A potent PPARα agonist stimulates mitochondrial fatty acid β-oxidation in liver and skeletal muscle

ANNE MINNICH, NIAN TIAN, LISA BYAN, AND GLENDA BILDER

Department of Cardiovascular Biology, Aventis Pharmaceuticals
Research and Development, Collegeville, Pennsylvania 19426-0994

Received 19 May 2000; accepted in final form 20 October 2000

Minnich, Anne, Nian Tian, Lisa Byan, and Glenda Bilder. A potent PPARα agonist stimulates mitochondrial fatty acid β-oxidation in liver and skeletal muscle. Am J Physiol Endocrinol Metab 280:E270–E279, 2001.—The proposed mechanism for the triglyceride (TG) lowering by fibrate drugs is via activation of the peroxisome proliferator-activated receptor-α (PPARα). Here we show that a PPARα agonist, ureido-fibrate-5 (UF-5), ~200-fold more potent than fenofibrate acid, exerts TG-lowering effects (37%) in fat-fed hamsters after 3 days at 30 mg/kg. In addition to lowering hepatic apolipoprotein C-III (apoC-III) gene expression by ~60%, UF-5 induces hepatic mitochondrial carnitine palmitoyltransferase I (CPT I) expression. A 3-wk rising-dose treatment results in a greater TG-lowering effect (70%) at 15 mg/kg and a 2.3-fold elevation of muscle CPT I mRNA levels, as well as effects on hepatic gene expression. UF-5 also stimulated mitochondrial ([3H]palmitate β-oxidation in vitro in human hepatic and skeletal muscle cells 2.7- and 1.6-fold, respectively, in a dose-related manner. These results suggest that, in addition to previously described effects of fibrates on apoC-III expression and on peroxisomal fatty acid (FA) β-oxidation, PPARα agonists stimulate mitochondrial FA β-oxidation in vivo in both liver and muscle. These observations suggest an important mechanism for the biological effects of PPARα agonists.

fibrates; carnitine palmitoyltransferase I; nuclear receptors; gene expression; peroxisome proliferator-activated receptor

THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR) is so named because of its initial identification as the molecular mediator of the peroxisome proliferation response to a number of chemical stimuli, including fibrates, in mice (15, 17). The regulation of genes involved in peroxisomal fatty acid (FA) β-oxidation by PPARα activators is well recognized (23). However, the peroxisomal response is likely to be rodent specific and may not occur in humans (14, 31).

Elevated plasma triglyceride (TG) concentrations constitute an independent risk factor for coronary artery disease (11, 13). Fibrate drugs and FAs are believed to be weak PPARα agonists, and PPARα is likely to mediate the hypolipidemic effects of fibrate TG-lowering therapy. One demonstrated mechanism for the TG-lowering effects of fibrates that is likely to occur in humans is via reduction of hepatic apolipoprotein C-III (apoC-III) transcription and synthesis (28). Because apoC-III is thought to inhibit very low density lipoprotein (VLDL)-TG hydrolysis by lipoprotein lipase and to inhibit uptake of VLDL remnants (1, 26, 32), a reduction in apoC-III synthesis would be predicted to result in a lowering of plasma TG concentrations.

In addition to apoC-III, molecular studies have implicated PPARα in the regulation of a number of genes involved in mitochondrial FA β-oxidation; however, the PPAR responsiveness of such genes in vivo is less well studied. Carnitine palmitoyltransferase I (CPT I) catalyzes the transfer of FA from CoA to carnitine, allowing the initial transport of fatty acids into mitochondria for β-oxidation. Its activity and expression are highly regulated and rate limiting. Eicosapentaenoic acid and fenofibrate administration increased mitochondrial CPT I and II activities in rabbits (9). Shunting of FAs toward β-oxidation would be expected to result in decreased substrate availability for TG synthesis in liver, presumably resulting in a reduction of VLDL-TG secretion. Although fibrate drugs and FAs are believed to exert their effects on gene regulation via PPARα activation, TG lowering and regulation of mitochondrial FA β-oxidation genes have not been directly demonstrated with the use of a potent, bona fide PPARα agonist. We used a ureido-fibrate analog (UF-5), shown to stimulate microsomal FA-ω hydroxylation and to lower VLDL cholesterol in rats (12), for this purpose.

