Mechanisms of the Triglyceride- and Cholesterol-Lowering Effect of Fenofibrate in Hyperlipidemic Type 2 Diabetic Patients

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In humans, the precise mechanisms of the hypolipidemic action of fenofibrate, a peroxisome proliferator–activated receptor-α agonist, remain unclear. To gain insight on these mechanisms, we measured plasma lipids levels, lipids synthesis (hepatic de novo lipogenesis and cholesterol synthesis), and mRNA concentrations in circulating mononuclear cells (RT-PCR) of hydroxymethylglutaryl (HMG)-CoA reductase, LDL receptor, LDL receptor–related protein (LRP), scavenger receptor class B type I (SR-BI), ABCAI, and liver X receptor (LXR)-α in 10 control subjects and 9 hyperlipidemic type 2 diabetic patients. Type 2 diabetic subjects were studied before and after 4 months of fenofibrate administration. Fenofibrate decreased plasma triglycerides ($P < 0.01$) and total cholesterol ($P < 0.05$) concentrations and slightly increased HDL cholesterol ($P < 0.05$). Hepatic lipogenesis, largely enhanced in diabetic subjects (16.1 ± 2.1 vs. 7.5 ± 1.6% in control subjects, $P < 0.01$), was decreased by fenofibrate (9.8 ± 1.5%, $P < 0.01$). Fractional cholesterol synthesis was normal in diabetic subjects (3.5 ± 0.4 vs. 3.3 ± 0.5% in control subjects) and was unchanged by fenofibrate (3.5 ± 0.5%). Absolute cholesterol synthesis was, however, increased in diabetic subjects before and after fenofibrate ($P < 0.05$ vs. control subjects). HMG-CoA reductase, LDL receptor, LRP, and SR-BI mRNA concentrations were not different in type 2 diabetic and control subjects and were unchanged by fenofibrate. LXR-α mRNA levels were increased ($P < 0.05$) by fenofibrate. ABCAI mRNA concentrations, which were decreased in diabetic subjects ($P < 0.05$) before fenofibrate, were increased ($P < 0.05$) by fenofibrate to values comparable to those of control subjects. The plasma triglyceride–lowering effect of fenofibrate is explained in part by a decrease in hepatic lipogenesis, the moderate fall in total plasma cholesterol is not explained by a reduction of whole-body cholesterol synthesis, and the increase in LXR-α and ABCAI mRNA levels suggests that fenofibrate stimulated reverse cholesterol transport. *Diabetes* 51:3486–3491, 2002

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Received for publication 4 June 2002 and accepted in revised form 6 September 2002.

ASR, absolute synthetic rate; DNL, de novo lipogenesis; FFA, free fatty acid; FSR, fractional synthetic rate; HMG, hydroxymethylglutaryl; LPL, lipoprotein lipase; LRP, LDL receptor–related protein; LXR, liver X receptor; PPAR, peroxisome proliferator–activated receptor; SR-BI, scavenger receptor class B type I.

Fibrates have been used for the treatment of hypertriglyceridemia or mixed hyperlipidemia for >30 years. Their main action is to lower plasma triglyceride levels, but they also reduce total and LDL cholesterol concentrations and induce a moderate increase in HDL cholesterol. It is now known that fibrates act by stimulating the activity of peroxisome proliferator–activated receptor (PPAR)-α, a member of the PPAR subfamily of nuclear receptors (1). PPAR-α is mainly expressed in liver, kidney, heart, and muscles (2,3), as well as in cells of the arterial wall, monocytes, macrophages (4), and lymphocytes (5). It controls the transcription of regulatory genes of fatty acids and cholesterol metabolism. The decrease in plasma triglycerides induced by fibrates has been attributed to an inhibition of the synthesis and secretion of triglycerides by the liver and a stimulation of the degradation of triglyceride-rich lipoproteins (6). This increased clearance of triglycerides results from a stimulation of the expression of lipoprotein lipase (LPL) (7,8) and a decreased expression and concentration of apolipoprotein CIII (9), an inhibitor of LPL activity. The mechanisms of the action of fibrates on cholesterol metabolism remained unclear until it appeared that PPAR-α activation modified the expression of several key genes controlling HDL cholesterol metabolism (10). It was first recognized that PPAR-α stimulated the expression of human apolipoprotein A-I and A-II genes (10). More recently, Chinetti and colleagues (11,12) demonstrated that PPAR-α activation stimulated the expression in differentiated human macrophages of CD36 and LImPII analogous 1 (CLA-1)/scavenger receptor class B type I (SR-BI) and ABCAI, which both play key roles in the reverse transport of cholesterol (13,14). SR-BI is a cell surface receptor that binds HDL with high affinity and mediates the selective uptake by liver and steroidogenic tissues of cholesterol esters from HDL. SR-BI could also play a role in the cellular efflux of cholesterol. ABCAI exports unesterified cholesterol and phospholipids from cells to nascent HDL and therefore has a key role in the control of the first step of reverse cholesterol transport. However, most of these data on the mechanisms of action of fibrates were obtained in vitro or in rodent animals. There are differences in the tissular expression and activity of PPAR-α between rodents and humans, as well as large differences in the regulation of lipoproteins metabolism, particularly HDL cholesterol. Hamsters are considered a more appropriate

