased expression of PPAR γ 2 mRNA, as well as an increased ratio of PPAR γ 2/ γ 1 splice variants that is proportional to the BMI; (c) a low calorie diet specifically down-regulates the expression of PPAR γ 2 mRNA in adipose tissue of obese humans; (d) insulin and corticosteroids synergistically induce PPAR γ mRNA after *in vitro* exposure to isolated human adipocytes; and (e) the *in vivo* modulation of PPAR γ 2 mRNA levels is an additional level of regulation for the control of adipocyte development and function, and could provide a molecular mechanism for alterations in adipocyte number and function in obesity. (*J. Clin. Invest.* 1997. 99:2416–2422.) Key words: PPAR γ • obesity • nutrition • hormones • gene regulation

Introduction

Obesity, defined as a state of pathologically excessive adipose tissue mass (1), has major adverse medical consequences, largely due to its association with non-insulin-dependent diabetes, hypertension, hyperlipidaemia, and cardiovascular diseases (2). The development of obesity requires the continuous differentiation of new adipocytes throughout life (1). This process of adipocyte differentiation from preadipocytes has been shown to be controlled by members of the peroxisome proliferator-activated receptor (PPAR γ),¹ adipocyte determination and differentiation-dependent factor-1 (ADD1)/SREBP1, and CCAAT/enhancer-binding protein (C/EBP) nuclear receptor families (3–7). PPAR γ is a dominant activator of fat cell differentiation acting through transactivation of adipose specific genes, including those that encode for proteins involved in lipid storage and metabolism (8–10). Forced expression of PPAR γ 2 and C/EBP α in fibroblasts (4) and myoblasts (11) is capable of differentiating these cells into adipocytes. PPAR γ has been shown to have the highest adipogenic activity among the members of the PPAR family (12).

Two different PPAR γ splice variants, PPAR γ 1 and PPAR γ 2, have been cloned in the mouse (5) and recently in humans (13, 14). These two forms differ only in the NH₂ terminus where PPAR γ 2 has an additional 30 amino acids. In the mouse, the two PPAR γ splice variants are derived from the

Address correspondence to Jeffrey S. Flier or Antonio J. Vidal-Puig, Endocrine Division, Beth Israel Deaconess Medical Center, Research North, 99 Brookline Avenue, Boston, MA 02215. Phone: 617-667-2151; FAX: 617-667-2927; E-mail: JFlier@BIDMC.Harvard.edu Avidalpu@BIDMC.Harvard.edu

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1. Abbreviations used in this paper: ADD1, adipocyte determination and differentiation-dependent factor-1; BMI, body mass index; C/EBP, CCAAT/enhancer-binding protein; DXM, dexamethasone; H-ALBP, human adipocyte lipid-binding protein; I, insulin; PPAR γ , peroxisome proliferator-activated receptor; TGL, troglitazone; TZD, thiazolidinediones.

same gene by alternative promoter usage and differential mRNA splicing (5, 15). It has been recently shown that prostanoids (16, 17), which are potent inducers of adipose tissue differentiation (18), are the natural ligands for PPAR γ mediating the ligand dependent activation of the PPAR γ receptor. Thiazolidinediones (TZD) are a new class of synthetic drugs that increase sensitivity to insulin (19), and have also been shown to be ligands for PPAR γ receptors. The activity of TZDs to improve the insulin resistance in muscle and liver that is observed in patients with non-insulin-dependent diabetes mellitus, could be mediated through a direct activation of PPAR γ receptors located in these tissues, or through signals generated in adipose tissue (20). For this reason it is important to clarify the tissue distribution of these receptors in human tissues.

Given the key role that PPAR γ plays in adipose tissue differentiation, regulated gene expression, and as the receptor for insulin-sensitizing drugs and given the possibility that the PPAR γ 1 and γ 2 splice variants have nonidentical functions, it is critical to study the in vivo regulation of both PPAR γ splice variants and their potential dysregulation in altered physiological states such as obesity. In this study we address this issue by quantitating the expression of PPAR γ 1 and γ 2 mRNAs in human muscle and fat tissue and in isolated adipocytes, in a variety of physiological and pathophysiological states.

Methods

Subjects. We studied 14 lean subjects (9 women/5 men; mean \pm SD body mass index [BMI] 23.3 ± 0.6 kg/m², mean age 32.5 ± 2.7 yr) and a group of 24 obese and morbid obese subjects (17 women/7 men; BMI 43.5 ± 3.08 kg/m², age 35.9 ± 3.12 yr). Obesity was defined as a BMI ≥ 27.3 for men (21). Women were considered lean when BMI < 27 , women were considered obese with a BMI > 27 . Comparison of BMI between both groups was statistically significant ($P < 0.0001$). No differences were observed comparing the BMI of men versus women. None of the subjects were taking any medication or had any evidence of metabolic disease other than obesity and all reported a stable body weight for at least 3 mo before the study.

