Regulation of PPAR γ Gene Expression by Nutrition and Obesity in Rodents

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Abstract

The orphan nuclear receptor, peroxisome proliferator-activated receptor (PPAR) γ, is implicated in mediating expression of fat-specific genes and in activating the program of adipocyte differentiation. The potential for regulation of PPAR γ gene expression in vivo is unknown. We cloned a partial mouse PPAR γ cDNA and developed an RNase protection assay that permits simultaneous quantitation of mRNAs for both γ1 and γ2 isoforms encoded by the PPAR γ gene. Probes for detection of adipocyte P2, the obese gene product, leptin, and 18S mRNAs were also employed. Both γ1 and γ2 mRNAs were abundantly expressed in adipose tissue. PPAR γ1 expression was also detected at lower levels in liver, spleen, and heart; whereas, γ1 and γ2 mRNA were expressed at low levels in skeletal muscle. Adipose tissue levels of γ1 and γ2 were not altered in two murine models of obesity (gold thioglucose and ob/ob), but were modestly increased in mice with toxigen-induced brown fat ablation uncoupling protein diphtheria toxin A mice. Fasting (12–48 h) was associated with an 80% fall in PPAR γ2 and a 50% fall in PPAR γ1 mRNA levels in adipose tissue. Western blot analysis demonstrated a marked effect of fasting to reduce PPAR γ protein levels in adipose tissue. Similar effects of fasting on PPAR γ mRNAs were noted in all three models of obesity. Insulin-deficient (streptozotocin) diabetes suppressed adipose tissue γ1 and γ2 expression by 75% in normal mice with partial restoration during insulin treatment. Levels of adipose tissue PPAR γ2 mRNA were increased by 50% in normal mice exposed to a high fat diet. In obese uncoupling protein diphertheria toxin A mice, high fat feeding resulted in de novo induction of PPAR γ2 expression in liver. We conclude (a) PPAR γ2 mRNA expression is most abundant in adipocytes in normal mice, but lower level expression is seen in skeletal muscle; (b) expression of adipose tissue γ1 or γ2 mRNAs is increased in only one of the three models of obesity; (c) PPAR γ1 and γ2 expression is downregulated by fasting and insulin-deficient diabetes; and (d) exposure of mice to a high fat diet increases adipose tissue expression of PPAR γ (in normal mice) and induces PPAR γ2 mRNA expression in liver (in obese mice). These findings demonstrate in vivo modulation of PPAR γ mRNA levels over a fourfold range and provide an additional level of regulation for the control of adipocyte development and function. (J. Clin. Invest. 1996. 97:2553–2561.) Key words: peroxisome proliferator-activated receptor • obesity • nutrition • insulin • gene regulation

Introduction

Obesity, defined as a state of pathologically increased adipose cell mass (1), is a major health problem in wealthy societies and is associated with increased risk of developing diabetes, hypertension, and hyperlipidemia. Increased mortality (2) is not only associated with morbid obesity as recent reports have shown that modestly increased body weight is an important determinant of mortality among middle-aged women (3). Although the adipocyte has previously been viewed as a passive participant in the generation of obesity, this cell has recently been recognized as having a more active role in the regulation of energy homeostasis and body composition (1). Thus there is rapidly advancing knowledge about the mechanisms that direct adipocyte differentiation and adipocyte-specific gene expression. Studies of the proximal promoter region of the adipocyte P2 (aP2) gene (which encodes an intracellular lipid-binding protein specifically expressed in adipocytes), have identified binding sites for transcription factors AP-1 and CCAAT/enhancer binding protein (4–8). These factors, especially CCAAT/ enhancer binding protein, appear to be involved in terminal adipocyte differentiation, but may not have a specific role in the initiation of the adipocyte program or tissue specificity. Analysis of another region located 5.4 kb upstream from the aP2 transcriptional start site revealed a 500-bp sequence which was shown to mediate differentiation-dependent and tissue-specific aP2 expression (9–11). Included in this region are enhancers, adipocyte regulatory elements-6 and -7, that contain imperfect versions of a DR-1 consensus sequence that is known to bind to orphan nuclear receptors in the peroxisome proliferator-activated receptor (PPAR) family. Three PPAR genes, PPAR α (12), PPAR δ (NUC1) (13), and PPAR γ (9, 14), encode different members of this family of orphan receptors.