TABLE 1
Hormones and metabolites concentrations measured in the postabsorptive state

	Control subjects	Diabetic patients before fenofibrate	Diabetic patients after fenofibrate
Body weight (kg)	62.7 ± 2.7	94.0 ± 3.7*	93.7 ± 5.7*
BMI (kg/m^2)	21.1 ± 0.6	30.7 ± 1.6*	30.1 ± 1.3*
Glucose (mmol/l)	4.46 ± 0.09	8.82 ± 1.32*	7.86 ± 0.92*
Insulin (mU/l)	6.8 ± 1.3	18.5 ± 3.6*	20.5 ± 3.7*
Glucagon (ng/l)	167 ± 29	191 ± 33	195 ± 36
FFAs (μmol/l)	367 ± 50	478 ± 57	335 ± 56†
Triglycerides (mmol/l)	0.81 ± 0.09	3.68 ± 0.72*	2.18 ± 0.45*‡
Total cholesterol (mmol/l)	4.98 ± 0.28	5.94 ± 0.46§	5.52 ± 0.45†
Free cholesterol (mmol/l)	1.10 ± 0.05	1.73 ± 0.17*	1.44 ± 0.12†§
LDL cholesterol (mmol/l)	3.29 ± 0.29	3.69 ± 0.32	3.51 ± 0.40
HDL cholesterol (mmol/l)	1.55 ± 0.11	0.95 ± 0.09*	1.02 ± 0.08†§

Data are means ± SE. *$P < 0.01$ vs. control subjects; †$P < 0.05$, ‡$P < 0.01$ vs. patients before fenofibrate; §$P < 0.05$ vs. control subjects.

model of lipoprotein metabolism but also have some divergent responses to fibrates, such as decreases in HDL cholesterol concentrations and apoprotein-AI expression instead of the increases seen in humans (15). Data on the mechanism of action of fenofibrate in human subjects, particularly in hyperlipidemic patients, are scarce and limited, to our knowledge, to measurements of the concentrations and kinetics of apoprotein B100 and apoprotein A (10). In the present report, we investigated the effect of fenofibrate administration in diabetic patients with hypertriglyceridemia or mixed hyperlipidemia. Cholesterol synthesis and hepatic lipogenesis were measured with deuterated water, and the expression, as appreciated by mRNA levels, of key regulatory genes of lipid metabolism was measured in circulating mononuclear cells.