To study the effect of weight loss, seven additional obese subjects (six women/one man; BMI 40.4 ± 5.2 kg/m², age 37 ± 13 yr) were fed 800 calorie (35% protein, 50% carbohydrates, 15% fat) liquid protein diets (Optifast 800; Sandoz Nutrition Corp., Minneapolis, MN). Blood was drawn and subcutaneous adipose tissue biopsies taken at baseline, when the subjects had 10% reduction in body weight, and again after 4 wk of maintenance of the reduced body weight. Fasting serum insulin, serum glucose, triglyceride, cholesterol, and leptin were measured in blood samples.

Muscle tissue biopsies obtained from lean normal controls (seven women/one man; BMI 24.72 ± 0.76 kg/m²; intraoperative glycemia 78.37 ± 6.09 mg/dl), obese (seven women/one man; BMI 41.55 ± 3.39 kg/m²; intraoperative glycemia 96.5 ± 5.7 mg/dl) and diabetic patients were studied (seven women/one man; BMI 48.39 ± 3.4 kg/m²; intraoperative glycemia, 170 ± 23.9 mg/dl).

Tissues. Abdominal subcutaneous adipose and muscle tissue biopsies were obtained from intraoperative procedures or by needle liposuction (22) after an overnight fast. The tissue was transported to the laboratory in saline and immediately frozen in liquid nitrogen. Total RNA was obtained by guanidinium thiocyanate-phenol chloroform extraction (23). Protocols involving adipose biopsies have been approved by the institutional Review Board at Thomas Jefferson University (Philadelphia, PA). To study PPAR γ mRNA expression in muscle, samples of skeletal muscle mRNA from lean, obese, and diabetic subjects were used. Protocols involving muscle biopsies were approved by East Carolina University Board (Greenville, NC). All subjects gave informed consent. Muscle (20 μ g), liver (25 μ g), heart

(20 μ g), and adipose tissue (15 μ g) total mRNA aliquots from human, mouse, and rat were used for the tissue distribution study.

Isolation and culture of adipocytes. Adipose tissue was digested with collagenase and the adipocytes isolated as described (24). Isolated adipocytes (2-ml packed cells) were incubated in 10 ml of DMEM/F-12 (1:1) medium containing 10% fetal bovine serum, antibiotics (Penicillin 20 U/ml; streptomycin 20 μ g/ml), and test compounds as described in the results. The cells were cultured in suspension in 125 ml polycarbonate erlenmeyer flasks and left undisturbed in a standard CO₂ incubator. To terminate the experiment the cells and the medium were transferred to a 50-ml propylene centrifuge tube and centrifuged at 150 g for 2 min. The medium was removed from below the cells and 1 ml of guanidinium isothiocyanate solution added. The mixture was vortexed to ensure disruption of the cells and the lipid extracted into 5 ml of chloroform. RNA in the aqueous phase was then isolated by standard techniques (23).

RNA extraction and analysis. Total RNA was extracted from adipose tissue and muscle using the method of Chomczynski and Sacchi (23). RNA was quantified by absorbance at 260 nm in a spectrophotometer and its integrity was assessed after electrophoresis in nondeaturing 1% agarose gels by ethidium bromide staining. Aliquots of human adipose (15 μ g) and muscle tissue RNA (20 μ g) were used for determination of PPAR γ . Aliquots (10 and 15 μ g) of muscle tissue RNA were used for determination of adipsin and human adipocyte lipid binding protein (H-ALBP), respectively (25). Cyclophilin was determined simultaneously with PPAR γ . Aliquots of 200 ng were used for measurement of 18S as internal control assays. Aliquots of 5 μ g total RNA from isolated adipocytes in culture were used in the in vitro studies.

