Coordinate Regulation of the Expression of the Fatty Acid Transport Protein and Acyl-CoA Synthetase Genes by PPARα and PPARγ Activators

Geneviève Martin, Kristina Schoonjans, Anne-Marie Lefebvre, Bart Staels, and Johan Auwerx

From the U.325 INSERM, Département d’Atherosclérose, Institut Pasteur, 59019 Lille, France

Intracellular fatty acid (FA) concentrations are in part determined by a regulated import/export system that is controlled by two key proteins, i.e., fatty acid transport protein (FATP) and acyl-CoA synthetase (ACS), which respectively facilitate the transport of FAs across the cell membrane and their esterification to prevent their efflux. The aim of this investigation was to analyze the expression pattern of FATP and ACS and to determine whether their expression was altered by agents that affect FA metabolism through the activation of peroxisome proliferator-activated receptors (PPAR) such as the fibrates and thiazolidinediones. PPAR mRNA was ubiquitously expressed, with highest levels being detected in adipose tissue, heart, brain, and testis. Fibrate treatment, which is known to preferentially activate PPARα, induced FATP mRNA levels in rat liver and intestine and induced ACS mRNA levels in liver and kidney. The antidiabetic thiazolidinedione BRL 49653, which is a high-affinity ligand for the adipocyte-specific PPARγ form, caused a small induction of muscle but a robust induction of adipose tissue FATP mRNA levels. BRL 49653 did not affect liver FATP and had a tendency to decrease heart FATP mRNA levels. ACS mRNA levels in general showed a similar pattern after BRL 49653 as FATP except for the muscle where ACS mRNA was induced. This regulation of FATP and ACS expression by PPAR activators was shown to be at the transcriptional level and could also be reproduced in vitro in cell culture systems. In the hepatocyte cell lines AML-12 or Fa 32, fenofibric acid, but not BRL 49653, induced FATP and ACS mRNA levels, whereas in the 3T3-L1 preadipocyte cell line, the PPARγ ligand induced FATP and ACS mRNA levels quicker than fenofibric acid. Inducibility of ACS and FATP mRNA by PPARα or γ activators correlated with the tissue-specific distribution of the respective PPARs and was furthermore associated with a concomitant increase in FA uptake. Most interestingly, thiazolidinedione antiabetic agents seem to favor adipocyte-specific FA uptake relative to muscle, perhaps underlying in part the beneficial effects of these agents on insulin-mediated glucose disposal.

Transmembrane transport of FAs is still poorly understood despite intense investigation. Uncharged molecules and weak acids such as fatty acids can cross membranes rapidly thanks to their lipid solubility. The rate of movement is controlled by mass action and can be enhanced by proteins such as fatty acid-binding protein that act as a cytoplasmic “sink.” Recently, however, several studies provided evidence that in addition to these nonfacilitated systems, facilitated transport also contributes to FA transport (1). Several proteins were hypothesized to be acting as FA transporters. Among these, three deserve further attention. First, plasma membrane fatty acid-binding protein, a protein related to the mitochondrial isoform of aspartate aminotransferase, has been suggested to increase FA uptake in cells (2). Since this protein has not yet been cloned, it is difficult to determine its exact role in FA transport processes. The second protein, fatty acid translocase is an 88-kDa membrane protein that has been cloned in mouse and is homologous to the human CD36 cell surface antigen (3). Although CD36 has been shown to bind FAs and might be involved in signal transduction after binding of a specific ligand (long chain fatty acids), it is until now not clear whether it is a transport protein. The only candidate for a long chain FA transporter for which functionality has been directly demonstrated is the fatty acid transport protein (FATP) (4). FATP is a 63-kDa plasma membrane protein with six predicted membrane-spanning domains that has been cloned using a functional expression cloning technique. It increased oleic acid uptake in FATP-transfected 3T3-L1 cells by >3-fold. Interestingly FATP is suggested to act in concert with acyl-CoA synthetase (ACS), an enzyme that prevents efflux of the incorporated fatty acids by their conversion into acyl-CoA derivatives and hence rendering the FA uptake process unidirectional. Furthermore, FATP shows a limited region of homology at the protein level (11 amino acids), with ACS shown to bind FAs and might be involved in signal transduction after binding of a specific ligand (long chain fatty acids), it is until now not clear whether it is a transport protein. The only candidate for a long chain FA transporter for which functionality has been directly demonstrated is the fatty acid transport protein (FATP) (4). FATP is a 63-kDa plasma membrane protein with six predicted membrane-spanning domains that has been cloned using a functional expression cloning technique. It increased oleic acid uptake in FATP-transfected 3T3-L1 cells by >3-fold. Interestingly FATP is suggested to act in concert with acyl-CoA synthetase (ACS), an enzyme that prevents efflux of the incorporated fatty acids by their conversion into acyl-CoA derivatives and hence rendering the FA uptake process unidirectional. Furthermore, FATP shows a limited region of homology at the protein level (11 amino acids), with ACS leading to the hypothesis that this common region might reflect a common function, such as a binding site (4). These 11 amino acids residues have also been found to be conserved in the rat and the yeast FATP homologues (5).