RESEARCH DESIGN AND METHODS

Materials. Deuterated water (99% mole percent excess) was from Cambridge Isotope Laboratory (Andover, MA). Chemicals and reagents were from Sigma (St. Louis, MO), Boehringer (Mannheim, Germany), or Pierce (Rockford, IL).
Subjects. After full explanation of the nature, purpose, and possible risks of the study, informed written consent was obtained from 10 healthy volunteers and 9 type 2 diabetic patients with hypertriglyceridemia (6 patients) or mixed hyperlipidemia (3 patients). The control group consisted of six women and four men (aged 20–51 years, BMI 18–25 kg/m^2). No control subject had a personal or familial history of diabetes, dyslipidemia, or obesity or was taking any medication; all had normal physical examination and normal plasma glucose and lipid concentrations (Table 1). Subjects with unusual dietary habits were excluded. All diabetic patients were overweight (BMI 27–34 kg/m^2). Four diabetic patients were treated by diet alone, two by metformin, two by sulfonylurea, and one by a combination of metformin and sulfonylurea. These treatments were not modified during the study. No diabetic patient took any hypolipidemic drug during the 6 months preceding the study.
Protocols. The protocol of the study was approved by the Ethical Committee of Lyon, and the study was conducted according to the French Huriet law. Tests in women were performed during the first 10 days of the menstrual cycle to account for the known variations of lipogenesis during the menstrual cycle (there are no menstrual variations for cholesterol synthesis) (16). The control subjects were studied only one time. They consumed their usual diet during the days preceding the study. Diabetic patients were studied twice, before and after at least 4 months of treatment with fenofibrate (200-mg micronized capsule once daily with breakfast). During the month preceding the first test and until the end of the study, they consumed a weight-maintaining diet, with 50% of energy intake as carbohydrate. In the evening before the test, the subjects drank a loading dose of deuterated water (3 g/kg body water; one-half after the evening meal and one-half at 10:00 P.M.). Then, until the end of the test the next morning, they drank only water enriched with ^2H_2O (4.5 g ^2H_2O/l drinking water). The next morning at 7:30 A.M., in the postabsorptive state after an overnight fast, an indwelling catheter was placed in a forearm vein and three blood samples were drawn at 15-min intervals for the measurement of deuterium enrichments in plasma water, plasma cholesterol, and palmitate of plasma triglycerides. Blood was also collected for the separation of circulating mononuclear cells and the measurement of plasma glucose, insulin, glucagon, and free fatty acid (FFA) and lipid concentrations.

Analytical procedures

Metabolite and hormone concentrations. Metabolites were assayed with enzymatic methods on neutralized perchloric extracts of plasma (glucose) or on plasma (FFAs and triglycerides) (17). Plasma insulin and glucagon concentrations were determined by radioimmunoassay. Total, unesterified, and HDL cholesterol were measured as previously described (18,19). LDL cholesterol was calculated using the equation of Friedewald, except in subjects with plasma triglycerides >4.5 mmol/l.
Deuterium enrichments. Plasma lipids were extracted by the method of Folch et al. (20). Free cholesterol and triglycerides were separated by thin-layer chromatography and scraped off the silica plates. Cholesterol was eluted from silica with ether before preparing its trimethylsilyl derivative (21). The methylated derivative of the palmitate of triglycerides was prepared according to Morrison and Smith (22). Deuterium enrichment determinations were performed as previously described (21,23) on a gas chromatograph (HP5890; Hewlett-Packard, Palo Alto, CA) equipped with a 25-m fused silica capillary column (OV1701; Chrompack, Bridgewater, NJ) and interfaced with a mass spectrometer (HP5971A; Hewlett-Packard) operating in the electronic impact ionization mode (70 eV). Helium was the carrier gas. Ions 368–370 were selectively monitored for cholesterol and ions 270–272 for palmitate. Deuterium enrichment in plasma water was measured by the method of Yang et al. (24). Special care was taken to obtain comparable ion peak areas between standard and biological samples, adjusting the volume injected or diluting the sample when necessary. Enrichment values are expressed as mole percent excess.
mRNA concentrations in circulating mononuclear cells. Mononuclear cells were immediately isolated by centrifugation of whole venous blood on a Ficoll gradient at 4°C as described (25) and stored at −80°C. Total RNA was prepared from frozen samples as described previously (26). Total RNA was then quantified by electrophoresis on 1% agarose gel of serial dilutions compared with known amounts of standard RNA (Boehringer Mannheim). LDL receptor–related protein (LRP) (18), SR-BI/CLA-1, and liver X receptor (LXR)-α mRNA concentrations were measured by RT-PCR using β-actin or cyclophilin as internal standard. Sequences of the primers are shown in Table 2. LDL receptor and hydroxymethylglutaryl (HMG)-CoA reductase mRNA copy numbers were determined by competitive RT-PCR. The detailed procedure has been previously reported (19). The results were expressed as copy number per μg of total cellular RNA. ABCA1 mRNA concentrations were also measured by competitive RT-PCR. A 449-bp cDNA fragment was synthesized by RT-PCR from human adipose tissue total RNA using specific primers (Table 2). The 404-bp ABCA1 competitor was obtained by deleting a 45-bp fragment by deletion PCR. To validate the competitive RT-PCR assay, RNA corresponding to part of the ABCA1 mRNA, including the target sequence, was synthesized by in vitro transcription (Riboprobe System; Promega, Charbonnières, France), and known amounts of the synthesized mRNA were quantified by competitive RT-PCR assay to perform a dose-response curve (27). For measurements of ABCA1 mRNA levels in biological samples, a specific first-strand cDNA was first synthesized from 0.1 μg total RNA with 2.5 units thermostable reverse transcriptase (Tth DNA polymerase; Promega) in 10 mmol/l Tris-HCl, pH 8.3, 90 mmol/l KCl, 1 mmol/l $MnCl_2$, 0.2 mmol/l de-