RNA analysis was performed using the solution hybridization nuclease protection method (26, 27). A partial human PPAR γ , adipsin and H-ALBP cDNA probes were generated by reverse transcriptase-PCR using total RNA from human fat and subsequently ligated into a TA cloning vector (Invitrogen Corp., San Diego, CA and Promega Corp., Madison, WI). For the human PPAR γ probe two primers (5'-AAC TGC GGG GAA ACT TGG GAG ATT CTC C 3' and 5'-AAT AAT AAG GTG GAG ATG CAG GCT CC 3') were used. The hPPAR γ probe spans a 341-bp region that includes 83 bp of the hPPAR γ 2 transcript and 258 bp common to both hPPAR γ 1 and hPPAR γ 2. The adipsin probe was prepared using two primers (5'-GCG CAC CTG GCG CAG GCG TCC TG 3' and 5'-GCA CTG CGC GCA GCA CGT CGT A3') designed to amplify 165 bp PCR product. H-ALBP probe was prepared using primers (5'-AGT CAA GAG CAC CAT AAC CTT AGA 3' and 5'-CCT TGG CTT ATG CTC TCT CAT AA 3') designed to amplify a 170-bp PCR product (25). Antisense PPAR γ probe and sense RNA reference were prepared using HindIII and XbaI, respectively. Antisense adipsin and H-ALBP probes were prepared using NcoI. Identity and orientation of these probes was confirmed by sequencing. A 103-bp cDNA human cyclophilin probe (Ambion, Inc., Austin, TX) and a previously described (28) 75-bp cDNA corresponding to 18S ribosomal RNA (gift of M. Jakubowski, Beth Israel Hospital, Boston, MA) were used as an internal control. In the tissue distribution experiment a previously (28) described mouse and rat PPAR γ probe and mouse reference standard curve was used. RNA transcripts for hPPAR γ , mPPAR γ , adipsin, H-ALBP, cyclophilin, and ribosomal fraction 18S were quantitated using previously described solution hybridization RNase protection assay methods (28). Protected bands were visualized by autoradiography and quantitated by Phosphorimager analysis using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Statistical analysis. Regression analysis of data obtained using known amounts of target reference RNAs was used to generate standard curves. Levels of PPAR γ and 18S were calculated and expressed as attomoles mRNA. All results are presented as means \pm SE (unless specifically noted). Statistical significance was assessed by ANOVA, unpaired Student's *t* test, and correlations using Pearson's coefficient. All analyses were done with Statview statistical package. The level of significance was set at $P < 0.05$.

Results

Tissue distribution of PPAR γ splice variants expression. We developed a sensitive RNase protection assay that allows for precise and specific quantitation of mRNAs encoding the human PPAR γ 2 and γ 1 splice variants. Performing reverse transcriptase-PCR with total RNA isolated from human adipose tissue as template, and using a 5' primer corresponding to the mouse sequence and a 3' primer from hPPAR γ 1 sequence we

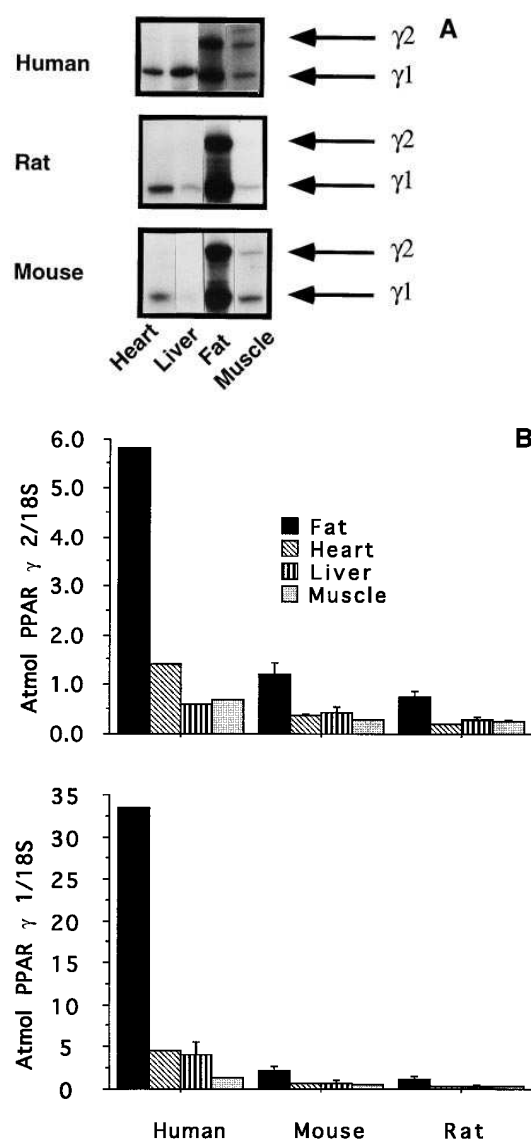


Figure 1. PPAR γ mRNA expression in human, mouse, and rat tissues. (A) RNase protection was used to assess the expression of PPAR γ 1 and γ 2 mRNAs in adipose tissue, muscle, liver, and heart of human, mouse, and rat. Total RNA from adipose tissue (15 μ g), liver (25 μ g), heart (20 μ g), and skeletal muscle (20 μ g) was analyzed. The upper band of 341 bp in human and 273 bp in mouse and rat corresponds to PPAR γ 2. The lower band of 258 bp in human and 185 bp in mouse and rat correspond to PPAR γ 1. (B) Comparison of the number of attomoles of PPAR γ 2 and γ 1/18S in human, mouse, and rat tissues. The pattern of tissue distribution is conserved among these three species, however human tissues express more molecules of PPAR γ mRNA/per 18S than mouse or rat.

