Plasma mannose-binding lectin is stimulated by PPARα in humans

Maryam Rakshandehroo,1,2# Rinke Stienstra,1,2# Nicole J. de Wit,1,2 Marjolijn C. E. Bragt,1,3 Martin Haluzik,4 Ronald P. Mensink,1,3 Michael Müller,1,2 and Sander Kersten1,2

1The Netherlands Nutrigenomics Center, Top Institute Food and Nutrition; 2Nutrition, Metabolism, and Genomics group, Division of Human Nutrition, Wageningen University, Wageningen; 3Department of Human Biology, School for Nutrition, Toxicology, and Metabolism, Maastricht University, Maastricht, The Netherlands; and 4Clinical Department of Endocrinology and Metabolism, Charles University, Prague, Czech Republic

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Rakshandehroo M, Stienstra R, de Wit NJ, Bragt MC, Haluzik M, Mensink RP, Müller M, Kersten S. Plasma mannose-binding lectin is stimulated by PPARα in humans. Am J Physiol Endocrinol Metab 302: E595–E602, 2012. First published January 3, 2012; doi:10.1152/ajpendo.00299.2011.—The peroxisome proliferator-activated receptor-α (PPARα) is a major transcriptional regulator of lipid metabolism in liver and represents the molecular target for hypolipidemic fibrate drugs. Effects of PPARα on lipid metabolism are partially mediated by circulating proteins such as FGF21 and ANGPTL4. The present study was undertaken to screen for and identify circulating proteins produced by human liver that are under the control of PPARα. Toward that aim, primary human hepatocytes were treated with the synthetic PPARα agonist Wy-14643 and whole genome expression data selected for secreted proteins. Expression of FGF21, ANGPTL4, and mannose-binding lectin (MBL), a soluble mediator of innate immunity and primary component of the lectin branch of the complement system, was markedly upregulated by Wy-14643 in primary human hepatocytes. Mice express two MBL isomers, Mbl1 and Mbl2. Mbl1 mRNA was weakly induced by Wy-14643 in primary mouse hepatocytes and remained unaltered by Wy-14643 in mouse liver. Mbl2 mRNA was unchanged by Wy-14643 in primary mouse hepatocytes and was strongly reduced by Wy-14643 in mouse liver. Remarkably, plasma Mbl1 levels were increased by chronic PPARα activation in lean and obese mice. Importantly, in two independent clinical trials, treatment with the PPARα agonist fenofibrate at 200 mg/day for 6 wk and 3 mo increased plasma MBL levels by 73 (P = 0.0016) and 86% (P = 0.017), respectively. It is concluded that hepatocyte gene expression and plasma levels of MBL are stimulated by PPARα and fenofibrate in humans, linking PPARα to regulation of innate immunity and complement activation in humans and suggesting a possible role of MBL in lipid metabolism.

THE LIVER IS A KEY ORGAN in the control of carbohydrate and lipid metabolism via its ability to metabolize, store, and release glucose and triglycerides. Disturbances in liver metabolic function may lead to fatty liver, thereby increasing the risk for development of steatohepatitis. Disturbed liver function may also negatively impact circulating lipid levels and contribute to dyslipidemia. Studies in the past two decades have shown that hepatic glucose and lipid metabolism are governed via a number of elaborate transcriptional regulatory networks involving, among others, the transcription factors sterol regulatory element-binding protein, carbohydrate response element-binding protein, forkhead box (Fox)α1, Foxa1, Foxa2, and C/EBPα as well as several members of the nuclear receptor superfamily (3, 10, 12). One nuclear hormone receptor that plays a key role in the regulation of hepatic lipid metabolism is the peroxisome proliferator-activated receptor-α (PPARα) (28). Lack of PPARα in mice leads to acute energy shortage in liver upon fasting and is characterized by defective ketone body formation, hypoglycemia, elevated plasma free fatty acids, and severe hepatic steatosis (14, 21, 23). The effects of PPARα deletion can be ascribed to defective expression of a large number of genes in numerous lipid metabolic pathways, including fatty acid uptake, fatty acid transport, peroxisomal and mitochondrial fatty acid oxidation, ketogenesis, and triglyceride hydrolysis (28, 29).

