Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids.

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The peroxisome proliferator activated receptor (PPAR gamma) 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 gamma gene expression in vivo are unknown. We have cloned a partial human PPAR gamma cDNA, and established an RNase protection assay that permits simultaneous measurements of both PPAR gamma1 and PPAR gamma2 splice variants. Both gamma1 and gamma2 mRNAs were abundantly expressed in adipose tissue. PPAR gamma1 was detected at lower levels in liver and heart, whereas both gamma1 and gamma2 mRNAs were expressed at low levels in skeletal muscle. To examine the hypothesis that obesity is associated with abnormal adipose tissue expression of PPAR gamma, we quantitated PPARgamma mRNA splice variants in subcutaneous adipose tissue of 14 lean and 24 obese subjects. Adipose expression of PPARgamma 2 mRNA was increased in human obesity (14.25 attomol PPAR gamma2/18S 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 gamma1/18S mRNA expression. There was a strong positive correlation (r = 0.70, P < 0.001) between the ratio of PPAR gamma2/gamma1 and the body mass index of these patients. We also observed sexually dimorphic expression with […]

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Effects of Obesity, Weight Loss, and Regulation by Insulin and Glucocorticoids
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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 γ1 and PPAR γ2 splice variants. Both γ1 and γ2 mRNAs were abundantly expressed in adipose tissue. PPAR γ1 was detected at lower levels in liver and heart, whereas both γ1 and γ2 mRNAs were expressed at low levels in skeletal muscle. To examine the hypothesis that obesity is associated with abnormal adipocyte gene expression, we quantitated PPARγ mRNA splice variants in subcutaneous adipose tissue of 14 lean and 24 obese subjects. Adipose expression of PPAR γ2 mRNA was increased in human obesity (14.25 attomol PPAR γ2/18S 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 γ1/18S mRNA expression. There was a strong positive correlation (r = 0.70, P < 0.001) between the ratio of PPAR γ2/γ1 and the body mass index of these patients. We also observed sexually dimorphic expression with increased expression of both PPAR γ1 and PPAR γ2 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 γ2 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 γ1 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 expression 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 γ2/γ1 splice variants that is proportional to the BMI; (c) a low calorie diet specifically down-regulates the expression of PPAR γ2 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 γ2 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 γ2 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 γ1 and PPAR γ2, have been cloned in the mouse (5) and recently in humans (13, 14). These two forms differ only in the NH2 terminus where PPAR γ2 has an additional 30 amino acids. In the mouse, the two PPAR γ splice variants are derived from the

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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 PPARγ mediating the ligand dependent activation of the PPARγ 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 PPARγ 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 PPARγ 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 PPARγ plays in adipose tissue differentiation, regulated gene expression, and as the receptor for insulin-sensitizing drugs and given the possibility that the PPARγ1 and γ2 splice variants have nonidentical functions, it is critical to study the in vivo regulation of both PPARγ 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 PPARγ1 and γ2 mRNAs in human muscle and fat tissue and in isolated adipocytes, in a variety of physiological and pathophysiologic states.

**Methods**

**Subjects.** We studied 14 lean subjects (9 women/5 men; mean±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 diet (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 24.7±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 guanidium thiocyanate-phenol chloroform extraction (23). Protocols involving adipose biopsy have been approved by the institutional Review Board at Thomas Jefferson University (Philadelphia, PA). To study PPARγ 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 µg), liver (25 µg), heart (20 µg), and adipose tissue (15 µg) 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 µg/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 nondenaturing 1% agarose gels by ethidium bromide staining. Aliquots of human adipose (15 µg) and muscle tissue RNA (20 µg) were used for determination of PPARγ. Aliquots (10 and 15 µg) of muscle tissue RNA were used for determination of adipin and human adipocyte lipid binding protein (H-ALBP), respectively (25). Cyclophilin was determined simultaneously with PPARγ. Aliquots of 200 ng were used for measurement of 18S as internal control assays. Aliquots of 5 µg total RNA from isolated adipocytes in culture were used in the in vitro studies.

