

# Negative Regulation of Human Fibrinogen Gene Expression by Peroxisome Proliferator-activated Receptor $\alpha$ Agonists via Inhibition of CCAAT Box/Enhancer-binding Protein $\beta$ \*

Received for publication, March 30, 2001, and in revised form, June 11, 2001  
Published, JBC Papers in Press, June 20, 2001, DOI 10.1074/jbc.M102839200

Philippe Gervois<sup>‡§\*\*\*</sup>, Ngoc Vu-Dac<sup>‡</sup>, Robert Kleemann<sup>§</sup>, Maaïke Kockx<sup>§</sup>, Guillaume Dubois<sup>‡</sup>, Bernard Laine<sup>¶</sup>, Vladimir Kosykh<sup>||</sup>, Jean-Charles Fruchart<sup>‡</sup>, Teake Kooistra<sup>§</sup>, and Bart Staels<sup>‡\*\*\*</sup>

From the <sup>‡</sup>Département d'athérosclérose, U.545 INSERM, Institut Pasteur de Lille and Faculté de Pharmacie, Université de Lille II, 59019 Lille, France, the <sup>§</sup>Gaubius Laboratory, TNO-Prevention and Health, P. O. Box 2215, 2301 CE Leiden, The Netherlands, <sup>¶</sup>U.459 INSERM, Laboratoire de Biologie Cellulaire, Faculté de Médecine H. Warembourg, 59045 Lille Cédex, France, and the <sup>||</sup>Cardiology Research Complex, 721552 Moscow, Russia

Fibrinogen is a coagulation factor and an acute phase reactant up-regulated by inflammatory cytokines, such as interleukin 6 (IL-6). Elevated plasma fibrinogen levels are associated with coronary heart diseases. Fibrates are clinically used hypolipidemic drugs that act via the nuclear receptor peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ). In addition, most fibrates also reduce plasma fibrinogen levels, but the molecular mechanism is unknown. In this study, we demonstrate that fibrates decrease basal and IL-6-stimulated expression of the human fibrinogen- $\beta$  gene in human primary hepatocytes and hepatoma HepG2 cells. Fibrates diminish basal and IL-6-induced fibrinogen- $\beta$  promoter activity, and this effect is enhanced in the presence of co-transfected PPAR $\alpha$ . Site-directed mutagenesis experiments demonstrate that PPAR $\alpha$  activators decrease human fibrinogen- $\beta$  promoter activity via the CCAAT box/enhancer-binding protein (C/EBP) response element. Co-transfection of the transcriptional intermediary factor glucocorticoid receptor-interacting protein 1/transcriptional intermediary factor 2 (GRIP1/TIF2) enhances fibrinogen- $\beta$  gene transcription and alleviates the repressive effect of PPAR $\alpha$ . Co-immunoprecipitation experiments demonstrate that PPAR $\alpha$  and GRIP1/TIF2 physically interact *in vivo* in human liver. These data demonstrate that PPAR $\alpha$  agonists repress human fibrinogen gene expression by interference with the C/EBP $\beta$  pathway through titration of the coactivator GRIP1/TIF2. We observed that the anti-inflammatory action of PPAR $\alpha$  is not restricted to fibrinogen but also applies to other acute phase genes containing a C/EBP response element; it also occurs under conditions in which the stimulating action of IL-6 is potentiated by dexamethasone. These findings identify a novel molecular mechanism of negative gene regulation by PPAR $\alpha$  and reveal the direct implication of PPAR $\alpha$  in the modulation of the inflammatory gene response in the liver.

Elevated plasma fibrinogen levels have been consistently associated with occlusive vascular disorders, and several investigations have prospectively related fibrinogen to myocardial infarction and stroke outcomes (1–3). Fibrinogen is synthesized in hepatocytes and secreted into the blood as a dimeric molecule, with each half composed of three nonidentical polypeptides (A $\alpha$ , B $\beta$ , and  $\gamma$ ) linked by disulfide bonds. The three polypeptides are encoded by three distinct genes clustered on the long arm of chromosome 4 (4). The three genes are arranged in the order of  $\gamma$ ,  $\alpha$ ,  $\beta$ , with the gene for the  $\beta$ -chain transcribed in the opposite direction. The three genes contain promoter elements with TATA and CAAT boxes and a number of regulatory elements that confer liver-specific and cytokine-induced expression, including hepatic nuclear factor 1 in the promoter region of A $\alpha$  and B $\beta$  genes (5, 6) and interleukin 6 (IL-6)<sup>1</sup> responsive elements 5' to all three genes (7–12). Induction of fibrinogen biosynthesis in response to trauma and inflammation is mainly mediated by IL-6 and occurs at the transcriptional level. In humans, fibrinogen- $\beta$ -chain synthesis is considered to be the rate-limiting chain for assembly and secretion of mature fibrinogen (13, 14). IL-6 induction of human fibrinogen- $\beta$  transcription involves two juxtaposed specific elements (7, 8, 15). The first element is an IL-6 response element, and the second is a binding site for the CCAAT box/enhancer-binding protein (C/EBP) family of transcription factors. These two distinct elements are both required for maximal induction by IL-6.

Among drugs affecting plasma fibrinogen levels, fibric acid derivatives are reported as negative regulators of fibrinogen (16). The rationale behind the use of fibrates in reducing cardiovascular events is based on their ability to attenuate hypertriglyceridemia and hypercholesterolemia, both of which are established risk factors for cardiovascular diseases (17, 18). Fibrates exert their effects on lipid and lipoprotein metabolism via activation of the nuclear receptor peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) (19). We have previously demonstrated the involvement of PPAR $\alpha$  as a mediator of the negative regulation of fibrinogen gene expression by fibrates (20), but the exact molecular mechanism remained unresolved.

PPAR $\alpha$  belongs to the PPAR subfamily of nuclear receptors that activate gene expression in response to ligands following

\* This work was supported by grants from the Fondation pour la Recherche Médicale and by European Community Marie Curie Fellowship QLK1-CT-1999-51206 (to P. G.), Netherlands Organization for Scientific Research Grant NOW:902-23-181 (to M. K.), and Netherlands Heart Foundation Grant NHS 99.110 (to R. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\*\* To whom correspondence may be addressed. Tel.: 33(0)3-20-87-73-88; Fax: 33-(0)3-20-87-73-60; E-mail: Bart.Staels@pasteur-lille.fr or philippe.gervois@pasteur-lille.fr.

<sup>1</sup> The abbreviations used are: IL, interleukin; APRG, acute phase response gene; C/EBP, CCAAT box/enhancer-binding protein; GRIP, glucocorticoid receptor-interacting protein; GST, glutathione S-transferase; LBD, ligand binding domain; PBS, phosphate-buffered saline; PPAR, peroxisome proliferator-activated receptor; RE, response element; TIF, transcriptional intermediary factor.

dimerization with the retinoid X receptor. PPAR/retinoid X receptor heterodimers bind to specific sequences localized in the promoter region of target genes termed peroxisome proliferator response elements. Several lines of evidence suggest that in addition to their hypolipidemic effect (21), fibrates may exert direct anti-atherogenic activity through an anti-inflammatory activity at the level of the vascular wall. For instance, several clinical studies, such as BECAIT and LOCAT, revealed that fibrate treatment causes a slower progression of coronary atherosclerosis that is independent of any significant lowering of atherogenic lipoprotein concentrations (22, 23). Furthermore, it has been reported that fibrates decrease plasma concentrations of inflammatory cytokines, such as tumor necrosis factor  $\alpha$  and IL-6, in patients with angiographically established atherosclerosis (24, 25). Interestingly, PPAR $\alpha$  has been demonstrated to act as a negative regulator of the vascular inflammatory gene response by antagonizing the activity of the transcription factors NF- $\kappa$ B and AP-1 (26). In line with these findings, PPAR $\alpha$  knockout mice exhibit a prolonged inflammatory response compared with wild type mice (27).