Several aspects of intracellular lipid and FA metabolism in cells are subjected to transcriptional control by the peroxisome proliferator-activated receptor (PPAR) family. PPARs are members of the superfamily of nuclear hormone receptors that function as ligand-dependent transcription factors. Three receptor subtypes of PPAR termed α, δ (or β), and γ, have been identified (6–15). These receptors heterodimerize with the retinoid X receptor and alter the transcription of target genes after binding to peroxisome proliferator response elements (PPREs), which consist of a hexameric nucleotide direct repeat of the recognition motif (TGACCT) spaced by 1 nucleotide

The abbreviations used are: FA, fatty acid; FATP, FA transport protein; ACS, acyl-CoA synthetase; PPAR, peroxisome proliferator-activated receptor; BSA, bovine serum albumin; LPL, lipoprotein lipase.
(DR-1). Several genes with a crucial role in FA metabolism have been shown to contain a peroxisome proliferator response element in their upstream regulatory sequences (reviewed in Refs. 16 and 17). Interestingly, the transcriptional activity of the PPAR subtypes is enhanced by a multitude of chemical compounds including fatty acids, thiazolidinedione antidiabetic agents, prosta standins, peroxisome proliferators, and fibrate hypolipidemic drugs. In addition to activating PPARs, some of these compounds have been shown to be direct ligands for them. PPARγ directly binds antidiabetic thiazolidinediones (18, 19) and prostaglandin derivatives (18, 20) but not the other activators, whereas PPARα binds leukaotriene B4 and the powerful peroxisome proliferator Wy 14643 (21).

In view of the convergence of FA import and PPARs in lipid and energy metabolism, we investigated the effects of two distinct chemical classes of PPAR activators, i.e. fibrates (PPARα-specific) and the antidiabetic thiazolidinediones (PPARγ-specific) on tissue-specific FATP gene expression. Fibrate treatment induced FATP and ACS expression strongest in liver, whereas BRL 49653, the high affinity ligand for PPARγ, had no effect on liver but induced adipocyte FATP and ACS expression in adipose tissue. The induction of FATP and ACS by PPAR activators was at the level of transcription and was associated with concomitant changes in transcription. Interestingly, the stronger effects of BRL 49653 on fatty acid import in adipose tissue relative to the muscle might limit FA uptake and oxidation in the muscle, an effect associated with an improvement in muscle glucose disposal.

EXPERIMENTAL PROCEDURES

Materials—BRL 49653 and fenofibrate were kind gifts of Dr. De Chaffoy de Courcelles (Janssen Research Foundation, Beerse, Belgium) and Dr. Alan Edgar (Laboratoires Fournier, Daix, France), respectively.

Animal Studies—Animal studies were carried out in compliance with French and European union specifications regarding the use of laboratory animals. Male Wistar rats (90 days old) were treated for 7 days with fenofibrate (Laboratoires Fournier) mixed at the indicated concentrations and times (by mass) in standard rat chow. The food intake of the rats was measured and charcoal-treated fetal calf serum, insulin, transferrin, and selenium (Collaborative Research), dexamethasone (0.1 μM), and gentamycin (50 μg/ml). Fenofibrate and BRL 49653 (both in Me2SO) were added to the medium at the appropriate concentrations and times.

FIG. 1. Tissue distribution of FATP mRNA expression in mouse (A) and rat (B). Twenty μg of total RNA from the respective tissues was analyzed by Northern blot hybridization for FATP and 36B4 mRNA expression. RNA extraction and analysis was performed as described under “Experimental Procedures.”

Regulation of FATP Expression by PPAR Activators

Dawley rats were group-housed and accustomed to a 12:12 h day-night ratio illumination cycle (light from 8 am to 8 pm). Rats were divided in groups of a minimum of three animals each and were treated for either 7 or 14 days. The first group received BRL 49653 (5 mg/kg/day) by gavage. The second group of animals received 0.5% w/v of fenofibrate (± 0.5 g/kg/day) mixed with their food, whereas the third group of animals served as controls and received 10% carboxymethylcellulose (vehicle for gavage) by gavage. In a separate experiment, adult C57Bl6 male mice were either fed during 14 days with a control chow (n = 3) or the same chow containing 0.5% w/v of fenofibrate. At the end of the treatment period, all animals were weighed and sacrificed by exsanguination under ether anesthesia between 8 and 10 a.m. Epididymal adipose tissue (in rats) and liver (in rats and mice) was removed, weighed, rinsed with 0.9% NaCl, and frozen in liquid nitrogen until RNA preparation.

Cell Culture—The mouse hepatoma and preadipocyte cell lines Fa 32 (rat), ob 1771 (mouse) (22), and 3T3-L1 (mouse; ATCC) were maintained in Dulbecco’s modified Eagle’s minimal essential medium and supplemented with 10% delipidated and charcoal-treated fetal calf serum, l-glutamine, and antibiotics unless stated otherwise. AML-12 mouse hepatocytes (23) were maintained in Dulbecco’s modified Eagle’s minimal essential medium/Ham’s F-12 supplemented with 10% delipidated and charcoal-treated fetal calf serum, insulin, transferrin, selenium (Collaborative Research), dexamethasone (0.1 μM), and gentamycin (50 μg/ml). Fenofibrate and BRL 49653 (both in Me2SO) were added to the medium at the appropriate concentrations and times.

