Clofibrate Increases Hepatic Triiodothyronine (T₃)- and Thyroxine (T₄)-Glucuronosyltransferase Activities and Lowers Plasma T₃ and T₄ Concentrations in Pigs

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ABSTRACT:

In rats, clofibrate acts as a microsomal enzyme inducer and disrupts the metabolism of thyroid hormones by increasing hepatic glucuronidation of thyroxine. Whether similar effects occur in the pig has not yet been investigated. This study was performed to investigate the effect of clofibrate treatment on metabolism of thyroid hormones in pigs. To this end, an experiment with 18 pigs, which were assigned to two groups, was performed. One group received a control diet, and the other group was fed the same diet supplemented with 5 g of clofibrate/kg for 28 days. Pigs treated with clofibrate had higher hepatic activities of T₃- and T₄-UDP glucuronosyltransferases (UGT) and lower concentrations of total and free T₄ and total T₃ in plasma than control pigs (P < 0.05). Weights and histology of the thyroid gland (epithelial height, follicle lumen diameter) did not differ between the two groups, but pigs treated with clofibrate had higher mRNA concentrations of various genes in the thyroid responsive to thyroid-stimulating hormone (TSH) such as TSH receptor, sodium iodine symporter, thyroid peroxidase, and cathepsin B than control pigs (P < 0.05). Pigs treated with clofibrate also had lower hepatic mRNA concentrations of proteins involved in plasma thyroid hormone transport [thyroxine-binding globulin (P < 0.10), transthyretin (P < 0.05), and albumin (P < 0.05)] and thyroid hormone receptor α₁ (P < 0.05) than control pigs. In conclusion, this study shows that clofibrate treatment induces a strong activation of T₃ and T₄-UGT in pigs, leading to increased glucuronidation and markedly reduced plasma concentrations of these hormones, accompanied by a moderate stimulation of thyroid function.

Fibrates are synthetic agonists of peroxisome proliferator-activated receptor-α (PPARα), a nuclear receptor also activated by natural ligands like free fatty acids or some eicosanoids. Activation of PPARα leads to up-regulation of transcription of several genes involved mainly in mitochondrial and peroxisomal β-oxidation, ketogenesis, and glucogeneosis (Mandard et al., 2004). Fibrates have been in clinical use as hypolipidemic agents for several decades. Several studies in rodents and cell culture systems have shown that fibrates, like many other drugs (e.g., phenobarbital, 3-methylcholanthrene, polychlorinated biphenyl, tetrachlorobiphenyl, pregnenolone-16α-carbonitrile, or dexamethasone), induce UDP glucuronosyltransferases (UGT) (Beestra et al., 1991; Saito et al., 1991; Barter and Klaassen, 1992a, 1994; Visser et al., 1993a,b; Jennitz et al., 2000; Viollon-Abadie et al., 2000; Vansell and Klaassen, 2002). UGT, consisting of UGT1 and UGT2 isoforms, are localized in the endoplasmatic reticulum of hepatocytes and extrahepatic tissue and belong to the enzymes of phase II metabolism. With broad and overlapping substrate specificities, the UGT isoenzymes catalyze the glucuronidation of differential functional groups, using UDP-glucuronic acid as the cofactor (Miners and Mackenzie, 1991; Mackenzie et al., 1997). Thyroid hormones thyroxine (T₄) and triiodothyronine (T₃) are substrates of hepatic UGT, and glucuronidation of these hormones is the main metabolic pathway for deactivateing them (Jennitz et al., 2000).

In rats, several of the drugs acting as inducers of microsomal enzymes have been shown to produce hypertrophy and hyperplasia of thyroid follicular cells, most probably through increased deactivation of thyroid hormones by UGT, leading to a reduction of serum T₄ and possibly T₃ (Beestra et al., 1991; Saito et al., 1991; Barter and Klaassen 1992a, 1994). In mice, in contrast to rats, clofibrate treatment did not alter T₃- and T₄-UGT activities and plasma concentrations of thyroid hormones (Viollon-Abadie et al., 1999). These studies show species-specific differences in the effects of clofibrate on hepatic thyroid hormone metabolism (i.e., glucuronidation of thyroid hormones).