amplified a 341-bp PCR fragment that contained 83 bp of hPPAR γ 2 and 258 bp common to hPPAR γ 1 and 2. To verify that the cloned sequence was complementary to human PPAR γ mRNA, a ribonuclease protection assay was performed. When the 341-bp hPPAR γ cDNA fragment was used as a probe for an RNase protection assay with human adipose tissue total RNA two protected bands of the appropriate size for PPAR γ 1 and γ 2 were detected. Compared to the high level in fat, much lower levels of PPAR γ 1 mRNA were observed in liver, skeletal muscle and heart (Fig. 1, A and B). The level of PPAR γ 2 mRNA signal in muscle was 10–25% of that seen in adipose tissue and the levels in skeletal muscle of lean, obese, and diabetic patients were similar (data not shown). To determine whether the PPAR γ 2 signal in skeletal muscle could be the result of fat cell contamination, aliquots of skeletal muscle RNA were assayed for human adipsin and H-ALBP mRNA (Fig. 2) expression. Adipsin was chosen because it is highly expressed in adipocytes (although it is probably not completely specific given that it can be detected in mouse muscle [29]). H-ALBP mRNA (25) is the human homologue of the mouse aP-2 (30), a gene that is highly expressed in adipose tissue and, probably similar to aP-2, would be induced by the activation of PPAR γ . The comparison among lean, obese, and diabetic human muscle samples revealed no differences in adipsin mRNA expression, suggesting that if any contamination of muscle tissue with fat was present, similar degrees of fat contamination would be present in all samples. As expected, low levels of H-ALBP mRNA were detected in RNA from muscle (5.3%) compared with adipose tissue (25). The ratio of PPAR γ 2/H-FABP in muscle mRNA was higher (1.31) than the same ratio in adipose tissue mRNA (0.256). Similarly the ratio of PPAR γ 2/adipsin in muscle RNA was higher (1.3) than this ratio in fat mRNA (0.48). The amount of PPAR γ 2 in muscle was there-

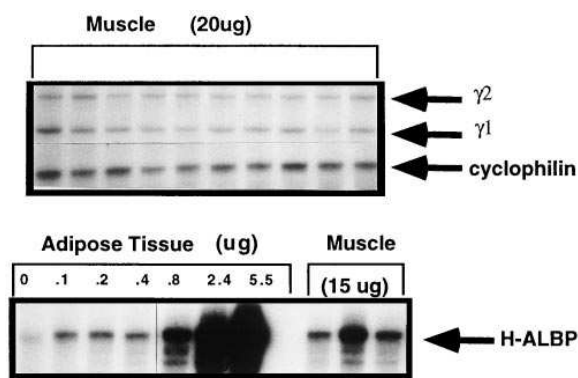


Figure 2. Expression of PPAR γ splice variants and H-ALBP in human skeletal muscle. This autoradiogram shows the results of RNase protection analysis of 20 μ g of muscle total RNA. In the top panel, PPAR γ 2 and γ 1 mRNA are visualized as two protected bands of 341 and 258 bp, respectively. The expression level of the cyclophilin (103 bp) was determined and used as an internal control. The bottom panel shows assessment of H-ALBP (165 bp) expression in muscle. Total muscle RNA samples (15 μ g) were analyzed by RNase protection assay to determine levels of H-ALBP. The autoradiogram shows results for three lean controls. Analysis of H-ALBP mRNA using different amounts of total RNA from human white adipose tissue indicates muscle expression of H-ALBP is very low (5.3%) relative to expression in adipose tissue.

fore 5.1- or 2.7-fold greater than that which could be attributed to fat contamination of muscle, unless it was the case that adipocytes present in muscle had a higher ratio of PPAR γ /H-FABP and PPAR γ /adipsin than adipocytes from adipose tissue. An additional argument against the PPAR γ signal in muscle being secondary to fat contamination is the fact that muscle and adipose tissue have different ratios of PPAR γ splice variants, with the ratio PPAR γ 2/ γ 1 being greater in muscle than in adipose tissue (0.972 ± 0.34 vs 0.335 ± 0.2 , $P < 0.001$). Together, these data strongly suggest that PPAR γ 2 mRNA is expressed in skeletal muscle, although considerably less than the level in fat. Finally, we compared the number of attomoles of PPAR γ 2 and γ 1/18S among human, mouse, and rat tissues (Fig. 1 B). The pattern of tissue distribution is very similar among these three species, however human tissues express an increased number of molecules of PPAR γ mRNA/per 18S than do those of mouse or rat.

Effect of obesity and dieting on PPAR γ expression. To explore the hypothesis that obesity is associated with abnormal adipose tissue expression of PPAR γ , we studied total RNA from subcutaneous adipose tissue of 14 lean subjects (9 women/5 men) and 24 obese subjects (18 women/7 men). The protected RNA bands corresponding to both PPAR γ 1 and PPAR γ 2 transcripts were quantitated, normalized for differences in RNA input (18S RNA and cyclophilin), and plotted against standard curves that were generated using known amounts of target PPAR γ RNA. Adipose expression of PPAR γ 2/18S mRNA was clearly increased in human obesity (14.25 attomoles PPAR γ 2/18S in obese females vs 9.9 in lean, $P = 0.011$) (Fig. 3, A and B). This increase was observed in both male ($P = 0.0039$) and females. In contrast, levels of PPAR γ 1/18S were similar or slightly decreased in obese patients. Interestingly there was a strong positive correlation ($r = 0.70$; $P = 0.001$) between the ratio PPAR γ 2/ γ 1 and the BMI of these patients (Fig. 3 C). We also observed sexually dimorphic expression with increased expression of both PPAR γ 1 (38.0 ± 1.74 vs 31.1 ± 2.2 , $P = 0.03$) and PPAR γ 2 mRNA (12.8 ± 0.835 vs 8.05 ± 0.744 , $P = 0.0023$) in the subcutaneous adipose tissue of women compared to men with similar BMI.

