PPARγ Ligand Troglitazone Lowers Cholesterol Synthesis in HepG2 and Caco-2 Cells *via* a Reduced Concentration of Nuclear SREBP-2

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Cholesterol synthesis in animal cells is regulated by sterol regulatory element-binding protein (SREBP)-2. The objective of this study was to investigate whether activation of peroxisome proliferator-activatedreceptor (PPAR)-γ influences the SREBP-2 dependent cholesterol synthesis in liver and intestinal cells. Therefore, HepG2 and Caco-2 cells were incubated with and without 10 or 30 μ*M* of troglitazone, a synthetic PPARγ agonist, for 4 hrs. Incubation with 10 or 30 μ*M* of troglitazone caused a significant, dose-dependent reduction of cholesterol synthesis in both HepG2 and Caco-2 cells ($P < 0.05$). HepG2 and Caco-2 cells incubated with 10 or 30 μ*M* of troglitazone had also lower mRNA concentrations and lower nuclear protein concentrations of SREBP-2 than untreated control cells ($P < 0.05$). mRNA concentrations of the SREBP-2 target genes HMG-CoA reductase and LDL receptor were also reduced in HepG2 and Caco-2 cells treated with 30 μ*M* of troglitazone compared to control cells ($P < 0.05$). In conclusion, this study shows that PPARγ activation by troglitazone lowers the cholesterol synthesis in HepG2 and Caco-2 cells by reducing the concentration of nuclear SREBP-2 and successive downregulation of its target genes involved in cholesterol synthesis. Exp Biol Med 231:1365–1372, 2006

Key words: sterol regulatory element-binding protein (SREBP)-2; peroxisome proliferator-activated receptor (PPAR)-γ; HepG2; Caco-2; cholesterol synthesis

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Cholesterol is a crucial component of cellular membranes and acts also as a precursor of steroid hormones, bile acids, or vitamin D_3 in some tissues. However, excess amounts of cholesterol can be cytotoxic. Therefore, cholesterol synthesis is normally tightly regulated by a feedback mechanism to maintain the appropriate cholesterol level. Cholesterol synthesis is catalyzed by a group of microsomal enzymes, including HMG-CoA synthase and reductase as the rate-limiting enzymes. Their transcriptional regulation is mainly controlled by sterol regulatory element-binding protein (SREBP)-2. SREBPs belong to a large class of transcription factors containing bHLH-Zip domains (reviewed in 1). After synthesis in membranes of the endoplasmatic reticulum (ER), SREBPs form a complex with SREBP-cleavage activating protein (SCAP). When cells are depleted of sterols, SCAP escorts SREBPs from ER to Golgi. Within the Golgi, two resident proteases, site-1 protease and site-2 protease, sequentially cleave the SREBPs, release the amino-terminal bHLH-Zip-containing domain from the membrane, and allow it to translocate to the nucleus and activate transcription of their target genes. Three isoforms of SREBP have been characterized: SREBP-1a, -1c, and -2. While SREBP-1c, the predominant isoform in adult liver, preferentially activates genes required for fatty acid synthesis, SREBP-2 preferentially activates the LDL receptor gene and various genes required for cholesterol synthesis, such as HMG-CoA reductase (2). SREBP-1a is an activator of both the cholesterol and fatty acid biosynthetic pathways, but it is present in much lower amounts in liver than the other two forms (3). Recently, insulin-induced genes (Insig)-1 and -2 were identified as membrane proteins that reside in the ER and play a central role in the regulation of SREBP cleavage (4, 5). When intracellular sterol concentrations are increased, SCAP binds to Insigs, an action which prevents the translocation of the SREBP-SCAP complex from ER to Golgi and the proteolytic activation of SREBP. As a result, the synthesis of cholesterol and fatty acids declines. Gene expression of Insig-1 is positively controlled by transcrip-

tionally active SREBP, providing a feedback mechanism which regulates lipid homeostasis, while Insig-2 expression is negatively regulated by insulin (5, 6). Recently, it has been shown that mice with disruption of both Insig-1 and Insig-2 genes had a markedly increased expression of genes involved in the synthesis of cholesterol and fatty acids and overaccumulated cholesterol and triglycerides in the liver (7).

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that function as important regulators of cell differentiation and energy homeostasis and act *via* a PPAR response element (PPRE) in the promoter region of target genes. There are several subtypes of PPAR which are expressed in a tissue-specific manner. PPARγ is expressed primarily in adipose tissue and is an adipogenic factor that regulates the expression of genes associated with lipid metabolism (8, 9). PPARγ is also expressed in many other tissues, such as liver or small intestine, albeit at a much lower level than in adipose tissue (10, 11).