RNA analysis was performed using the solution hybridization nucleic acid protection method (26, 27). A partial human PPARγ, adipin and H-ALBP cDNA probes were generated by reverse transcription-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 PPARγ probe two primers (5’AAC TGC GGG GAA ACT TGG GAG ATT CTC C 3’ and 5’ AAT AAT AAG GTG GAG ATG CAG CCT CC 3’) were used. The hPPARγ probe spans a 341-bp region that includes 83 bp of the hPPARγ2 transcript and 258 bp common to both hPPARγ1 and hPPARγ2. The adipin probe was prepared using two primers (5’ GGC CAC CTG CCG CAG GCC TCG TTC 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 GAC CAT AAC CTT AGA 3’ and 5’ CTT TGG ATT ATG CTC TCT CAT AA 3’) designed to amplify a 170-bp PCR product (25). Antisense PPARγ probe and sense RNA reference were prepared using HindIII and XbaI, respectively. Antisense adipin and H-ALBP probes were prepared using Ncol. 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 described (28) described mouse and rat PPARγ probe and mouse reference standard curve was used. RNA transcripts for hPPARγ, mPPARγ, adipin, 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 PPARγ and 18S were calculated and expressed as attomoles mRNA. All results are presented as means±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.

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Results

Tissue distribution of PPARγ splice variants expression. We developed a sensitive RNase protection assay that allows for precise and specific quantitation of mRNAs encoding the human PPARγ2 and γ1 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 hPPARγ1 sequence we amplified a 341-bp PCR fragment that contained 83 bp of hPPARγ2 and 258 bp common to hPPARγ1 and 2. To verify that the cloned sequence was complementary to human PPARγ mRNA, a ribonuclease protection assay was performed. When the 341-bp hPPARγ 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 PPARγ1 and γ2 were detected. Compared to the high level in fat, much lower levels of PPARγ1 mRNA were observed in liver, skeletal muscle and heart (Fig. 1, A and B). The level of PPARγ2 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 PPARγ2 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 PPARγ.

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 PPARγ2/H-FABP in muscle mRNA was higher (1.31) than the same ratio in adipose tissue mRNA (0.256). Similarly the ratio of PPARγ2/adipsin in muscle RNA was higher (1.3) than this ratio in fat mRNA (0.48). The amount of PPARγ2 in muscle was there-

Figure 1. PPARγ mRNA expression in human, mouse, and rat tissues. (A) RNase protection was used to assess the expression of PPARγ1 and γ2 mRNAs in adipose tissue, muscle, liver, and heart of human, mouse, and rat. Total RNA from adipose tissue (15 μg), liver (25 μg), heart (20 μg), and skeletal muscle (20 μg) was analyzed. The upper band of 341 bp in human and 273 bp in mouse and rat corresponds to PPARγ2. The lower band of 258 bp in human and 185 bp in mouse and rat correspond to PPARγ1. (B) Comparison of the number of attomoles of PPARγ2 and γ1/18S in human, mouse, and rat tissues. The pattern of tissue distribution is conserved among these three species, however human tissues express more molecules of PPARγ mRNA per 18S than mouse or rat.