In the present work, we delineated the molecular mechanism of fibrinogen gene regulation in more detail, and we extended our previous observations in rodents to the human situation. We demonstrate that the nuclear receptor PPAR $\alpha$  is also crucial for the negative regulation of the human fibrinogen- $\beta$  gene expression by PPAR $\alpha$  agonists and that this occurs under both basal and inflammatory conditions. Evidence is provided that the suppressive effect of PPAR $\alpha$  requires the integrity of the C/EBP response element and is independent of the IL-6 response element. PPAR $\alpha$  does not interact directly with C/EBP. Instead, we found that transcriptional intermediary factor glucocorticoid receptor-interacting protein 1/transcriptional intermediary factor 2 (GRIP1/TIF2) is a novel positive regulator of fibrinogen- $\beta$  transcription and that the sequestration of GRIP1/TIF2 by PPAR $\alpha$  constitutes a molecular mechanism by which negative regulation of fibrinogen- $\beta$  by PPAR $\alpha$  agonists takes place. Finally, we observed that the PPAR $\alpha$  inhibitory effect may be extended to acute phase response genes other than the fibrinogen- $\beta$  gene.

#### EXPERIMENTAL PROCEDURES

**Reagents**—Fenofibric acid was a kind gift of Dr. A. Edgar (Laboratoires Fournier, Daix, France); ciprofibrate and bezafibrate were from Sanofi-Synthelabo (Aramon, France) and Roche Molecular Biochemicals, respectively. Wy 14,643 was from Chemsyn (Lenexa, KS). Human recombinant IL-6 was purchased from Tebu (Le Perray-en-Yvelines, France). Dexamethasone was from Sigma.

**Cell Culture**—Human hepatocytes, isolated by collagenase perfusion, and HepG2 cells, obtained from the European Collection of Animal Cell Culture (Porton Down, Salisbury, United Kingdom) were cultured exactly as described previously (28).

**Fibrinogen Measurement**—Fibrinogen concentrations in conditioned medium were measured by an enzyme-linked immunosorbent assay procedure as previously described (20).

**RNA Extraction**—Total RNA extraction and Northern blot analysis were performed as described (29) using a 1930-base pair *EcoRI/PstI* fragment of the human fibrinogen- $\beta$  and human acidic ribosomal phosphoprotein 36B4 (30) cDNA probes. Probes for human fibrinogen- $\alpha$ , fibrinogen- $\gamma$ , and serum amyloid A were generated by reverse transcription-polymerase chain reaction.

**Plasmids**—pSG5-hPPAR $\alpha$  and pSG5-hPPAR $\alpha$  $\Delta$ LBD were described previously (31). phFib- $\beta$  was generated by amplification and cloning of a 400-base pair genomic fragment corresponding to nucleotides -400 to +13 of the human fibrinogen- $\beta$  promoter pGL3 reporter vector. Specific mutations in either the IL-6 or C/EBP $\beta$  response elements previously described (8) were generated by site-directed mutagenesis using the quick mutagenesis kit (Stratagene) and phFib- $\beta$  as template, giving rise to phFib- $\beta$  M1 and phFib- $\beta$  M3 plasmids, respectively.

**Transfections**—HepG2 cells were transiently transfected using the calcium phosphate precipitation method with reporter and expression plasmids, as stated in the figure legends. The total amount of DNA was

kept constant by complementation with corresponding empty vector mock DNA. After a 4-h incubation period, cells were washed with phosphate-buffered saline (PBS) and refed with Dulbecco's modified Eagle's medium supplemented with 0.2% fetal calf serum and Wy 14,643 or vehicle and IL-6 as indicated in the figure legends. Cells were harvested after 24-h incubation and collected for the determination of the luciferase activity performed using a luciferase assay system (Promega Corp., Madison, WI).

**Cell Extracts**—HepG2 cells were washed twice with ice-cold PBS, scraped off in 1 ml of ice-cold PBS, and collected by centrifugation for 5 min at 500  $\times$  g at 4  $^{\circ}$ C. The pellet was resuspended in 100  $\mu$ l of ice-cold lysis buffer (1% Nonidet P-40, 0.5% sodium desoxycholate, 0.1% SDS in PBS), protease inhibitors were freshly added (5  $\mu$ g/ml leupeptin, 5  $\mu$ g/ml pepstatin, 5 mg/ml EDTA- $\text{Na}_2$ , 1 mM benzamide, 5  $\mu$ g/ml aprotinin, and 0.5 mM phenylmethylsulfonyl fluoride), and the suspension was vigorously vortexed. The cell extract was centrifuged (5 min at 10,000  $\times$  g and 4  $^{\circ}$ C), and the supernatant was transferred to new tubes, aliquoted, and stored at -80  $^{\circ}$ C.

**Western Blotting**—Electrophoresis of samples was performed on 10% SDS-polyacrylamide gels (Minigel system, Bio-Rad) under reducing conditions (10 mM dithiothreitol). Proteins were blotted onto nitrocellulose membrane. Nonspecific binding sites were blocked with 10% skim milk powder diluted in TNT buffer (20 mM Tris, 55 mM NaCl, 0.1% Tween), overnight at 4  $^{\circ}$ C. The membrane was probed with primary antibody diluted in 5% skim milk-TNT for 4 h at room temperature. Membrane was washed and incubated with peroxidase-conjugated anti-rabbit antibody, followed by a subsequent six washes of 10 min. The bands were visualized using SuperSignal $\text{\textcircled{R}}$  West Dura substrate.

**In Vitro Protein-Protein Interaction Assay (GST Pull-down)**—0.5  $\mu$ g of GST-GRIP1/TIF2(536-1121) bound to glutathione-Sepharose 4B beads was incubated with 5  $\mu$ l of *in vitro* synthesized [ $^{35}$ S]methionine-labeled protein in the presence or absence of 100  $\mu$ M Wy-14643 (dissolved in  $\text{Me}_2\text{SO}$ ) in a total volume of 200  $\mu$ l of incubation buffer (20 mM Hepes, pH 7.8, 100 mM KCl, 10 mM  $\text{MgCl}_2$ , 10% glycerol, 0.1% Nonidet P-40, 0.1% Triton X-100, 0.1% bovine serum albumin, 1 mM dithiothreitol, 1  $\mu$ g/ml of each aprotinin, leupeptin, and pepstatin) and gently rotated at 4  $^{\circ}$ C. After centrifugation, the beads were washed four times for 15 min with incubation buffer without bovine serum albumin, resuspended in 30  $\mu$ l of 1 $\times$  Laemmli buffer, boiled for 5 min, and centrifuged. The supernatant was loaded on a SDS-polyacrylamide gel electrophoresis gel. After drying the gel, input and bound proteins were analyzed with a phosphorimager apparatus equipped with ImageQuant software.