Recently, Tontonoz et al. (11) cloned a binding factor for adipocyte regulatory element-6 and showed it to be an isoform of PPAR γ (PPAR γ2). Forced expression of PPAR γ2 or γ1 in fibroblasts was sufficient to drive the determination of an adipocyte cellular lineage (11). Furthermore, ectopic expression of PPAR γ and CCAAT/enhancer binding protein in cultured myoblasts results in a switch from myogenesis to adiposo-

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1. Abbreviations used in this paper: aP2, adipocyte P2; GTG, gold thioglucose; PPAR, peroxisome proliferator-activated receptor; STZ, Streptozotocin; UCP-DTA, uncoupling protein diphertheria toxin A.
mRNAs in a variety of physiologic and pathophysiologic states.

Although PPAR-γ2 mRNA is expressed very early in the course of adipocyte differentiation in vitro (9, 17), nothing is known about the physiologic regulation of PPAR-γ gene expression in vivo. Given the key role that PPAR-γ seems to play in adipose tissue differentiation, it is critical that physiological regulation of PPAR-γ gene expression or its potential dysregulation in altered physiologic states, including obesity, be characterized. The results of this study address this issue by quantitation of the in vivo expression of both PPAR-γ1 and γ2 mRNAs in a variety of physiologic and pathophysiologic states in rodents.

Methods

Animals: experimental protocols
Care of mice was according to institutional guidelines. Mice were weaned at age 19 d and housed in conventional cages. They were maintained with a 12-h light/12-h dark photoperiod in a humidity- and temperature-controlled room (24°C). Water and food were available ad libitum, except when noted.

For experiments performed with normal (lean) mice alone, a single inbred strain was employed (FVB-N; Taconic Farms Inc., Germantown, NY). Three different mouse models of genetic or acquired forms of obesity were also studied. Mice with the ob/ob genotype, mice on the C57BL/6J background, and lean controls were obtained from The Jackson Laboratories (Bar Harbor, ME). Gold-thioglucose (GTG) mice that were generated by double injection of gold thioglucose administered to 3-wk-old male Swiss-Albino mice (18) (body wt 67.6±0.678 g) and untreated (lean) Swiss-Albino mice (body wt 39.80±2 R 0.202 g) were kindly provided by Y. Le Marchand-Brustel, Institut National de la Santé et de la Recherche Médicale, Nice, France.

Obese transgenic FVB-N mice with brown adipose tissue deficiency, uncoupling protein dipeptidase toxin A (UCP-DTA), were generated by brown fat ablation via toxigene expression as previously described (19). Matched lean littermate controls were studied in parallel.

Unless otherwise noted, mice received a regular chow diet (Amway chow 5008;Ralston Purina Co., St. Louis, MO) containing 6.5% (wt/wt) fat (17.3% of calories), 47% (wt/wt) carbohydrates (51% of calories) and 23.5 (wt/wt) protein (27.6% of calories). A group of UCP-DTA mice and age-sex-matched lean FVB-N mice were fed the regular chow diet or a diet with higher fat content (Adjusted Calories Western-Type diet 88137; Teckell Premier Laboratory Diets, Madison, WI), containing 21% (wt/wt) fat (anhydrous milk fat, 40.8% of calories), 42.9% (wt/wt) carbohydrate (70% of which was sucrose, 42.2% of calories), and 19.8% (wt/wt) protein (17% of calories) beginning at age 19 d for a period up until age 12 wk. Detailed characterization of these animals was previously published (20). The effects of fasting and refeeding were studied using normal FVB-N mice or obese mice (UCP-DTA, ob/ob, and GTG) with corresponding matched lean controls. For these studies, mice were deprived of chow for the indicated time periods but were allowed ad libitum access to water.

For the induction of insulin-deficient diabetes mellitus, Streptozotocin (STZ, Zanosar®; The Upjohn Co., Kalama, MI) was administered via intraperitoneal injection (170–180 mg/kg in 0.9% NaCl) to normal male FVB-N mice (12 wk). Diabetic mice (age-matched controls) were studied 6 d after STZ treatment. A separate group of STZ-diabetic animals received treatment with insulin (1.5 U of NPH, Eli Lilly and Co., Indianapolis, IN) twice a day, beginning 15 d after STZ administration to up to 13 d until glucose levels were normalized.