PPARs are ligand-activated transcription factors that share a common mode of action via heterodimerization with the nuclear retinoid X receptor and subsequent binding to DNA response elements in promoters of target genes. Three different PPAR subtypes are known: PPARα, PPARβ/δ, and PPARγ. Whereas PPARα and PPARβ/δ are expressed in numerous tissues, including liver, PPARγ is expressed mainly in adipose tissue, macrophages, and colon (6). Consistent with its role in lipid metabolism, PPARα and PPARβ/δ can be activated by numerous fatty acids and fatty acid derivatives (26). In addition, PPARα serves as a molecular target for the fibrate class of drugs used in the treatment of (diabetic) dyslipidemia. More recently, fibrates have also been considered as a therapeutic option for nonalcoholic fatty liver disease with so far limited success (8, 9).

Recently, it was shown that PPARα governs the hepatic production of secreted proteins fibroblast growth factor 21 (FGF21) and angiopoietin-like protein 4 (ANGPTL4) (20, 24). Whereas FGF21 has been shown to serve as partial mediator of the PPARα-induced starvation response in mice and regulator of ketogenesis (1, 16), ANGPTL4 is now well established as a potent regulator of plasma triglyceride levels via inhibition of lipoprotein lipase (18). The present study was undertaken to screen for and identify circulating proteins produced by human liver that are under the control of PPARα. Our results indicate that hepatocyte gene expression and plasma levels of mannose-binding lectin (MBL), the main component of the lectin branch of the complement system (17), are positively regulated by PPARα in humans.

MATERIALS AND METHODS

Fenofibrate intervention trials. For the first fenofibrate study, serum was sampled from 11 obese females with type 2 diabetes mellitus and serum triglyceride concentrations ≥2.0 mmol/l at baseline and after a 3-mo treatment with micronized fenofibrate (200 mg/day lipanyl). Full details of this study can be found elsewhere (13). A complete description of the second fenofibrate intervention trial has been published elsewhere (2). A summary is provided below.

# M. Rakshandehroo and R. Stienstra are joint first authors of this article.

Address for reprint requests and other correspondence: S. Kersten, Nutrition, Metabolism, and Genomics Group, Wageningen University, P. O. Box 8129, 6700 EV Wageningen, The Netherlands (e-mail: sander.kersten@wur.nl).
Subjects with body mass index (BMI) >27 kg/m² were recruited via posters and advertisements in local newspapers. On the first screening visit, fasting blood was sampled for analyses of serum lipids and lipoprotein, and height and body weight were determined. Furthermore, subjects had to complete a medical and general questionnaire. Exclusion criteria were BMI <27 kg/m², impaired kidney and liver function, serum total cholesterol >8 mmol/l, serum triglycerides >4 mmol/l, taking medication that could influence the study outcome or interfere with fenofibrate treatment, use of fish oil supplements, consumption of plant sterol or stanol-enriched food products, having donated blood within 1 mo prior to the start of the study, having a diagnosis of any long-term medical condition (e.g., diabetes, cardiovascular diseases, epilepsy), or experiencing strong symptoms of allergy. After screening, 26 subjects met all of our inclusion criteria and started this study. Subjects received oral and written information about the nature and risk of the experimental procedures before their written informed consent was obtained before the start of the study. The study was approved by the Medical Ethics Committee of Maastricht University.