**In Vivo Protein-Protein Interaction Assay (Co-immunoprecipitation)**—For binding of endogenous hPPAR $\alpha$  to GRIP1/TIF2 a freshly isolated piece of human liver of about 1 g was homogenized in ice-cold PBS containing proteinase inhibitor mixture (Roche Molecular Biochemicals) and stored at -80  $^{\circ}$ C. Thawed homogenates were centrifuged at 10,000  $\times$  g for 10 min to recover soluble proteins. Samples were diluted 10-fold in PBS/protease inhibitors and rotated at 4  $^{\circ}$ C for 6 h in the presence of 4  $\mu$ g/ml primary rabbit anti-hPPAR $\alpha$  antibody (Santa Cruz Biotechnology) or rabbit preimmune serum, respectively. Complexes were immunoprecipitated by antibody/protein A-Sepharose (Amersham Pharmacia Biotech) at 4  $^{\circ}$ C for 2 h and washed once in PBS/protease inhibitors and three times in protease inhibitor-containing 50 mM Tris-HCl, pH 8.0, 170 mM NaCl, 0.5% Nonidet P-40, 50 mM NaF. Co-immunoprecipitated proteins were analyzed by immunoblotting.

#### RESULTS

**Fibrates Down-regulate Fibrinogen- $\beta$  Expression and Reduce Fibrinogen Secretion in Human Hepatocytes**—The regulation of fibrinogen biosynthesis is mainly transcriptional and is stimulated by IL-6. Fibrinogen- $\beta$  is considered the rate-limiting chain for fibrinogen biosynthesis. Therefore, we studied the effect of fibrates on the regulation of human fibrinogen- $\beta$  expression in primary hepatocytes under basal and IL-6-induced conditions. In the absence of IL-6, basal fibrinogen mRNA levels were decreased by treatment with fenofibric acid, whereas control 36B4 mRNA was unaffected (Fig. 1). The addition of IL-6 led to enhanced expression of fibrinogen- $\beta$  mRNA. When cells were treated with both IL-6 and fenofibric acid, fibrinogen expression was strongly lowered.

In order to check whether this inhibitory effect is not restricted to fenofibric acid, fibrinogen- $\beta$  expression was analyzed in HepG2 cells treated with various other fibrates for

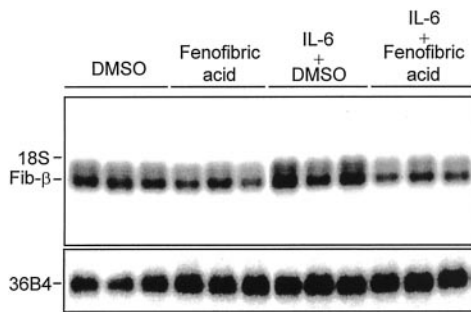


FIG. 1. **Effect of fenofibric acid on fibrinogen- $\beta$  expression in human primary hepatocytes under basal and IL-6-stimulated conditions.** Human hepatocytes were isolated and treated for 3 h with 100  $\mu$ M fenofibric acid or vehicle (*DMSO*, dimethyl sulfoxide) and subsequently stimulated with IL-6 for 21 h (25 ng/ml) as indicated. Total RNA (10  $\mu$ g) was subjected to Northern blot analysis using human fibrinogen- $\beta$  (*Fib- $\beta$* ) (top panel) or 36B4 (bottom panel) cDNA probes.

24 h. Treatment with each fibrate resulted in a down-regulation of fibrinogen- $\beta$  expression in both the presence and the absence of IL-6 (Fig. 2*a*). The lowering effect also occurred at the protein level because secretion of fibrinogen was diminished by fibrate treatment; it also occurred in the presence of IL-6 (Fig. 2*b*). Treatment of HepG2 cells with increasing concentrations of Wy 14,643 resulted in a dose-dependent inhibition of basal and IL-6-induced fibrinogen- $\beta$  mRNA levels (Fig. 3). These experiments demonstrate that PPAR $\alpha$  agonists suppress human fibrinogen- $\beta$  mRNA levels and fibrinogen secretion under both basal and IL-6-stimulated conditions.

**Fibrinogen- $\beta$  Repression by Fibrates Occurs at the Transcriptional Level through Activation of PPAR $\alpha$** —To elucidate whether fibrates can suppress the expression of the fibrinogen- $\beta$ -chain gene at the transcriptional level, a 400-base pair promoter fragment of the human fibrinogen- $\beta$  gene, which contains the essential regulatory elements for basal and inducible promoter activity (8), was cloned in front of a luciferase reporter gene giving rise to phFib- $\beta$ . This reporter construct was transiently transfected into HepG2 cells that were subsequently treated with different fibrates in the presence or absence of IL-6. As shown in Fig. 4*a*, basal promoter activity decreased when cells were treated with fibrates and was enhanced 6-fold when cells were incubated with IL-6. Furthermore, prior treatment of the cells with fibrates resulted in a consistent inhibition of fibrinogen- $\beta$  transcription induced by IL-6 (Fig. 4*a*). Co-transfection of PPAR $\alpha$  reduced fibrinogen- $\beta$  promoter activity in both control and IL-6-treated cells, an effect that was further enhanced by the presence of Wy 14,643 (Fig. 4*b*). These results indicate that the repressive effect of fibrates on human fibrinogen- $\beta$  expression occurs at the transcriptional level through activation of PPAR $\alpha$ .

**PPAR $\alpha$  Functions as a Repressor of C/EBP $\beta$ -mediated Fibrinogen- $\beta$  Gene Transactivation**—IL-6 induction of fibrinogen- $\beta$  promoter is mediated by two distinct cis-acting elements (7, 8). One of these elements is an IL-6 response element (RE)-like site, and the other is a consensus binding site for members of the C/EBP family of transcription factors. To delineate whether one of these elements is involved in PPAR $\alpha$ -mediated repression of fibrinogen- $\beta$  gene transcription, we performed transient transfection experiments using the 400-base pair fibrinogen promoter reporter constructs carrying mutations in either the C/EBP $\beta$  or IL-6 response elements (Fig. 5). As described above, phFib- $\beta$  activity was repressed by activated PPAR $\alpha$  in both the presence and absence of IL-6. Mutation of the IL-6 RE core site in the fibrinogen- $\beta$  promoter construct (phFib- $\beta$  M1) led to a decreased basal transcriptional activity and to a loss of IL-6 responsiveness. Furthermore, its

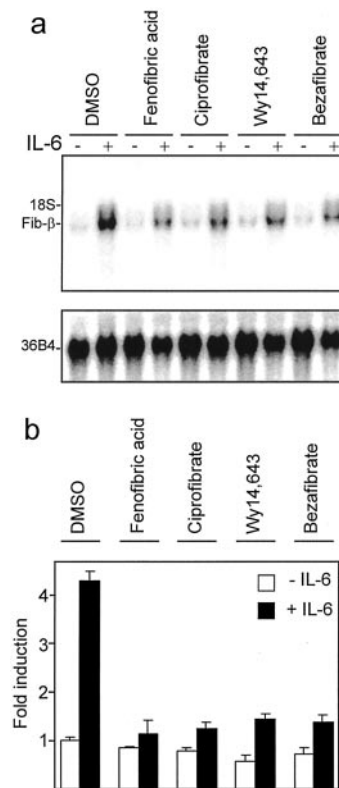


FIG. 2. **Effect of fibrates on fibrinogen- $\beta$  expression under basal and IL-6-stimulated conditions in HepG2 cells.** Cells were treated for 3 h with fenofibric acid (250  $\mu$ M), ciprofibrate (250  $\mu$ M), Wy 14,643 (250  $\mu$ M), bezafibrate (250  $\mu$ M), or vehicle (*DMSO*, Me<sub>2</sub>SO) and then stimulated for 21 h with IL-6 (25 ng/ml) or vehicle as indicated. *a*, total RNA (10  $\mu$ g) was subjected to Northern blot analysis using human fibrinogen- $\beta$  (top panel) or 36B4 (bottom panel) cDNA probes. *b*, fibrinogen concentrations were measured in culture medium by enzyme-linked immunosorbent assay and expressed as mean  $\pm$  S.D.