Tissues and RNA extraction
Perigonadal fat pads and other tissues were surgically dissected, rapidly removed, snap frozen in liquid nitrogen, and stored at −80°C until processed. For preparation of isolated mouse adipocytes, freshly obtained perigonadal fat was minced and digested with collagenase. Adipocytes (supernatant) were separated from stromal-vascular cells (pellet) by centrifugation. Cultured 3T3-F442A adipocytes incubated with and without insulin (5 μg/ml) were prepared as previously described (21). Total RNA was extracted using the method of Chomczynski and Sacchi (22). Total RNA from rat liver and hepatic Ito cells was kindly supplied by Scott Friedman (University of California, San Francisco, CA). RNA was quantified by absorbance at 260 nm in a spectrophotometer (Lambda 2; Perkin-Elmer Spectrometer, South Plainfield, NJ) and its integrity was assessed after electrophoresis in nondenaturing 1% agarose gels by ethidium bromide staining. 15-μg aliquots of adipose tissue RNA were used for determination of PPAR-γ and ob gene mRNA expression. Two aliquots (200 ng each) were removed from each sample for measurement in 18S and aP2 internal control assays. 30-μg aliquots of skeletal-muscle and heart RNA were used for determination of PPAR-γ, 10-μg aliquots of skeletal-muscle and heart total RNA were used for determination of aP2. 200-ng aliquots were removed from each sample for measurement in 18S and aP2 (adipose tissue) internal control assays. 25-μg aliquots of total RNA from liver were used for measurement of PPAR-γ, ob, and aP2 mRNA. 200-ng aliquots were used in the 18S assay. RNA aliquots of 20 μg, 15 μg, and 200 ng were used for the tissue distribution study.

RNA analysis
The solution-hybridization nuclease protection method was used because it offers the highest specificity and quantitative accuracy and is the most suitable method for multiplex mRNA analysis and use with a large number of samples (23).

Specific probe templates. A partial PPAR-γ cDNA probe was generated by reverse transcriptase-PCR using total RNA from mouse fat as follows: two primers (5’-AACCGAGGATTTGAACACTGCGGAGATCTCC-3’ and 5’GGGTTACGCAACCACTTGGTTCGGCTCCT-3’) were designed to amplify a region that includes 90 bp of the 3’ end of the PPAR-γ transcript and 185 bp common to both PPAR-γ1 and PPAR-γ2. The PPAR-γ cDNA PCR-product was ligated into the Pst-I and EcoRI restriction sites of the PGEM-3 (2) cloning vector polylinker. A linearized template for the antisense probe and sense RNA reference was prepared using HindIII and EcoRI, respectively. Use of the antisense probe in an RNase protection assay permits quantitation of PPAR-γ mRNA transcripts as protected bands of either 273 bp (PPAR-γ2) or 185 bp (PPAR-γ1). The mouse ob probe was prepared from a previously described cDNA clone (24). A 290 bp Pst-I/HindIII cDNA fragment spanning the region from base 110 to 400 in the published sequence (25) was cloned into the pBluescript KS cloning vector polylinker. Template for the antisense probe was prepared by linearizing the vector with HindIII; template for reference RNA was prepared using XbaI. A 450-bp fragment of the mouse aP2 cDNA (gift of Bruce Spiegelman, Dana Farber Cancer Institute, Boston) was subcloned into pBluescript SK. Template for the antisense probe was obtained using XbaI; template for sense RNA reference was obtained using Sall. A 75 bp cDNA corresponding to 185 ribosomal RNA (cloned into pBluescript KS, gift of M. Jakubowski, Beth Israel Hospital, Boston, MA) was used as an internal control to correct for variations in RNA amount. The 18S ribosomal fraction was chosen because it is unlikely to fluctuate under conditions that affect the expression of mRNAs, since it is transcribed by a distinct polymerase. For sense template, the vector was cut with EcoRI and the antisense RNA (cloned into pBluescript KS, gift of M. Jakubowski, Beth Israel Hospital, Boston, MA) was used as an internal control to correct for variations in RNA amount.
Protein analysis

Production of PPAR γ antibody. The entire coding region of the PPAR γ was obtained by PCR using oligonucleotides 5’ATGAGC- TCAATGGGTAAAC3’ and 5’ATCTCGAGCTAATACAAGTC3’. Restriction sites SstI and XhoI were incorporated at the 5’ and 3’ ends, respectively, and ligated into the polyethylene terephthalate-30a vector (Novagen, Inc., Madison, WI). The resulting polyethylene terephthalates-PPARγ2 was used to generate 5 mg of His-tagged PPAR γ2 as described by Novagen instruction. Purified His-PPAR γ2 was used to generate a rabbit polyclonal antibody (Research Genetics, Rockville, MD).