FIG. 2. Effect of fenofibrate on FATP mRNA levels. A, graph showing the effects fenofibrate mixed with food in the indicated concentrations on FATP mRNA levels. RNA extraction and analysis was performed as described under “Experimental Procedures.” R.A.U., relative absorbance units. B, effect of increasing amounts of fenofibrate on liver FATP expression in rat. Twenty μg of total liver RNA was analyzed by Northern blot hybridization for FATP and 36B4 mRNA expression as indicated under “Experimental Procedures.” C, effect of fenofibrate on liver FATP expression in mouse. Twenty μg of total liver RNA tissues was analyzed by Northern blot hybridization for FATP and 36B4 mRNA expression as indicated under “Experimental Procedures.”
indicated. Control cells received vehicle only.

3T3-L1 cells were differentiated by a treatment of 2 days with dexamethasone (0.1 mM), isobutylmethylxanthine (0.25 mM), and insulin (0.4 mM); the cells were then maintained for an additional 8 days with insulin until complete differentiation.

Preparation of Albumin-bound Fatty Acids—Radiolabeled \(^{14}\text{C}\)oleate fatty acid was mixed in water at 40 °C, albumin (BSA; fraction V, fatty acid-free, Sigma) was then added from a concentrated stock (20 g/100 ml) to give a final molar ratio of 1/1 by gentle mixing. Hanks' solution was added to obtain a final solution. Incubation was carried out at 37 °C for 45 min.

Fatty Acid Uptake Assay—The measurement of uptake of \(^{1}\text{H}\) labeled oleate was mixed in water at 40 °C, albumin (BSA; fraction V, fatty acid-free, Sigma) was then added from a concentrated stock (20 g/100 ml) to give a final molar ratio of 1/1 by gentle mixing. Hanks' solution was added to obtain a final solution. Incubation was carried out at 37 °C for 45 min.

FATP mRNA Is Ubiquitously Expressed—To determine whether FATP expression was ubiquitous or restricted to certain tissues, we hybridized both a mouse (Fig. 1A) and a rat (Fig. 1B) multiple tissue Northern blot with a radiolabeled FATP probe. In both rat and mouse, adipose tissue, heart, brain, and testis showed the highest level of expression. Intestine and muscle show intermediate levels of expression, and low levels are expressed in the liver, kidney, lung, and spleen.

Fenofibrate, a PPARa Activator, Induces FATP mRNA in Vivo—In addition to being building blocks and energy substrates, fatty acids are also important signaling molecules. Besides being activated by peroxisome proliferators and certain thiazolidinediones, the transcriptional activity of PPARs can be activated by fatty acids (reviewed in Refs. 16 and 17). Therefore, we were interested in analyzing whether activation of these PPARs would affect FA uptake in general and FATP expression in particular. To address this issue we first assessed the effect of fibrates, potent PPARa activators, on adipose tissue RNA using the primers 382 (ATGCGGGCTCCTGGAGCAGGACAGCC) and 399 (CTGCGTGTCAGGCAGGATGCTTCAGGCC) into pBS-KS. The insert was sequenced and found to be identical to the reported mouse FATP sequence. The rat ACS probe corresponds to the EcoRV restriction fragment of the rat ACS cDNA. The human acidic ribosomal phosphoprotein 36B4 (25) was used as a control probe.

RESULTS

FATP mRNA Is Ubiquitously Expressed—To determine whether FATP expression was ubiquitous or restricted to certain tissues, we hybridized both a mouse (Fig. 1A) and a rat (Fig. 1B) multiple tissue Northern blot with a radiolabeled FATP probe. In both rat and mouse, adipose tissue, heart, brain, and testis showed the highest level of expression. Intestine and muscle show intermediate levels of expression, and low levels are expressed in the liver, kidney, lung, and spleen.

Fenofibrate, a PPARa Activator, Induces FATP mRNA in Vivo—In addition to being building blocks and energy substrates, fatty acids are also important signaling molecules. Besides being activated by peroxisome proliferators and certain thiazolidinediones, the transcriptional activity of PPARs can be activated by fatty acids (reviewed in Refs. 16 and 17). Therefore, we were interested in analyzing whether activation of these PPARs would affect FA uptake in general and FATP expression in particular. To address this issue we first assessed the effect of fibrates, potent PPARa activators, on the expression of the fatp gene in rats. Rats were hence treated with different doses of fenofibrate (14 days treatment at the doses 0.005, 0.05, and 0.5% by mass) mixed in food. Next, RNA was isolated from various organs and analyzed by Northern blot hybridization. In liver, FATP mRNA levels increased gradually.
Regulation of FATP Expression by PPAR Activators

**Fig. 4.** Tissue-selective induction of FATP (A) and ACS (B) mRNA in rat liver, adipose tissue, skeletal muscle, and heart by fenofibrate and BRL 49653, respectively. A, expression of FATP mRNA in liver, epididymal adipose tissue, skeletal muscle, and heart of animals treated with fenofibrate (FF, 0.5% (w/w)) or BRL 49653 (5 mg/kg/day) during 7 days. The blots were stripped and rehybridized with the human acidic ribosomal protein 36B4 control cDNA. B, expression of ACS mRNA in liver, epididymal adipose tissue, skeletal muscle, and heart of animals treated with fenofibrate (FF, 0.5% (w/w)) or BRL 49653 (5 mg/kg/day) during 7 days. The blots were stripped and rehybridized with the human acidic ribosomal phosphoprotein 36B4 control cDNA. Animal treatment and preparation and analysis of RNA is described under “Experimental Procedures.” C, bar graph summarizing the regulation of ACS and FATP mRNA in liver, epididymal adipose tissue, skeletal muscle, and heart of animals treated with or without BRL 49653. R.A.U., relative absorbance units. Values statistically significant from controls (Mann-Whitney; p < 0.05) are indicated by an asterisk.