Recently, it has been shown that activation of PPARγ by rosiglitazone induces the expression of Insig-1 in white adipose tissue of diabetic rats, which in turn inhibits the processing of SREBPs and lipogenesis. It has been suggested that this is a feedback mechanism to control lipogenesis in adipose tissue (6). In contrast, treatment of macrophages with the PPARγ ligands troglitazone and pioglitazone upregulated the expression of the SREBP2 target genes HMG-CoA synthase and HMG-CoA reductase (12). This finding suggests that PPARγ activation stimulated gene expression or processing of SREBP-2. Thus, the effects of PPARγ activation on the processing of SREBPs and expression of their target genes may be tissue-specific. In the human body, liver and intestine are the major sites of cholesterol biosynthesis (13, 14). Newly synthesized cholesterol in these tissues contributes to the plasma pool of cholesterol. Thus, changes in hepatic and intestinal cholesterol synthesis will directly alter plasma cholesterol concentrations (15). To our knowledge, it has not yet been investigated whether activation of PPARγ influences the activation of SREBP-2 in liver and intestine and the expression of its target genes. In the current study, we investigated the effect of troglitazone, a synthetic PPARγ agonist, on gene expression and nuclear concentrations of SREBP-2 and on expression of its target genes LDL receptor and HMG-CoA reductase. We further examined the effect of troglitazone on the cholesterol synthesis. As models we used HepG2 cells, a human hepatoma cell line, and Caco-2 cells, a human colon carcinoma cell line, which are accepted models for the study of cholesterol metabolism in liver and intestine (16–19). Furthermore, these *in vitro* systems enable us to study the specific effects of troglitazone on the parameters to be studied without the interfering whole-body effects of troglitazone occurring in animals.

Materials and Methods

Chemicals. Cell culture media, supplements and trypsin/EDTA, and Trizol reagent were from Invitrogen (Karlsruhe, Germany). Sterile plastic wares for cell culture were products of Greiner Bio-One (Frickenhausen, Germany). Troglitazone was from Calbiochem (San Diego, CA). DMSO, TEMED, protease inhibitor mix, SYBR Green I, TRIS, KCl, $MgCl_2$, $CaCl_2$, glucose, and Triton X-100 were supplied by Sigma-Aldrich (Deisenhofen, Germany). Acrylamid/bisacrylamid, ammonium persulfate, methanol, n-hexane, diethyl ether, acetic acid, HEPES, NaCl, dNTP solution, and primer oligonucleotides were from Roth (Karlsruhe, Germany), and SDS from Merck-Schuchardt (Hohenbrunn, Germany). Reverse transcriptase was supplied by MBI Fermentas (St. Leon-Rot, Germany), and Taq polymerase by Promega (Mannheim, Germany). The nitrocellulose blotting membrane was from Pall (Pensacola, FL), and the ECL-reagent kit from Amersham-Pharmacia (Freiburg, Germany). The anti-SREBP-2 antibody (rabbit polyclonal IgG) was from Santa Cruz Biotechnology (Santa Cruz, CA), the anti-β-actin antibody (rabbit polyclonal IgG) was from Abcam Ltd. (Cambridge, UK), and the horseradish peroxidase-conjugated anti-rabbit IgG antibody was from Sigma-Aldrich (Deisenhofen, Germany). Radioactive [1,2-^{14}C] acetate (specific activity 108 mCi/mmol) was from Hartmann Analytic (Braunschweig, Germany), and TLC sheets (Si 60 aluminum sheets) were from VWR International (Darmstadt, Germany). Bicinchoninic acid assay reagent was a product of Interchim (Montlucon, France). Autoradiography film for Western blot analysis (Agfa Cronex) was from Roentgen Bender (Baden-Baden, Germany).

Cell Culture. HepG2 (ACC 180) and Caco-2 (ACC 169) cells were obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cells were cultured in 75 cm^2 culture flasks up to a density of 90% confluence. HepG2 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and 0.5% gentamicin (10 mg/mL). Caco-2 cells were grown in minimum essential medium supplemented with 10% fetal bovine serum, 1% MEM nonessential amino acids and 0.5% gentamicin (10 mg/mL). Cells from passages 10–42 for HepG2 and 12–64 for Caco-2 were used for this study. For all experiments, HepG2 and Caco-2 cells were seeded at a density of 105,300 and 195,000 cells/cm^2, respectively, in 6 or 24 well plates and incubated for 2 days until reaching 70%–90% confluence (HepG2) or 7 days for full differentiation (Caco-2). Subsequently, cells were incubated with 10 μ*M* or 30 μ*M* of troglitazone for 4 hrs at 37°C, 5% CO_2 in the incubation buffer (25 m*M* HEPES/Tris, 140 m*M* NaCl, 5.4 m*M* KCl, 1.8 m*M* $CaCl_2$, 0.8 m*M* $MgSO_4$, 5 m*M* glucose, pH 7.5). The concentration of the solvent DMSO in the incubation buffer was maximum 0.5%. The same amount of DMSO was added to control cells (vehicle control). The viability of the cells was checked