SDS-PAGE analysis and Immunoblotting. Mouse fat pads were frozen in liquid nitrogen and stored at −80°C until processed. Protein extracts were obtained after complete polytron homogenization in extraction buffer and 10,000 rpm centrifugation at 4°C for 30 min. The extraction buffer was a solution of PBS, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS. Protease inhibitors were added fresh (100 μg/ml PMSF, 5 μg/ml pepstatin, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 5 μg/ml E64, 10 μg/ml calpain inhibitor-1, 100 mM sodium orthovanadate, and 100 mM sodium fluoride). Protein concentration was assessed by biocinchonic acid technique (Pierce, Rockford, IL). Proteins were separated via SDS-PAGE, electroblotted onto polyvinylidifluoride membranes (Millipore Corp., Bedford, MA), and immunoblotted using the PPAR γ2 antibody.

A modified SDS-PAGE protocol was used to obtain better resolution.

Figure 1. PPAR γ mRNA expression in mouse tissues. RNase protection was used to assess the expression of PPAR γ1 and γ2 mRNAs in mouse adipose tissue as well as several other tissues derived from normal FVB mice. Total RNA (15μg samples) from isolated adipocytes (cells) or the stromal-vascular cellular fraction (pellet) of epididymal fat pads was analyzed as depicted in the left panel. The right panel depicts the expression pattern of PPAR γ in other tissues: gut and ovary (10 μg RNA, each), liver (20 μg), skeletal muscle (10 and 25 μg), lung (10 μg), and heart muscle (10 and 25 μg). Protected bands of 273 bases correspond to PPAR γ1, bands of 185 bases correspond to PPAR γ2.

Table 1. Summary of mRNA expression in mouse tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>PPAR γ1</th>
<th>PPAR γ2</th>
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<tbody>
<tr>
<td>Adipose tissue</td>
<td>1.2 ± 0.5</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td>Gut</td>
<td>0.8 ± 0.3</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Liver</td>
<td>0.9 ± 0.4</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>1.5 ± 0.8</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>Lung</td>
<td>0.7 ± 0.3</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Heart</td>
<td>0.9 ± 0.4</td>
<td>0.6 ± 0.3</td>
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Results

Tissue distribution of PPAR γ isoform expression

We developed a sensitive RNase protection assay that allows for precise and specific quantitation of mRNAs encoding PPAR γ2 and γ1 isoforms. When we examined RNA extracted from mouse adipose tissue, similar amounts of both PPAR γ2 and PPAR γ1 mRNAs were detected. To examine which isoform(s) were expressed in adipocytes per se, we analyzed RNA extracted from both isolated mouse adipocytes in mice with three forms of obesity. Expression of PPAR γ1 and γ2 mRNAs in mouse white fat RNA (epididymal fat pads) was assessed in mice with three forms of obesity (ob/ob, n = 4, GTG, n = 3, UCP-DTA, n = 5) in the fed state. Results are expressed as the mean ± SEM percentage of mean values obtained using adipose tissue RNA derived from equal numbers of the appropriate age-sex-matched lean control mice. *UCP-DTA vs control, P < 0.05.
and residual stromal-vascular cells present in the pellet after adipocyte isolation. As shown in Fig. 1, the stromal-vascular fraction contained only PPAR γ mRNA, whereas mature adipocytes expressed similar high levels of both PPAR γ1 and PPAR γ2 mRNAs.

In comparison with levels in white adipose tissue, both PPAR γ1 and γ2 mRNAs were expressed at similar levels in brown adipose tissue; both isoforms were also detected in RNA from the adrenal gland (not shown). PPAR γ1 mRNA was expressed in spleen and also at low levels in liver, skeletal muscle, and heart. A less intense PPAR γ2 mRNA signal (13% of that seen in adipose tissue) was also detected in skeletal muscle (Fig. 1). To determine whether the PPAR γ2 signal in skeletal muscle was entirely the result of contamination with fat cells, 10-μg aliquots of skeletal muscle and heart total RNA were assayed for aP2 mRNA expression. Very low levels of aP2 mRNA were detected in both tissues. Levels in heart (a tissue that does not have PPAR γ2 mRNA expression) were higher than in skeletal muscle. If the PPAR γ2 signal detected in 30 μg of muscle mRNA was entirely due to fat contamination, 4.1 μg of adipose tissue mRNA would be needed. This amount of adipose tissue generates a much more intense signal of aP2 mRNA than we detected. These data strongly suggest that PPAR γ2 mRNA is expressed to some degree in skeletal muscle, although considerably less than is seen in fat.