starting from 0.05% fenofibrate and reached a maximal 4.2-fold induction at the highest dose of 0.5% fenofibrate (Fig. 2, A and B). A representative Northern blot showing the induction of FATP mRNA in the liver is depicted in Fig. 2B. Next, the response of FATP mRNA levels to fibrate was studied in intestine, skeletal muscle, heart, and kidney. Only intestinal FATP mRNA expression was slightly induced (2-fold) by the highest dose of fibrate treatment, whereas muscle, kidney, and heart FATP mRNA expression remained unchanged. Also in mice, administration of fenofibrate (0.5%) induced FATP mRNA levels in liver (15-fold) (Fig. 2C).

Parallels between the Fibrate Effects on FATP and ACS—Several proteins are hypothesized to enhance fatty acid uptake into cells. In contrast to FATP, which acts as an FA transport protein, ACS prevents the efflux from the imported FAs by converting them into acyl-CoA derivatives, which can subsequently be used in both anabolic and catabolic pathways. Therefore, we next analyzed whether there was a parallel between the induction of FATP and ACS after fibrate treatment (Fig. 3). Fenofibrate induced liver and kidney ACS mRNA expression, whereas no change in ACS expression was observed in heart and intestine. Therefore both FATP and ACS mRNA levels seem to be coordinately regulated in liver and heart, since fibrates affect both parameters in a similar fashion. The regulation of ACS and FATP in the kidney and intestine seems to be divergent, since in these tissues only one of the respective mRNAs is regulated by fibrate treatment.

**PPARα Activators Induce FATP mRNA in Adipose Tissue**—In addition to the well-established effects of peroxisome proliferators such as the different fibrates on PPARα activity, we next tested the effects of the PPARα-selective ligand BRL 49653 on FATP and ACS expression in various rat tissues after administration of these compounds. Fenofibrate (0.5% (w/w), ±0.5 g/kg/day) induced FATP and ACS mRNA in rat liver (Fig. 4, A and B), confirming our previous observations (26). In contrast, treatment with fenofibrate did not change FATP and ACS mRNA levels significantly in adipose tissue, skeletal muscle, or heart (Fig. 4, A and B). Administration of 5 mg/kg/day of BRL 49653 was associated with the expected decrease in serum triglyceride levels (from 167 to 88 mg/dl). Furthermore, this treatment with BRL 49653 resulted in a significant induction of adipose tissue FATP (7-fold) and ACS (7-fold) mRNA levels (Fig. 4). This induction of FATP and ACS mRNA by BRL 49653 was observed in epididymal (Fig. 5) and omental (data not shown) adipose tissue. In perirenal adipose tissue, however, only FATP but not ACS mRNA was induced (Fig. 5). We observed a 1.6- and 3.1-fold induction of respective levels of FATP and ACS mRNA in skeletal muscle after BRL 49653 administration. BRL 49653 did not significantly influence the expression of FATP or ACS in liver, whereas FATP mRNA levels showed a tendency to decrease in the heart after BRL 49653 treatment.

The Induction of FATP and ACS Expression by Fenofibrate Is at the Transcriptional Level—To analyze whether the induction of FATP and ACS mRNAs occurred at the transcriptional level, a nuclear run-on assay was performed on liver nuclei obtained from fenofibrate-treated rats (Fig. 6). In comparison with control liver nuclei, the rate of FATP and ACS transcription was respectively 3- and 3.5-fold higher in nuclei from fenofibrate-treated animals. The transcription rate of acyl-CoA oxidase, a key enzyme in the peroxisomal β oxidation pathway, a positive control for fibrate action, was induced (5-fold), whereas the glyceraldehyde phosphate dehydrogenase gene, a negative control, did not change.

**BRL 49653 Induces FATP mRNA Specifically in Preadipocyte Cells, whereas Fibrates Induce FATP mRNA in Cells of Hepatic Origin**—To study the cellular mechanism of this induction, we investigated the regulation of the FATP gene expression by fibrates and BRL 49653 in hepatocyte (Fig. 7), adipocyte (Fig. 8), and muscle cells cell lines. FATP and ACS mRNA were measured in mouse AML-12 and rat Fa 32 liver-derived cell lines. A strong induction of expression of both FATP and ACS mRNA levels was seen after treatment of these liver-derived lines with fenofibric acid. Fenofibric acid induced both mRNAs optimally within 24 h (Fig. 7A) at a dose of 250 μM (Fig. 7B). The results of dose response and time course of FATP and ACS induction after treatment with fibrates seem to show an apparent difference between Fa 32 and AML-12 cells. The reason for this apparent difference in induction of FATP and ACS in the two cell lines is most likely caused by the difference in basal levels of FATP, which in Fa 32 cells is barely detectable. In contrast, under basal conditions, AML-12 expresses...
Regulation of FATP Expression by PPAR Activators

Fig. 5. BRL 49653 induces FATP and ACS mRNA in different adipose tissue depots. Expression of ACS and FATP mRNA in epididymal (A), and perirenal (B) adipose tissue of animals treated with BRL 49653 (5 mg/kg/day during 7 days). The blots were stripped and rehybridized with the human acidic ribosomal phosphoprotein 36B4 control cDNA. Animal treatment and preparation and analysis of RNA is described under “Experimental Procedures.”