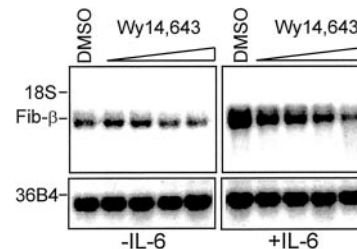
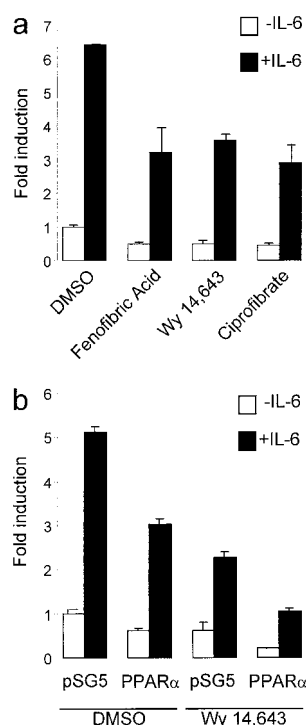


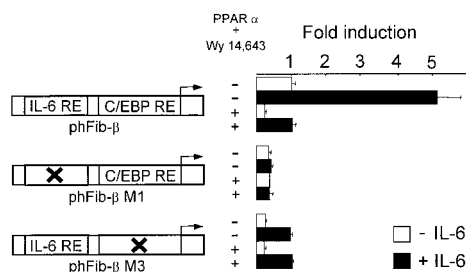
FIG. 3. **Dose-dependent effect of Wy 14,643 on fibrinogen expression in HepG2 cells.** Cells were treated for 3 h with increasing concentrations of Wy 14,643 (3, 10, 30, and 100  $\mu$ M) and stimulated for 21 h or not with IL-6 (25 ng/ml) as indicated. Total RNA (10  $\mu$ g) was subjected to Northern blot analysis using human fibrinogen- $\beta$  (top panel) or 36B4 (bottom panel) cDNA probes. *DMSO*, Me<sub>2</sub>SO.

activity was unaffected by activated PPAR $\alpha$ . Mutation in the C/EBP binding site of fibrinogen- $\beta$  promoter (phFib- $\beta$  M3) resulted in a weaker basal transcriptional activity and in a diminished IL-6 inducibility. Interestingly, activated PPAR $\alpha$  did not influence transcriptional activity of phFib- $\beta$  M3 in either the absence or presence of IL-6. These results point to a crucial role of the IL6-RE in both basal and IL-6-induced fibrinogen- $\beta$  promoter activity, whereas the C/EBP binding site alone does not respond to IL-6 but rather controls the overall transcriptional activity. Furthermore, PPAR $\alpha$  does not interfere directly with the IL-6 pathway but diminishes the overall activity of fibrinogen- $\beta$  promoter by antagonizing C/EBP $\beta$ -mediated activation of fibrinogen- $\beta$  gene transcription.

To determine whether PPAR $\alpha$  could directly interfere with the C/EBP $\beta$ -mediated activation of fibrinogen- $\beta$  transcription,

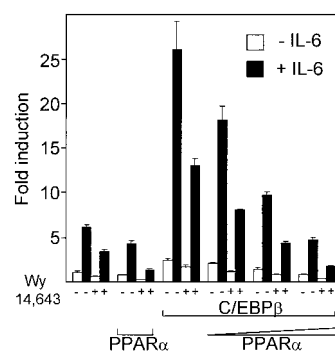


**FIG. 4. Effect of fibrates on human fibrinogen- $\beta$  promoter activity.** HepG2 cells were transfected with the human fibrinogen- $\beta$  promoter reporter plasmid (1  $\mu$ g) and stimulated (*black columns*) or not (*white columns*) with IL-6 (25 ng/ml) for 24 h. *a*, cells treated with 10  $\mu$ M of various PPAR $\alpha$  agonists or vehicle as indicated. *b*, cells were co-transfected with phFib $\beta$  and 200 ng of pSG5-hPPAR $\alpha$  or pSG5 vectors and treated with Wy 14,643 (10  $\mu$ M) or vehicle for 24 h. Luciferase activities are expressed as mean  $\pm$  S.D. DMSO, Me<sub>2</sub>SO.



**FIG. 5. Mapping of the PPAR $\alpha$  response element in the human fibrinogen- $\beta$  promoter.** HepG2 cells were transfected with 1  $\mu$ g of either human fibrinogen- $\beta$  promoter reporter construct phFib- $\beta$ , phFib- $\beta$ -M1, or phFib- $\beta$ -M3 reporter constructs as indicated, and subsequently stimulated with (*black columns*) or without (*white columns*) IL-6 (25 ng/ml). +, cells co-transfected with pSG5-hPPAR $\alpha$  and activated with Wy 14,643 (10  $\mu$ M); -, cells were co-transfected with pSG5 vector and vehicle-treated. Luciferase activities are expressed as mean  $\pm$  S.D.

we analyzed the effect of PPAR $\alpha$  on C/EBP $\beta$ -induced fibrinogen- $\beta$  promoter activity. As described above, overexpression of PPAR $\alpha$  decreases basal and IL-6-induced activity of fibrinogen- $\beta$  promoter, an effect that was enhanced in the presence of Wy 14,643 (Fig. 6). Transfection of C/EBP $\beta$  resulted in a 3-fold induction of basal fibrinogen- $\beta$  promoter activity, and luciferase activity was further increased upon addition of IL-6. In the absence of IL-6, co-transfection of a constant amount of C/EBP $\beta$  and increasing amounts of PPAR $\alpha$  led to a dose-dependent inhibition of fibrinogen- $\beta$  transactivation, an effect that was amplified by the presence of Wy 14,643 (Fig. 6). Interestingly, PPAR $\alpha$  also repressed in a dose-dependent manner fibrinogen- $\beta$  transcriptional activity induced by C/EBP $\beta$  in the presence of IL-6 stimulation. Again, this action of PPAR $\alpha$  was much more pronounced in the presence of

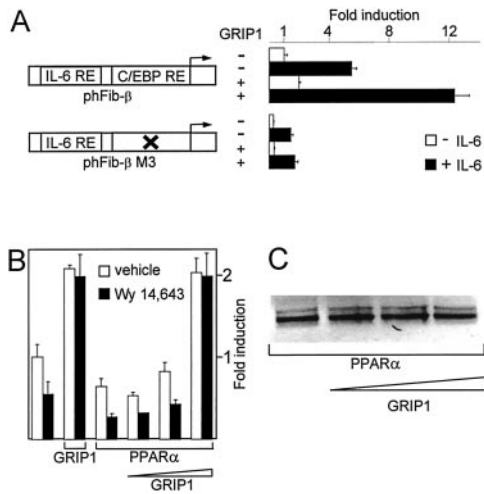


**FIG. 6. Inhibition of C/EBP $\beta$ -induced fibrinogen- $\beta$  promoter activity by PPAR $\alpha$ .** HepG2 cells were transfected with the human fibrinogen- $\beta$  promoter reporter plasmid (1  $\mu$ g) in the presence of PPAR $\alpha$  and/or C/EBP $\beta$  expression vectors. Increasing amounts of pSG5-hPPAR $\alpha$  (0.5 $\times$ , 1 $\times$ , and 2 $\times$ ) were added to a constant amount (100 ng) of pC/EBP $\beta$ . Cells were stimulated (*black columns*) or not (*white columns*) with IL-6 (25 ng/ml) and treated with 10  $\mu$ M Wy 14,643 (+) or Me<sub>2</sub>SO (-). Luciferase activities are expressed as mean  $\pm$  S.D.