To determine the effect of weight loss on PPAR γ expression, seven additional obese subjects (6 women/1 man) were fed a low calorie diet. The expression of PPAR γ 2 decreased (25%) after a 10% reduction in body weight (BMI 35.1 ± 4.5 kg/m², $P < 0.05$, as compared to preweight loss value) and returned to pretreatment levels after 4 wk of maintenance of reduced body weight (34.7 ± 4.5 , $P < 0.05$ as compared to preweight loss value) (Fig. 4, A and B). PPAR γ 1 mRNA abundance, in contrast, did not show modifications. These body weight changes secondary to diet were associated with decreased fasting serum insulin levels (16.1 ± 4.5 μ U/ml preweight loss vs 11.9 ± 5.0 μ U/ml after weight loss, $P < 0.05$), serum leptin (50.2 ± 25.9 vs 24.2 ± 16.9 , $P < 0.05$), serum triglycerides (158 ± 78 vs 85 ± 18 mg/dl, $P < 0.05$) and total cholesterol (189 ± 41 vs 166 ± 41 , $P < 0.05$). The increase of PPAR γ 2 observed during the maintenance weight phase was not associated with increases in TG, cholesterol, or insulin, however a moderate increase (24.2 ± 6.3 vs 32.4 ± 9.0 , $P = 0.04$) in leptin was observed.

In vitro studies of human PPAR γ , hormonal, and pharmacological regulation. The observation that expression of PPAR γ 2 mRNA is increased in obesity (a state characterized by hyperinsulinemia) and is regulated by changes in nutrition sug-

gested the possibility of hormonal regulation. In fact, our previous data on the expression of PPAR γ mRNA in the streptozotocin mouse model (31) suggested that insulin could play an important role in the expression of PPAR γ . However, that in vivo experiment did not assess the effect of other adipogenic hormones such as corticosteroids. Here, we assessed this issue with an in vitro model to evaluate the effect of insulin and dexamethasone (DXM) on the expression of PPAR γ mRNA in isolated human adipocytes in short-term culture. We also assessed whether troglitazone, a synthetic TZD ligand for the PPAR γ receptor, had an effect on the levels of the