TABLE 2
Sequences of the primers used

mRNA species	Sense primers	Antisense primers
HMG-CoA reductase	5′-TACCATGTCAGGGGTACGTC-3′	5′-CAAGCCTAGAGACATAATCAC-3′
LDL recepter	5′-CAATGTCTCACCAAGCTCTG-3′	5′-TCTGTCTCGAGGGGTAGCTG-3′
LRP	5′-ATCTTGGCCACGTACCTGAG-3′	5′-CGAGTTGGTGGCATAGAGAT-3′
LXR-α	5′-GAGGGCTGCAAGGGATTCTT-3′	5′-GTTACACTGTTGCTGGGCAG-3′
SR-BI	5′-TCGCTCATCAAGCAGGAGGT-3′	5′-GCCCAGAGTCGGAGTTGTTG-3′
ABCAI	5′-CAGGAGGTGATGTTTCTGACCA-3′	5′-TTGGCTGTTCTCCATGAAGGTC-3′
Cyclophilin	5′-GCTCTGAGCACTGGAGAGAA-3′	5′-GGTGATCTTCTTGCTGGTCTGC-3′
β-actin	5′-GACGAGGCCCAGAGCAAGAGA-3′	5′-GGGTGTTGAAGGTCTCAAACA-3′

oxynucleoside triphosphate, and 15 pmol of the specific antisense primer in a final volume of 20 μl. The reaction was carried out for 10 min at 32°C, 3 min at 60°C, and 15 min at 70°C followed by 5 min at 99°C. After chilling, the whole RT reaction was then added to 80 μl of a PCR mix (10 μl Tris-HCl, pH 8.3, 100 mmol/l KCl, 25 mmol/l $MgCl_2$, 75 mmol/l EGTA, and 5% glycerol) containing 0.2 mmol/l deoxynucleosidetriphosphates, 5 units *Taq* polymerase (Life Technologies, Cergy Pontoise, France), 45 pmol of the corresponding sense primer, and 30 pmol of the antisense primer. Sense primers were labeled in the 5′ position with Cy-5 fluorescent dye. Four 20-μl aliquots were then transferred using a multichannel pipette in a 96-well plate, with each well containing 5 μl of defined working solutions of the competitor cDNA. The PCR conditions were 2 min at 94°C followed by 40 cycles (1 min at 94°C, 1 min at 58°C, and 1 min at 72°C) and finally 10 min at 72°C. The PCR products were then analyzed with an automated laser fluorescence DNA sequencer (ALFexpress; Pharmacia, Uppsala, Sweden) in 4% denaturating polyacrylamide gels. The amounts of PCR products (competitor and target) were calculated by integrating peak areas using The Fragment Manager software from Pharmacia. The initial concentration of target mRNA was determined at the competition equivalence point as previously described (27).