The fat-fed hamster represents a potentially important model of non-diabetic hypertriglyceridemia. Unlike other rodents, hamsters respond to fat feeding with a greater than twofold increase in plasma TG levels (29). Lipoprotein metabolism in hamsters may more closely reflect that of humans than of rats or mice (29), but hamsters are not generally considered responsive to classical fibrates (16). Here we describe the effects of UF-5 on TG metabolism in vivo in a hamster model of hypertriglyceridemia and on FA catabolism in vitro.

PPARα-mediated responses have been traditionally studied in liver, but human and rat skeletal muscle

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
expresses high levels of PPARα (21), and in humans, skeletal muscle may be the major site of PPARα expression. We therefore hypothesized that a potent PPARα agonist would stimulate mitochondrial FA β-oxidation in muscle as well as in liver.

METHODOLOGIES

Chemicals. 2-(4-[(4-fluorophenyl)-N-heptylureido)-ethyl]phenoxy)-2-methylpropionic acid was synthesized as previously described (12). Fenofibrate was obtained by hydrolysis of fenofibrate methyl ester (Sigma, St. Louis, MO). Wy-14643 was from BioMol (Plymouth Meeting, PA). Etopoxir was kindly provided by Dr. H. P. O. Wolf (Allensbach, Germany).

In vivo protocol. Male Golden Syrian hamsters (Harlan Sprague Dawley, Madison, WI), weighing 120–135 g, were group housed with a 12:12-h light-dark cycle. Hamsters were placed on a high-fat high-cholesterol diet (0.05% cholesterol, 10% coconut oil; Dyets, Bethlehem, PA) for ~2 wk before treatment with UF-5 or vehicle and continued on this diet throughout the treatment period. UF-5 was prepared by sonication with vehicle (0.5% methylcellulose, 0.2% Tween 80) and administered twice daily by gavage in a rising-dose fashion (7.5, 15, and 30 mg/kg, n = 7 animals/group) with each dose given for a 1-wk period, resulting in a total of 3 wk of treatment with incremental doses. Vehicle was administered at 5 ml/kg. In a second study, vehicle, UF-5 (7.5, 15, and 30 mg/kg), and fenofibrin acid (30, 60, and 120 mg/kg) were administered twice daily to fat-fed hamsters (n = 6/group) with a similar 3-wk rising-dose protocol. Blood samples, removed under CO2 narcosis, were obtained at specified times throughout the studies and were analyzed for triglycerides. *-CATCAAGAAGGTGGTGAAGC-3 (forward) and 760–964 of the rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) coding sequence was cloned by PCR with primers 5’-CATCAAGAAGGTGGTGAAGC-3 (forward) and 5’-ACCCTGTGGCTGTAGCCATA-3 (reverse) into PCRII and excised with EcoRI and XhoI from the Sigma Diagnostic assay kit according to the manufacturer’s instructions.

Cell culture. HepG2 cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA) and cultured in DMEM-10% FCS-1% penicillin-streptomycin. Human primary skeletal muscle cells were obtained from Clonetics (San Diego, CA) and cultured in SkBM, supplemented with SkGM Singlequots.

Northern blotting. RNA was extracted from hamster soleus muscle or liver with Trizol reagent according to the manufacturer’s protocol. Total RNA was subjected to Northern blotting onto Nytran membranes (Schleicher & Schuell, Keene, NH). A probe for rat apoC-III was cloned exactly as previously described (28). Human liver and muscle CPT I probes (accession nos. R28631 and W85710, respectively) were excised from pTT73 by EcoRI I/Not I and EcoRI IV/PCl I digestion, respectively. A probe corresponding to nucleotides 760–964 of the rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) coding sequence was cloned by PCR with primers 5’-CATCAAGAAGGTGGTGAAGC-3 (forward) and 5’-ACCCTGTGGCTGTAGCCATA-3 (reverse) into PCRII and excised with EcoRI and XhoI.