After the inclusion of 26 subjects, seven subjects dropped out. Ten men and 10 women completed the trial. Subjects were 52 ± 12 yr old (means ± SD), with a body weight of 98 ± 19 kg for men and 95 ± 20 kg for women, a BMI of 31 ± 5 kg/m² for men and 34 ± 5 kg/m² for women, and a waist circumference of 118 ± 13 cm for men and 116 ± 11 cm for women. Serum concentrations of total cholesterol were 6.23 ± 1.18 mmol/l, of LDL cholesterol 3.97 ± 1.09 mmol/l, of HDL cholesterol 1.52 ± 0.44 mmol/l, of triglycerides 1.63 ± 0.59 mmol/l, and of glucose 5.34 ± 0.73 mmol/l. Systolic blood pressure was 131 ± 14 mmHg, and diastolic pressure was 91 ± 8 mmHg. Four subjects smoked. According to the National Cholesterol Education Program Adult Treatment Panel III guidelines for diagnosing the metabolic syndrome, subjects had on average 2.2 ± 1 criteria of the metabolic syndrome, and seven of 20 subjects could be diagnosed as having the metabolic syndrome.

The study had a randomized, double-blind, placebo-controlled crossover design. Each subject enrolled in random order in a fish oil, a fenofibrate, and a placebo period for 6 wk, with a washout period of ≥2 wk between the intervention periods. This article focuses exclusively on the effect of fenofibrate. Subjects consumed two capsules that provided 200 mg/day micronized fenofibrate (lipanthyl; Fournier Laboratories, Dijon, France) together with eight placebo-matching fish oil capsules (containing 80% high oleic sunflower oil). Subjects were instructed to ingest one-half of the capsules before breakfast and the other half before dinner with a glass of water. Subjects were restricted in their fish consumption to a maximum of 1 portion/wk.

In weeks 5 and 6 of each intervention period, subjects arrived in the morning after an overnight fast and after abstention from drinking alcohol the preceding day. Venous blood samples were collected in BD vacutainer tubes (Becton Dickinson). Serum was obtained by clotting the blood for 30 min, followed by 30 min centrifugation at 2,000 g. EDTA plasma was obtained by centrifugation at 2,000 g for 30 min at 4°C directly after sampling. Serum and plasma aliquots were snap-frozen and stored at −80°C until analysis.

Animal experiments. Male SV129 PPARα/−/− mice and corresponding wild-type mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and further expanded at our local animal facility. Mice were 2–3 mo old at the start of the experiments. Animals were fasted for 4 h (starting at 5 AM) before receiving an oral gavage of Wy-14643 (400 μl of 10 mg/ml Wy-14643 dissolved in 0.5% carboxymethyl cellulose). Control mice received only carboxymethyl cellulose. Each group contained 4–5 mice. Six hours after the gavage, mice were euthanized. In another experiment, wild-type and PPARα/−/− mice were fed with Wy-14643 for 5 days by mixing it in their Chow (0.1%, n = 4/group). Alternatively, wild-type and PPARα/−/− mice maintained on a high-fat diet (D12451; Research Diets, New Brunswick, NJ) for 21 wk were fed Wy-14643 mixed in their feed for the last 5 days (0.1%, n = 5/group). For the fasting experiment, male mice (n = 4–5/group) were either fed or fasted for 24 h. At the end of each experiment, mice were anesthetized with a mixture of isofluorane (1.5%), nitrous oxide (70%), and oxygen (30%). Mice were euthanized by cervical dislocation, and livers were removed and directly frozen into liquid nitrogen and stored at −80°C. The animal experiments were approved by the Local Committee for Care and Use of Laboratory Animals at Wageningen University.

Hepatocyte isolation and culture. Primary human hepatocytes from six donors were purchased from Lonza Bioscience (Verviers, Belgium). Details of isolation and procedure are described elsewhere (27). Briefly, cells were isolated from surgical liver biopsies by the two-step collage-nase perfusion method. Mouse hepatocytes were isolated as described previously from six different strains of mice: NMRI, SV129, FVB, DBA, BALB/c, and C57BL/6j (22, 27). Cells were incubated in fresh medium in the presence or absence of Wy-14643 (10 μM) dissolved in DMSO for 6 and 24 h, followed by RNA isolation.