In rodents, PPARα agonists not only induce many genes involved in various metabolic pathways such as β-oxidation, ketogenesis, and glucogeneosis but also cause severe peroxisome proliferation in the liver, hepatomegaly, and hepatocarcinogenesis (Peters et al., 2005). In contrast to rodents, PPARα agonists do not induce peroxisome proliferation or tumor in the liver of many other species, such as guinea pigs, swine, monkeys, and humans, although they retain a hypotriglyceridemic effect in these species (Holden and Tugwood, 1999). In nonproliferating species, expression of PPARα in the liver is much lower, and the response of many genes to PPARα activation is weaker than in proliferating species (Cheon et al., 2005). It is known that
PPARα activation can modulate metabolizing enzymes of phase I and II biotransformation (Rushmore and Kong, 2002; Zhou et al., 2005). Moreover, it has been shown that some UGT isoforms (UGT1A9, UGT2B4) are PPARα target genes (Barbier et al., 2003a,b). Therefore, nonproliferating species could respond differently from proliferating species to clofibrate with respect to induction of UGT (i.e., UGT involved in glucuronidation of thyroid hormones). To our knowledge, the effect of clofibrate on the hepatic thyroid hormone metabolism has not yet been investigated in vivo in a nonproliferating species.

The aim of our study was to investigate the effects of clofibrate treatment on hepatic thyroid hormone metabolism (i.e., activities of T3- and T4-UGT in pigs, representing a nonproliferating species). Therefore, as well as determining hepatic activities of T3- and T4-UGT, we also measured plasma concentrations of thyroid hormones, thyroid weights, thyroid epithelial cell height, and follicle lumen diameter and gene expression levels of several thyroidal genes involved in thyroid hormone biosynthesis [thyroid-stimulating hormone (TSH) receptor, sodium iodide symporter, thyroid peroxidase, dual oxidase 2, thyroglobulin, cathepsin B, and type II iodothyronine deiodinase]. We also investigated the effect of clofibrate on mRNA expression of genes involved in thyroid hormone transport (transferrin, thyroxine-binding globulin, and albumin), peripheral conversion of thyroid hormones (Type I iodothyronine deiodinase), and thyroid hormone signaling (thyroid hormone receptor α1) in the liver.

Materials and Methods

Chemicals. Bilirubin, Brij 56, clofibrate, dibuthioleit, p-nitrophenol (pNP), 6-propyl-2-thiouracil, cholic acid, T3, T4, Triton X-100, and UDP-glucuronic acid (UDPGA) were obtained from Sigma (Deisenhofen, Germany). 125I-T3 (3076 pCi/μg) and 125I-T4 (1500 μCi/μg) were obtained from Amersham Biosciences (Freiburg, Germany); and bicinchoninic acid protein assay reagent was from Interchim (Montelucon, France).

Animals and Treatments. Eighteen male 8-week-old crossbred [(German Landrace X Large White) X Pietrain] pigs, bred in the local animal facility, were used. They weighed between 11.0 and 13.5 kg. They were individually housed in a room maintained at 23°C and 50 to 60% relative humidity with light from 6:00 AM to 6:00 PM. On the day before the start of the experimental feeding period, all the pigs were weighed and assigned to two groups with light from 6:00 AM to 6:00 PM. On the day before the start of the experimental feeding period, all the pigs were weighed and assigned to two groups with 1.5 MJ metabolizable energy and 700 g of crude protein/kg. The diet of the treatment group was supplemented with 5 g of clofibrate/kg. To standardize feed intake, each pig within the experiment received 700 g of the diet daily, which was completely consumed by all the animals in the experiment. The clofibrate dosage in the treated pigs was 220 mg/kg b.wt./day. The pigs had free access to water via nipple drinking systems. The experimental diets were administered for 28 days. All the experimental procedures described followed established guidelines for the care and use of laboratory animals and were approved by the local veterinary office.

Sample Collection. After completion of the feeding period, the animals were killed under a light anesthesia. Blood was collected into heparinized polyethylene tubes. Liver and thyroid gland were dissected and weighed. Plasma was obtained by centrifugation of the blood (1100g; 10 min). All the samples were stored at −80°C pending analysis.