Table 1. Effect of UF-5 on plasma triglyceride levels in the fat-fed hamster

<table>
<thead>
<tr>
<th>Study</th>
<th>Body Weight, g</th>
<th>Liver Weight, g</th>
<th>Soleus Muscle Weight, g</th>
<th>Plasma TG, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>135 ± 5</td>
<td>137 ± 3</td>
<td>137 ± 6</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>135 ± 5</td>
<td>141 ± 3</td>
<td>143 ± 4</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>139 ± 5</td>
<td>137 ± 3</td>
<td>134 ± 4</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>7.3 ± 0.3</td>
<td>15.8 ± 0.5</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>UF-5 on days</td>
<td>0</td>
<td>137 ± 3</td>
<td>137 ± 3</td>
<td>137 ± 6</td>
</tr>
<tr>
<td></td>
<td>135 ± 5</td>
<td>196 ± 17</td>
<td>196 ± 22</td>
<td>196 ± 22</td>
</tr>
<tr>
<td></td>
<td>135 ± 5</td>
<td>152 ± 23</td>
<td>152 ± 23</td>
<td>152 ± 23</td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td>7.7 ± 0.5</td>
<td>8.2 ± 0.3</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>131 ± 6</td>
<td>197 ± 10*</td>
<td>197 ± 10*</td>
<td>197 ± 10*</td>
</tr>
<tr>
<td></td>
<td>129 ± 8</td>
<td>214 ± 29*</td>
<td>214 ± 29*</td>
<td>214 ± 29*</td>
</tr>
</tbody>
</table>

Hamsters were treated with rising doses of ureido-fibrate-5 (UF-5) twice daily or vehicle, as described in METHODS. UF-5 was administered by gavage at 7.5 mg/kg on days 1–7, 15 mg/kg on days 8–14, and 30 mg/kg on days 15–21. Blood was obtained, and triglycerides were measured as described in METHODS after 1 wk of treatment with each dose. After the 3rd dose (30 mg/kg), hamsters were not fasted before blood sampling. Values are means ± SE; n = 7 animals/group. For the 3-day study, plasma triglyceride levels were measured in 3 groups of nonfasted hamsters treated with vehicle or 15 or 30 mg/kg UF-5 twice daily for 3 days. Values are means ± SE; n = 5 hamsters/group. Terminal (3-day) values only are shown. TG, triglycerides. *P < 0.05 vs. vehicle.
sham, Buckinghamshire, England). Blots were hybridized with probes (as indicated in Figs. 1–5) with ExpressHyb (Clontech, Palo Alto, CA) and washed according to the manufacturer’s protocol. After exposure of membranes to X-ray film, signals were quantitated by densitometry (Personal Densitometer SI, Molecular Dynamic, Sunnyvale, CA). Blots were stripped for reprobing by boiling 2 × 10 min in 0.5% SDS.

**Western blotting.** Cell proteins were extracted from 100-mm dishes with PBS-1% Triton X-100–5 mg/ml NaEDTA-1 mM phenylmethylsulfonyl fluoride. Extracts were sonicated and centrifuged at 20,000 g for 15 min at 4°C. Proteins were resolved by electrophoresis on 10% SDS-PAGE gels, transferred onto nitrocellulose membranes, and probed with rabbit polyclonal antibodies against human PPARα (6) or PPARγ (kind gift of Bart Staels) by use of the Western Breeze (Novex, San Diego, CA) hybridization/detection system. pcDNA3.1-hPPARα and -γ positive controls were transcribed and translated in vitro with the Promega (Madison, WI) system as per manufacturer’s instructions. Protein was

---

**Fig. 1.** Effect of peroxisome proliferator-activated receptor-α (PPARα) agonist treatment on gene expression in liver and muscle in fat-fed hamsters. Liver (A) and soleus muscle (B) total RNA (15 μg) from hamsters treated with rising doses of ureido-fibrate-5 (UF-5) and vehicle (Table 1) was subjected to Northern blotting, as described in METHODS. Blots were probed with rat apolipoprotein C-III (apoC-III, A) and human liver carnitine palmitoyltransferase I (CPT I) and human muscle CPT I (B) and then stripped and probed with rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH). A: lanes 1–7, vehicle; lines 8–14, UF-5. B: lanes 1–5 and 6–12 are from vehicle- and UF-5-treated hamsters, respectively.
quantitated by the Bradford method with the Bio-Rad (Hercules, CA) reagent according to manufacturer's instructions, with BSA as standard.