Affymetrix microarray analysis. RNA isolation and subsequent processing for microarray were carried out as described previously (27). Hybridization, washing, and scanning of Affymetrix Gene Chip human genome U133 2.0 plus and mouse genome 430 2.0 arrays were done according to standard Affymetrix protocols. Analysis of the microarray data was as described previously (27). Genes encoding secreted proteins were selected using Gene Ontology Classification, SignalP, and ngLOC (n-gram-based Bayesian classifier) predicting tools. The raw microarray data sets have been submitted to Gene Expression Omnibus (GSE17254).

Real-time quantitative PCR. One microgram of total RNA was used for reverse transcription with iScript (Bio-Rad, Veenendaal, The Netherlands). cDNA was amplified on a Bio-Rad CFX384 Real Time System using Sensimix (Bioline; GC Biotech, Alphen aan de Rijn, The Netherlands). Cyclophilin was used as a housekeeping gene. PCR primer sequences were taken from the PrimerBank and ordered from Eurogentec (Seraing, Belgium). Primer sequences used were hMBL (forward: GCAAACAGAATGCGACGTATC; reverse: CTGGAACTTGGACA- CACAAGGC), mMBL (forward: CTGTGGCTATCCCCAGGAT; reverse: TACGTACATGAACTGCCCTT), and mMBL2 (forward: TACGTGGTTATGTACGAGAC; reverse: GTGTCACGTTCATCTTTCGCC). Other primer sequences are available upon request.

Tissue expression profiling: expression profile of MBL2 mRNA in human tissues. One microgram of FirstChoice Human Total RNA Survey Panel (Ambion) was reverse transcribed and used for quantitative PCR (qPCR) using primers specific for human MBL2. Expression levels are expressed relative to liver, which showed the highest expression level (100%).

Fig. 1. Peroxisome proliferator-activated receptor-α (PPARα) regulates mannose-binding lectin (MBL) expression in human hepatocytes. A: heat map illustrating the relative induction of genes encoding secreted proteins in response to 24-h Wy-14643 treatment in human hepatocytes (left) and relative changes in expression of corresponding mouse orthologs in mouse hepatocytes (right). The mouse strains are indicated at the top. All genes were changed significantly (P < 0.05) and ranked based on mean fold change (MFC). Expression levels in the DMSO-treated cells were set at 1. B: relative induction of fibroblast growth factor 21 (FGF21), angiotensin-like protein 4 (ANGPTL4), and MBL2 by Wy-14643 (50 μM) in human primary hepatocytes. C: 50 bp up- and downstream of the transcriptional start site of the human MBL gene was examined for the presence of putative PPARα response elements (PRES) using a previously published algorithm (15). D: relative induction of MBL2 by Wy-14643 (50 μM) in human primary hepatocytes. The sample cells were used for analysis of MBL2 protein in medium and cell lysate using kits from Hycult Biotechnology (E) or Bioporte (F). G: relative induction of Fgf21, Angptl4, MBL1, and MBL2 by Wy-14643 (10 μM) in mouse primary hepatocytes obtained from 6 mice of different strains. Error bars represent SE. *Significantly different according to Student’s t-test.
Plasma/serum analysis. Human MBL levels were determined by the use of a commercially available MBL Oligomer ELISA kit using biotinylated monoclonal detection antibody, following the instructions of the manufacturer (Bioporto Diagnostics, Copenhagen, Denmark). Subjects characterized as MBL deficient (plasma MBL <20 ng/ml) were excluded from subsequent analyses. Human MBL levels in medium and lysates of primary hepatocytes were determined using MBL ELISA kits from Bioporto and Hycult (Sanbio, Uden, The Netherlands). Both kits detect MBL multimers. Plasma ANGPTL4 was measured as described previously (19). Plasma FGF21 was determined using a commercially available ELISA (Biovendor, Brno, Czech Republic). Levels of Mbl1 in mouse plasma were determined using a kit from Hycult.

Statistical analysis. Statistically significant differences were calculated using Student’s t-test or two-way ANOVA. The cutoff for statistical significance was set at a P value of ≤0.05. Plasma MBL levels were log-transformed before statistical analysis.