PPAR γ expression in rodent models of obesity

To explore the hypothesis that obesity might be associated with abnormal adipose tissue expression of PPAR γ, we compared three different rodent models that represent genetic (ob/ob), acquired (GTG), and transgenic (UCP-DTA) forms of obesity with their lean controls (also matched for age and sex). The protected RNA bands corresponding to both PPAR γ1 and PPAR γ2 transcripts were quantitated, normalized for mi-
nor differences in RNA input (18S RNA), and plotted against standard curves generated using known amounts of target PPAR γ RNA. When the data derived from obese mice were expressed as a percentage of values obtained using the corresponding lean controls, no differences in the amount of either PPAR γ isoform mRNAs were detected in ob/ob or GTG obese mice (Fig. 2). In contrast, UCP-DTA mice showed a modest increase in expression of PPAR γ1 and γ2 mRNA (57% and 59%, p = 0.023 and p = 0.0158, respectively) compared to lean FVB controls. Thus, when expressed relative to total RNA amount, obesity is not associated with substantial changes in adipocyte PPAR γ gene expression.

**Effect of fasting and refeeding on PPARγ expression**

As shown in Fig. 3 A, initial results obtained with female FVB mice that were food deprived for 24 h indicated that the expression of PPAR γ2 in adipose tissue was markedly reduced with a more modest decrease in PPAR γ1 expression. As expected, levels of 18S RNA (per microgram of total RNA) remained constant. In a second series of experiments that were conducted using male FVB mice, we observed that fasting was associated with a progressive decline in PPAR γ2 expression, reaching a maximum 82% reduction after 48 h (Fig. 3 C). PPAR γ1 mRNA levels were reduced by 60% after 48 h of fasting. After 24 h of refeeding, PPAR γ2 mRNA levels were partially restored to normal. Immunoblotting analysis of adipose tissue protein extracts using a PPAR γ2 antibody supported the RNA data. Thus, fasting was associated with a marked decrease in PPAR γ2 protein levels (Fig. 3 B).

To determine the extent of changes in PPAR γ gene expression induced by fasting relative to changes in the expression of other fat-specific genes, RNase protection analysis of ob and aP2 mRNA expression in adipose tissue was performed using the same RNA samples used for PPAR γ analysis. As shown in Fig. 3 D, ob mRNA levels were dramatically reduced by fasting and partially restored by refeeding, following a pattern similar to that for PPAR γ2. The specificity of these changes was confirmed since aP2 mRNA levels were unaffected under these experimental conditions (not shown). These findings indicate that both PPAR γ2 and ob mRNA expression are tightly regulated in parallel by in vivo nutritional deprivation.

In addition, we assessed the effects of fasting (24 h) on PPAR γ2 mRNA expression in adipose tissue from the three obese mouse models. Fasting was associated with significantly reduced PPAR γ2 mRNA expression in both ob/ob and GTG mice (Fig. 3 E). A similar trend was evident in UCP-DTA mice as well. These results suggest that regulation of PPAR γ gene expression by fasting is preserved in obesity.

We evaluated whether similar regulation of PPAR γ mRNA expression was observed in skeletal muscle or heart. No differences were observed in the expression of PPAR γ1 mRNA in heart or skeletal muscle during fasting or refeeding compared to the fed state (data not shown). In contrast, PPAR γ2 mRNA in skeletal muscle showed nutritional regulation, modestly decreasing after 48 h fasting and increasing with refeeding (25% fall, P = 0.05).

**Effects of insulin on PPAR γ gene expression: the insulin-deficient streptozotocin model**

Normal FVB mice were treated with STZ to induce insulin-deficient diabetes mellitus. The mean value of plasma glucose levels (fed) in untreated control mice was 179.8 mg/dl. The mean glucose level in STZ-treated mice was 314.5 mg/dl. More extreme hyperglycemia (longer term diabetes or higher dose STZ) was associated with a marked decrease in fat pad size, which precluded RNA extraction. Several mice with longer term diabetes received insulin therapy to restore glucose levels to normal (mean 108 mg/dl). Expression of PPAR γ1 and γ2 mRNAs in adipose tissue was examined by RNase protection assay and quantitated by phosphoimaging technology. As shown in Fig. 4, diabetes was associated with an 80% reduction in the level of expression of PPAR γ2. There was a less pronounced (60–70%) decline in the level of PPAR γ1 mRNA levels (P < 0.05, not shown). Insulin administration restored expression of PPAR γ1 and γ2 mRNAs to levels not significantly different from those seen in the nondiabetic animals. These data demonstrate that in the insulinopenic diabetic state, there is a reduction in the expression of both isoforms of PPAR γ and that PPAR γ mRNA levels can be restored to normal by insulin replacement.