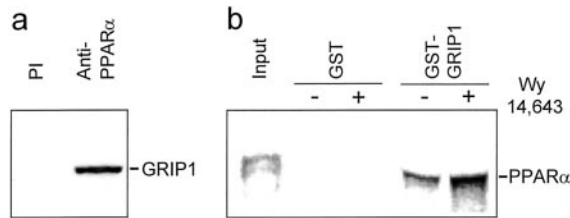
Wy 14,643. Taken together, these results demonstrate that PPAR $\alpha$  counteracts C/EBP $\beta$ -induced activation of the fibrinogen- $\beta$  promoter.

**GRIP1/TIF2 Alleviates the Repressive Effect of PPAR $\alpha$  on Fibrinogen- $\beta$  Transcription**—Next, we addressed the question whether PPAR $\alpha$  inhibits C/EBP induction of fibrinogen- $\beta$  promoter activity through direct protein-protein interaction or by competition for a common co-factor. Because GST fusion protein pull-down assays and yeast two hybrid analysis failed to detect a direct interaction between PPAR $\alpha$  and C/EBP $\alpha$  as reported by Hollenberg *et al.* (36), it is unlikely that PPAR $\alpha$  and C/EBP $\beta$  directly interact. Therefore, we hypothesized that interference with coactivator might be a mechanism of gene repression by PPAR $\alpha$ . Interestingly, a member of the TIF family has been previously identified as a co-factor interacting with both C/EBP and glucocorticoid receptor to regulate expression of the  $\alpha$ 1-acid glycoprotein gene, another acute phase protein (32). In addition, GRIP1/TIF2 has been described as a transcriptional mediator for the ligand-dependent activation function of nuclear receptors (33). We first analyzed whether fibrinogen- $\beta$  transcriptional activity could be affected by GRIP1/TIF2. Transfection of GRIP1/TIF2 in HepG2 cells increased fibrinogen- $\beta$  reporter activity and enhanced stimulation of fibrinogen transcription in the presence of IL-6 (Fig. 7A). GRIP1/TIF2 had no effect on fibrinogen- $\beta$  promoter mutated in its C/EBP binding site (phFib- $\beta$  M3), showing that C/EBP binding site integrity is required. To investigate whether GRIP1/TIF2 plays a role in PPAR $\alpha$ -mediated repression of fibrinogen transcription, GRIP1/TIF2 and PPAR $\alpha$  expression were co-transfected together with the fibrinogen- $\beta$  reporter vector. Wy 14,643 treatment failed to repress transcription when GRIP1/TIF2 was overexpressed (Fig. 7B). In addition, co-transfection of increasing amounts of GRIP1/TIF2 expression vector with a constant amount of PPAR $\alpha$  expression vector led to the abolishment of PPAR $\alpha$  inhibitory effect on fibrinogen- $\beta$  transactivation; this also occurred in the presence of PPAR $\alpha$  ligand. Transfected cell extract subjected to electrophoresis demonstrated that the abolishment of PPAR $\alpha$  action by GRIP1/TIF2 was not linked to an indirect effect on PPAR $\alpha$  expression vector (Fig. 7C). These data highlight that GRIP1/TIF2 potentiates C/EBP-mediated fibrinogen- $\beta$  transcription and strongly suggest that PPAR $\alpha$  exerts its repressive effect through titration of this co-factor.

**PPAR $\alpha$  Physically Interacts with GRIP1/TIF2**—To evaluate whether sequestration of GRIP1/TIF2 by PPAR $\alpha$  also occurs under physiological conditions, association between endoge-



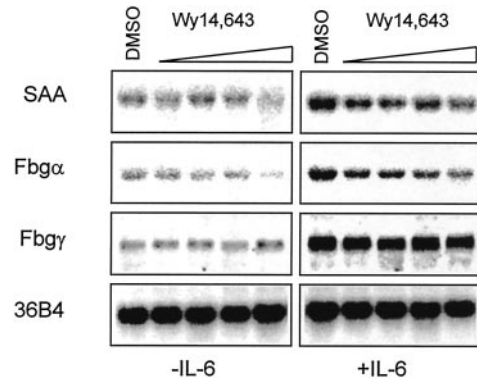
**FIG. 7. GRIP1/TIF2 inhibits the repression of human fibrinogen promoter activity by PPAR $\alpha$ .** *A*, HepG2 cells were transfected with 1  $\mu$ g of either human fibrinogen- $\beta$  promoter phFib- $\beta$  or phFib- $\beta$ -M3 reporter constructs as indicated and subsequently stimulated with (black columns) or without (white columns) IL-6 (25 ng/ml). +, cells co-transfected with pSG5-GRIP1/TIF2; -, cells were co-transfected with pSG5 vector. *B*, HepG2 cells were transfected with the human fibrinogen- $\beta$  promoter reporter plasmid (1  $\mu$ g) in the presence of PPAR $\alpha$  and/or GRIP1/TIF2 expression vectors. Increasing amounts of pSG5-GRIP1/TIF2 (0.5 $\times$ , 1 $\times$ , and 2 $\times$ ) were added to a constant amount (100 ng) of pSG5-hPPAR $\alpha$ . Cells were treated with 10  $\mu$ M Wy 14,643 or vehicle (Me<sub>2</sub>SO). Luciferase activities are expressed as mean  $\pm$  S.D. *C*, analysis of PPAR $\alpha$  protein levels by Western blotting of protein extract from HepG2 cells transfected with a constant amount of PPAR $\alpha$  and increasing amounts of GRIP1/TIF2 expression vectors.



**FIG. 8. GRIP1/TIF2 and PPAR $\alpha$  interact *in vitro* and *in vivo*.** *a*, co-immunoprecipitation was performed on protein extract prepared from fresh human liver. Anti-PPAR $\alpha$  antibody was used to precipitate endogenous PPAR $\alpha$  and GRIP-1. *b*, GST pull-down assay using GST-GRIP1/TIF2(536–1121) and *in vitro* synthesized <sup>35</sup>S-labeled PPAR $\alpha$  in the presence or absence of Wy 14,643 as indicated. The input represents 20% of the amount of PPAR $\alpha$  protein.

nous proteins expressed at physiological levels in regular human hepatocytes was assessed by co-immunoprecipitation. Endogenous PPAR $\alpha$ -GRIP1/TIF2 complexes were precipitated from human liver protein extracts (Fig. 8*a*). Specific co-immunoprecipitation of GRIP1/TIF2 was detected by anti-GRIP1/TIF2 Western blot when anti-PPAR $\alpha$  but not control antibody was used for precipitation (Fig. 8*a*).

