THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 16, Issue of April 16, pp. 16154–16160, 2004

Printed in U.S.A.

Global Suppression of IL-6-induced Acute Phase Response Gene Expression after Chronic *in Vivo* Treatment with the Peroxisome Proliferator-activated Receptor-α Activator Fenofibrate*

Received for publication, January 13, 2004
Published, JBC Papers in Press, February 5, 2004, DOI 10.1074/jbc.M400346200

Philippe Gervois‡§¶, Robert Kleemann‡§‖, Antoine Pilon, Frédéric Percevault**, Wolfgang Koenig‡‡, Bart Staels**, and Teake Kooistra‡**

*From the ‡Gaubius Laboratory, TNO-Prevention and Health, Zernikedreef 9, P. O. Box 2215, 2301 CE Leiden, The Netherlands, the **Département d'Athérosclerose, U.545 INSERM, Institut Pasteur de Lille and Faculté de Pharmacie, Université de Lille II, 59006 Lille cedex, France, and the ‡‡Department of Internal Medicine II – Cardiology, University of Ulm, D89081 Ulm, Germany*

The peroxisome proliferator-activated receptor α (PPARα), which is highly expressed in liver, plays key roles in lipid metabolism and inflammation. Interleukin-6 (IL-6) is the principal inducer of acute phase response (APR) gene expression. In the present study, we demonstrate that chronic treatment with the PPARα agonist fenofibrate fully prevents the IL-6-induced APR gene expression in wild-type but not in PPARα-deficient mice. PPARα prevents the IL-6-induced expression of the positive APR genes fibrinogen-α, -β, -γ, haptoglobulin, and serum amyloid A and the IL-6-induced suppression of the negative APR gene, major urinary protein. Furthermore, the effect of PPARα on the APR gene expression does not simply consist in a delayed systemic response to IL-6 but occurs directly at the transcriptional level. This global suppression of acute phase gene transcription may be explained by two PPARα-dependent *in vivo* effects: 1) PPARα activation results in the down-regulation of the IL-6 receptor components gp80 and gp130 in the liver, thereby reducing the phosphorylation and activation of the downstream transcription factors STAT3 and c-Jun that transduce the IL-6 signal; and 2) PPARα reduces the basal expression of the transcription factors CCAAT enhancer-binding protein-α, -β, -δ, which are responsible for immediate and maintained transcription of APR genes. A similar global effect of fenofibrate on acute phase protein expression is observed in hyperlipidemic patients chronically treated with fenofibrate, which displayed decreased plasma concentrations of the positive APR proteins fibrinogen, C-reactive protein, serum amyloid A, plasminogen, and α2-macroglobulin and increased plasma concentrations of the negative APR albumin, underlining the clinical significance of our findings.

* This work was supported by the European Community Marie Curie Fellowship QLK-1-CT-1999-51206 (to P. G.) and FEDER/ Conseil Régional Nord/Pas-de-Calais (Genopole Grant 01360124) and the Leducq Foundation (to B. S.). 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.

§ Both authors contributed equally to the work.

¶ To whom correspondence should be addressed: Laboratoire de Biochimie, Faculté de Pharmacie, 3, rue du Professeur Laguesse, BP83, 59006 Lille cedex, France. Tel.: 33-3-20-96-40-40; Fax: 33-3-20-96-49-11; E-mail: pgervois@pharma.univ-lille2.fr.

‖ Supported by Grant 99.110 from the Dutch Heart Foundation and Grant 016.036.061 from the Dutch Organization for Scientific Research.

The acute phase response (APR)[1] is a generalized response of the organism to multiple disturbances of its physiological homeostasis. Inflammatory processes are the main causes for the initiation of these defense mechanisms (1). As part of systemic inflammatory reactions, interleukin 6 (IL-6) regulates APR genes in liver cells including C-reactive protein (*CRP*), fibrinogen, serum amyloid A (*SAA*), α2-macroglobulin, and albumin (2–4). Elevated levels of IL-6 and liver APR genes that are a reflection of the inflammatory state have been reported in patients with acute coronary syndrome (5, 6).

IL-6 actions are mediated by a specific cell surface IL-6 receptor (IL-6R), an 80-kDa glycoprotein (gp80), and a signal-transducing molecule, glycoprotein gp130, which is also the signaling molecule for various IL-6 family cytokines (7, 8). IL-6 binds to its cognate receptor, and the IL-6/IL-6R forms a complex with a gp130 homodimer. Ligand-induced oligomerization of receptor subunits leads to activation and phosphorylation of signal transducer and activator of transcription 3 (STAT3) and of linker proteins, which propagate the signal to other pathways and cause activation of immediate early response genes, such as c-*jun* (9). STAT3 and c-Jun cooperatively activate APR gene transcription (10–12) and act in concert with various isoforms of the transcription factor CAAT enhancer-binding protein (C/EBP) (13) to up-regulate APR protein expression (14–17).

Fibrates are hypolipidemic drugs that efficiently normalize hypertriglyceridemia and hypercholesterolemia. In addition, fibrates may also lower fibrinogen and CRP plasma levels and affect other plasma APR proteins (18–20). Fibrates exert their action via activation of the nuclear receptor peroxisome proliferator-activated receptor α (PPARα). PPARα regulates transcription of target genes via binding of PPARα with its heterodimeric partner retinoid X receptor to specific response elements. This pathway is mainly involved in the regulation of genes that play a role in lipid and lipoprotein metabolism and may, in large part, explain the normolipidemic action of fibrates (21). Furthermore, PPARα has been demonstrated to act as a negative regulator of genes involved in the inflammatory response by antagonizing the activity of transcription factors, such as NF-κB and AP-1, partly by direct interaction with proteins such as p65 and c-Jun (20, 22). In line with these findings, impaired expression of PPARα has been found to

[1] The abbreviations used are: APR, acute phase response; PPARα, peroxisome proliferator-activated receptor α; IL, interleukin; IL-6R, IL-6 receptor; C/EBP, CAAT enhancer-binding protein; CRP, C-reactive protein; SAA, serum amyloid A; ACO, acyl-CoA oxidase; HG, haptoglobulin; MUP, major urinary protein; Fbg, fibrinogen; STAT, signal transducers and activators of transcription.

sustain the inflammatory response (23).