Total RNA Preparation and cDNA Synthesis. Total RNA from liver and thyroid tissue was isolated by TRIzol reagent (Invitrogen, Karlsruhe, Germany) following the manufacturer’s protocol, resuspended in diethyl pyrocarbonate-treated water, and stored at −80°C until use. The concentration of total RNA was determined by ultraviolet absorbance at 260 nm. The quality of all the RNA samples was assessed by agarose gel electrophoresis. cDNA was prepared from total RNA (1.2 μg) by reverse transcription using M-MuLV reverse transcriptase (MBI Fermentas, St. Leon-Rot, Germany) and oligo(dT)12 primers (Operon, Cologne, Germany).

Semiquantitative Polymerase Chain Reaction. Expression analysis for semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was normalized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal standard. cDNA templates (2 μl) were used in a final volume of 20 μl containing 0.2 μM concentration of the corresponding primers (Roche, Karlsruhe, Germany) (see Table 1), 1.5 mM magnesium chloride, 1X PCR buffer, 1 U Taq polymerase (Gene Craft, Luedinghausen, Germany), and 0.2 mM deoxyribonucleoside triphosphates (Roche). Each PCR cycle comprised denaturation for 30 s at 94°C, annealing for 30 s at 60°C (see Table 1), and elongation for 1 min at 72°C, followed by a final extension period for 10 min at 72°C. Number of cycles for each primer pair was tested previously. Cycle number was as follows: genes of thyroid gland: GAPDH, 23; sodium iodide symporter, 25; dual oxidase 2, 42; cathepsin B, 35; TSH receptor, 28; Type II iodothyronine deiodinase, 40; and thyroglobulin, 33; hepatic genes: GAPDH, 32; acyl CoA oxidase (ACO), 32; carnitine palmitoyltransferase 1 (CPT-1), 32; albumin, 20; thyroid hormone receptor α1, 35; thyroxine-binding globulin, 42; transferrin, 30; and type I iodothyronine deiodinase, 40. A water control was included in all the PCRs for detection of contamination, and dilutions of the isolated total RNA corresponding to the cDNA synthesis were used as template to exclude impurities caused by genomic DNA. A volume of 10 μl per PCR was submitted to agarose gel electrophoresis (1.5%). Ethidium bromide-stained gels were digitized for quantification (apparatus and software from Syngene, Cambridge, UK).

Preparation of Hepatic Micromeres.One gram of liver was homogenized in a medium (10 ml) containing 0.25 M sucrose and 0.1 M phosphate buffer...
(pH 7.4) using a Potter-Elvehjem homogenizer. Homogenates were centrifuged at 1000 g for 10 min at 4 °C, and the supernatant was centrifuged at 15,000 g for 15 min. The microsomal pellet was obtained by centrifugation of the 15,000 g supernatant at 105,000 g for 60 min. Microsomal pellets were suspended in the homogenization medium, and the protein concentration was determined with the bicinchoninic acid reagent according to the supplier’s protocol using bovine serum albumin as standard.

Enzyme Assays. The activity of pNPG-UGT in hepatic microsomes was assayed by the method of Thurman et al. (1981). The assay mixture consisted of 50 mM potassium phosphate buffer (pH 7.4), 0.2 mg of bovine serum albumin/ml, 1 mM magnesium chloride, 0.5 mM dithiothreitol, 0.5 mg of Triton X-100/ml, 0.2 mM pNP, 0.2 mM UDPGA, and 0.25 mg of microsomal protein/ml. The final volume of the assay was 200 µl. A blank was incubated without UDPGA. The reaction was stopped after 30 min at 37°C by addition of 1 ml of 0.1 M sodium hydroxide solution. The pNP glucuronidation was quantified by measuring the decrease of absorbance at 400 nm. The concentration of pNP glucuronidation was calculated using a molar extinction coefficient of 18,300/cm.

Activity of bilirubin-UGT in hepatic microsomes was measured in an assay mixture containing 0.1 M Tris-hydrochloride (pH 7.8), 0.1 mM bilirubin, and 5 mM UDPGA in a final volume of 200 µl. Suspended microsomes were preincubated with 20 mg/ml sodium cholate (1:1, v/v) for 10 min at 4°C. The reaction was started by addition of 1 mg of microsomal protein/ml for 60 min at 37°C. The amount of bilirubin glucuronide formed during the incubation was quantified with a commercial kit (DiaSys Diagnostic Systems, Holzheim, Germany).