We also performed *in vitro* experiments to investigate whether protein-protein interaction between PPAR $\alpha$  and GRIP1/TIF2 is ligand-dependent. GST pull-down experiments revealed that interaction between GRIP1/TIF2 and PPAR $\alpha$  is enhanced in the presence of PPAR $\alpha$  ligand (Fig. 8*b*), which is in agreement with the transfection results. Because PPAR $\alpha$  ligands can potentiate the interaction between PPAR $\alpha$  and GRIP1/TIF2, we analyzed whether PPAR $\alpha$  ligand binding domain (LBD) is required for the transcriptional repression of fibrinogen- $\beta$  promoter activity by PPAR $\alpha$ . We therefore compared the activity of wild type PPAR $\alpha$  with that of the recently identified PPAR $\alpha$  truncated isoform (31) lacking the entire LBD (PPAR $\alpha$ - $\Delta$ LBD) on fibrinogen- $\beta$  transcription. Transcriptional activity of fibrinogen- $\beta$  was not affected by co-transfec-



**FIG. 9. Dose-dependent effect of Wy 14,643 on gene expression of acute phase proteins in HepG2 cells.** Cells were treated for 3 h with increasing concentrations of Wy 14,643 (3, 10, 30, and 100  $\mu$ M) and stimulated for 21 h or not with IL-6 (25 ng/ml) as indicated. Total RNA (10  $\mu$ g) was subjected to Northern blot analysis using human serum amyloid A (SAA), fibrinogen- $\alpha$  (Fbg $\alpha$ ), fibrinogen- $\gamma$  (Fbg $\gamma$ ), or 36B4 cDNA probes.

tion of PPAR $\alpha$ - $\Delta$ LBD, in contrast to PPAR $\alpha$  wild type (data not shown). Altogether, these experiments demonstrate that PPAR $\alpha$  negatively regulates fibrinogen- $\beta$  through sequestration of GRIP1/TIF2 and that functional interference between PPAR $\alpha$  and GRIP1/TIF2 requires the presence of the LBD of PPAR $\alpha$ .

**PPAR $\alpha$  Down-regulates Positive Acute Phase Response Genes Other Than Fibrinogen- $\beta$** —To determine a broader regulatory pattern of the PPAR $\alpha$  regulatory process, we examined the effect of Wy 14,643 on the expression of other acute phase genes, such as serum amyloid A, fibrinogen- $\alpha$ , and fibrinogen- $\gamma$ , in HepG2 cells. The expression of serum amyloid A and fibrinogen- $\alpha$  were decreased following treatment with Wy 14,643 in a dose-dependent manner under both basal and IL-6-stimulated conditions (Fig. 9). By contrast, both basal and IL-6-stimulated expression of fibrinogen- $\gamma$  gene (which contains an IL-6 response element but lacks a C/EBP response element) were unaffected by treatment with Wy 14,643. These results demonstrate that the PPAR $\alpha$  repressive action is not restricted to the isolated case of fibrinogen- $\beta$  but also applies to other acute phase genes containing a C/EBP response element.

Because glucocorticoids potentiate the action of IL-6 on the regulation of acute phase genes, we sought to determine whether PPAR $\alpha$  could modulate this effect. HepG2 cells were stimulated by IL-6 and dexamethasone in the presence of Wy 14,643. As shown in Fig. 10, the low basal expression of the genes tested was only slightly affected by dexamethasone treatment. By contrast, the IL-6-stimulated expression of serum amyloid A, fibrinogen- $\alpha$ , fibrinogen- $\beta$ , and fibrinogen- $\gamma$  was enhanced by the presence of dexamethasone. Interestingly, the potentiation of IL-6 action by dexamethasone was counteracted by treatment with Wy 14,643 (Fig. 10, right panel). Altogether, these results indicate that PPAR $\alpha$  also controls the expression of acute phase genes stimulated by IL-6 in combination with dexamethasone.

## DISCUSSION

High circulating plasma fibrinogen levels are associated with an increased risk for myocardial infarction and stroke. Therefore, factors that down-regulate fibrinogen expression may be of importance in the prevention of cardiovascular diseases. In previous studies, we showed that PPAR $\alpha$  regulates basal levels of plasma fibrinogen and established that PPAR $\alpha$  mediates the fibrate-induced suppression of fibrinogen expression in rodents (20). We now report that PPAR $\alpha$  also negatively regulates fibrinogen expression in humans under both basal and IL-6-

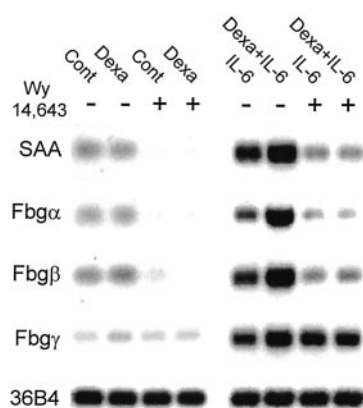


FIG. 10. Effect of Wy 14,643 on gene expression of acute phase proteins induced by dexamethasone and IL-6. Cells were treated for 3 h with Wy 14,643 (100  $\mu$ M) and incubated for 21 h with or without IL-6 (25 ng/ml) in the presence or absence of dexamethasone (10  $\mu$ M) as indicated. Total RNA (10  $\mu$ g) was subjected to Northern blot analysis using human serum amyloid A (SAA), fibrinogen- $\alpha$  (*Fbg* $\alpha$ ), fibrinogen- $\beta$  (*Fbg* $\beta$ ), fibrinogen- $\gamma$  (*Fbg* $\gamma$ ), or 36B4 cDNA probes.

stimulated conditions. We furthermore elucidate the molecular mechanism of this action. We demonstrated that PPAR $\alpha$  interferes with the C/EBP $\beta$  but not the IL-6 pathway. Remarkably, we identified GRIP1/TIF2 as a positive factor of fibrinogen- $\beta$  gene regulation and found that titration of GRIP1/TIF2 by PPAR $\alpha$  may explain the negative regulation of fibrinogen- $\beta$  expression by fibrates. Finally, we observed that the inhibitory effect of PPAR $\alpha$  can be extended to other acute phase response genes containing a C/EBP response element.

Because fibrates suppress fibrinogen- $\beta$  expression at the transcriptional level through activation of PPAR $\alpha$  under both basal and IL-6-induced conditions, we sought to identify the cis-regulatory elements involved in PPAR $\alpha$  action. Functional analysis of the promoter of the human fibrinogen- $\beta$ -chain in HepG2 cells revealed the existence of two response elements that are crucial for full induction by IL-6 (7). The first element, present in several promoters of acute phase response genes (34, 35), is the so-called IL-6 RE. Transfection of a fibrinogen- $\beta$  promoter construct carrying mutations in the IL-6 RE led to a loss of IL-6 inducibility. The second important response element consists of a C/EBP binding site. As previously reported, we found that C/EBP $\beta$  induces fibrinogen- $\beta$  transcriptional activity (7). Site-directed mutagenesis of the C/EBP $\beta$  binding site did not affect IL-6 inducibility but rendered the fibrinogen- $\beta$  promoter unresponsive to activated PPAR $\alpha$ . These observations indicate that PPAR $\alpha$  does not directly interfere with the IL-6 transduction pathway but rather interferes with the C/EBP $\beta$  activation function of the fibrinogen- $\beta$  promoter. Notably, whereas combination of C/EBP $\beta$  expression and IL-6 stimulation conferred full induction of fibrinogen- $\beta$  transcription, co-transfection of PPAR $\alpha$  reduced the overall promoter activity in a dose-dependent manner but was unable to abolish IL-6 inducibility. Functional antagonism between PPAR and C/EBP factors has also been described for the regulation of the leptin promoter (36). In this study, it was observed that PPAR $\gamma$  down-regulates leptin expression by inhibiting C/EBP $\alpha$ -mediated transactivation, although direct interaction between PPAR and C/EBP was not shown.