We previously showed that PPARα agonists attenuate the IL-6-induced expression of human fibrinogen genes *in vitro* in hepatocytes pretreated for 4 h with PPARα activators (24). In the present work, we demonstrate that chronic *in vivo* treatment with the PPARα activator fenofibrate results in the total suppression of the IL-6-induced APR response gene expression in a PPARα-dependent manner. The suppressive effect involves both negative and positive APR genes. The inhibitory effect of PPARα activation occurs at the level of transcription as shown by nuclear run-on experiments. An explanation for this global transcriptional effect is provided by the PPARα-dependent down-regulation of the IL-6 receptor components gp80 and gp130 and by the PPARα-dependent down-regulation of APR protein transcription factors controlling APR gene transcription. Finally, fenofibrate treatment of hyperlipidemic human subjects resulted in a similar global effect on positive and negative APR protein expression, thereby underlining the clinical significance of our findings.

EXPERIMENTAL PROCEDURES

Animal Studies—Animal studies were performed in compliance with European Community specifications regarding the use of laboratory animals. Experimental conditions have been described previously (19). Briefly, male Sv/129 homozygous PPARα wild-type (+/+) and PPARα-deficient (−/−) mice (10–12 weeks of age) (a kind gift of Dr. Frank Gonzalez) were fed for 17 days with either a standard mouse chow or mouse chow containing 0.2% (w/w) fenofibrate (three mice per treatment). Mice were subjected to intraperitoneal injection of IL-6 (0.5 μg/mice) or saline buffer 4 h before sacrifice. Animals were killed by exsanguination under ether anesthesia. Livers were removed immediately, weighed, rinsed with 0.9% (w/v) NaCl, and subjected to nucleus isolation. For RNA analysis, a similar second experiment with longer IL-6 treatment (24 h) was performed with four animals/group. For analysis of fenofibrate effect on gp130 protein at different time periods (1, 3, 7, 14 days), a time course experiment was performed with either a standard chow or mouse chow containing 0.2% (w/w) fenofibrate (three mice per treatment). For immunoblotting, livers were homogenized in phosphate-buffered saline containing proteinase inhibitors (Roche Diagnostics), centrifuged (5 min, 13,000 rpm) at 4 °C, and soluble proteins were boiled in Laemmli electrophoresis buffer for immunoblot analysis.

Preparation of Nuclei and Run-on Assays—Nuclei were prepared from fresh mice livers as described by Gorski et *al.* (25). Transcription run-on assays were performed as described by Nevins (26). Equivalent amounts of labeled nuclear RNA were hybridized for 48 h at 42 °C to 1 μg of purified cDNA probes immobilized on Hybond C Extra filters (Amersham Biosciences). The following cDNAs were spotted: a mouse fibrinogen Aα-chain cDNA probe, a mouse fibrinogen Bβ-chain cDNA probe, a mouse fibrinogen γ-chain cDNA probe, a rat acyl-CoA oxidase (ACO) cDNA probe, a mouse glyceraldehyde-3-phosphate deshydrogenase cDNA probe, a mouse serum amyloid A cDNA probe, a mouse haptoglobulin cDNA probe, a major urinary protein cDNA probe, and a human acidic ribosomal phosphoprotein 36B4 cDNA probe. After hybridization, filters were washed at room temperature for 10 min in 0.5× SSC and 0.1% (w/v) SDS and washed twice for 30 min at 65 °C and then subsequently exposed to an x-ray film (X-OMAT-AR, Eastman Kodak Co.).

RNA Analysis—Total RNA extraction and Northern blot analysis were performed as described (27) using as cDNA probes: mouse fibrinogen Aα-chain, mouse fibrinogen Bβ-chain, mouse fibrinogen γ-chain, rat ACO, serum amyloid A, haptoglobulin, major urinary protein, and human acidic ribosomal phosphoprotein 36B4. RNA expression of gp80, gp130, and 28S was analyzed by real time quantitative PCR using SYBR Green technology on a MX4000 apparatus (Stratagen). PCR was performed with the following oligonucleotides: forward, 5′-AAG CAG CAG GCA ATG TTA CC-3′, and reverse, 5′-CAT AAA TAG TCC CCA GTG TCG-3′ for gp80; forward, 5′-AGG GGA AGA ATA TGC TGT GC-3′, and reverse, 5′-AAG TGC CAT GCT TTG ACT GG-3′ for gp130; forward, 5′-AAA CTC TGG TGG AGG TCC GT-3′, and reverse, 5′-CTT ACC AAA AGT GGC CCA CTA-3′ for 28S. Quantification of gp80 and gp130 mRNA expression was corrected using ribosomal protein 28S gene expression as a internal control.

Western Blotting—For SDS-PAGE, samples were electrophoresed as described (24). Proteins were blotted onto Immobilon-P polyvinylidene fluoride transfer membranes (Millipore, Bedford, MA). Blots were blocked with 5% (w/v) skim milk powder (Merck) diluted in 20 mM Tris (pH 7.4), 55 mM NaCl, 0.1% (v/v) Tween 20. Blots were incubated with the following primary antibodies (Santa Cruz Biotechnology, Heerhugowaard, The Netherlands): a goat anti-C/EBPα (sc-9315)antibody, a rabbit anti-C/EBPβ (sc-150) antibody, a rabbit anti-C/EBPδ (sc-636) antibody, a rabbit anti-c-Jun (sc-45), a rabbit anti-phosphoSer73 c-Jun (sc-7981) antibody, a rabbit anti-STAT3 (sc-7179) antibody, a goat anti-phospho Tyr705 STAT3 (sc-7993) antibody, a rabbit anti-IL-6R (sc-660) followed by horseradish peroxidase-conjugated secondary antibodies. For detection of gp130, the Santa Cruz Biotechnology antibody set gp130 (sc-656) in combination with the pre-adsorbed horseradish peroxidase-coupled secondary antibody (sc-2305) was used. Goat anti-β-actin antibodies (sc-1615) were used for control. All antibodies were diluted in 20 mM Tris (pH 7.4), 55 mM NaCl, 0.1% (v/v) Tween 20, 5% (w/w) bovine serum. The Super Signal West Dura extended duration substrate (Pierce) and the luminescent image work station (Roche Diagnostics) were used for visualization.