To further characterize the effect of insulin on PPAR γ gene expression, we examined the levels of γ1 and γ2 mRNAs in fully differentiated 3T3F442A adipocytes cultured in media with or without 5 μg/ml insulin for 21 d. Cells incubated with insulin showed a 2.0- and 1.7-fold increase in the expression of PPAR γ2 (P < 0.01) and PPAR γ1 (P < 0.05), respectively (data not shown). Taken together, the results described above indicate that insulin could have a key role as a specific stimulus of PPAR γ gene expression in adipose cells.

**High fat vs low fat diet**

**Effect of a high fat diet on PPAR γ expression in adipose tissue** (Fig. 5). The PPAR γ isoform is known to be activated by fatty acids (27, 28). PPAR γ has also been suggested to be activated by certain polyunsaturated fatty acids (11). In addition to binding and transactivation, which might potentially be mediated by lipids, we sought to determine whether dietary fat might regulate PPAR γ at the level of gene expression. To address this issue, we studied both normal and obese (UCP-DTA) FVB mice that had received either regular chow (17% calories from fat) or a diet high in fat (41% of calories) from age 19 d to 12 wk. RNase protection analysis revealed that exposure of normal FVB mice to the high fat diet resulted in a modest increase in both PPAR γ1 (33%, P < 0.05) and PPAR γ2 expression by STZ diabetestes and insulin. Three groups of male FVB mice were compared: normal FVB mice (control, n = 5), mice rendered diabetic by treatment with streptozocin (STZ-diabetes, n = 8), and STZ-diabetic mice which had received insulin for several days to normalize plasma-glucose levels (STZ-diabetes + insulin, n = 4). PPAR γ2 mRNA expression in epididymal fat pads was assessed. Results are expressed as amol PPAR γ2 mRNA/μg 18S RNA. *diabetic vs control, P = 0.018; **insulin treatment vs STZ-diabetes, P = 0.024.
of dietary fat (Fig. 5A). The levels detected in liver were estimated to be 0.1% of aP2 mRNA levels in white fat (Fig. 6B). These findings exclude the possibility that liver RNA from high fat-fed obese mice was contaminated with adipose tissue RNA, and confirm the specificity of the PPAR \( \gamma_2 \) mRNA induction in obese mouse liver by the high fat diet.

**Discussion**

The PPAR family of nuclear receptors is comprised of three distinct isoforms (\( \alpha, \delta, \gamma \)) with different and specific tissue distribution. PPAR \( \alpha \) is expressed predominantly in tissues that undergo peroxisomal proliferation such as liver, kidney, and heart and is activated by several hypolipidemic drugs and fatty acids, resulting in induction of the expression of enzymes involved in peroxisomal fatty acid oxidation (27, 29, 30). PPAR \( \delta \) is expressed ubiquitously, is activated by fatty acids, and has no known specific ligand(s) (31). Although it has been suggested that PPAR \( \delta \) could play an important role in the adipogenic effect of overfeeding (32), no clear physiologic function has been described (13). PPAR \( \gamma \) is a relatively new member of the PPAR family that was recently suggested to have a key role in the regulation of gene expression in adipose tissue. Thus, PPAR \( \gamma \) has been implicated in mediating the expression of fat-specific genes and in activating the program of adipocyte differentiation in vitro (9, 11). Consequently, activation of PPAR \( \gamma \) could constitute an important part of the molecular mechanism behind the adipogenic effect of overfeeding. In overexpression studies, it has most recently been shown that PPAR \( \gamma \) has the greatest adipogenic action, that PPAR \( \alpha \) also has some ability to stimulate adipogenesis, but PPAR \( \delta \) has no such ability even when activated with multiple compounds (33). Since PPAR \( \gamma \) is the most important member of this family for regulation of adipogenesis, the aim of the present work was to determine the physiological regulation of PPAR \( \gamma \) gene expression in vivo and its potential dysregulation in altered physiologic states including obesity and insulin-deficient streptozocin-induced diabetes.