Our studies on the regulatory mechanisms of fibrinogen expression have focused on the  $\beta$ -chain gene because of findings from whole animal and cell culture studies indicating that the  $\beta$ -chain synthesis is rate-limiting compared with  $\alpha$ - and  $\gamma$ -chains (14, 37). However, the possibility cannot be excluded that under certain experimental conditions or in different species, regulation of  $\alpha$  and  $\gamma$  fibrinogen genes are equally impor-

tant (9). In that respect, it may be significant that also the  $\alpha$ -fibrinogen gene appears to be regulated by a C/EBP-dependent pathway (7, 8, 10). By contrast, the fact that no C/EBP-like binding site gene has been identified in  $\gamma$ -fibrinogen might explain why its expression is hardly suppressed by fibrates (20). Interestingly, we observed that fibrinogen- $\alpha$  expression is down-regulated by PPAR $\alpha$  ligand, whereas fibrinogen- $\gamma$  expression was unaffected by PPAR $\alpha$  activation in both the presence and the absence of IL-6, thus corroborating our findings about the requirement of C/EBP binding site in the repressive action of PPAR $\alpha$ .

Because it has recently been reported that TIF1 $\beta$  co-operates with C/EBP $\beta$  to induce the expression of  $\alpha$ 1-acid glycoprotein gene (32), another acute phase response gene, we asked ourselves whether titration of such a coactivator could also be the mechanism by which PPAR $\alpha$  exerts its repressive effect on fibrinogen- $\beta$  transcription. Results from transient transfection experiments revealed that GRIP1/TIF2 enhances fibrinogen- $\beta$  transcription and thereby counteracts the inhibitory effect of PPAR $\alpha$ . Moreover, co-immunoprecipitated PPAR/GRIP complexes were shown to exist *in vivo* in human liver, and GST-pull down experiments showed that the functional interference PPAR/GRIP1/TIF2 was due to a direct ligand-stimulated physical interaction. We therefore identified a novel positive regulator of fibrinogen- $\beta$  transcription and demonstrated that PPAR $\alpha$  mediates the repressive effect of fibrates on fibrinogen expression through sequestration of the coactivator GRIP1/TIF2.

Our findings that PPAR $\alpha$  can diminish the functional activity of C/EBP through binding to the coactivator GRIP1/TIF2 and thereby down-regulates fibrinogen expression may be also of relevance for other acute phase response genes (APRGs) because numerous APRGs are regulated by C/EBP transcription factors (38, 39). Indeed, for some of these genes, *e.g.*  $\alpha$ 1-glycoprotein and fibrinogen- $\alpha$ , it has been shown that PPAR $\alpha$  activators decrease their mRNA levels. Here we report that PPAR $\alpha$  inhibitory effect is not restricted to fibrinogen- $\beta$  gene but may be extended to serum amyloid A and fibrinogen- $\alpha$  under both basal and inflammatory conditions.

Our observation that the stimulatory effect of IL-6 on the expression of acute phase genes is potentiated in the presence of dexamethasone is in agreement with previous reports (38, 40, 41). This potentiating effect of dexamethasone has been ascribed to interaction between C/EBP, glucocorticoid receptor, and TIF in the context of  $\alpha$ 1-acid glycoprotein gene (32). Our finding that PPAR $\alpha$  activation also prevents the dexamethasone-potentiated IL-6 effect on several acute phase genes is in accordance with a mechanism in which PPAR $\alpha$  binds the coactivator GRIP1/TIF2, whether it is complexed with glucocorticoid receptor or not.

Involvement of PPAR $\alpha$  in various other anti-inflammatory mechanisms suggests that PPAR $\alpha$  may also have a role in the regulation of APRGs that are not under control of C/EBP-dependent signaling pathways. For example, AP-1 and NF- $\kappa$ B sites also participate in the activation of certain APRGs (42, 43). Because PPAR $\alpha$  can also interfere negatively with AP-1 and NF- $\kappa$ B transcription complex (25, 26, 44), it is possible that PPAR $\alpha$  is also implicated in the negative regulation of APRGs regulated by these transcription factors. This hypothesis is corroborated by the findings that exposure of rodents to peroxisome proliferators leads to the down-regulation of diverse liver-specific genes, including acute phase response genes (45). Involvement of PPAR $\alpha$  in several cytokine signaling pathways through different mechanisms suggests a general role for PPAR $\alpha$  in the regulation of acute phase response in the liver. PPAR $\alpha$  has also been reported to be a negative regulator of

vascular gene response by inhibition of inflammatory mediators involved in atherogenesis (25, 26). Indeed, PPAR $\alpha$  activators lower plasma concentrations of IL-6, tumor necrosis factor  $\alpha$ , and interferon  $\gamma$  in patients with atherosclerosis (24, 25) and suppress cytokine-stimulated IL-6 production in human aortic smooth muscle cells (25), inducible nitric-oxide synthase activity in murine macrophages (46), and vascular cell adhesion molecule-1 expression in endothelial cells (47). Altogether, these data indicate that PPAR $\alpha$  plays an important role in the control of inflammation.

The positive association between plasma fibrinogen and cardiovascular diseases is dependent on a relatively small variation in fibrinogen concentrations. Small changes in PPAR $\alpha$  activity may be sufficient to bring about such a small variation in fibrinogen concentrations. First, PPAR $\alpha$  activity is modulated by the level of expression of PPAR $\alpha$ , which is reported to vary among individuals (31). Second, PPAR $\alpha$  activity depends on the quality and the quantity of PPAR $\alpha$  activators. Existing drugs, such as fibrates, and also certain naturally occurring dietary fatty acids are activators of PPAR $\alpha$  and may thereby influence fibrinogen expression. These data highlight the necessity to further understand the regulation of fibrinogen synthesis to allow a rational approach to lower fibrinogen plasma levels.

The results of the present study demonstrate that fibrates down-regulate human fibrinogen- $\beta$  via negative interference with C/EBP $\beta$  as a result of titration of GRIP1/TIF2 by PPAR $\alpha$  and that the repressive action of PPAR $\alpha$  may also be applicable to other acute phase response genes. This novel mode of action of PPAR $\alpha$  agonists further adds to the anti-inflammatory potential of PPAR $\alpha$  and is complementary to its beneficial effect on lipid and lipoprotein metabolism. These observations underscore the importance of PPAR $\alpha$  as an attractive target for therapeutic strategies for preventing cardiovascular diseases.

**Acknowledgments**—We thank Sebastien Playe and Odile Vidal for excellent technical assistance and Jean Dallongeville for helpful discussions. We are grateful to M. Stallcup for providing the GRIP1/TIF2 plasmid constructs.