**Cellular FA β-oxidation assay.** The rate of cellular β-oxidation of [9,10(n)-3H]palmitic acid (52 Ci/mmol, Amersham) was measured as 3H2O release, as previously described (20). For cell incubations, [3H]palmitic acid was used at a final concentration of 22 μM in Hanks' balanced salt solution-0.5% free FA-free BSA (Sigma) by dilution with unlabeled palmitic acid (Sigma). Cell DNA content was quantitated with pico Green double-strand DNA quantitation reagent (Molecular Probes, Eugene, OR) according to the manufacturer's instructions. Linearity of palmitate β-oxidation with time and cell number was established by plating increasing numbers of HepG2 cells and measuring cellular FA β-oxidation after 48 h of growth. After 1- or 2-h incubation with [3H]palmitate,

Fig. 2. Effect of short-term UF-5 treatment on liver and muscle gene expression in hamsters. A: hepatic apoC-III and CPT I mRNA levels. B: soleus muscle CPT I mRNA levels. A and B: lanes 1–5, vehicle; lanes 6–10, UF-5 15 mg/kg; lanes 11–15, UF-5, 30 mg/kg.
cell supernatants were assayed for palmitate β-oxidation product. On the basis of these data, cells were plated at $1.2 \times 10^5$ cells/well, and a 2-h incubation with substrate was used for experiments. UF-5 was prepared as 100 mM stock in DMSO and added once every 24 h. In some experiments, 40 μM etomoxir (2) was included either as a 24-h preincubation or during the latter 24 h to inhibit CPT I.

Statistics. Data are presented as means ± SE. Means were compared with Student’s t-test. In Figs. 1–5 and Tables 1 and 2, a P value of < 0.05 was considered significant.

RESULTS

Effects of UF-5 on FA and TG metabolism in fat-fed hamsters. Recent reports (4, 12) demonstrated that UF-5 is a potent PPARα activator with an EC50 value of 400 and 30 nM for human and mouse PPARα, respectively, in cell-based transactivation assays.

Administration of UF-5 at 15 or 30 mg/kg twice daily for 1 wk to hamsters fed a high-fat diet resulted in ~50 and 70% reduction in fasting and nonfasting plasma TG levels, respectively (Table 1). UF-5 concentrations well in excess of EC100 in plasma (2,639 ± 502 ng/ml, n = 7) and liver (39.9 ± 5.3 μg/g) were achieved. Hepatic apoC-III mRNA levels in hamsters treated for 3 wk with rising doses of UF-5 were reduced by 63% when corrected for GAPDH compared with vehicle-treated hamsters (Fig. 1A). To assess whether UF-5 treatment affects expression of mitochondrial FA β-oxidation genes, blots of total RNA from liver and soleus muscle were hybridized with tissue-specific CPT I probes. These yielded bands of ~5 and 3 kb, respectively, as reported (34). UF-5 treatment significantly increased hamster hepatic CPT I expression by 1.4-fold compared with vehicle (Fig. 1B). Muscle CPT I was upregulated 2.3-fold in UF-5- compared with vehicle-treated hamsters (Fig. 1B).

Effects of treatment duration on UF-5-induced lipid metabolism changes. To see how the treatment duration affects TG lowering and gene expression, fat-fed hamsters were treated for 3 days with UF-5 or vehicle. TG lowering with 30 mg/kg treatment of the nonfasted hamster was 37% after 3 days, or ~50% of the effect of the rising-dose treatment (Table 1). However, suppression of hepatic apoC-III levels was similar to that achieved after the rising-dose treatment (56 vs. 63%; Fig. 2A), and stimulation of hepatic CPT I expression was even greater (160 vs. 37%; Fig. 2A). Muscle-type CPT I to-GAPDH mRNA ratio was unaffected in hamsters treated for 3 days with 30 mg/kg UF-5 (Fig. 2B). To see whether any effect might have been underestimated due to upregulation of GAPDH by UF-5, blots were stripped and reprobed with a murine S10 corresponding to a ribosomal protein. Muscle CPT I/S10 mRNA levels were not significantly different among any of the treatment groups (Fig. 2B).

Effects of UF-5 compared with fenofibric acid on lipid metabolism in hamsters. Next we compared the effects of fenofibric acid (FF), a very weak PPARα activator (4), to UF-5 in vivo. As expected, administration of rising doses of UF-5 to fat-fed hamsters lowered plasma TG concentrations by >50%, whereas FF at the doses tested did not (Table 2). UF-5, but not FF treatment, also lowered plasma LDL cholesterol (Table 2), consistent with putative inhibitory effects on VLDL secretion. Hepatic apoC-III mRNA levels in hamsters treated for 3 wk with rising doses of UF-5 or FF were reduced by ~50% when corrected for GAPDH, compared with vehicle-treated hamsters (Fig. 3A). Soleus muscle CPT I mRNA levels were increased approximately twofold in UF-5-treated hamsters but were not affected in FF-treated animals (Fig. 3B).