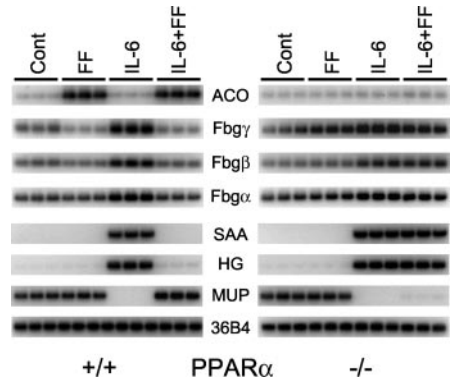

FIG. 1. **Fenofibrate controls IL-6 mediated APR gene expression via PPARα.** Wild-type (+/+) and PPARα-deficient mice (−/−) were treated with 0.2% (w/w) fenofibrate (*FF*) or not (*Cont*) for 17 days and stimulated or not for 4 h with IL-6 as indicated (three mice per group). Total RNA extracted from liver was subjected to Northern blot analysis (20 μg) using rat *ACO*, mouse *Fbgα*, mouse *Fbgβ*, mouse *Fbgγ*, *SAA*, *HG*, *MUP*, or acidic ribosomal phosphoprotein 36B4cDNA probes. Triplicates represent three mice per treatment.

Clinical Study—Mildly hyperlipidemic patients ($n = 38$) received 250 mg of fenofibrate (Knoll AG, Mannheim, Germany) per day for 4 weeks. After a 12-h overnight fast, venous blood samples were taken under standardized conditions before and after 4 weeks of treatment with fenofibrate. Fibrinogen was determined by the Clauss method (28). Plasminogen activity (in percentage) was measured by a chromogenic assay (Chromogenix, Milano, Italy). For α2-macroglobulin, albumin, and C-reactive protein, a nephelometric method was used (Beckman, Munich, Germany). Serum amyloid A was measured by a commercially available enzyme-linked immunosorbent assay (Laboserv, Giessen, Germany). Data was analyzed using the standard univariate repeated measures model (20). A one-sided p value <0.05 was regarded as significant.

RESULTS

PPARα Activation Prevents IL-6-induced APR Response at the mRNA Level in Vivo—To study the effect of chronic PPARα activation on IL-6-induced APR gene expression *in vivo*, we determined mRNA levels of APR genes in livers of wild-type and PPARα-deficient mice, untreated (control) or treated for 17 days with fenofibrate and subsequently stimulated or not by IL-6 for 4 h (Fig. 1) (three mice per treatment). The positive APR genes fibrinogen (*Fbg*), serum amyloid A (*SAA*), and haptoglobulin (*HG*) and the negative APR gene major urinary protein (*MUP*) were analyzed together with the established hepatic PPARα target gene *ACO* (29) and 36B4, which served as internal control. As expected, *ACO* mRNA levels were induced in fibrate-treated wild-type but not in PPARα-deficient mice (Fig. 1). 36B4 mRNA levels remained constant under all conditions. Basal mRNA levels of the fibrinogen α, β, and γ genes coding for the three non-identical polypeptide chains, Aα, Bβ, and γ, were decreased by fenofibrate treatment in wild-

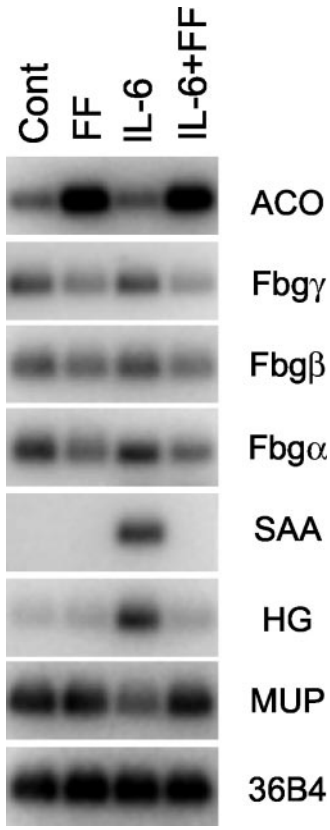

FIG. 2. **Fenofibrate prevents the induction of APR gene expression by IL-6 for 24 h.** Mice were treated with 0.2% (w/w) fenofibrate (*FF*) or not (*Cont*) for 2 weeks and stimulated or not for 24 h with IL-6 as indicated (four mice per group). Total RNA extracted from liver was subjected to Northern blot analysis (20 μg). Rat *ACO*, mouse *Fbgα*, mouse *Fbgβ*, mouse *Fbgγ*, *SAA*, *HG*, *MUP*, or acidic ribosomal phosphoprotein 36B4cDNA probes. Each lane was loaded with a pool of RNA from four mice.