Analysis of tissue distribution revealed that both PPAR \( \gamma_1 \) and \( \gamma_2 \) mRNAs were abundantly expressed in white and
brown adipose tissue. When the adipose tissue was analyzed as separate fractions, mRNA corresponding to both isoforms was abundantly present in mature isolated adipocytes. PPAR γ2 was not detected in the stromal-vascular fraction from adipose tissue, although high levels of PPAR-γ1 mRNA were present. These data clearly show that not only is PPAR γ2 expressed in adipose cells, but the PPAR-γ1 isoform is also detected in substantial amounts. This differs somewhat from the observation of Tontonoz et al. (11) that PPAR γ2 is the only isoform expressed in cultured adipocytes, perhaps representing a difference between tissues and cultured cells. Recent data reported by Tontonoz et al. showed that transfection of a truncated form of PPAR γ2 that lacks 127–NH₂-terminal amino acids (retaining the DNA and ligand binding domains) mediates adipogenic differentiation of fibroblasts at even a higher rate than wild-type PPAR γ2 and furthermore, overexpression studies indicate that both PPAR γ2 and PPAR γ1 are competent to stimulate adipogenesis (11). Taken together, these observations suggest that both PPAR γ2 and PPAR γ1 might play an important role in the initiation or determination of the adipocyte program in vivo. The physiologic relevance of low level PPAR-γ1 mRNA expression in muscle, heart, liver, spleen, and kidney remains to be determined. However, some recent evidence suggests that thiazolidinediones, a new class of insulin sensitizers with proven antidiabetic activity, improve hepatic (34) and muscle (35) insulin resistance. Recent reports also show that thiazolidinedione derivatives are high affinity ligands for PPAR γ (36). Therefore, the insulin sensitizing effect of thiazolidinediones in muscle and liver may be due to activation of the PPAR γ1 and γ2 isoforms that we have detected in these tissues. Similarly, the activation of PPAR γ1 in muscle and liver by its endogenous ligand could have an important role in maintaining the insulin sensitivity of these tissues.

Adipose tissue levels of PPAR γ1 and γ2 mRNA were not altered in the ob/ob mice or in GTG-induced obesity, and were only modestly increased in obese brown adipose tissue-deficient UCP-DTA mice. These findings suggest that the increased adiposity characteristic of these animal models is not likely to result from altered PPAR γ gene expression. On the other hand, the contribution of new fat cell formation to obesity (adipocyte hyperplasia) may well require the presence of PPAR γ. This role could readily be subserved by PPAR-γ1 expressed in preadipocytes or adipocyte precursors since the stromal-vascular fraction of adipose tissue is known to contain these cells (37). To the extent that micromolar fatty acid concentrations may activate PPAR-γ (31), a vicious cycle might ensue whereby obesity promotes increases in free fatty acid levels, which lead to further adipogenesis via PPAR γ transactivation.

In contrast to the relative lack of altered PPAR γ expression associated with obesity, fasting provoked a substantial decrease in the levels of both PPAR γ1 and γ2 mRNAs and corresponding protein levels in adipose tissue from normal mice. Similar effects of fasting on PPAR γ mRNAs were noted in all three models of obesity. Thus, PPAR γ expression is physiologically regulated by food intake and this regulation is conserved in the obese animal models that we studied. It is important to note that aP2 mRNA expression was not significantly altered by fasting. This finding indicates that effects on PPAR γ mRNA levels do not reflect a generalized decrease in fat tissue gene expression. Given that PPAR γ2 is likely to play a prominent role in the upregulation of aP2 expression, which occurs during adipocyte differentiation (9), the lack of change in aP2 mRNA at a time when PPAR γ2 mRNA and PPAR γ protein levels have decreased by severalfold is somewhat surprising. PPAR γ is likely to modulate the expression of many other genes in adipose tissue, although the only other known example is phosphoenolpyruvate carboxykinase which mediates glycerol synthesis (38). It is plausible that PPAR γ-responsive genes that influence metabolism within the fat cell (such as phosphoenolpyruvate carboxykinase) may be regulated not only by changes in the circulating or intracellular levels of potential PPAR γ ligands, but also by changes in the levels of PPAR γ expression such as we observed with fasting.