To investigate whether the TG lowering and weight loss effects of UF-5 were related to anorectic effects, food consumption was measured in this study. In fact, food consumption increased significantly in UF-5-treated hamsters after 2 and 3 wk of treatment (Table 2).

Table 2. Comparison of UF-5 and fenofibric acid effects on lipid metabolism in hamsters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight, g</th>
<th>Liver Weight, g</th>
<th>Plasma Glucose, mg/dl</th>
<th>Plasma TG, mg/dl</th>
<th>Food Consumption, g</th>
<th>Plasma TC, mg/dl</th>
<th>LDL-C</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>156 ± 2.5</td>
<td>80 ± 2.8</td>
<td>81 ± 14</td>
<td>8.3 ± 0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>158 ± 3.8</td>
<td>95 ± 3.9</td>
<td>88 ± 24</td>
<td>10.1 ± 0.4*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>160 ± 3.2</td>
<td>101 ± 6.3</td>
<td>78 ± 22</td>
<td>7.8 ± 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>154 ± 3.8</td>
<td>4.5 ± 0.3</td>
<td>93 ± 27</td>
<td>8.2 ± 0.4</td>
<td>126 ± 7</td>
<td>24 ± 4</td>
<td>88 ± 3</td>
<td></td>
</tr>
<tr>
<td>UF-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>146 ± 3.9†</td>
<td>84 ± 3.0</td>
<td>83 ± 4</td>
<td>8.1 ± 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>136 ± 4.9</td>
<td>89 ± 4.4</td>
<td>52 ± 10†</td>
<td>7.5 ± 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>136 ± 4.7</td>
<td>108 ± 1.3</td>
<td>25 ± 7†</td>
<td>10.9 ± 1.1*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>125 ± 5.88</td>
<td>13.8 ± 0.5</td>
<td>43 ± 3†</td>
<td>10.9 ± 1.4*</td>
<td>89 ± 7</td>
<td>9 ± 2†</td>
<td>74 ± 4</td>
<td></td>
</tr>
<tr>
<td>Fenofibrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>155 ± 5.4†</td>
<td>75 ± 2.3</td>
<td>85 ± 7</td>
<td>8.9 ± 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>138 ± 5.3</td>
<td>87 ± 4.4</td>
<td>110 ± 11†</td>
<td>9.2 ± 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>135 ± 4.9</td>
<td>109 ± 3.9</td>
<td>87 ± 17</td>
<td>6.1 ± 0.5*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>122 ± 5.48</td>
<td>7.7 ± 0.4</td>
<td>111 ± 5.2</td>
<td>6.9 ± 0.9*</td>
<td>117 ± 29</td>
<td>29 ± 19</td>
<td>85 ± 9</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 hamsters/group. Vehicle, UF-5 (7.5, 15, and 30 mg/kg), and fenofibric acid (fenofibrate; 30, 60, and 120 mg/kg) were administered to fat-fed hamsters in a rising-dose protocol as described in METHODS and Table 1. All blood samples were taken after a 17-h fast. For each dose, food consumption represents the mean of measurements after 4 and 10 doses. TC, plasma total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. *P < 0.05 vs. baseline; †P < 0.05 vs. vehicle.
UF-5 stimulates mitochondrial FA-$eta$-oxidation in vitro. These results suggested that, in addition to previously described effects of fibrates on apoC-III expression and on peroxisomal FA $\beta$-oxidation, PPAR$\alpha$ agonists might also exert TG-lowering effects through stimulation of mitochondrial FA $\beta$-oxidation. To further explore the ability of PPAR$\alpha$ agonists to stimulate FA $\beta$-oxidation in vivo, we studied cell types representative of tissues carrying out high rates of FA $\beta$-oxidation, namely HepG2 and hSKMC. To assess the suitability of the cell types for these experiments, the presence of PPAR$\alpha$ mRNA and protein was first established. mRNA for hPPAR$\alpha$ was in greater abundance in human skeletal muscle tissue than in liver (Fig. 4A). HepG2 and hSKMC expressed PPAR$\alpha$ mRNA (Fig. 4A) and also contained immunoreactive PPAR$\alpha$ protein (Fig. 4B) in hSKMC until 7 passages.