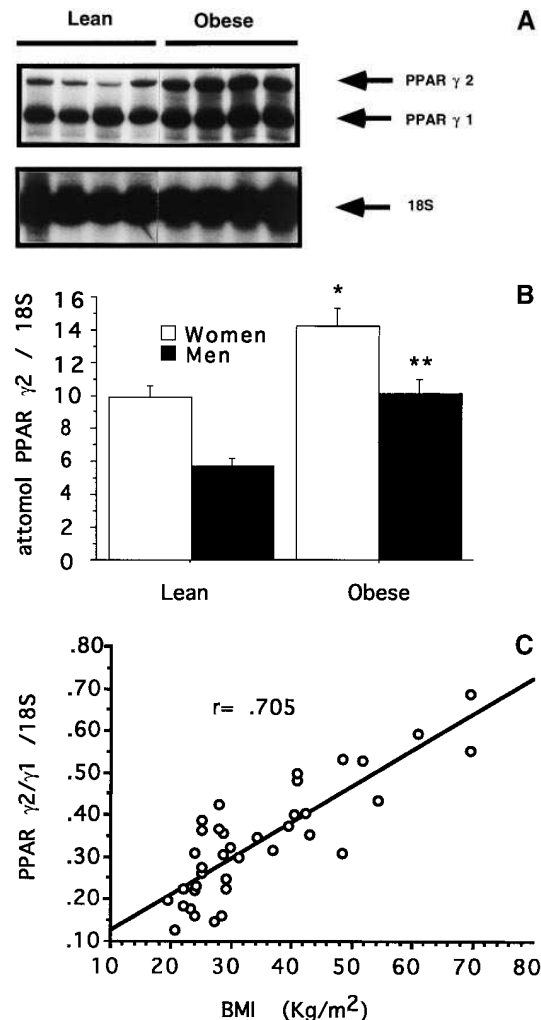


Figure 3. Effect of obesity on adipose tissue PPAR γ mRNA expression. (A) Obesity is associated with increased PPAR γ 2 mRNA expression in adipose tissue. This autoradiogram shows results of RNase protection assay obtained using total RNA (15 μ g) extracted from subcutaneous adipose tissue obtained from four obese (BMI > 27.3) and four lean women (BMI < 27.3). The expression level of 18S ribosomal RNA (75-bp protected band) was determined and used as an internal control to correct for minor variations in total RNA amount. (B) Increased expression of PPAR γ 2/18S in obese patients compared with lean controls. Results are expressed as attomoles of PPAR γ 2/attomole of 18S (mean \pm SE). Statistically significant differences were found in women ($P = 0.011$, $n = 26$) and men ($P = 0.0039$, $n = 12$). (C) Positive correlation ($r = 0.70$; $P = 0.001$) between the ratio of PPAR γ 2/ γ 1/18S and the BMI ($n = 38$).

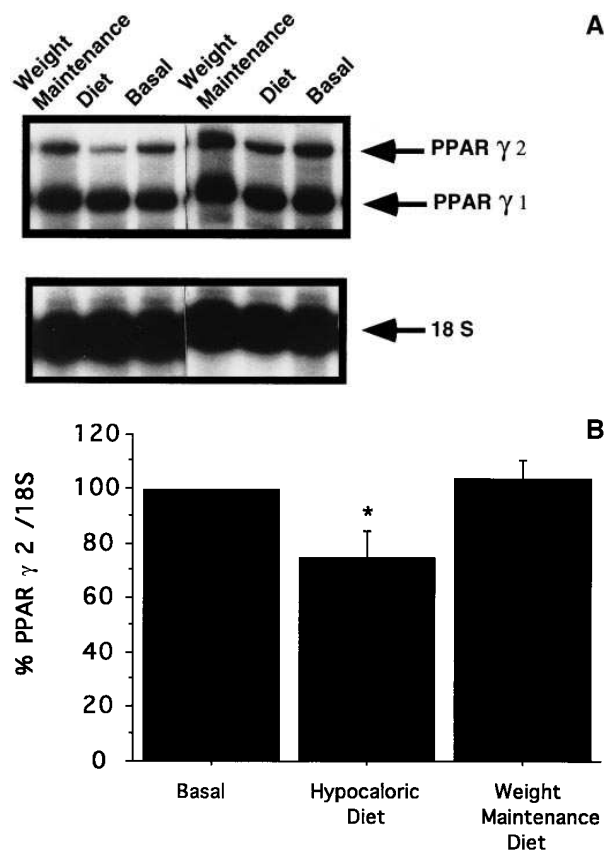


Figure 4. Effect of weight loss on PPAR γ expression. (A) This autoradiogram shows results of RNase protection assay using total RNA (15 μ g) extracted from subcutaneous adipose tissue obtained from two obese women in the basal state, after a 10% reduction in body weight and after 4 wk of maintenance of reduced body weight. The expression level of 18S ribosomal RNA (75-bp protected band) was determined and used as an internal control to correct for variations in total RNA amount. (B) Evolution of the PPAR γ 2 mRNA expression in obese patients under no dietary intervention, under hypocaloric diet (800 Kcal/d) and during weight maintenance diet. Results are expressed as mean \pm SEM percentage of mean values obtained previously to dietary intervention. * P = 0.025. n = 7.

mRNA encoding the receptor. We tested the effects of 10^{-7} M DXM, 10^{-7} M insulin (I), 10^{-7} M DXM + I, 10μ M/l troglitazone (TGL) and TGL + I on the expression of PPAR γ mRNA in isolated human adipocytes in a 24-, 48-, 72-h time course experiment. Insulin and troglitazone either alone or in combination had no effect in PPAR γ mRNA expression under these conditions. However, after 72 h with DXM alone, and with DXM + insulin there were marked increases in the expression of PPAR γ 1 and γ 2 splice variants mRNA (Fig. 5, A and B).

Discussion

The family of peroxisomal proliferator activated receptors is comprised of three gene products (α , δ , γ), each of which has different and specific tissue distribution and function (32, 33). One of these gene products, PPAR γ , is induced very early in the process of adipose differentiation (5). Two different PPAR γ