RESULTS

MBL expression in liver is regulated by PPARα in a human-specific manner. To screen for circulating proteins produced by liver that may be implicated in lipid metabolism, primary human hepatocytes were treated with the PPARα agonist Wy-14643 for 24 h, followed by Affymetrix microarray analysis. Differentially expressed genes encoding secreted proteins were selected using gene ontology classification. Besides established secreted targets of PPARα such as ANGPTL4 and FGF21, expression of MBL2 was upregulated significantly by PPARα activation in all donors (Fig. 1A). In fact, MBL2 represented the most highly induced gene encoding a secreted protein. MBL2 represents the primary component of the lectin branch of the complement system, which forms an integral part of the innate immune system (33). Other inflammation-related genes encoding secreted proteins that were induced by PPARα activation in primary human hepatocytes included CC chemokines CCL14, CCL15, and CCL16.

Induction of FGF21, ANGPTL4, and MBL2 mRNA by Wy-14643 in primary human hepatocytes was confirmed by qPCR and was already observed after 6 h of PPARα activation (Fig. 1B). In silico analysis of the region immediately surrounding the MBL2 transcriptional start site did not reveal the presence of any putative strong PPAR response elements (PPREs; Fig. 1C). The closest medium-strength PPREs were identified ∼10 kb up- and downstream of the MBL2 transcriptional start site. Whereas MBL2 mRNA was induced by Wy-14643 (Fig. 1D), levels of (multimeric) ANGPTL4 protein in human hepatocyte medium and cell lysate were not changed following Wy-14643 treatment, which was observed using two independent commercial ELISAs (Fig. 1, E and F).

Mice express two MBL isomers, Mbl1 and Mbl2. Consistent with the microarray data, Mbl2 showed a tendency toward reduced expression after Wy-14643 treatment in mouse hepatocytes, whereas Mbl1 expression was modestly but significantly increased by 24-h Wy-14643 treatment (Fig. 1G). qPCR also verified the induction of Fgf21 and Angptl4 by Wy-14643 in mouse hepatocytes (Fig. 1G). The data indicate that MBL2 mRNA is upregulated specifically by PPARα in human hepatocytes, whereas no changes in (multimeric) MBL protein levels could be detected.

Tissue expression profiling indicated that MBL2 is expressed exclusively in liver (see Fig. 2A and also http://biogps.org/). To further explore regulation of Mbl1 and Mbl2 in mouse liver, we studied the effect of PPARα activation by fasting and short- and long-term administration of Wy-14643 in wild-type and PPARα−/− mice. Whereas the expression changes of Fgf21 and Angptl4 clearly indicated upregulation by PPARα (Fig. 2, B–D), expression patterns of Mbl1 and Mbl2 were more complicated. Expression of Mbl1 was unaltered by Wy-14643 treatment and reduced in PPARα−/− mice, especially during fasting (Fig. 2, B–D), whereas Mbl2 expression was suppressed by Wy-14643 and fasting in a PPARα-dependent manner (Fig. 2, B–D). These data indicate that PPARα is important for maintaining basal Mbl1 expression in mouse liver. In contrast, PPARα negatively regulates Mbl2 expression in mouse liver.

Since Mbl1 and Mbl2 are expressed exclusively in liver (http://biogps.org/), changes in gene expression in mouse liver may directly impact circulating Mbl levels. Plasma Mbl1 levels were modestly but significantly reduced in PPARα−/− mice, especially after fasting (Fig. 3A). Surprisingly, Wy-14643 markedly increased plasma Mbl1 levels in lean mice in a PPARα-dependent manner (Fig. 3B). Similar results were obtained in obese mice (Fig. 3C). Mbl2 was not detectable in plasma of Sv129 mice, the background strain for the PPARα−/− mice (data not shown). Furthermore, Wy-14643 increased abundance of Mbl1 protein in mouse liver (Fig. 3D). Since Wy-14643 did not affect hepatic Mbl1 mRNA, the increase in plasma and liver Mbl1 levels likely occur via a posttranscriptional type of mechanism.