$[^{3}H]$palmitate $\beta$-oxidation was linear between 1 and 2 h and over $3.0 \times 10^4$-$2.5 \times 10^5$ HepG2 cells/well under the experimental conditions tested. UF-5 stim-
regulated FA β-oxidation in HepG2 and in human primary skeletal muscle cells (Fig. 5, left) in a dose-dependent manner. Maximal stimulation was ~2.7- and 1.6-fold in HepG2 and hSKMC, respectively. Stimulation by 200 μM FF was 9% (n = 4, P < 0.06) over DMSO in HepG2 cells. To assess the extent to which stimulation of FA β-oxidation by UF-5 was due to an increase in mitochondrial vs. peroxisomal β-oxidation, etomoxir, a CPT I inhibitor, was used. Etomoxir almost completely inhibited both basal and UF-5-stimulated palmitate β-oxidation (Fig. 5, right), indicating that PPARα agonist-stimulated FA β-oxidation in HepG2 cells is mainly mitochondrial.

**DISCUSSION**

It is widely believed that the lipid-lowering effects of fibrates are mediated through PPARα (22). Fibrates have been shown retrospective of their clinical efficacy to be very weak PPARα agonists, with unmeasurable binding affinities to PPARα and with EC_{50} values in cell-based reporter assays in the tens of micromolar range (4). It has been hypothesized that more potent, directed PPARα agonists would exert more powerful TG-lowering effects (27). The data reported herein support the hypothesis with a potent bona fide PPARα agonist, UF-5 (4), in a fat-fed hamster model of non-diabetic hypertriglyceridemia. Greater than 50% plasma TG lowering with UF-5 was seen at doses an order of magnitude lower than those typically necessary for clinically used fibrates. These effects are likely to be mediated through PPARα, given that selectivity for murine PPAR isoform activation of UF-5 is 26-fold for PPARα over PPARγ (4).

A further objective of this study was to explore the potential mechanism for TG lowering of PPARα agonists. Consistent with the effects of fenofibrate in rats (28), we observed a sharp reduction in hepatic apoC-III expression in fat-fed hamsters. In addition to effects on hepatic apolipoprotein expression, we show here that PPARα agonist treatment influences CPT I expression. Two CPT I isoforms, liver (3, 7) and muscle (33, 34), have been cloned and characterized. Previous molecular evidence has implicated PPARα in regulation of the muscle isoform. The promoter of human muscle-type
CPT I is stimulated by FAs, a response mediated by a PPRE to which PPARα binds (19, 35). Olate increases CPT I expression in cardiac myocytes (2). More limited information also implicates PPAR in regulation of the liver isoform; clofibrate and FAs increase CPT I mRNA expression in fetal rat hepatocytes (5). In this study, UF-5 treatment of fat-fed hamsters upregulated both hepatic and muscle CPT I.

PPARα is also implicated in regulation of other genes involved in mitochondrial FA β-oxidation. The promoters of medium-chain acyl-CoA dehydrogenase and rat mitochondrial HMG-CoA synthase are PPAR responsive (10, 24). Expression of acyl-CoA synthase (18, 25) and fatty acid transfer protein (8, 18) is induced by fibrates in rats. PPAR responsiveness of these genes in vivo in mice has also been modeled with etomoxir, which inhibits CPT I and is presumed to activate PPARα indirectly by causing accumulation of cellular FA (2, 10). Further evidence for the importance of PPARα regulation of some of these genes was provided by the absence of their regulation in PPARα knockout mice (2). These studies, in combination with the CPT I upregulation by UF-5 in vivo, suggest that stimulation of mitochondrial FA β-oxidation represents an additional TG-lowering mechanism of PPARα agonists. Consistent with this, UF-5 markedly stimulated palmitate β-oxidation in human hepatic and skeletal muscle cells in the present study. Although UF-5 is a potent activator of human PPARγ (4), the effects of UF-5 are not likely due to PPARγ activation, because PPARγ immunoreactive protein was undetectable in these cells. The reason for the high (micromolar-range) concentrations necessary to achieve this relative to PPARα activation potency (~50 nM for human PPARα) is not clear. However, a similar dissociation between apparent activation potency and functional effects in untransfected cells has been observed for fenofibrate effects on gene regulation (28, 30).