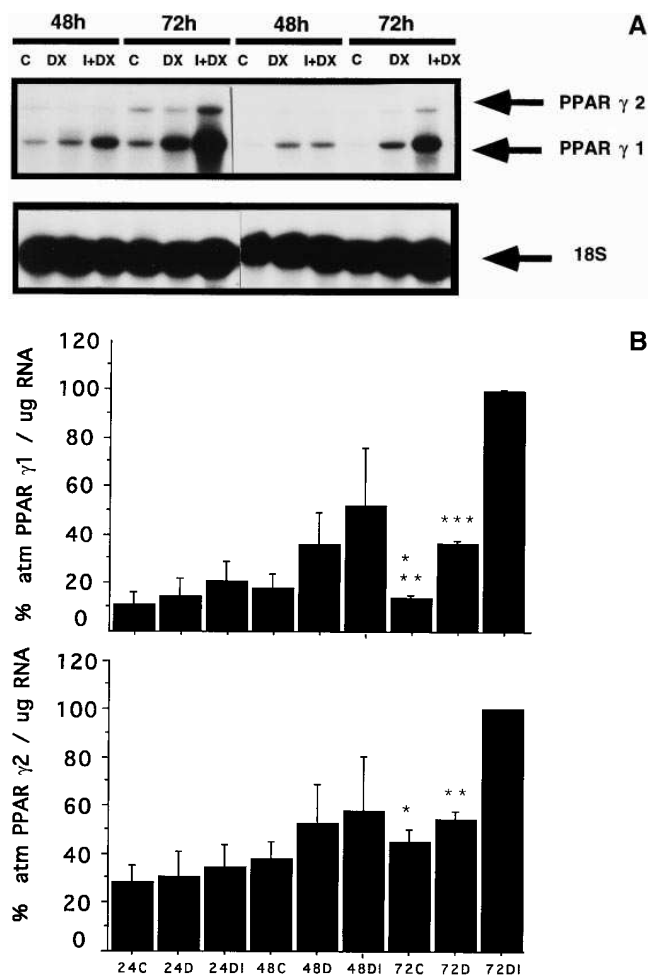


Figure 5. Effect of insulin and/or dexamethasone on PPAR γ mRNA expression. (A) Induction of PPAR γ mRNA by dexamethasone and insulin. This autoradiogram shows results of RNase protection analysis from two separate experiments, obtained using RNA (5 μ g) extracted from isolated human adipocytes in culture treated with dexamethasone \pm insulin for 48 and 72 h. (B) Time course experiment of the effects of 10^{-7} M dexamethasone (DXM), and DXM + 10^{-7} M insulin (I), on the expression of PPAR γ mRNA in isolated human adipocytes at 24, 48, 72 h. Results are expressed as mean \pm SEM percentage of mean values of the highest induction obtained per microgram RNA. Analysis of percentage of attomoles of PPAR γ 1/ μ g RNA revealed: *72 h Control (C) vs 72 h DXM, P = 0.0071; **72 h C vs 72 h DXM + I. P = 0.002; ***72 h DXM vs 72 h DXM + I. P = 0.006. Analysis of percentage of PPAR γ 2/ μ g RNA, *72 h C vs 72 h DXM + I, P = 0.0092; 72 h DXM vs 72 h DXM + I, P = 0.0061.

splice variants have been identified, differing in their 30 NH₂-terminal amino acids. Both splice variants are preferentially expressed in adipose tissue (5, 28, 34). Forced expression of PPAR γ 2 in fibroblasts (5) and myoblasts (11) is sufficient to differentiate these cells into adipocytes. The discovery of new natural (16, 17) and artificial (35, 36) ligands for PPAR γ with potent adipogenic activity is further evidence of the important role of these receptors in adipose tissue differentiation. Indeed it has been suggested that PPAR γ is not only a key factor required for adipocyte differentiation but also for maintaining the expression of fat-specific genes and the adipocyte phenotype (37). In previous work in the rodent, we reported that

PPAR γ mRNA and protein levels are regulated in vivo by fasting, insulin-deficient diabetes, and a diet rich in fatty acids (28). We also showed that transgenic ablation of brown adipose tissue which causes marked obesity was associated with increased PPAR γ mRNA (28). In the present study, we report the nutritional regulation of the human PPAR γ mRNA and its potential dysregulation in obesity. We also assessed the expression of PPAR γ in some key tissues for fuel homeostasis.

Although previous reports have shown expression of PPAR γ mRNA in human tissues (13, 38) our results demonstrate that there are human homologues of both splice products, PPAR γ 1 and γ 2 of mouse, and these have a tissue distribution pattern similar to that of mouse and rat. Both mRNA splice variants, PPAR γ 1 and γ 2 are expressed at high levels in adipose tissue. Much lower levels are expressed in muscle tissue, with similar amounts of both mRNA splice variants present in skeletal muscle of lean, obese, and diabetic patients. Using partial sequences of adipsin and H-ALBP as probes in an RNase protection assay, we provided evidence that this muscle expression of PPAR γ is unlikely to be a simple consequence of fat contamination of muscle. However, these data do not rule out the unlikely possibility that the PPAR γ that we detect in muscle is due to adipocytes in muscle tissue that have different relative expression of PPAR γ /adipsin or PPAR γ /H-FABP compared with adipocytes in fat tissue. Ultimately in situ hybridization may be required to confirm absolutely that PPAR γ expression in muscle tissue is occurring in myocytes rather than adipocytes in and around muscle.