Fig. 6. The induction of FATP and ACS by fibrates is at the transcriptional level. Transcription rates were determined for the FATP, ACS, acetyl-CoA oxidase (ACO) and glyceraldehyde-3-phosphate dehydrogenase genes in rat liver nuclei obtained from control, or BRL 49653 (BRL)- or fenofibrate-treated rats (FF). A pUC-20 template was used as a control (C). Densitometric scanning of the results is depicted at the left panel. GAPDH, glyceraldehyde-3-phosphate dehydrogenase, is used for relative values; ACO, acetyl-CoA oxidase; BS, BlueScript.

much higher levels of FATP transcript. This difference between these cells will result in an overestimation of the induction of the FATP in Fa 32 cells, explaining the discordance between relative levels of induction between FATP and ACS in the two cell lines.

To examine FATP and ACS regulation in adipocyte-like cell lines, 3T3-L1 preadipocyte cells were used. First, we analyzed the effects of BRL 49653 on nondifferentiated 3T3-L1 preadipocyte cells. As shown in Fig. 8A, a limited effect of BRL 49653 was observed in undifferentiated 3T3-L1 cells. In differentiated 3T3-L1 adipocytes, FATP and ACS mRNA levels were induced 5- and 9-fold after 4 days of treatment with BRL 49653. The ob 1771 preadipocyte cell line (22) was also analyzed (Fig. 8). The addition of BRL 49653 also induced FATP and ACS mRNA levels in this cell line (Fig. 8), whereas fenofibric acid had only a weak effect (data not shown).

Finally the effects of both BRL and fenofibric on L6 muscle cells were analyzed. Unlike in adipocyte or hepatocyte cell lines, no change in L6 ACS and FATP mRNA levels were detected upon treatment with either fenofibrate or BRL 49653 (data not shown).

Induction of FATP or ACS mRNA Levels Results in a Change in FA Uptake into Cells—To verify whether changes in mRNA levels of FATP and ACS were correlated with alterations in fatty acid uptake, we analyzed [14C]oleic acid uptake in Fa 32 and AML-12 hepatic cells, L6 muscle cells, and 3T3-L1 preadipocytes. As shown in Fig. 9, fatty acid uptake of [14C]oleic acid significantly increased after treatment of the liver-derived AML-12 cells with fenofibric acid and after treatment of the differentiated adipocyte-like 3T3-L1 cells with BRL 49653. In Fa 32 cells, fatty acid uptake was also increased (data not shown). The increase, although statistically significant, was however less pronounced than in AML-12 cells. As expected in view of the absence of a major regulation of ACS and FATP mRNA in muscle cells, no effects of either fenofibric acid nor BRL 49653 were observed on FA uptake in L6 muscle cells. The regulation of FA uptake was hence completely consistent with the regulation of respective mRNA levels of FATP and ACS in the various cell models.

DISCUSSION

Both ACS and FATP have been suggested to play a crucial role in the transport of fatty acids into the cell (4). FATP acts as a fatty acid transport protein, whereas ACS prevents efflux of the newly imported fatty acids by their esterification with coenzyme A. Fatty acids are important cellular components that can function both as metabolic substrates or as signaling molecules, by functioning as second messengers and triggering signal transduction pathways or by directly activating transcription factors such as the PPAR family of nuclear receptors. Since FATP and ACS control, in part, the intracellular availability of FAs, important PPAR activators, the aim of the present investigation was to perform detailed analysis of FATP and ACS expression and to establish whether FATP and ACS expression themselves might be subject to control by PPARs.

In the liver, one of the major organs susceptible to peroxisomal proliferation, FATP gene transcription is strongly induced upon fibrate treatment. This strong induction is not surprising if one takes the strong induction of peroxisomal β oxidation into account after fibrate treatment. FATP is likely to be responsible in part for the increased FA import necessary to sustain...
this increased β oxidation. Furthermore, a striking parallelism exists between the induction by fibrates of a number of genes involved in fatty acid import in the liver. In fact, the mRNAs for lipoprotein lipase (LPL) (27), ACS (this paper and Refs. 27 and 28), and FATP genes are all induced after fibrate treatment in the liver. This coinduction of genes seems to prime the cells for more efficient β oxidation. Induced liver LPL expression will increase lipolysis in the vascular bed of the liver, generating more fatty acids, which are then avidly taken up by the cells thanks to the higher levels of FATP expression. Efflux of these imported fatty acids is prevented by induced ACS levels, which in addition, primes them for subsequent metabolism. Therefore it seems that fibrates not only induce β oxidation but also induce genes important for supplying the cells with the extra fatty acids they need to sustain this increase in β oxidation.