Figure 2. Expression of PPARγ splice variants and H-ALBP in human skeletal muscle. This autoradiogram shows the results of RNase protection analysis of 20 μg of muscle total RNA. In the top panel, PPARγ2 and γ1 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 μg) 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 PPARγ/adipsin that 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 γ2/γ1 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 γ2 mRNA is expressed in skeletal muscle, although considerably less than the level in fat. Finally, we compared the number of attomoles of PPAR γ2 and γ1/18S 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 γ1 and PPAR γ2 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 γ2/18S mRNA was clearly increased in human obesity (14.25 attomoles PPAR γ2/18S 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 γ1/18S 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 γ2/γ1 and the BMI of these patients (Fig. 3 C). We also observed sexually dimorphic expression with increased expression of both PPAR γ1 (38.0±1.74 vs 31.1±2.2, P = 0.03) and PPAR γ2 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 caloric diet. The expression of PPAR γ2 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 γ1 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 μU/ml pre-weight loss vs 11.9±5.0 μU/ml post 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 γ2 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 expression.** The observation that expression of PPAR γ2 mRNA is increased in obesity (a state characterized by hyperinsulinemia) and is regulated by changes in nutrition suggested 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 γ2 mRNA expression in adipose tissue. This autoradiogram shows results of RNase protection assay obtained using total RNA (15 μg) 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 γ2/18S in obese patients compared with lean controls. Results are expressed as attomoles of PPAR γ2/attomole of 18S (mean±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 γ2/γ1/18S and the BMI (n = 38).
mRNA encoding the receptor. We tested the effects of $10^{-7}$ M DXM, $10^{-7}$ M insulin (I), $10^{-7}$ M DXM + I, 10 µM/3 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 γ1 and γ2 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 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 γ2 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-1 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-1 mRNA (28). In the present study, we report the nutritional regulation of the human PPAR-1 mRNA and its potential dysregulation in obesity. We also assessed the expression of PPAR-1 in some key tissues for fuel homeostasis.

Although previous reports have shown expression of PPAR-1 mRNA in human tissues (13, 38) our results demonstrate that there are human homologues of both splice products, PPAR-1a and -1b, and these have a tissue distribution pattern similar to that of mouse and rat. Both mRNA splice variants, PPAR-1a and -1b 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 adipin and H-ALBP as probes in an RNase protection assay, we provided evidence that this muscle expression of PPAR-1 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-1 that we detect in muscle is due to adipocytes in muscle tissue that have different relative expression of PPAR-1/adipin or PPAR-1/H-FABP compared with adipocytes in fat tissue. Ultimately in situ hybridization may be required to confirm absolutely that PPAR-1 expression in muscle tissue is occurring in myocytes rather than adipocytes in and around muscle.

The physiologic relevance of the low level of PPAR-1 mRNA splice variants observed in muscle and liver remains to be determined. However it is possible that thiazolidinediones, high affinity ligands for PPAR-1, which possess adipogenic, insulin sensitizing, and antiangiogenic activity (39-41) might influence hepatic and muscle insulin resistance through the activation of these relatively nonabundant receptors. Similarly, the activation of PPAR-1 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-1a and -1b 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-1 in muscle may be capable of mediating some actions of TZD's in this tissue, it remains likely that activation of PPAR-1 in adipose tissue results in the generation of signals that affect insulin sensitivity in muscle and liver.

Our results clearly show that expression of PPAR-1 mRNA is increased in adipose tissue of obese men and women, and that the ratio of PPAR-1 mRNA 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-1 in muscle compared to fat. It is well established that PPAR-1 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 increased expression of PPAR-1 mRNA as well as an increased ratio -1/1 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-1 possesses a ligand-independent NH2-terminal activation domain, and this activity is greater for the NH2 terminus of PPAR-1 than PPAR-1 (Werman, A., and J.S. Flier, unpublished data). These data also raise the question of whether the increase in PPAR-1 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-1 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-1 mRNA is nutritionally regulated, but the rebound observed during the weight maintenance phase suggests a tendency to up-regulation of PPAR-1 independent of food intake or body weight that could be of pathogenic importance. However, the rebound in the expression of PPAR-1 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-1 and -1 are controlled by distinct promoter elements, it will be important to identify the factors that selectively result in increased PPAR-1 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 hyperglycemic 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-1 expression in cultured adipocytes have shown a synergistic effect of insulin and corticosteroids to induce PPAR-1 and -1 splice variants that is proportional to the BMI are increased expression in the pathogenesis of altered adipocyte number and function in obesity.
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