The physiologic relevance of the low level of PPAR γ mRNA splice variants observed in muscle and liver remains to be determined. However it is possible that thiazolidinediones, high affinity ligands for PPAR γ , which possess adipogenic, insulin sensitizer, and antidiabetic activity (39–41) might influence hepatic and muscle insulin resistance through the activation of these relatively nonabundant receptors. Similarly, the activation of PPAR γ in muscle and liver by endogenous ligands might play a role in maintaining the insulin sensitivity of these tissues. Although the tissue distribution pattern of PPAR γ 2 and γ 1 is conserved among species, the absolute number of receptors when related to the molecules of 18S mRNA appears to be higher in human than murine tissues. Although our findings raise the possibility that PPAR γ in muscle may be capable of mediating some actions of TZD's in this tissue, it remains likely that activation of PPAR γ in adipose tissue results in the generation of signals that affect insulin sensitivity in muscle and liver. TNF- α is one example of a protein produced in adipose tissue that modulates systemic insulin resistance and whose expression is influenced by TZD (42, 43). In the same way the activation of PPAR γ in adipose tissue could cause a decrease in free fatty acids, thereby improving insulin resistance in other tissues (20).

Our results clearly show that expression of PPAR γ 2 mRNA is increased in adipose tissue of obese men and women, and that the ratio of PPAR γ 2/ γ 1 is directly correlated with their BMI. We did not observe similar changes in muscle of lean, obese, or diabetic patients suggesting that the regulation of PPAR γ 2 is different in muscle compared to fat. It is well established that PPAR γ is a key transcription factor in adipogenesis, both as a direct regulator of fat specific genes and as a "master" director that can trigger the entire program of adipogenesis (20). The implications of the observation that adipose tissue-derived RNA from obese humans displays in-

creased expression of PPAR γ 2 mRNA as well as an increased ratio γ 2/ γ 1 splice variants that is proportional to the BMI are currently unknown, but could be related to the expansion of the adipose tissue mass observed in these patients. This possibility is reinforced by our recent observation that PPAR γ possesses a ligand-independent NH₂-terminal activation domain, and this activity is greater for the NH₂ terminus of PPAR γ 2 than PPAR γ 1 (Werman, A., and J.S. Flier, unpublished data). These data also raise the question of whether the increase in PPAR γ 2 mRNA is secondary to obesity or increased food intake, or is due to an independent defect leading to its up-regulation. When we studied the effect of weight loss on PPAR γ 2 mRNA expression, we observed a 25% fall after a 10% reduction in body weight. However this was followed by an increase to pretreatment levels after 4 wk of weight maintenance. Thus, similar to our observations in the mouse (28), human PPAR γ 2 mRNA is nutritionally regulated, but the rebound observed during the weight maintenance phase suggests a tendency to up-regulation of PPAR γ 2 independent of food intake or body weight that could be of pathogenic importance. However, the rebound in the expression of PPAR γ 2 during weight maintenance could result from a recovery of steady-state lipid stores, compared to the active delipidation process that is associated with active weight loss. Nevertheless, since PPAR γ 1 and γ 2 are controlled by distinct promoter elements, it will be important to identify the factors that selectively result in increased PPAR γ 2 mRNA in obesity.

In a number of rodent models of obesity, adrenalectomy has a major effect to reduce obesity that is reversed by administration of corticosteroids. The obesity syndrome is characteristically associated with hyperinsulinemia and many of the monogenic obesity syndromes in rodents also develop a hyperglucocorticoid state. Patients affected with Cushing's syndrome (hypercortisolism) typically present with truncal obesity, in contrast to patients with adrenal insufficiency who have a characteristic lean phenotype (44). Our studies of the in vitro regulation of human PPAR γ expression in cultured adipocytes have shown a synergistic effect of insulin and corticosteroids to induce PPAR γ 1 and γ 2. This synergistic effect of insulin and dexamethasone in vitro suggests that their adipogenic activity could be mediated in part through PPAR γ induction. However, because this in vitro induction is predominantly of PPAR γ 1, it is unlikely that corticosteroids alone could determine the specific induction of PPAR γ 2 observed in adipose tissue of obese patients.

In summary, we have used a sensitive RNase protection assay to detect and quantitate for the first time, levels of PPAR γ 1 and γ 2 mRNAs in human tissues. As in rodents, human PPAR γ mRNA expression is most abundant in adipose tissue, although expression of both splice variants is clearly seen, albeit at lower levels, in skeletal muscle. Adipose tissue-derived RNA from obese humans has increased expression of PPAR γ 2 mRNA, as well as an increased ratio of PPAR γ 2/ γ 1 splice variants that is proportional to the BMI. A low calorie diet specifically down-regulates the expression of PPAR γ 2 mRNA in adipose tissue of obese humans, but this increases again during weight maintenance. In vitro, two adipogenic hormones, insulin and corticosteroids, show a synergistic effect to induce PPAR γ mRNA in isolated adipocytes. In vivo modulation of human PPAR γ 2 mRNA by obesity and nutrition suggests a possible role for PPAR γ expression in the pathogenesis of altered adipocyte number and function in obesity.

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