T3- and T4-UGT activities were determined in separate assays using a modified version of the method of Beetstra et al. (1991) by incubating 1 µM T3 or T4, respectively, and 0.1 µCi of 14C-labeled T3 or T4 in reaction mixture containing 75 mM Tris-hydrochloride (pH 7.8), 7.5 mM magnesium chloride, 0.25 mg of Brij 56/ml, 5 mM UDPGA, and 1 mM 6-propyl-2-thiouracil. The reaction was started by addition of 1 mg of microsomal protein/ml at 37°C. Blanks were performed in the absence of UDPGA. After 30 min, reactions were terminated by addition of 200 µl of ice-cold methanol, and the mixtures were centrifuged at 3500 g for 8 min. Fifty microliters of the supernatants was injected into a high-performance liquid chromatograph for separation of T3 or T4 glucuronides formed during the incubation with a modified version of the method of Jennitz et al. (2000). The high-performance liquid chromatography equipment consisted of a 1100 series pump (isocratic), an autosampler, a LiChrospher 100 RP 18e column (125 × 4 mm, 5-µm particle size) with matching guard column (4 × 4 mm; Agilent Technologies, Waldbronn, Germany). The mobile phase consisted of 50 mM potassium dihydrogen phosphate and methanol (43:57, v/v, pH 7.0). For separation of T3-glucuronide, the flow rate was 0.8 ml/min. For separation of T4-glucuronide, the flow rate was 1.25 ml/min. Fractions containing T3- or T4-glucuronide, respectively, were collected with a fraction collector 203 (Gilson International, Bad Camberg, Germany). The radioactivity of the fractions was counted to calculate T3- and T4-UGT activities.

Histology of Thyroid Gland. Samples of thyroid glands were fixed by immersion in 10% neutral buffered formalin, processed for embedding into paraffin wax, and cut into 4-µm sections. For light microscopy, the sections were stained with hemalum and eosin. The epithelial cell height was measured using 4 cells per follicle in 100 follicles of each thyroid. The lumen diameter was measured in 10 sections for 10 follicles per section of each thyroid. All the pictures were digitized, and the parameters were measured using the Lucia G (Nikon, Düsseldorf, Germany) software (release 4.81).

Analysis of Plasma Hormones. The plasma concentrations of free and total T4 and total T3 were measured with radioimmunoassay kits (MP Biomedicals, Eschwege, Germany).

Statistics. The results were analyzed using Minitab (State College, PA) statistical software (release 13). Statistical significance of differences between control group and treatment group was evaluated using Student’s t test. Mean values were considered significantly different for P < 0.05.

Results

Initial and final body weights after an experimental period of 28 days were similar in both groups of pigs (Table 2). Animals treated with clofibrate had heavier livers (P < 0.05) and higher concentrations of microsomal protein in the liver (P < 0.05) than control pigs (Table 2). Relative hepatic mRNA concentration of the PPARα target genes ACO and CPT-1 was higher (P < 0.05) in pigs treated with clofibrate than in control pigs (ACO: 1.39 ± 0.27 versus 1.00 ± 0.35; CPT-1: 1.60 ± 0.13 versus 1.00 ± 0.12; mean ± S.D., n = 9 for each group). Moreover, concentrations of total and free T4 and total T3 in plasma were markedly lower in pigs treated with clofibrate than in control pigs (P < 0.05), whereas the T4/T3 ratio did not differ between both groups of pigs (Table 2).

Pigs treated with clofibrate had a higher activity of bilirubin-UGT in the liver than control pigs (1.08 ± 0.05 versus 0.44 ± 0.02 nmol/min/mg; mean ± S.D., n = 9 for each group; P < 0.05). The activity of hepatic pNP-UGT was lower in pigs treated with clofibrate than in control pigs (44 ± 5 versus 70 ± 8 nmol/min/mg; mean ± S.D., n = 9 for each group; P < 0.05). Activities of hepatic T3- and T4-UGT were higher in pigs treated with clofibrate than in control pigs (P < 0.05) (Fig. 1).