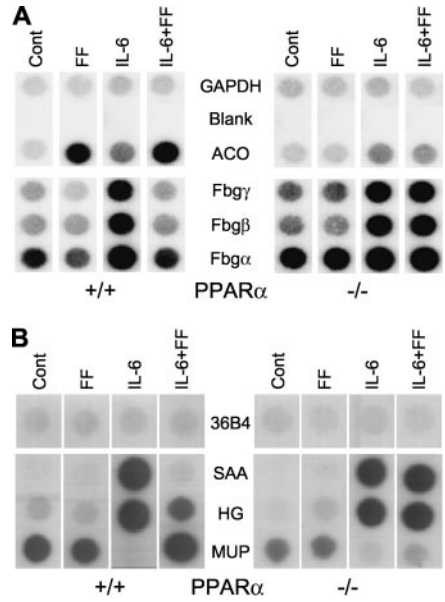

FIG. 3. **Effect of chronic fenofibrate treatment on IL-6-induced transcription of positive and negative acute phase genes in wild-type *versus* PPARα-deficient mice.** Wild-type (+/+) and PPARα-deficient mice (−/−) were treated with 0.2% (w/w) fenofibrate (*FF*) or not (*Cont*) for 17 days and stimulated or not for 4 h with IL-6 as indicated. Nuclear run-on assays were performed on nuclei isolated from livers. *A*, the autoradiogram shows glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as a control, membrane background (*Blank*), *ACO*, *Fbgα* chain, *Fbgβ* chain, and *Fbgγ* chain. *B*, the autoradiogram shows acidic ribosomal phosphoprotein 36B4 as a control, *SAA*, *HG*, and *MUP*.

type but not in PPARα-deficient mice (Fig. 1). Wild-type mice stimulated with IL-6 responded with a strong induction of the mRNA expression of the three fibrinogen genes. Interestingly, this IL-6 effect was fully suppressed by fenofibrate treatment in wild-type mice, whereas IL-6-stimulated mRNA levels of the three fibrinogen genes were unaffected by fenofibrate treatment in PPARα-deficient mice.

To determine whether the suppressive effect of PPARα on IL-6-induced APR gene expression is global, the influence of fenofibrate on other positive as well as negative APR gene expression was also analyzed. In wild-type and PPARα-deficient mice, IL-6 treatment increased mRNA levels of the positive APR genes *SAA* and *HG* and decreased the negative APR gene *MUP* (Fig. 1). Fenofibrate treatment inhibited IL-6-stimulated mRNA expression of the *SAA* and *HG* genes in the livers of wild-type mice, whereas, as was similar for fibrinogen, no effect was observed in PPARα-deficient mice. Remarkably, the down-regulation of *MUP* by IL-6 was completely prevented by fenofibrate treatment in wild-type but not in PPARα-deficient mice (Fig. 1). Together, these results indicate that chronic PPARα activation has a global effect on the hepatic expression of both positive and negative acute phase genes *in vivo*.

To determine whether PPARα activation also delays IL-6 modulation of APR genes at a later time point, the effect of chronic fenofibrate treatment was examined on mRNA levels of APR genes in livers of wild-type mice (four mice per treatment) stimulated or not with IL-6 for 24 h (Fig. 2). Control and fenofibrate groups without IL-6 stimulation exhibit a comparable effect as that reported for higher levels (Fig. 1). Activation of all three fibrinogen genes by IL-6 was almost totally abolished after 24 h IL-6 treatment, whereas activation of *SAA* and *HG* was still detectable at this time point and whereas *MUP* mRNA expression partially recovered after 24 h of IL-6 treatment (Fig. 2). Interestingly, fenofibrate treatment prevented the effects of IL-6 stimulation for 24 h in a similar manner as after 4 h of IL-6 stimulation. These results demonstrate that the systemic response to IL-6 treatment is not delayed but prevented upon PPARα activation *in vivo*.

Suppressive Effect of PPARα on IL-6-induced APR Occurs at the Transcriptional Level in Vivo—To determine whether the effect of PPARα on APR gene expression occurs at the transcriptional level *in vivo*, nuclear run-on transcription assays were performed on nuclei prepared from livers of wild-type and PPARα-deficient mice, pretreated or not with fenofibrate and stimulated or not with IL-6 for 4 h (Fig. 3). As expected, the transcription rate of ACO was enhanced in fenofibrate-treated wild-type mice but not in PPARα-deficient mice, and IL-6 treatment slightly increased ACO transcription independent of the presence or absence of PPARα (Fig. 3*A*). As a negative control, glyceraldehyde-3-phosphate dehydrogenase transcription levels remained constant under all conditions.

Basal transcription of the fibrinogen α and γ genes and to a lesser extent of the fibrinogen β gene was decreased by fenofibrate treatment (Fig. 3*A*). In wild-type mice, the transcription rate of the three fibrinogen genes was strongly enhanced upon IL-6 stimulation, an effect that was fully abolished by fenofibrate treatment. As observed at the mRNA levels, the basal transcription rates of the three fibrinogen genes was elevated in PPARα-deficient mice when compared with wild-type mice. The absence of effect of fenofibrate treatment in PPARα-deficient mice demonstrated that PPARα is required for fenofibrate inhibition of IL-6-mediated fibrinogen gene transcription *in vivo*.

The suppressive effect of PPARα on the IL-6-induced APR also occurs at the transcriptional level for both the positive

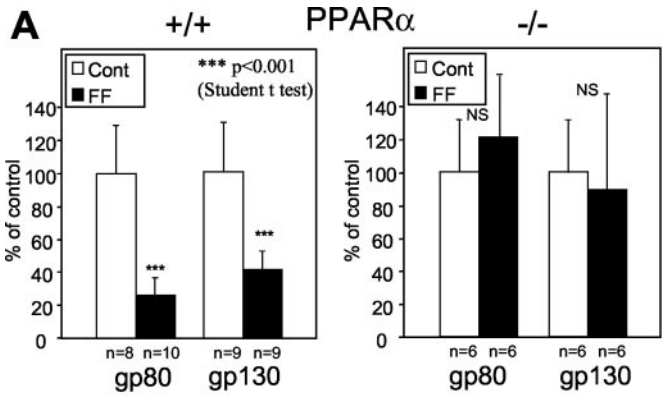

FIG. 4. **PPARα activation represses the expression of the IL-6 receptor complex components gp80 and gp130 *in vivo*.** RNA was extracted from livers of the indicated number of mice (*n*) treated with 0.2% (w/w) fenofibrate (*FF*) or not for 17 days. *A*, *gp80* and *gp130* mRNA expression was measured by quantitative reverse transcriptase-PCR. Results are mean ± S.D. of three independent experiments and analyzed using student test. *B*, Western blot analysis of *gp130* from proteins extracted from liver of mice treated with 0.2% (w/w) fenofibrate or not for 17 days. β-actin was used for control to confirm equal loading.