Human MBL plasma levels are increased by fenofibrate. We next aimed to study the impact of PPARα on FGF21, ANGPTL4, and MBL in humans. To that end, plasma levels were measured in 19 overweight subjects before and after 6 wk of treatment with the PPARα agonist fenofibrate (200 mg/day). Consistent with upregulation of FGF21 and ANGPTL4 mRNA by Wy-14643 in human hepatocytes, plasma FGF21 (413%, P < 0.001) and ANGPTL4 (69%, P < 0.001) were increased significantly by fenofibrate treatment (Fig. 4A). Baseline plasma MBL levels showed marked interindividual variation, which is characteristic of MBL and is related to differences in MBL haplotypes. Despite the large variation, fenofibrate raised plasma MBL in all subjects (Fig. 4A), with a mean increase of 73% (P = 0.0016).

To substantiate induction of plasma MBL levels by PPARα activation in humans, serum levels of MBL protein were measured in 11 obese females with type 2 diabetes before and after 3 mo of treatment with PPARα agonist fenofibrate (200 mg/day). Consistent with the results from the other study, fenofibrate raised serum MBL in all but one subject (Fig. 4B), with a mean increase of 86% (P = 0.017). These data demonstrate clearly that plasma MBL levels are increased by PPARα activation in humans.

To examine whether the changes in plasma levels of MBL, FGF21, and ANGPTL4 by fenofibrate may be interconnected, we plotted the relative increase of one circulating factor against the relative increase of another factor. No significant correlations were observed (data not shown), indicating that inductions of plasma levels of PPARα targets upon fenofibrate treatment do not appear to be correlated.

DISCUSSION

MBL is an important player in complement cascade activation as part of the first-line host defense. In the present study we show that 1) MBL mRNA is strongly upregulated by PPARα in human but not mouse hepatocytes, 2) plasma MBL...
levels are increased by chronic PPARα activation in humans, and 3) plasma Mbl1 levels are increased by chronic PPARα activation in mice.

MBL recognizes and binds to conserved carbohydrate structures present on the surface of microorganisms (17, 33). MBL binding results in activation of the lectin pathway of the complement system by the action of MBL-associated serine proteases, which associate with circulating MBL in their inactive proenzyme forms (32). Alternatively, MBL acts as an opsonin, leading to stimulation of phagocytosis by binding to...
cell surface receptors present on phagocytic cells. Studies have shown that, in addition to being a key regulator of hepatic lipid metabolism, PPARα has a major impact on inflammatory pathways (31). The pronounced induction of MBL2 mRNA by PPARα in human liver fits within the role of PPARα as important regulator of inflammation and innate immunity (5).

Currently, little is known about factors controlling plasma MBL levels. Although MBL levels in serum are known to be determined largely by polymorphisms in the MBL gene, differences in plasma MBL of up to 10-fold can be found between individuals despite identical genotypes (30). Also, little is known about regulation of MBL gene expression. Specific expression of MBL in liver has been suggested to be mediated by HNF3 (forkhead box protein A) based on the presence of a specific response element in the MBL promoter and its ability to bind HNF3 in vitro (25). However, extensive evidence for regulation of MBL by HNF3 is currently lacking.

Our data indicate that MBL is differentially regulated by PPARα between mouse and human. Interestingly, whereas Wy-14643 markedly induced MBL2 mRNA in human primary hepatocytes, MBL protein levels in cell lysate and medium as determined by two different commercial MBL assays were not increased by Wy-14643 treatment. The lack of effect of Wy-14643 on MBL protein levels in cell lysate and medium may be related to the fact that both MBL ELISA assays specifically measure MBL multimers. Alternatively, it is possible that synthesis of MBL protein is not increased by Wy-14643 despite the increase in MBL2 mRNA.