## REFERENCES

- Heinrich, J., Balleisen, L., Schulte, H., Assmann, G., and van de Loo, J. (1994) *Arterioscler. Thromb.* **14**, 54–59
- Meade, T. W., Mellows, S., Brozovic, M., Miller, G. J., Chakrabarti, R. R., North, W. R., Haines, A. P., Imeson, J. D., and Thompson, S. G. (1986) *Lancet* **2**, 533–537
- Wilhelmsen, L., Svardsudd, K., Korsan-Bengtson, K., Larsson, B., Welin, L., and Tibblin, G. (1984) *N. Engl. J. Med.* **311**, 501–505
- Kant, J. A., Fornace, A. J., Saxe, D., Simon, M. I., McBride, O. W., and Crabtree, G. R. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82**, 2344–2348
- Courtois, G., Baumhueter, S., and Crabtree, G. R. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 7937–7941
- Morgan, J. G., Courtois, G., Fourel, G., Chodosh, L. A., Campbell, L., Evans, E., and Crabtree, G. R. (1988) *Mol. Cell. Biol.* **8**, 2628–2637
- Anderson, G. M., Shaw, A. R., and Shafer, A. (1993) *J. Biol. Chem.* **268**, 22650–22655
- Dalmon, J., Laurent, M., and Courtois, G. (1993) *Mol. Cell. Biol.* **13**, 1183–1193
- Hu, C. H., Harris, J. E., Davie, E. W., and Chung, D. W. (1995) *J. Biol. Chem.* **270**, 28342–28349
- Liu, Z., and Fuller, G. M. (1995) *J. Biol. Chem.* **270**, 7580–7586
- Mizuguchi, J., Hu, C. H., Cao, Z., Loeb, K. R., Chung, D. W., and Davie, E. W. (1995) *J. Biol. Chem.* **270**, 28350–28356
- Zhang, Z., Fuentes, N. L., and Fuller, G. M. (1995) *J. Biol. Chem.* **270**, 24287–24291
- Roy, S. N., Mukhopadhyay, G., and Redman, C. M. (1990) *J. Biol. Chem.* **265**, 6389–6393
- Yu, S., Sher, B., Kudryk, B., and Redman, C. M. (1983) *J. Biol. Chem.* **258**, 13407–13410
- Huber, P., Laurent, M., and Dalmon, J. (1990) *J. Biol. Chem.* **265**, 5695–5701
- Sirtori, C. R., and Colli, S. (1993) *Cardiovasc. Drugs Ther.* **7**, 823
- Hokanson, J. E., and Austin, M. A. (1996) *J. Cardiovasc. Risk* **3**, 213–219
- Anonymous (1994) *Circulation* **89**, 1329–1345
- Gervois, P., Pineda-Torra, I., Fruchart, J.-C., and Staels, B. (2000) *Clin. Chem. Lab. Med.* **38**, 3–11
- Kockx, M., Gervois, P., Poulain, P., Derudas, B., Peters, J. M., Gonzalez, F. J., Princen, H. M. G., Kooistra, T., and Staels, B. (1999) *Blood* **93**, 2991–2998
- Staels, B., Dallongeville, J., Auwerx, J., Schoonjans, K., Leitersdorf, E., and Fruchart, J.-C. (1998) *Circulation* **98**, 2088–2093
- Frick, M. H., Syvanne, M., Nieminen, M. S., Kauma, H., Majahalme, S., Virtanen, V., Kesaniemi, Y. A., Pasternack, A., and Taskinen, M. R. (1997) *Circulation* **96**, 2137–2143
- Ruotolo, G., Ericsson, C. G., Tettamanti, C., Karpe, F., Grip, L., Svane, B., Nilsson, J., de Faire, U., and Hamsten, A. (1998) *J. Am. Coll. Cardiol.* **32**, 1648–1656
- Madej, A., Okopien, B., Kowalski, J., Zielinski, M., Wysocki, J., Szygula, B., Kalina, Z., and Herman, Z. S. (1998) *Int. J. Clin. Pharmacol. Ther.* **36**, 345–349
- Staels, B., Koenig, W., Habib, A., Merval, R., Lebret, M., Pineda Torra, I., Delerive, P., Fadel, A., Chinetti, G., Fruchart, J. C., Najib, J., Maclouf, J., and Tedgui, A. (1998) *Nature* **393**, 790–793
- Delerive, P., De Bosscher, K., Besnard, S., Vanden Berghe, W., Peters, J. M., Gonzalez, F. J., Fruchart, J.-C., Tedgui, A., Haegeman, G., and Staels, B. (1999) *J. Biol. Chem.* **274**, 32048–32054
- Devchand, P. R., Keller, H., Peters, J. M., Vazquez, M., Gonzalez, F. J., and Wahli, W. (1996) *Nature* **384**, 39–43
- Staels, B., Vu-Dac, N., Kosykh, V. A., Saladin, R., Fruchart, J.-C., Dallongeville, J., and Auwerx, J. (1995) *J. Clin. Invest.* **95**, 705–712
- Staels, B., and Auwerx, J. (1992) *Development* **115**, 1035–1043
- Masiakowski, P., Breathnach, R., Bloch, J., Gannon, F., Krust, A., and Chambon, P. (1982) *Nucleic Acids Res.* **10**, 7895–7903
- Gervois, P., Pineda Torra, I., Chinetti, G., Grotzinger, T., Dubois, G., Fruchart, J.-C., Fruchart-Najib, J., Leitersdorf, E., and Staels, B. (1999) *Mol. Endocrinol.* **13**, 1535–1549
- Chang, C. J., Chen, Y. L., and Lee, S. C. (1998) *Mol. Cell. Biol.* **18**, 5880–5887
- Voegel, J. J., Heine, M. J., Zechel, C., Chambon, P., and Gronemeyer, H. (1996) *EMBO J.* **15**, 3667–3675
- Fowlkes, D. M., Mullis, N. T., Comeau, C. M., and Crabtree, G. R. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 2313–2316
- Hattori, M., Abraham, L. J., Northemann, W., and Fey, G. H. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 2364–2368
- Hollenberg, A. N., Susulic, V. S., Madura, J. P., Zhang, B., Moller, D. E., Tontonoz, P., Sarraf, P., Spiegelman, B. M., and Lowel, B. B. (1997) *J. Biol. Chem.* **272**, 5283–5290
- Yu, S., Kong, V., Kudryk, B., and Redman, C. (1987) *Thromb. Res.* **46**, 281–293
- Baumann, H., Jahreis, G. P., Morella, K. K., Won, K. A., Pruitt, S. C., Jones, V. E., and Prowse, K. R. (1991) *J. Biol. Chem.* **266**, 20390–20399
- Baumann, H., Morella, K. K., Campos, S. P., Cao, Z., and Jahreis, G. P. (1992) *J. Biol. Chem.* **267**, 19744–19751
- Baumann, H., Firestone, G. L., Burgess, T. L., Gross, K. W., Yamamoto, K. R., and Held, W. A. (1983) *J. Biol. Chem.* **258**, 563–570
- Baumann, H., Prowse, K. R., Marinkovic, S., Won, K. A., and Jahreis, G. P. (1989) *Ann. N. Y. Acad. Sci.* **557**, 280–295
- Nonaka, M., and Huang, Z. M. (1990) *Mol. Cell. Biol.* **10**, 6283–6289
- Ron, D., Brasier, A. R., McGehee, R. J., and Habener, J. F. (1992) *J. Clin. Invest.* **89**, 223–233
- Ricote, M., Li, A. C., Willson, T. M., Kelly, C. J., and Glass, C. K. (1998) *Nature* **391**, 79–82
- Corton, J. C., Fan, L. Q., Brown, S., Anderson, S. P., Bocos, C., Cattley, R. C., Mode, A., and Gustafsson, J. A. (1998) *Mol. Pharmacol.* **54**, 463–473
- Colville-Nash, P. R., Qureshi, S. S., Willis, D., and Willoughby, D. A. (1998) *J. Immunol.* **161**, 978–984
- Marx, N., Sukhova, G. K., Collins, T., Libby, P., and Plutzky, J. (1999) *Circulation* **99**, 3125–3131

**Negative Regulation of Human Fibrinogen Gene Expression by Peroxisome Proliferator-activated Receptor  $\alpha$  Agonists via Inhibition of CCAAT Box/Enhancer-binding Protein  $\beta$**

Philippe Gervois, Ngoc Vu-Dac, Robert Kleemann, Maaïke Kockx, Guillaume Dubois, Bernard Laine, Vladimir Kosykh, Jean-Charles Fruchart, Teake Kooistra and Bart Staels

*J. Biol. Chem.* 2001, 276:33471-33477.

doi: 10.1074/jbc.M102839200 originally published online June 20, 2001

---

Access the most updated version of this article at doi: [10.1074/jbc.M102839200](https://doi.org/10.1074/jbc.M102839200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 47 references, 28 of which can be accessed free at <http://www.jbc.org/content/276/36/33471.full.html#ref-list-1>