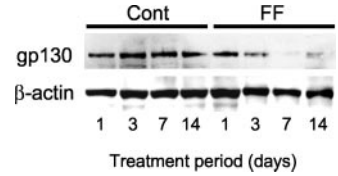

FIG. 5. **Time-dependent effect of PPARα activation on hepatic gp130 protein levels *in vivo*.** Mice were treated with 0.2% (w/w) fenofibrate (*FF*) or not (*Cont*) for different time periods. Animals were sacrificed after 1, 3, 7, and 14 days of diet treatment. Liver samples were prepared for Western blotting, and 30 μg of protein was electrophoresed per lane. β-actin was used for control to confirm equal loading.

APR genes (*SAA* and *HG*) and the negative APR gene (*MUP*) (Fig. 3*B*). Again, the involvement of PPARα in the action of fenofibrate was demonstrated using PPARα-deficient mice in which fenofibrate had no effect on the transcriptional regulation of both positive and negative APR genes (Fig. 3*B*). Together, these results indicate that PPARα exerts its global effect on the hepatic expression of both positive and negative APR genes at the level of transcription.

PPARα Represses the Expression of IL-6 Receptor Complex Components in Vivo—The broad effect on positive and negative APR proteins prompted us to analyze whether PPARα acts at the level of upstream receptor proteins required for IL-6 signal transduction, *i.e.* gp80 and gp130. The levels of gp80 and gp130 mRNA were determined in livers of mice treated with fenofibrate for 17 days by quantitative reverse transcriptase-PCR. A marked decrease of both gp80 and gp130 mRNA expression was observed in wild-type mice upon fenofibrate treatment (Fig. 4*A*). This effect occurred in a PPARα-dependent fashion since mRNA expression of both IL-6 receptor components were unaffected in livers of PPARα-deficient mice treated with fenofibrate (Fig. 4*A*). Next, the level of gp80 and gp130 proteins was analyzed by Western blot. Although the expression of gp80 was very weak and too low to be detectable by Western blot, the gp130 subunit was reduced in a PPARα-dependent fashion (Fig. 4*B*). Results from a time course experiment with fenofibrate indicated that the protein levels of gp130 already slightly decreased after 3 days of treatment and strongly decreased on further prolonged treatment (Fig. 5).

Finally, it was analyzed whether fenofibrate treatment also reduced gp130 protein levels in non-hepatic tissues, *i.e.* heart, kidney, muscle, and spleen of mice treated with fenofibrate for 2 weeks. Although gp130 protein was expressed in all tissues tested (Fig. 6), a down-regulation was observed only in liver but not in the other tissues tested. Altogether, these findings demonstrate that PPARα activation decreases the expression of hepatic IL-6 receptor system proteins required for IL-6 signal transduction, which, in part, explains the global suppressive effect of fenofibrate on IL-6-mediated APR gene expression. Also, we identify the IL-6 receptor subunits gp80 and gp130 as two novel PPARα-regulated genes.

Activated PPARα Lowers Protein Concentrations of Transcription Factors Mediating the IL-6-induced APR in Vivo—The gp130 subunit transduces the IL-6 signal to downstream transcription factors and mediates Tyr-705 phosphorylation, which results in activation of STAT3 (7) and subsequent transcription of STAT3-regulated APR genes (30). Recently, it has been reported that c-Jun and phosphorylated c-Jun enhance IL-6-mediated activation of transcription (11, 12). We therefore analyzed whether chronic PPARα activation by fenofibrate could affect the level of STAT3 and c-Jun protein and their activated forms phospho-STAT3 and phospho-c-Jun in the livers of wild-type and PPARα-deficient mice treated or not for 17 days with fenofibrate and subsequently stimulated for 4 h with IL-6.

STAT3 protein levels were similar in wild-type and PPARα-deficient and unchanged upon IL-6 stimulation (Fig. 7*A*). However, phosphorylated STAT3 levels were decreased in wild-type mice treated with fenofibrate but not in PPARα-deficient mice, which is in accordance with the observed PPARα-dependent down-regulation of the gp130 receptor subunit. Fenofibrate treatment only slightly reduced basal c-Jun levels in wild-type mice. However, a strong reduction of phosphorylated c-Jun levels was observed in wild-type mice exposed to fenofibrate. By contrast, this reduction was not observed in PPARα-deficient mice (Fig. 7*A*).

The protein concentration of C/EBP transcription factors determines the transcriptional regulation of many IL-6-responsive APR genes (14). C/EBPs are induced by IL-6 and act in concert with STAT3 and c-Jun to promote APR gene expression (13). Interestingly, analysis of the effect of fenofibrate on C/EBP expression levels in wild-type and PPARα-deficient mice revealed that the basal protein expression levels of the isoforms C/EBPα, -β, and -δ were markedly reduced in livers of wild-type mice treated for 17 days with fenofibrate (Fig. 7*B*) in the presence and absence of IL-6. This down-regulatory effect was not observed in PPARα-deficient mice and thus fully dependent on the presence of PPARα.

Together, these data show that PPARα activation reduces both the phosphorylation status of transcription factors STAT3 and c-Jun that transduce the IL-6 signal and the basal expression levels of cooperatively acting C/EBP transcription factors *in vivo*. Reduced availability of native and activated forms of transcription factors required for IL-6-induced APR gene transcription may explain the global suppression of the acute phase

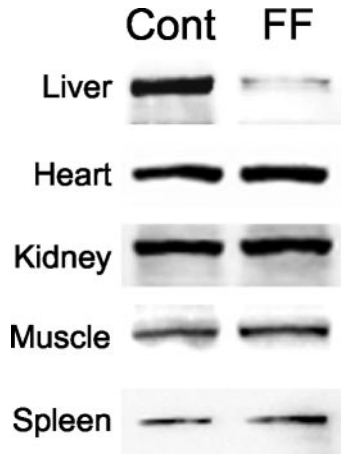

FIG. 6. **Effect of PPARα activation on gp130 protein in different tissues.** Mice were treated with 0.2% (w/w) fenofibrate (*FF*) or not (*Cont*) for 2 weeks. Western blot analysis of gp130 from proteins extracted from liver, heart, kidney, muscle, and spleen is shown.