In the intestine, FATP was also induced by fibrates, albeit to a lesser extent. The FATP induction in this tissue shows a striking parallel to the induction of CD36 after fibrate treatment in this tissue (29). In contrast to the liver and intestine, heart FATP mRNA levels did not vary substantially under fibrate treatment. The basal levels of FATP expression were, however, very high in heart, which almost exclusively uses fatty acids as energy source. In this tissue, fatty acids are, however, constitutively metabolized to provide energy necessary for the contraction of the heart muscle. Unresponsiveness of FATP expression in the heart to hormonal control could hence be physiologically significant, since the continued function of the heart is far too crucial to allow any form of major regulation of a transporter vital to its energy supply. This would suggest that in the heart, the FATP promoter is maximally active, resulting in a high level of constitutive FATP gene expression, which would be consistent with the high basal levels of FATP mRNA in this tissue. Similar to the heart, less extreme changes were observed in adipose tissue FATP mRNA after fibrate treatment. The absence of an effect of fibrates on FATP expression in adipose tissue is most likely due to the lower levels of PPARα relative to PPARγ. For kidney and intestine, the regulation of ACS and FATP by fibrates is discordant. This is consistent with the less crucial functions lipids play in kidney and intestinal metabolism. Kidney expressed only low levels of FATP mRNA, and its expression was furthermore refractory to induction by fibrates. Relative to heart and liver, the kidney utilizes relatively little fatty acids, and therefore a coordinate import mechanism is of lesser importance. In intestine, fatty acids are primarily absorbed, but they are less actively metabolized than in heart and liver. Therefore, intestine apparently has an actively regulated transport mechanism, as evidenced by regulation of the expression of both FATP and FAT, another transport protein that is also expressed and highly regulated in this organ (29). Since fatty acids are less actively metabolized and rather resecreted under the form of lipoproteins, there is less need for their conversion to acyl-CoA derivatives and hence less need for coordinated regulation of ACS together with these transport proteins suggested to be implicated in fatty acid transport.

The demonstration of the inducibility of the FATP and ACS
genes by PPARγ ligands such as the thiazolidinedione BRL 49653 has important implications for adipocyte physiology. PPARγ has been shown to promote preadipocyte determination as well as terminal differentiation (13, 30), and its mRNA is itself induced in the earliest steps of adipocyte differentiation before the induction of early marker genes for adipocyte differentiation. Many of these genes induced during adipocyte differentiation encode proteins involved in lipid storage and metabolism. The increase in FATP and ACS expression in differentiated adipocyte-like cells caused by PPARγ ligands will result in an increased delivery of fatty acids to the adipocytes, which possibly sustains a positive regulatory feedback loop involving continued PPARγ activation of the FATP and ACS (28) genes and aimed at promoting and maintaining the mature adipocyte phenotype. In fact, in addition to the thiazolidinediones, certain fatty acid-derived prostaglandin derivatives, whose delivery to the cell is increased by FATP, bind to and/or activate PPARγ (19, 20, 31). This hypothesis is supported by the observation that fatty acids (including arachidonic acid-derived prostaglandins) and fatty acid analogues induce the expression of adipocyte-specific genes and enhance adipocyte conversion (30, 32–35). In addition to being potent PPAR activators (7, 12, 31, 36, 37), fatty acids will provide the necessary building blocks for triglyceride accumulation, ultimately enhancing adipocyte differentiation. The PPAR-mediated activation of FATP and ACS expression in cells of the adipogenic lineage might furthermore in part be responsible for the previously reported capacities of thiazolidinediones to induce adipocyte differentiation and induce the development of obesity (38–46). In this context, it is interesting to note that the PPARγ-mediated effects of BRL 49653 on FATP and ACS expression might act in concert with induced LPL expression and the reduced leptin mRNA and protein levels and the associated increase in caloric intake enhancing energy storage in the adipocytes observed with this compound (47, 48). Interestingly, FATP and ACS are not coordinately regulated in perirenal and epididymal adipose tissue stores. This differential regulation is consistent with the distinct metabolic nature of the different adipose tissue depots (49–51). Further studies are required to determine whether the role of FATP is a consequence of or is causative of the physiologic differences between the adipose tissue depots.

The tissue-selective effects of the various PPAR activators/ligands are highly intriguing and provide insight in their effects on triglyceride metabolism. Fibrate treatment induced FATP and ACS expression strongest in liver, whereas BRL 49653 had no effect on liver, but strongly induced adipocyte FATP and to a lesser extent ACS expression. The effects of fibrates (PPARα activators) on the liver and PPARγ ligands in adipose tissue correlates well with the tissue-specific expression of the respective receptors and suggests that the FATP and ACS genes show a tissue-selective activation similar to the one previously described for the LPL gene (27). In this context, we need however to address the discrepancy between ACS and FATP regulation after BRL 49653 administration in skeletal muscle. Muscle tissue expresses very low levels of PPARγ, which is consistent with the absence of an important regulatory effect of PPARγ activators in this tissue as observed for the LPL (27) or FATP (this study) genes. In this context, the induction of ACS expression by BRL 49653 is however difficult to explain. One must however bear in mind that not all of the effects of the thiazolidinediones are mediated via PPARγ activation, and it has been shown that these agents activate several other signaling pathways (52–54). Further investigations need to address whether ACS expression, unlike FATP or LPL expression, is subject to such a regulatory circuit.