Weights of thyroids, diameter of follicle lumen, and thyroid epithelial cell height did not differ between both groups of pigs (Table 3). Relative mRNA concentrations of TSH receptor, sodium iodide symporter, thyroid peroxidase, and cathepsin B were higher in thyroids of

### Table 2

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<thead>
<tr>
<th></th>
<th>Control</th>
<th>Clofibrate</th>
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<tr>
<td>Initial body weight (kg)</td>
<td>12.0 ± 1.1</td>
<td>11.9 ± 0.6</td>
</tr>
<tr>
<td>Final body weight (kg)</td>
<td>26.0 ± 1.5</td>
<td>25.2 ± 1.2</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>673 ± 63</td>
<td>779 ± 63*</td>
</tr>
<tr>
<td>Hepatic microsomal proteins (mg/g liver)</td>
<td>12.4 ± 1.8</td>
<td>15.4 ± 2.4*</td>
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</tbody>
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* P < 0.05 compared with control group.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Clofibrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid weight (g)</td>
<td>2.17 ± 0.19</td>
<td>2.39 ± 0.55</td>
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<tr>
<td>Follicle lumen diameter (µm)</td>
<td>84.9 ± 10.9</td>
<td>88.8 ± 14.2</td>
</tr>
<tr>
<td>Epithelial cell height (µm)</td>
<td>7.53 ± 0.14</td>
<td>7.64 ± 0.67</td>
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Data are reported as mean ± S.D. with nine animals per group.
pigs treated with clofibrate than in thyroids of control pigs (P < 0.05) (Fig. 2). mRNA concentration of type II iodothyronine deiodinase was lower in thyroids of pigs treated with clofibrate than in thyroids of control pigs (P < 0.05); those of dual oxidase 2 and thyroglobulin did not differ between both groups of pigs (Fig. 2).

Pigs treated with clofibrate had lower hepatic mRNA concentrations of transthyretin, albumin, and thyroid hormone receptor α1 than control pigs (P < 0.05) (Table 4). Hepatic mRNA concentration of thyroxine-binding globulin tended to be lower in pigs treated with clofibrate compared with control pigs (P < 0.10), whereas hepatic mRNA concentration of type I iodothyronine deiodinase did not differ between both groups of pigs.

Discussion

To our knowledge, this is the first study to investigate the effect of clofibrate on the hepatic metabolism of thyroid hormones in the pig. It is well known that pigs are a nonproliferating species, meaning that treatment with PPARα agonist causes no or only weak peroxisome proliferation in the liver. Interestingly, in this study clofibrate treatment caused a significant increase in liver weights of pigs, by about 15%. This is in disagreement with a recent study in which pigs did not show significantly increased liver weights after a 1-week treatment with a dose of clofibrate similar to those used in our study (Cheon et al., 2005). The difference in these results could be because of the longer treatment period in our study compared with that in the study of Cheon et al. (2005). Moreover, we observed a moderate up-regulation of the PPARα target genes ACO and CPT-1 in the liver of pigs treated with clofibrate, which indicates that clofibrate treatment caused PPARα activation in these pigs. Nevertheless, increases in liver weights and hepatic ACO and CPT-1 mRNA concentration were much lower than those observed in rodents treated with clofibrate. In rats and mice, feeding PPARα agonists increases liver weights by 50% or more and mRNA concentrations of ACO 5- to 10-fold compared with untreated controls (Kawashima et al., 1990; He et al., 2002; Frederiksen et al., 2004; Li et al., 2004).

Several families of UGT enzymes are expressed in the liver. To study the effect of clofibrate treatment on the induction of microsomal enzymes, we determined the activities of bilirubin- and pNP-UGT. The finding that clofibrate treatment strongly increases bilirubin-UGT is in accordance with studies in rats and mice (Visser et al., 1993a; Viollon-Abadie et al., 1999). The finding that clofibrate reduces the activity of pNP-UGT is also in accordance with a study in which clofibrate significantly reduced the activity of pNP-UGT in the liver of Wistar rats (Visser et al., 1993a). These observations suggest that clofibrate stimulated the microsomal enzyme system in pigs in a similar way as in rats. This study also shows that clofibrate treatment strongly increases the activity of T3 and T4-UGT in the liver, which in turn leads to a dramatic reduction of plasma T3 and T4 concentrations. Increased activity of T3-UGT had previously been observed in Wistar rats but not in mice treated with clofibrate (Visser et al., 1993a; Viollon-Abadie et al., 1999). The increased activity of T3-UGT in pigs treated with clofibrate, however, is in strong contrast to rats, in which clofibrate treatment did not increase T3-UGT activity (Visser et al., 1993a).