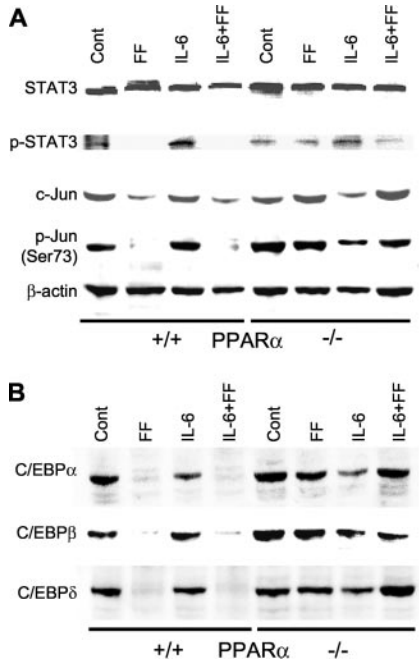

FIG. 7. **Effect of chronic PPARα activation on protein levels of transcription factors that transduce the IL-6 signal in the liver.** Western blot analysis was carried out on protein extracted from livers of wild-type and PPARα-deficient mice (−/−) treated with 0.2%(w/w) fenofibrate (*FF*) or not (*Cont*) for 17 days and stimulated or not for 4 h with IL-6 as indicated. *A*, analysis of STAT3, Tyr-705-phosphorylated STAT3 (*p-STAT3*), c-Jun, and Ser-73-phosphorylated c-Jun (*p-Jun*). *B*, analysis of C/EBPα, C/EBPβ, and C/EBPδ. Levels of β-actin are shown for confirmation of equal loading.

gene transcription by fenofibrate.

Treatment with Fenofibrate Regulates the Expression of Positive and Negative APR Proteins in Humans—To verify whether treatment with fenofibrate has a similar global quenching effect on positive and negative APR proteins in humans, plasma samples of patients with mild hyperlipidemia that had undergone a 4-week fenofibrate treatment were analyzed before and after the treatment. The influence of fenofibrate on plasma concentrations of marker proteins that are related to the APR is summarized in Table I. Significantly reduced plasma concentrations were observed for the positive APR proteins fibrinogen, CRP, SAA, α2-macroglobulin, and plasminogen, whereas the negative APR protein albumin was elevated by fenofibrate treatment. The data show that treatment with fenofibrate has a similar broad effect on the expression of positive and negative APR proteins in humans and underscore the clinical significance of our findings in mice.

DISCUSSION

Although PPARα is predominantly expressed in the liver and activities of PPARα comprise an anti-inflammatory action (31), the effect of PPARα on the APR is unclear. IL-6 is considered to be one of the most important inducers of the global hepatic APR reaction (1). We previously investigated the direct effect of acute activation of PPARα on the APR *in vitro* in hepatocytes. We here demonstrate that chronic activation of PPARα with fenofibrate fully suppresses IL-6-induced APR gene expression in mice *in vivo*. Evidence is provided that PPARα acts at the transcriptional level because IL-6-stimulated APR gene transcription was not suppressed in fenofibrate-treated PPARα-deficient mice. We found that PPARα is a global suppressor of the APR influencing the expression of both positive (IL-6-up-regulated) and negative (IL-6-down-regulated) APR genes, which can be explained by two PPARα-dependent effects. First, chronic PPARα activation down-regulates the expression of the IL-6 receptor components gp80 and gp130, leading to reduced phosphorylation, *i.e.* reduced activation of downstream transcription factors by IL-6. The down-regulation of gp130 protein expression by PPARα activation time-dependent and selectively occurs in liver but not in the other tissues tested. Second, PPARα down-regulates protein levels of synergistically acting transcription factors C/EBPα, -β, and -δ of APR genes. Importantly, the global suppression of APR gene transcription by fenofibrate in mice *in vivo* corresponds with a similar global effect in fenofibrate-treated humans, which display reduced plasma levels of several positive APR proteins and an increased plasma level of the negative acute phase protein serum albumin.

In wild-type and PPARα-deficient mice, IL-6 strongly induced the expression of positive APR genes fibrinogen-α, -β, and -γ, haptoglobulin, and *SAA*. The expression level of the negative APR protein MUP is reduced by IL-6, but its expression remained elevated in IL-6-stimulated, fenofibrate-treated wild-type animals. PPARα activation clearly interfered with this negative regulation of *MUP* as demonstrated in PPARα-deficient mice. To our knowledge, this is the first example of a gene whose cytokine-induced down-regulation is inhibited by PPARα. These IL-6 effects on APR gene expression, however, were fully suppressed in fenofibrate-treated wild-type mice but not in fenofibrate-treated PPARα-deficient mice, indicating that chronic PPARα activation *in vivo* can very potently counteract the modulation of IL-6-modulated APR gene expression in the liver. Moreover, we demonstrated that PPARα does not act by delaying the systemic response to IL-6 but that its effects occur at the transcriptional level for all genes studied. The broadness and strength of this chronic *in vivo* effect has been surprising, and haptoglobulin, *SAA*, and *MUP* represent novel PPARα-dependently regulated genes. The suppression of the IL-6-induced expression of fibrinogen genes α, β, and γ *in vivo* is in line with our previous *in vitro* observations (24) and underscores the broad inhibition of APR gene expression by PPARα. The global suppression of APR gene expression in the liver by PPARα adds to its activities in the vascular wall (32–34) and to the findings that PPARα-deficient mice exhibit an exacerbated response to lipopolysaccharide stimulation (32) and a prolonged inflammatory response (23). Thus, PPARα may represent a major target of interest for the control of hepatic cardiovascular risk factors. However, it remains to be investigated which hepatic PPARα-expressing cells are most responsive to fibrate treatment *in vivo* and contribute most to the observed effect.