Regulation similar to that seen with fasting was also observed in the insulin-deficient (streptozotocin) diabetic mice, where adipose tissue shows suppressed PPAR γ1 and γ2 mRNA expression with partial restoration during insulin treatment. Furthermore, insulin stimulation of 3T3F442A adipocytes induced an increase in the expression of PPAR-γ1 and γ2 mRNAs. These data suggest that insulin, a well known adipogenic factor, could directly or indirectly (21) induce PPAR γ expression in adipose tissue. The effect of insulin on another PPAR family member is apparently different. Thus, insulin has been demonstrated to have an inhibitory effect on PPAR-α mRNA expression in liver. In the absence of insulin, PPAR α is increased (39), activating peroxisomal β oxidation (30) and an alternative pathway for the use of fatty acids, involved in ketogenesis (40). These data together suggest that insulin could be an important hormone regulating different pathways for the utilization of fatty acids through the activation or inactivation of different PPAR family members in different tissues. In the fed state, insulin would stimulate the expression of PPAR γ in adipose tissue at the same time that it would tend to inhibit hepatic expression of PPAR α. In the fasted and insulin-deficient diabetic states, expression of PPAR γ in adipose tissue would be downregulated, while in liver, PPAR α would induce the expression of enzymes involved in fatty acid oxidation.

Interestingly, levels of leptin mRNA appeared to parallel the decline in PPAR γ gene expression during fasting. Furthermore, Frederich et al. (24) and MacDougald et al. (41) have demonstrated that leptin expression is markedly downregulated during insulin deficient diabetes. In addition, leptin mRNA levels are upregulated in vivo under hyperinsulinemic-euglycemic clamp conditions and may also be directly stimulated by insulin in vitro using isolated adipocytes (42). These observations suggest that changes in the level of PPAR γ expression could cause or contribute to the regulation of leptin expression, or that both genes are responding to a common pathway or transacting factor(s). Further studies will be required to address the hypothesis that the ob gene promoter may be regulated by PPAR γ.

The effects of high fat feeding were studied in both control and obese UCP-DTA mice. We observed that the PPAR γ2 mRNA in adipose tissue was modestly induced in fat-fed control mice to levels that were similar to obese mice on a normal diet. On the other hand, no further increase in adipose tissue PPAR γ expression was evident in fat-fed obese mice. Interestingly, these results appear to correlate with relative differences in fat cell size: FVB mice on the chow diet have mean adipocyte sizes of 0.4 μg lipid per cell, and in fat-fed normal FVB mice, mean adipocyte size is increased to 0.73 μg/cell. This increased
size is similar to mean values obtained with chow-fed UCP-DTA mice (0.8 µg/cell)(U. Frevert and B.B. Kahn, unpublished observations). Therefore, it is reasonable to speculate that PPARγ gene expression might be stimulated by increased cellular lipid content until a determined threshold is reached. This model of saturable metabolic regulation has been observed with other members of the PPAR family (43). Thus, it is possible that other factors may act to prevent further induction of PPARγ mRNA in the obese mice. Indeed, we previously observed that high fat feeding of obese mice causes a substantial increase in TNF α mRNA expression (20). This may serve as an adaptive mechanism to slow down the further accumulation of fat stores since TNF α is known to inhibit adipogenesis in vitro (44, 45). Further studies will be required to address the hypothesis that PPARγ gene expression could be inhibited through a TNF-mediated mechanism.

Most striking was the finding that high fat feeding results in de novo expression of PPARγ2 mRNA in liver with no apparent increase in low levels of PPARγ1 mRNA detected in this tissue. Since alternative promoters direct the synthesis of PPARγ1 vs γ2 mRNA synthesis (16), these data suggest that markedly increased levels of circulating fatty acids (which occur in this physiologic context) may serve to specifically activate the γ2 promoter in a tissue not normally expressing this isoform. Under these circumstances, we failed to detect expression of leptin mRNA and there was no change in trace levels of aP2 mRNA. Therefore, the level of PPARγ expression achieved in the liver of fat-fed obese mice (which was approximately 10% of that in white adipose tissue) was not sufficient to initiate the program of adipocyte differentiation.

In summary, we used a sensitive RNase protection assay to detect and quantitate levels of PPARγ1 and γ2 in mice. Levels of adipose tissue PPARγ1 or γ2 mRNAs were not affected by obesity in the ob and GTG animal models. However, ablation of brown adipose tissue was associated with a modest increase in PPARγ mRNA expression associated with marked obesity. The expression of adipose tissue PPARγ1 and γ2 mRNA and PPARγ protein were downregulated by fasting and insulin-deficient diabetes (along with leptin expression). Exposure of normal mice to a high fat diet resulted in the modest induction of PPARγ2 mRNA and enlargement of adipose cells to an extent that was comparable to chow-fed obese mice. Administration of a high fat diet to UCP-DTA obese mice did not induce a further increase in adipose tissue PPARγ expression, but it resulted in specific induction of hepatic PPARγ2 mRNA expression. These results suggest that in vivo modulation of PPARγ gene expression provides an additional level of regulation for the control of adipocyte development and function.

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