Calculations. The fractional contributions of cholesterol synthesis to the plasma free cholesterol pool and of hepatic lipogenesis to the plasma triglyceride–fatty acid pool were calculated from the deuterium enrichments in free cholesterol, in the palmitate of plasma triglycerides, and in plasma water, as previously described (21,28). In short, the deuterium enrichments that would have been obtained if endogenous synthesis were the only source of plasma cholesterol and triglyceride–fatty acid pool were calculated from plasma water enrichment. The comparison of the actual enrichments observed with these theoretical values gives the contribution, expressed as fractional synthetic rate (FSR), of endogenous synthesis to the pool of rapidly exchangeable free cholesterol and of plasma triglycerides during the time elapsed between the ingestion of the loading dose of deuterated water and blood sampling (12 h). The FSR of cholesterol was then transformed in an estimate of absolute synthetic rate (ASR), expressed in mg synthesized during the 12-h period of deuterated water ingestion. For this calculation, we first calculated the total pool M_1 of rapidly exchangeable cholesterol using the equation of Goodman et al. (29). M_1 comprises both free and esterified cholesterol, and we found deuterium enrichment in free cholesterol only. Therefore, we calculated the pool Mf_1 of rapidly exchangeable free cholesterol, estimating that the ratio in plasma of free-to-total cholesterol represents the ratio in the whole pool. ASR was then calculated as $ASR = FSR \times Mf_1$. The absolute value of plasma triglycerides pool provided by hepatic lipogenesis, TG*, expressed as mg/kg of body weight, was also calculated from the corresponding FSR, and the total plasma triglycerides pool M as $TG^* = FSR \times M$. M was calculated from the plasma triglycerides concentration, in mg/l, and from the plasma volume estimated to 45 ml/kg in control subjects with a BMI <30 kg/m^2 and to 37 ml/kg in subjects with a BMI >30 kg/m^2 (30).

Results are shown as means ± SE. Comparisons between values of the control and diabetic groups were performed using two-tailed Student's *t* test for unpaired data and comparison of values of the diabetic patients before and after fenofibrate treatment by two-tailed Student's *t* test for paired values.

RESULTS

Hormonal and metabolic parameters. Table 1 shows the BMI, body weight, metabolite, and hormone values for the control subjects and diabetic patients before and after fenofibrate administration. Diabetic patients had higher body weights and BMI ($P < 0.01$), which were unchanged during the period of treatment with fenofibrate. Glucose and insulin concentrations were higher ($P < 0.01$) in diabetic patients than in control subjects; the moderate decrease in glucose observed after fenofibrate administration failed to reach significance ($P < 0.10$). Plasma triglycerides ($P < 0.01$) and total ($P < 0.05$) and free ($P < 0.01$) cholesterol were higher and HDL cholesterol was lower ($P < 0.01$) in diabetic patients. Fenofibrate decreased triglycerides ($P < 0.01$) and total and free cholesterol ($P < 0.05$), whereas HDL cholesterol increased slightly ($P < 0.05$). FFAs were also decreased after fenofibrate ($P < 0.05$); however, FFA concentrations, either before or after fenofibrate, were not different from the values of control subjects.

Endogenous synthetic rates. There was a large increase ($P < 0.01$) in the fractional contribution of hepatic lipogenesis to the circulating pool of plasma triglycerides in the diabetic group (Table 3). Fenofibrate lowered this fractional contribution to values comparable to those observed in the control group. However, the absolute pool of plasma triglycerides provided by hepatic lipogenesis, although largely decreased by fenofibrate, remained higher ($P < 0.05$) in diabetic patients than in control

TABLE 3
Fractional and absolute contributions of hepatic lipogenesis and cholesterol synthesis to the circulating pool of triglycerides and cholesterol in controls subjects and diabetic patients before and after fenofibrate

	Control subjects	Diabetic patients before fenofibrate	Diabetic patients after fenofibrate
Hepatic lipogenesis (%)	7.5 ± 1.2	16.1 ± 2.1*	9.8 ± 1.5†
Plasma pool of triglycerides provided by lipogenesis (mg/kg)	2.5 ± 0.6	19.5 ± 4.9*	5.9 ± 1.2‡§
Cholesterol FSR	3.3 ± 0.5	3.5 ± 0.4	3.5 ± 0.5
Free cholesterol ASR (mg/12 h)	173 ± 19	270 ± 37‡	267 ± 28‡

Data are means ± SE. *$P < 0.01$ vs. control subjects; †$P < 0.01$ vs. patients before fenofibrate; ‡$P < 0.05$ vs. control subjects; §$P < 0.05$ vs. patients before fenofibrate.