In rats, T3 is accepted as a substrate by hepatic bilirubin-UGT (UGT1A1) and phenol-UGT (UGT1A6), and it was shown that increased activities of these enzymes were associated with increased glucuronidation of T4 in the liver (Beestra et al., 1991; Magdalou et al., 1993; Visser et al., 1993a,b; Viollon-Abadie et al., 2000; Vansell and Klaassen, 2002). The enzymes involved in glucuronidation of thyroid hormones in pigs have not yet been identified. The fact that activities of both bilirubin- and T4-UGT were increased suggests that in pig liver T3 was also glucuronidated by bilirubin-UGT, as happens in rats. It is probable that enzymes other than UGT1A1 and UGT1A6 can also be induced by clofibrate in rats and are involved in T4 conjugation (Jennitz et al., 2000). In rat liver, glucuronidation of T3, unlike glucuronidation of T4, is catalyzed by androsterone-UGT (Beestra et al., 1991; Visser et al., 1993b). The increased T3-UGT activity in pigs treated with clofibrate could therefore also have been caused by an increased activity of androsterone-UGT, although this was not assayed in this study. The UGT in pig liver have been less extensively investigated and have not yet been phenotyped. Therefore, it remains unknown which specific UGT were responsive for the increased glucuronidation of T3 and T4 in pigs treated with clofibrate.

It has been shown that activation of PPARα leads to transcriptional up-regulation of the CYP4A genes, which are also constituents of the microsomal biotransformation system in both proliferating and non-proliferating species (Lawrence et al., 2001; Cheon et al., 2005). It has further been shown that some UGT isoforms (UGT1A9, UGT2B4) are PPARα target genes (Barbier et al., 2003a,b). PPARα is naturally activated during fasting, and Visser et al. (1996) showed that food restriction resulted in increased bilirubin and thyroid hormone UGT activities in rats. These findings suggest that UGT catalyzing the glucuronidation of thyroid hormones may be transcriptionally up-regulated by activation of PPARα. It is well known that expression of PPARα in the liver is much lower in nonproliferating species and that the response of many genes to PPARα activation is weaker than in proliferating species. This is also true for up-regulation of microsomal CYP4A genes by treatment with PPARα agonists (Lawrence et al., 2001; Cheon et al., 2005). If PPARα plays a crucial role in the
activation of UGT catalyzing thyroid hormone glucuronidation, the effect of clofibrate on up-regulation of these enzymes in pigs would be expected to be much lower than in proliferating species such as rats or mice. But activation of T₃- and T₄-UGT by clofibrate was even stronger in pigs than reported for rats or mice (Visser et al., 1993a; Viollon-Abadie et al., 1999). These findings suggest that activation of PPARα does not play a key role in clofibrate-induced up-regulation of thyroid hormone UGT. Nevertheless, the role of PPARα in the regulation of thyroid hormone glucuronidation should be further investigated.

The increased activities of T₃ and T₄-UGT make it highly probable that the markedly reduced plasma concentrations of T₃ and T₄ in pigs treated with clofibrate are mainly caused by increased glucuronidation of these hormones in the liver. Because most T₃ is generated in peripheral tissues, mainly the liver, by deiodination of T₄, a reduced T₃ concentration could potentially be caused by an inhibition of type I iodothyronine deiodinase. Indeed, in the study of Visser et al. (1993a), clofibrate treatment of rats reduced the activity of that enzyme, which might be responsible for the reduced concentration of T₃ observed in their study. We did not determine the activity but only the mRNA concentration of that enzyme in the liver, which was not influenced by clofibrate treatment. Interestingly, in contrast to hepatic type I iodothyronine deiodinase, type II deiodinase in the thyroid showed a reduced mRNA concentration in pigs treated with clofibrate compared with control pigs. A reduced activity of type II deiodinase, which converts T₄ to T₃ in the thyroid, may play some role in the reduced T₃ concentration in plasma. However, because the thyroid produces less than 20% of total T₃ (Findlay et al., 2000), a reduced activity of type II deiodinase most probably plays a minor role in the reduced plasma concentration of T₃. The reduction of plasma concentrations of total T₃ (by 47% versus control), free T₃ (by 32% versus control), and total T₄ (by 35%) concentrations by clofibrate are also stronger than those observed in Wistar rats. In Wistar rats, a dose of 800 mg of clofibrate/kg b.wt./day reduced plasma T₃ concentration by 27% but did not reduce plasma total and free T₃ (Visser et al., 1993a). In mice, a dose of 300 mg of clofibrate/kg b.wt./day reduced plasma free T₄ concentration by 13% but did not significantly reduce plasma concentration of free T₃ (Viollon-Abadie et al., 1999). It is clear that different studies cannot be directly compared with each other, but these data nevertheless suggest that pigs could be even more sensitive to disruptions of thyroid hormone metabolism by clofibrate than rodents.