One remaining question is the relationship between PPARγ, thiazolidinediones, and insulin resistance. It is tempting to speculate that the increase of LPL, ACS, and FATP activity in adipose tissue is related to the anti diabetic effects of the thiazolidinediones. Due to the enhanced triglyceride clearance in adipose tissue, less triglycerides will become available to be hydrolyzed to fatty acids in the vascular bed of the muscle. Furthermore, relative to the strong induction of FATP and ACS in adipose tissue by BRL 49653, very limited inductions of both genes are observed in skeletal and heart muscle, favoring uptake of fatty acids in adipose tissue relative to muscle. In view of the inhibitory effects of fatty acids on insulin-mediated glucose metabolism (55), the decrease in fatty acids delivered to the muscle cells might be responsible for the improvement in insulin sensitivity of this tissue.

In conclusion, FATP and ACS mRNA levels can be regulated in a tissue-specific fashion by PPARα activators and PPARγ ligands. In adipose tissue, the increase in FATP, ACS, and LPL (27) production after treatment with thiazolidinediones will enhance the clearance of plasma triglycerides (27, 56) and provide the (pre)adipocytes with additional fatty acids, which can further stimulate the transactivation capacity of PPAR or which can be stored under form of triglycerides. In the liver, the enhanced production of FATP and ACS after fibrates together with the increase in β oxidation and the reduced production of apoCIII (57), may contribute to the hypolipidemic action of these compounds. This tissue-selective induction of FATP and ACS gene transcription by activators of different PPARs, demonstrates the feasibility of the development of highly specific PPAR subtype-specific agonists and antagonists, which can be used as drugs.

Acknowledgments—Dr. D. de Chaffoy de Courcels and Dr. J. C. Fruchart are acknowledged for stimulating discussions and suggestions and D. Cayet and O. Vidal for excellent technical assistance. We thank Drs. A. Edgar and de Chaffoy de Courcels for the gift of valuable materials.