TABLE 1
Changes in acute phase protein plasma concentrations of hyperlipidemic patients (n = 38) that were treated with fenofibrate (250 mg/day) for 4 weeks

Blood samples were taken from patients before and after 4 weeks of fenofibrate treatment. Changes in the plasma concentrations were indicated with trend arrows and presented as differences of means together with their standard errors (S.E.) and *P* values.

	Mean before	Mean after	Trend	Difference (SE)	Power *P*
Fibrinogen (g/L)	2.78	2.35	↓	-0.43 (0.1)	0.00003
SAA (mg/L)	18.2	11.8	↓	-6.4 (4.3)	0.07
CRP (mg/L)	5.2	4.3	↓	-0.9 (1.06)	0.0025
α2-Macroglobulin (mg/dl)	111.3	106.6	↓	-4.7 (1.5)	0.0015
Plasminogen (%)	116.0	109.0	↓	-7.0 (1.5)	0.000005
Albumin (g/L)	63.1	64.0	↑	+0.9 (0.4)	0.018

The global effect of PPARα on the expression of positive and negative APR gene expression suggested an upstream suppression of the IL-6 pathway. IL-6-induced APR genes share a common receptor system, consisting of gp80 and gp130, which initiates a signaling cascade leading to STAT3 activation by phosphorylation. In fenofibrate-treated wild-type mice, PPARα down-regulates mRNA expression levels of the IL-6R (gp80) by 70% and of the signal transducer gp130 by 60%. In line with this observation, we found a PPARα-dependent reduced expression of gp130 protein and, accordingly, reduced levels of phosphorylated STAT3. All these effects were not observed in PPARα-deficient mice, indicating that the down-regulation is PPARα-dependent, thereby identifying *gp80* and *gp130* as two novel PPARα-regulated genes. Of note, propagation of the IL-6 signal to other pathways also requires a functional gp130 subunit (11, 12), and the PPARα-dependent down-regulation of gp130 may cause the observed reduced phosphorylation, *i.e.* activation, of c-Jun.

Our finding that PPARα can down-regulate gp130 expression may also be of relevance for other cytokines of the IL-6 family since IL-6, IL-11, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and cardiotrophin (CT)-1 all share the gp130 receptor for signal transduction (8, 11). Not unexpectedly for such a central receptor, the *gp130* gene knockout is lethal, whereas mice deficient in one of the above cytokines display only relatively mild defects (35). Nevertheless, we found that PPARα-dependent repression of gp130 occurs in liver but not in heart, kidney, muscle, or spleen when PPARα is activated by fenofibrate. Depending on the tissue specificity of PPARα activation and properties of PPARα ligands, PPARα may influence processes triggered by the above cytokines such as hematopoiesis, leukocyte proliferation, blastocyst implantation, and osteoclast development (7).

Down-regulation of the gp80/gp130 receptor system may not fully explain the broad and potent activity of PPARα on APR gene regulation for two main reasons: first, since complete abolishment of gp130 protein expression only occurs after PPARα activation for at least 7 days, and second, since the promoter regions of APR genes display a high degree of divergence with respect to the types and number of response elements. C/EBPs are responsible for immediate and maintained activation of transcription of the IL-6-induced APR, and APR gene transcription is related to the concentrations of C/EBPs present in the cell (14–16). It has been reported that genetic ablation of a single C/EBP isoform does not lead to the complete abolishment of the APR (36), and it is suggested that lack of one particular C/EBP isoform is compensated by the other C/EBP isoforms. Our results show that the basal cellular protein concentrations of C/EBPα, -β, and -δ isoforms are PPARα-dependently decreased in wild-type mice by fenofibrate treatment. The observed reduction of all three C/EBP isoforms thus prevents any compensatory mechanism in these mice. In PPARα-deficient mice, the basal C/EBPα, -β, and -δ protein levels were comparable with the basal levels in wild-type mice and were, by contrast, not down-regulated by fenofibrate treatment. This PPARα-dependent down-regulation of C/EBPα and -δ is supported by data from Takata *et al.* (37), who demonstrated that PPARγ ligands suppress the expression and transcription of C/EBPδ protein through dephosphorylation of STAT3 in the vascular wall. It may be speculated that the reduced gp130 activity and thus reduced STAT3 activation in wild-type mice treated with fenofibrate may contribute to a down-regulation of C/EBPs in liver.

In humans, we found that fenofibrate treatment reduced the plasma concentrations of the positive APR proteins fibrinogen and CRP, an effect that has also been observed for other PPARα-activators, *i.e.* bezafibrate (18) and ciprofibrate (38). In this study, we demonstrate that treatment with fenofibrate has a much broader effect on plasma levels of APR proteins in humans and causes down-regulation of SAA, α2-macroglobulin, and plasminogen. We show for the first time that the plasma concentration of a negative APR protein, albumin, is increased in humans. This is in accordance with the transcriptional changes of the respective murine genes in fenofibrate-treated mice and suggests a comparable suppressive effect of PPARα on acute phase gene transcription in mice and humans.

In conclusion, PPARα activation thus impairs the IL-6 signaling pathway in the liver at two levels, the level of membrane receptors and the level of transcription factors, together resulting in a potent suppression of the IL-6-stimulated APR gene

expression. PPARα could be considered as a factor playing a determinant role in the control of hepatic inflammation at the molecular level and thus forms an attractive target to dampen deleterious effects of inflammation in liver.