TABLE 4
mRNA values in control subjects and diabetic patients before and after treatment with fenofibrate

	Control subjects	Diabetic patients before fenofibrate	Diabetic patients after fenofibrate
HMG-CoA reductase (10^4 copies/μg RNA)	793 ± 237	493 ± 61	451 ± 43
LDL receptor (10^4 copies/μg RNA)	34.0 ± 9.6	44.4 ± 5.6	49.5 ± 8.7
LRP/β-actin	0.38 ± 0.10	0.43 ± 0.12	0.50 ± 0.09
SR-BI/cyclophilin	0.48 ± 0.15	0.43 ± 0.09	0.45 ± 0.15
ABCA1 (10^4 copies/μg RNA)	4.5 ± 0.8	2.3 ± 0.6*	3.9 ± 0.1†
LXR-α/β-actin	0.38 ± 0.06	0.28 ± 0.12	0.53 ± 0.14†

Data are means ± SE. *$P < 0.05$ vs. control subjects; †$P < 0.05$ vs. patients before fenofibrate.

subjects. Cholesterol FSR was not increased in diabetic patients and was unchanged by fenofibrate. Since plasma cholesterol concentrations, and thus rapidly exchangeable pools, were increased in diabetic patients, cholesterol ASR was higher in diabetic patients than in control subjects before and after fenofibrate ($P < 0.05$).

mRNA concentrations (Table 4). We observed no significant differences between control subjects and diabetic patients in values for HMG-CoA reductase, LDL receptor, or LRP mRNA concentrations in circulating mononuclear cells, and these values were unaffected by fenofibrate. SR-BI mRNA levels were also comparable in control and diabetic subjects and unchanged by fenofibrate. There was a trend for lower LXR-α mRNA concentrations in diabetic patients, and ABCAI mRNA concentrations were significantly lower ($P < 0.05$). Fenofibrate increased LXR-α mRNA concentrations ($P < 0.05$) and raised ABCAI mRNA concentrations ($P < 0.05$) to values not different from those observed in control subjects.

DISCUSSION

The administration of fenofibrate to hyperlipidemic type 2 diabetic patients induced the expected modifications of plasma lipid levels, with a large decrease of triglyceride concentration, a moderate fall of total cholesterol, and an increase of HDL cholesterol levels. The decrease in circulating triglycerides has been attributed to a stimulation of the degradation of triglycerides through increased expression and activity of LPL and to a decrease of hepatic synthesis and secretion of triglycerides (6). In this report, we did not investigate plasma triglycerides clearance but we show a clear and important decrease of hepatic de novo lipogenesis (DNL) by fenofibrate, one of the pathways providing fatty acyl-CoA for liver triglycerides synthesis. In addition, we have indirect evidence for a decreased contribution of the reesterification of plasma FFAs to liver triglycerides synthesis. DNL was a minor contributor to hepatic triglycerides synthesis and secretion in normal subjects, in agreement with previous reports (23). Both the fractional and absolute contributions of DNL to these metabolic processes were largely increased in the diabetic patients before treatment. These contributions were lowered by fenofibrate, the fractional one returning to values comparable with those of control subjects, whereas the absolute contribution, although dramatically reduced, remained above control values. These results strongly suggest that the modifications of the expression of several key genes of liver fatty acids metabolism observed in fenofibrate-treated hamsters (15), i.e., inhibition of acetyl-CoA carboxylase and fatty acid synthase and stimulation of acyl-CoA oxidase expression, are also present in humans. Reesterification of plasma FFAs taken up by the liver is considered a main pathway of hepatic triglycerides synthesis (31). This pathway was probably also inhibited by fenofibrate, since inhibition of lipogenesis and stimulation of fatty acid oxidation divert hepatic fatty acid metabolism away from reesterification (32). In addition, plasma FFA levels were decreased by fenofibrate. Because the uptake of fatty acids by the liver is proportional to their concentration in plasma (33), it is probable that the total amount of plasma fatty acids delivered to the liver for oxidation or reesterification was also decreased. Several mechanisms could have contributed to this fall in plasma FFAs. First, fibrates have been reported to decrease hormone-sensitive lipase activity (34). Second, PPAR-α is highly expressed in human muscles (3). Its stimulation increases the expression of carnitine palmitoyl transferase I and malonyl-CoA decarboxylase, resulting, as in liver, in an increased oxidation of fatty acids (35–37). It is therefore probable that fatty acid uptake and oxidation by muscles were increased by fenofibrate in the diabetic patients investigated, resulting in increased FFA clearance.