REFERENCES
1. Higgins, C. F. (1994) Cell 79, 393–395
2. Berk, P. D., Wada, H., Horio, J., Potter, B. J., Sorrentino, D., Zhou, S.-L., Isola, L. M., Stump, D., Jiang, C.-L., and Thung, S. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3484–3488
3. Abumrad, N. A., El-Maghrabi, M. R., Amri, E.-Z., Lopez, E., and Grimaldi, P. A. (1993) J. Biol. Chem. 268, 17655–17661
4. Schaffer, J. E., and Lodish, H. F. (1994) Cell 79, 427–436
5. Faergeman, N. J., DiRusso, C., Elberger, A., Knuudsen, J., and Black, P. N. (1997) J. Biol. Chem. 272, 8531–8538
6. Isseman, I., and Green, S. (1990) Nature 347, 645–650
7. Gottlicher, M., Widmark, E., Li, Q., and Gustafsson, J. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4653–4657
8. Schmidt, A., Endo, N., Bottigley, S. J., Vogel, R., Shinar, D., and Rodan, G. A. (1992) Mol. Endocrinol. 6, 1634–1641
9. Dreyer, C., Keller, H., Mahfoudi, A., Laudet, V., Krey, G., and Wahli, W. (1993) Biol. Cell 77, 67–77
10. Sherr, T., Y. H. F., McFride, W., and Gonzalez, F. J. (1993) Biochemistry 32, 5598–5604
11. Zhu, Y., Alvare, K., Huang, Q., Rao, M. S., and Reddy, J. K. (1993) J. Biol. Chem. 268, 26187–26202
12. Kliewer, S. A., Forman, B. M., Blumberg, B., Ong, E. S., Borgmeyer, U., and Evans, R. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7355–7359
13. Tontonoz, P., Hu, E., Graves, B. A., Budavari, A. I., and Spiegelman, B. M. (1994) Genes Dev. 8, 1224–1234
14. Amri, E.-Z., Bonino, F., Ailhaud, G., Abumrad, N. A., and Grimaldi, P. A. (1995) J. Biol. Chem. 270, 2367–2371
15. Apelort, C., Pognonec, P., Saladin, R., Auwerx, J., and Bouloukos, K. (1995) Gene 162, 297–302
16. Schoonjans, K., Staels, B., and Auwerx, J. (1996) J. Lipid Res. 37, 907–925
17. Schoonjans, K., Staels, B., and Auwerx, J. (1996) J. Lipid Res. 37, 907–925
18. Schoonjans, K., Staels, B., and Auwerx, J. (1996) Biochim. Biophys. Acta 1302, 93–109
19. Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) Cell 83, 803–812
20. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkson, W. O., Wilson, T. M., and Kliever, S. A. (1995) J. Biol. Chem. 270, 12953–12956
21. Kliewer, S. A., Lenhard, J. M., Willson, T. M., Patel, I., Morris, D. C., and
Lehman, J. M. (1995) Cell 83, 813–819
21. Devchand, P. R., Keller, H., Peters, J. M., Vazquez, M., Gonzalez, F. J., and Wahl, W. (1996) Nature 384, 39–43
22. Negrel, R., Grimaldi, P., and Ailhaud, G. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 6054–6058
23. Wu, J. C., Merlino, G., and Ailhaud, G. (1978) Proc. Natl. Acad. Sci. U. S. A. 86, 674–678
24. Auwerx, J., Chait, A., and Deeb, S. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1133–1137
25. Masiakowski, P., Breathnach, R., Bloch, J., Gannon, F., Krust, A., and Chambon, P. (1982) Nucleic Acids Res. 10, 7895–7903
26. Schoonjans, K., Staels, B., Grimaldi, P., and Auwerx, J. (1993) Eur. J. Biochem. 216, 615–622
27. Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A. M., Heyman, R., Briggs, M., Cayet, D., Deeb, S., Staels, B., and Auwerx, J. (1996) EMBO J. 15, 5336–5344
28. Schoonjans, K., Watanabe, M., Suzuki, H., Mahfoudi, A., Krey, G., Wahl, W., Grimaldi, P., Staels, B., Yamamoto, T., and Auwerx, J. (1995) J. Biol. Chem. 270, 19269–19276
29. Poizier, H., Degrazia, P., Niot, I., Bernard, A., and Besnard, P. (1996) Eur. J. Biochem. 238, 368–373
30. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) Cell 79, 1147–1156
31. Yu, K., Bayona, W., Kallen, C. B., Harding, H. P., Ravera, C. P., McMahon, G., Brown, M., and Lazar, M. A. (1995) J. Biol. Chem. 270, 23975–23983
32. Gaillard, D., Negrel, R., Lagarde, M., and Ailhaud, G. (1989) Biochem. J. 257, 389–397
33. Amri, E.-Z., Bertrand, B., Ailhaud, G., and Grimaldi, P. (1991) J. Lipid Res. 32, 1449–1456
34. Distel, R. J., Robinson, G. S., and Spiegelman, B. M. (1992) J. Biol. Chem. 267, 3527–3531
35. Chawla, A., and Lazar, M. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1786–1790
36. Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K., and Wahl, W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2160–2164
37. Forman, B. M., Umesono, K., Chen, J., and Evans, R. (1995) Cell 81, 541–550
38. Hiragu, A., Sato, M., and Mitsu, H. (1988) J. Cell. Physiol. 134, 124–130
39. Ikeeda, H., Taketomi, S., Sugiyama, Y., Shimura, Y., Sobh, T., Meguro, K., and Fujita, T. (1999) Drug Res. 40, 156–162
40. Sparks, R. L., Strauss, E. E., Zygmunt, A. I., and Phelan, T. E. (1991) J. Cell. Physiol. 146, 101–109
41. Kletzien, R. F., Clarke, S. D., and Ulrich, R. G. (1992) Mol. Pharmacol. 41, 393–398
42. Castle, C. K., Colca, J. R., and Melchior, G. W. (1993) Arterioscler. Thromb. 13, 302–309
43. Sandouk, T., Reda, D., and Hofmann, C. (1993) Am. J. Physiol. 264, C1600–C1608
44. Hirshman, M. F., Fagnant, P. M., Horton, E. D., King, P. A., and Horton, E. S. (1995) Biochem. Biophys. Res. Commun. 208, 835–845
45. Tontonoz, P., Cayet, D., Deeb, S., Staels, B., and Auwerx, J. (1996) Mol. Pharmacol. 50, 1449–1456
46. De Vos, P., Lefebvre, A. M., Miller, S. G., Guerre-Millo, M., Wong, K., Saladin, R., Hamann, L., Staels, B., Briggs, M. R., and Auwerx, J. (1996) J. Clin. Invest. 98, 1004–1009
47. Zhang, B., Berger, J., Zhou, G., Elbrecht, A., Biewas, S., White-Carrington, S., Szalkowski, D., and Moller, D. E. (1996) J. Biol. Chem. 271, 31771–31774
48. Bjorntorp, P. (1996) Int. J. Obes. Relat. Metab. Disord. 20, 291–302
49. Marin, P., Lonn, B., Oden, B., Bengtsson, B. A., and Bjorntorp, P. (1996) J. Clin. Endocrinol. Metab. 81, 1018–1022
50. Shimomura, I., Takahashi, M., Tokunaga, K., Nakamura, T., Yamashita, S., Takemura, K., Yamamoto, T., Punahashi, T., and Matsuzawa, Y. (1996) Am. J. Physiol. 270, E995–1002
51. Buchanan, T. A., Meehan, W. P., Jeng, Y. Y., Yang, D., Chan, T. M., Nadler, J. L., Scott, S., Rude, R. K., and Haseu, W. A. (1995) J. Clin. Invest. 96, 354–360
52. Maegawa, H., Ide, R., Hasegawa, M., Ugi, S., Egawa, K., Iwanishi, M., Kikkawa, R., Shigeta, Y., and Kashigawa, A. (1995) J. Biol. Chem. 270, 7721–7730
53. Ren, J., Dominguez, L. J., Sowers, J. R., and Davidoff, A. J. (1996) Diabetes 45, 1822–1825
54. Randle, P. J., Garland, P. B., Hales, C. N., and Newsholme, E. A. (1961) Lancet 2, 785–788
55. Lefebvre, A.-M., Peinado-Onsurbe, J., Leitersdorf, I., Briggs, M. R., Paterniti, J. R., Fruchart, J.-C., Fievet, C., Auwerx, J., and Staels, B. (1997) Arterioscler. Thromb. Vasc. Biol. in press
56. Staels, B., Vu-Dac, N., Kosykh, V., Saladin, R., Fruchart, J., C., Dallongeville, J., and Auwerx, J. (1995) J. Clin. Invest. 95, 705–712