In mouse liver, gene expression of the closest mouse ortholog Mbl2 was downregulated by PPARα activation. Levels of Mbl2 in plasma of Sv129 mice were too low to make any inferences regarding the impact of PPARα on plasma Mbl2. Remarkably, expression of the closely related Mbl1 was modestly induced by PPARα activation in primary mouse hepatocytes and reduced in livers of PPARα−/− mice. Despite a lack of increase in Mbl1 mRNA in liver after 5-day Wy-14643 treatment, the treatment raised circulating and liver Mbl1 protein levels significantly. Considering that MBL is expressed exclusively in liver, it seems extremely unlikely that the increase in plasma Mbl1 by Wy-14643 in mice and the increase in plasma MBL by fenofibrate in human

**Fig. 3.** Plasma levels of Mbl1 are increased by PPARα activation in mice. A: levels of Mbl1 in plasma of fed and 24-h-fasted wild-type and PPARα−/− mice. B: levels of Mbl1 in plasma of wild-type and PPARα−/− mice fed a chow diet containing 0.1% WY for 5 days. C: levels of Mbl1 in plasma of wild-type and PPARα−/− mice fed a high-fat diet for 21 wk supplemented with 0.1% WY for the last 5 days. D: levels of Mbl1 in liver of wild-type and PPARα−/− mice fed a chow diet containing 0.1% WY for 5 days. Error bars represent SE. Differences were evaluated statistically using 2-way ANOVA. Significance (P value) of effect of G, T, and I between genotype and treatment is indicated.

**Fig. 4.** Fenofibrate treatment increases plasma levels of MBL, FGF21, and ANGPTL4. A: 19 overweight subjects were treated with fenofibrate treatment for 6 wk (200 mg/day micronized; lipanthyl). Changes in plasma FGF21, ANGPTL4, and MBL concentrations after fenofibrate treatment in individual subjects are indicated. B: 11 female subjects with type 2 diabetes mellitus were treated with fenofibrate for 3 mo (200 mg/day micronized; lipanthyl). Changes in plasma MBL concentrations after fenofibrate treatment in individual subjects are indicated. Note that plasma MBL concentrations are displayed in log scale. Subjects deficient in MBL were excluded from analysis. The effect of fenofibrate was evaluated statistically using paired t-test on log-transformed data (MBL).
are caused by upregulation of MBL gene expression in extrahepatic tissues. Because plasma levels are determined by the balance between rate of production and rate of clearance, the data suggest that induction of plasma MBL by PPARα activation may be related to impaired MBL clearance. Future studies will have to address possible regulation of MBL clearance by PPARα.

Previously, transcription of several genes has been reported to be induced specifically by PPARα in humans, including apolipoprotein (apo)A1, apoAI, and apoAIV (7). For each of these genes the loss of regulation in mouse was related to lack of conservation of the functional PPREs. It is impossible to determine whether a similar mechanism applies to MBL, because in silico screening of the region up- and downstream of the transcriptional start site of the MBL gene failed to yield any candidate PPREs in proximity to the transcriptional start site. Furthermore, since induction of Mbl1 and Mbl2 was not observed in mice, sequence conservation could not be used to narrow down candidate PPREs, making identification of the exact mechanism of regulation of MBL2 mRNA by PPARα a very complicated task. In our study, we found that every subject receiving fenofibrate exhibited an increase in plasma MBL. Although baseline differences in plasma MBL are related largely to polymorphisms in the MBL gene, our data suggest that some variation may be due to differences in PPARα activity and/or expression level. In this context, it is interesting to note that two other secreted proteins that were highly induced by PPARα activation in primary human hepatocytes, ANGPTL4 and FGF21, also show large inter-individual variations in their plasma level. Similarly to MBL, plasma levels of ANGPTL4 and FGF21 are increased by treatment with PPARα agonist (4, 11, 19), which was confirmed here.

In conclusion, MBL is a circulating target of PPARα in humans, suggesting that MBL may be involved in mediating effects of PPARα on innate immunity. Additionally, regulation by PPARα may imply a potential role of MBL in lipid metabolism and metabolic diseases, which we are currently exploring in more detail.

GRANTS

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DISCLOSURES

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS


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