REFERENCES

1. Streetz, K. L., Wustefeld, T., Klein, C., Manns, M. P., and Trautwein, C. (2001) *Cell. Mol. Biol.* (*Noisy-Le-Grand*) **47,** 661–673
2. Yudkin, J. S., Kumari, M., Humphries, S. E., and Mohamed-Ali, V. (2000) *Atherosclerosis* **148,** 209–214
3. Dhainaut, J. F., Marin, N., Mignon, A., and Vinsonneau, C. (2001) *Crit. Care Med.* **29,** S42–S47
4. Hoffmeister, A., Rothenbacher, D., Bazner, U., Frohlich, M., Brenner, H., Hombach, V., and Koenig, W. (2001) *Am. J. Cardiol.* **87,** 262–266
5. Ridker, P. M., Rifai, N., Pfeffer, M. A., Sacks, F. M., Moye, L. A., Goldman, S., Flaker, G. C., and Braunwald, E. (1998) *Circulation* **98,** 839–844
6. Ridker, P. M., Stampfer, M. J., and Rifai, N. (2001) *J. Am. Med. Assoc.* **285,** 2481–2485
7. Heinrich, P. C., Behrmann, I., Muller-Newen, G., Schaper, F., and Graeve, L. (1998) *Biochem. J.* **334,** 297–314
8. Taga, T., and Kishimoto, T. (1997) *Annu. Rev. Immunol.* **15,** 797–819
9. Kim, H., and Baumann, H. (1999) *Mol. Cell. Biol.* **19,** 5326–5338
10. Schaefer, T. S., Sanders, L. K., and Nathans, D. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 9097–9101
11. Schuringa, J. J., Timmer, H., Luttickhuizen, D., Vellenga, E., and Kruijer, W. (2001) *Cytokine* **14,** 78–87
12. Yoo, J. Y., Wang, W., Desiderio, S., and Nathans, D. (2001) *J. Biol. Chem.* **276,** 26421–26429
13. Lekstrom-Himes, J., and Xanthopoulos, K. G. (1998) *J. Biol. Chem.* **273,** 28545–28548
14. Baumann, H., Morella, K. K., Campos, S. P., Cao, Z., and Jahreis, G. P. (1992) *J. Biol. Chem.* **267,** 19744–19751
15. Zauberman, A., Lapter, S., and Zipori, D. (2001) *J. Biol. Chem.* **276,** 24719–24725
16. Kordula, T., and Travis, J. (1996) *Biochem. J.* **313,** 1019–1027
17. Zhang, D., Sun, M., Samols, D., and Kushner, I. (1996) *J. Biol. Chem.* **271,** 9503–9509
18. Jonkers, I. J., Mohrschladt, M. F., Westendorp, R. G., van der Laarse, A., and Smelt, A. H. (2002) *Am. J. Med.* **112,** 275–280
19. Kockx, M., Gervois, P. P., Poulain, P., Derudas, B., Peters, J. M., Gonzalez, F. J., Princen, H. M., Kooistra, T., and Staels, B. (1999) *Blood* **93,** 2991–2998
20. Staels, B., Koenig, W., Habib, A., Merval, R., Lebret, M., Torra, I. P., Delerive, P., Fadel, A., Chinetti, G., Fruchart, J. C., Najib, J., Maclouf, J., and Tedgui, A. (1998) *Nature* **393,** 790–793
21. Gervois, P., Torra, I. P., Fruchart, J. C., and Staels, B. (2000) *Clin. Chem. Lab. Med.* **38,** 3–11
22. 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
23. Devchand, P. R., Keller, H., Peters, J. M., Vazquez, M., Gonzalez, F. J., and Wahli, W. (1996) *Nature* **384,** 39–43
24. Gervois, P., Vu-Dac, N., Kleemann, R., Kockx, M., Dubois, G., Laine, B., Kosykh, V., Fruchart, J. C., Kooistra, T., and Staels, B. (2001) *J. Biol. Chem.* **276,** 33471–33477
25. Gorski, K., Carneiro, M., and Schibler, U. (1986) *Cell* **47,** 767–776
26. Nevins, J. R. (1987) *Methods Enzymol.* **152,** 234–241
27. Staels, B., and Auwerx, J. (1992) *Development* **115,** 1035–1043
28. Ernst, E., Resch, K. L., Saradeth, T., Maier, A., and Matrai, A. (1992) *J. Clin. Pathol.* **45,** 534–535
29. Desvergne, B., and Wahli, W. (1999) *Endocr. Rev.* **20,** 649–688
30. Alonzi, T., Maritano, D., Gorgoni, B., Rizzuto, G., Libert, C., and Poli, V. (2001) *Mol. Cell. Biol.* **21,** 1621–1632
31. Delerive, P., Fruchart, J. C., and Staels, B. (2001) *J. Endocrinol.* **169,** 453–459
32. Delerive, P., Gervois, P., Fruchart, J. C., and Staels, B. (2000) *J. Biol. Chem.* **275,** 36703–36707
33. Marx, N., Sukhova, G., Collins, T., Libby, P., and Plutzky, J. (1999) *Circulation* **99,** 3125–3131
34. Marx, N., Kehrle, B., Kohlhammer, K., Grub, M., Koenig, W., Hombach, V., Libby, P., and Plutzky, J. (2002) *Circ. Res.* **90,** 703–710
35. Yoshida, K., Taga, T., Saito, M., Suematsu, S., Kumanogoh, A., Tanaka, T., Fujiwara, H., Hirata, M., Yamagami, T., Nakahata, T., Hirabayashi, T., Yoneda, Y., Tanaka, K., Wang, W. Z., Mori, C., Shiota, K., Yoshida, N., and Kishimoto, T. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 407–411
36. Fattori, E., Sellitto, C., Cappelletti, M., Lazzaro, D., Bellavia, D., Screpanti, I., Gulino, A., Costantini, F., and Poli, V. (1995) *Ann. N. Y. Acad. Sci.* **762,** 262–273
37. Takata, Y., Kitami, Y., Yang, Z. H., Nakamura, M., Okura, T., and Hiwada, K. (2002) *Circ. Res.* **91,** 427–433
38. Rizos, E., Kostoula, A., Elisaf, M., and Mikhailidis, D. P. (2002) *Angiology* **53,** 273–277

Global Suppression of IL-6-induced Acute Phase Response Gene Expression after Chronic *in Vivo* Treatment with the Peroxisome Proliferator-activated Receptor-α Activator Fenofibrate

Philippe Gervois, Robert Kleemann, Antoine Pilon, Frédéric Percevault, Wolfgang Koenig, Bart Staels and Teake Kooistra

J. Biol. Chem. 2004, 279:16154-16160.
doi: 10.1074/jbc.M400346200 originally published online February 5, 2004