In this study, hamster soleus muscle CPT I mRNA levels were increased 2.3-fold in hamsters treated for 3 wk with rising doses of up to 30 mg/kg UF-5, but they were unaffected in animals treated with 30 mg/kg UF-5 for only 3 days. This result suggests that, although reduction of apoC-III and increase of CPT I expression in liver may contribute to short-term TG lowering in hamsters, stimulation of muscle FA β-oxidation does not. However, the data suggest that stimulation of muscle FA β-oxidation contributes to the greater TG-lowering effect of 30 mg/kg UF-5 after longer-term administration. Furthermore, fenofibrin acid administration at the doses tested lowered hepatic apoC-III to a similar extent as did efficacious doses of UF-5 but did not affect muscle CPT I gene expression and had no TG-lowering efficacy. These results, by dissociating TG lowering from suppression of hepatic apoC-III expression, provide further support for the importance of muscle CPT I upregulation in TG lowering in fat-fed hamsters. Increased FA β-oxidation did not decrease glucose utilization in muscle as suggested by the lack of effect of UF-5 on plasma glucose concentrations. In fact, the decrease in body weight seen in UF-5-treated hamsters suggests that PPARα activation increased energy expenditure in this animal model.

The relative contribution of FA β-oxidation and apoC-III regulation in TG lowering by PPARα agonists may have implications for lipoprotein and lipid metabolism. Reduction of apoC-III synthesis, by stimulating lipoprotein lipase activity, might be expected to increase the VLDL-TG fractional catabolic rate (1), thereby increasing LDL production and raising LDL levels. In contrast, stimulation of FA β-oxidation would be expected to reduce VLDL-TG production and therefore to lower LDL levels. In fact, UF-5 treatment significantly lowered LDL cholesterol by over 50%, consistent with the importance of the latter mechanism for TG lowering.

An additional effect of UF-5 in hamsters was a large increase in liver weight (Tables 1 and 2). This increase probably does not reflect fatty liver as judged by visual observation. Rather, hepatic hypertrophy undoubtedly results from peroxisome proliferation (as measured by increased cyanide-insensitive palmitoyl-CoA oxidation and by an increased immunoreactive bifunctional enzyme, not shown). In any case, the observed hepatic hypertrophy raises the possibility that the TG-lowering effect of UF-5 occurs at least partially through increased FA utilization in liver for cell membrane lipid synthesis. It is also possible that the increased hepatic CPT I expression reflects the need for an increase in cellular ATP production associated with liver hypertrophy. However, the following observations argue against these mechanisms: 1) UF-5 stimulated FA β-oxidation directly in liver cells with no associated cellular proliferation or hypertrophy; 2) the TG-lowering effects of less potent, clinically administered PPARα agonists such as fenofibrate are dissociated from liver hypertrophy in humans; and 3) this possibility does not apply to UF-5-induced increase in muscle FA β-oxidation, because UF-5 did not cause muscle hypertrophy (as judged by soleus muscle weights).

In summary, the present study supports the hypothesis that a potent PPARα agonist exerts marked TG-lowering effects. Stimulation of mitochondrial FA β-oxidation in liver and muscle appears to contribute to maximal hypolipidemic effects of PPARα activation. These results should be considered in the design and monitoring of similar pharmacological agents.

We thank Drs. Linda Merkel and Mark Perrone for helpful discussions; Colleen Charsky, Charles Hanning, and Tambra Peters for excellent technical assistance; and Elizabeth O’Connor, Kevin Darlington, and Henry Sarau for assistance with animal studies. We thank Dr. Ken Page for analysis of UF-5 levels and Dr. Zaid Jayyosi and Margaret Muc for measurements of liver peroxisomal enzymes. We are grateful to Drs. Robert Groneberg for chemical syntheses and Hong Zhu for providing the rat GAPDH probe. We acknowledge MSD Panlabs (Bothell, WA) for plasma HDL and LDL cholesterol determinations.

Present addresses: Anne Minnich and Nian Tian, Division of Respiratory Disease and Rheumatoid Arthritis, Aventis Pharmaceuticals, Bridgewater, NJ 08807; Lisa Byan, Guilford Pharmaceuticals, Baltimore, MD 21205; Glenda Bilder, Valleybrooke Corporate Center, Malvern, PA 19355.
REFERENCES