Reduced plasma concentrations of T₃ and T₄ are expected to increase the release of TSH from the pituitary gland. It has indeed been shown that microsomal enzyme inducers elevate TSH plasma concentrations in rodents, which in turn stimulates proliferation of epithelial cells in thyroid tissue as a result of increased glucuronidation of thyroid hormones (e.g., Curran and DeGroot, 1991; De Sandro et al., 1991; Saito et al., 1991; Liu et al., 1995). As no assay was available for measuring TSH concentration in plasma of the pigs, we investigated if the increased plasma concentration of TSH, which in turn stimulates proliferation of epithelial cells in thyroid tissue as a result of increased glucuronidation of thyroid hormones. These alterations were accompanied by moderately increased mRNA concentrations of various TSH-responsive enzymes in the thyroid gland, reduced hepatic mRNA concentrations of proteins involved in thyroid hormone transport, and thyroid hormone receptors. Because the pig represents a species that does not respond with peroxisome proliferation to treatment with PPARα agonists, the study shows that clofibrate treatment also disrupts the metabolism of thyroid hormones in nonproliferating species.

References
Beentjes JB, van Engelen JG, Karel P, van der Heek HJ, de Jong M, Docter R, Krenning EP, Hennemann G, Brouwer A, and Visser TJ (1991) Thyroxine and 3,3′,5-triiodothyronine are reduced by clofibrate treatment. The finding that thyroid weights, epithelial cells, and follicle lumen diameter were not increased by clofibrate was unexpected and suggests that stimulation of the thyroid was moderate, only increasing gene expression of TSH-responsive genes in the thyroid, whereas histological alterations (i.e., increased epithelial cell height) may take longer than 4 weeks to become evident.

The action of thyroid hormones like T₃ is mediated by thyroid hormone receptors that belong to the family of nuclear hormone receptors. The present study shows that clofibrate treatment reduces gene expression of thyroid hormone receptor α₁ in the liver of rats. This finding agrees with a recent study in which bezafibrate down-regulated thyroid hormone receptors in rat liver (Bonilla et al., 2001). That study further showed that down-regulation of thyroid hormone receptors was caused by activation of PPARα. Therefore, it is likely that in our study down-regulation of thyroid hormone receptor α₁ in pigs treated with PPARα was also caused by PPARα activation by clofibrate. Down-regulation of thyroid hormone receptor implies that the biological activity of T₃ may have been reduced in pigs treated with clofibrate.

Thyroxine-binding globulin, transthyretin, and albumin are the major plasma transport proteins in pigs (Janssen et al., 2002). These proteins are synthesized in the liver. We found in our study that gene expression of these proteins in the liver was reduced by clofibrate treatment of pigs. In studies by Motojima et al. (1992, 1997), the same effect of clofibrate on expression of transthyretin was observed in rats and several mouse strains, whereas there was no effect in PPARα-null mice. This suggests that down-regulation of transthyretin expression was induced by PPARα activation. Consequently, down-regulation of transthyretin and possibly also of thyroxine-binding globulin and albumin could be the result of PPARα activation by clofibrate. Our data suggest, although we did not measure concentrations of these proteins in blood, that clofibrate treatment lowers not only concentrations of thyroid hormones in plasma but also could reduce the transport capacity for thyroid hormones.

In conclusion, this study shows for the first time that clofibrate treatment induces a strong activation of T₃- and T₄-UGT in pigs, leading to increased glucuronidation and markedly reduced plasma concentrations of these hormones. These alterations were accompanied by moderately increased mRNA concentrations of various TSH-responsive enzymes in the thyroid gland, reduced hepatic mRNA concentrations of proteins involved in thyroid hormone transport, and thyroid hormone receptors. Because the pig represents a species that does not respond with peroxisome proliferation to treatment with PPARα agonists, the study shows that clofibrate treatment also disrupts the metabolism of thyroid hormones in nonproliferating species.
glucuronidated in rat liver by different uridine diphosphate-glucuronyltransferases. *Endocrinology* **126**:741–750.


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