The mechanisms of the decrease in plasma total cholesterol remain unclear. Guo et al. (15) found, in fenofibrate-treated hamsters, a decrease in the expression and activity of hepatic HMG-CoA synthase and HMG-CoA reductase and a decreased hepatic synthesis of cholesterol from acetate. Although the method they used for measuring cholesterol synthesis (bolus injection of labeled acetate) is debatable (38), these data suggested that the main mechanism for the cholesterol-lowering effect of fenofibrate was an inhibition of cholesterol synthesis. The results we obtained in type 2 diabetic patients contrast those obtained in hamsters: these patients had an increased cholesterol ASR that was not lowered by fenofibrate, and this treatment induced no decrease in HMG-CoA reductase mRNA levels. However, these mRNAs levels were not measured in hepatocytes but in circulating mononuclear cells. Although this was initially suggested (39), it is unclear whether these cells can be considered as representative of hepatocytes with respect to the expression of key regulatory genes of cholesterol metabolism (19). With respect to the lack of decrease of cholesterol ASR after treatment by fenofibrate, the method used for measuring cholesterol synthesis does not allow the delineation of respective contributions of liver and extra-hepatic tissues to the sampled pool of cholesterol. It remains possible that a decreased hepatic synthesis and secretion of cholesterol, incorporated into VLDL, was masked by an increased

influx of cholesterol from extra-hepatic tissues in the HDL pathway. Actually a decreased flux of cholesterol from liver to peripheral tissue through the VLDL-LDL pathway would be consistent with the observed modifications of triglyceride metabolism. A stimulation of reverse cholesterol transport is supported by the moderate increase in HDL cholesterol concentration. In addition to the stimulation of apolipoprotein A-I and A-II expression, fibrates have been shown to stimulate in vitro the expression of SR-BI and ABCAI (11,12). We found no evidence for a stimulation of SR-BI expression in circulating mononuclear cells, but ABCAI expression, decreased in diabetic patients, was restored to normal levels by fenofibrate. This effect, if present in other tissues, would favor an increased cholesterol efflux to nascent HDL and thus a stimulation of reverse cholesterol transport. To our knowledge, no PPAR-responsive element has been described in the ABCAI promoter. However, PPAR-responsive elements are present in the LXR promoter, and fibrates stimulate the activity of the LXR promoter in vitro (40). Our finding that fenofibrate increased LXR-α mRNA concentration in vivo in humans agrees with these in vitro results; it provides an explanation for the effect of fenofibrate on ABCAI, since LXR response elements are present in the ABCAI promoter (41,42) and LXR-α agonists stimulate ABCAI expression (43). A stimulation of LXR-α expression in other tissues, such as liver, would also stimulate reverse cholesterol transport through an increase in the expression of CETP (44). In addition, fenofibrate has been shown to increase the expression and activity of phospholipid transfer protein in mice (45). On the other hand, LXR-α has been shown to stimulate the expression of lipogenic genes in liver through both a direct action and a stimulation of SREBP (sterol regulatory element–binding protein)-1c expression (46). An increase during fenofibrate treatment of liver LXR-α expression could therefore have had a stimulatory action on the expression of the lipogenic pathway. Our results show that this effect, if present in humans, is clearly insufficient to counteract the inhibitory action of fibrates on liver lipogenesis.

In conclusion, our results show that the hypotriglyceridemic action of fenofibrate is mediated in part by an important decrease of hepatic lipogenesis, and probably by a reduction of the hepatic reesterification of plasma FFAs. Our results also suggest that fenofibrate stimulates the expression of key regulatory genes of reverse cholesterol transport in humans.

ACKNOWLEDGMENTS

This work was supported in part by a grant from Laboratoires Fournier.

The authors thank all the subjects who participated in this study and J. Peyrat and C. Maitrepierre for helping with the tests.

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