Table 1. Characteristics of the Specific Primers Used for RT-PCR Analysis

Gene	Forward and reverse primers	bp	Annealing temperature	NCBI GenBank
GAPDH (EC 1.2.1.12)	5′ GACCACAGTCCATGCCATCAC 3′ 5′ TCCACCACCCTGTTGCTGTAG 3′	247	60°C	NM002046
PPARγ	5′ GCAGGAGCAGAGCAAAGAGGTG 3′ 5′ AAATATTGCCAAGTCGCTGTCATC 3′	352	60°C	BT007281
SREBP-2	5′ CGCCACCTGCCCCTCTCCTTCC 3′ 5′ TGCCCTGCCACCTATCCTCTCACG 3′	390	65°C	BC056158
HMG-CoA reductase (EC 1.1.1.34)	5′ TACCATGTCAGGGGTACGTC 3′ 5′ CAAGCCTAGAGACATAAT 3′	453	60°C	NM000859
LDL receptor	5′ CCCCGCAGATCAACCCCCACTC 3′ 5′ AGACCCCCAGGCAAAGGAAGACGA 3′	369	60°C	BC014514
Insig-1	5′ GACAGTCACCTCGGAGAACCCCAC 3′ 5′ ACCGTGACGCCTCCTGAGAAAAATA 3′	335	60°C	AY112745
Insig-2	5′ ATAAATCATGCCAGTGCTAAAGTG 3′ 5′ TTACATTCGTACATTGCCAGTTG 3′	296	55°C	BC022475

by the MTT assay (20) for a time period of 30 mins (HepG2) or 90 mins (Caco-2) before the end of the incubation.

Analysis of Gene Expression. For analysis of gene expression, total RNA was extracted from cells using Trizol reagent. RNA was quantified by A_260 and its integrity verified by agarose gel electrophoresis using ethidium bromide for visualization. Total RNA and oligo dT were used for cDNA synthesis by reverse transcriptase. The concentration of cDNA was analyzed by real-time detection PCR using SYBR Green I (Rotorgene 2000; Corbett Research, Mortlake, Australia). The PCR reaction mixture contained in a final volume of 2 μL of the first strand cDNA, 26.7 pmol (20 pmol for HMG-CoA reductase and for LDL receptor) of the specific primers, 500 μ*M* dNTP, 2 μL 10 × buffer, 1.25 U Taq DNA polymerase, 3.5 m*M* $MgCl_2$, and 0.5 μl 10 × SYBR Green I. After an initial denaturation step (120 secs, 95°C), PCR was carried out for 40 cycles, each cycle comprising denaturation for 20 secs at 95°C, annealing for 30 secs at primer specific temperature and elongation for 40 secs at 72°C. Fluorescence was measured at 72°C for SREBP-2 and PPARγ, in the other cases at 80°C. A final melting curve guaranteed the authenticity of the target product. The primer oligonucleotides were selected using the Primerselect Software (DNA-Star Inc., Madison, WI) from database sequences. The sequences, product lengths, and annealing temperatures of the primers are shown in Table 1.

Western Immunoblotting Analysis. For determination of nuclear SREBP-2 protein cells were lysed in 150 m*M* NaCl, 20 m*M* Tris, 0.1% Triton X-100, 1% protease inhibitor mix, pH 7.5, after the incubation in 6-well plates. The protein content was determined by the bicinchoninic acid assay. First, 25–50 μg of the cellular protein were separated by electrophoresis on a 10% sodium dodecylsulfate polyacrylamide gel. Proteins on the gel were then transferred to a nitrocellulose membrane by semidry blotting (21). Bands corresponding to nuclear SREBP-2 and β-actin (as loading control) were visualized with enhanced chemiluminescence reagents and exposure to the autoradiography film. Films were analyzed with the Gel-Pro Analyzer software (Intas, Upland, CA).

Determination of Cholesterol Synthesis. After a preincubation of 2 hrs at 37°C, 5% CO_2 with the different concentrations of troglitazone fresh buffer with the same concentrations and 0.05 μCi [1,2-^{14}C] acetate (specific activity 108 mCi/mmol) was added in order to measure the newly synthesized cholesterol (22, 23). Cells were incubated for 2 hrs at 37°C, 5% CO_2. After incubation the cells were washed twice with cold PBS, and the culture plates were stored at –20°C pending analysis. The lipids were extracted twice with a mixture of hexane and isopropanol (3:2, v/v) (24). After removing the solvents in a vacuum centrifugal evaporator, the lipids were dissolved in 100 μl chloroform, 2 μL (Caco-2) or 4 μL (HepG2) of which were applied to 10 × 20 cm^2 TLC using a TLC spotter PS01 (Desaga, Heidelberg, Germany). Plates were developed with a mixture of hexane, diethyl ether, and acetic acid (80:20:3, v/v/v) (25). Lipid-bound radioactivity was detected and quantified by autoradiography (Fuji imager system, Tina 2 software; Raytest, Straubenhart, Germany).

Statistical Analysis. Means of treatments and control were compared by Student's *t* test using the Minitab Statistical Software (Minitab, State College, PA). Data were considered significantly different at $P < 0.05$.

Results

Cell Viability. Viability of Caco-2 cells was not affected by incubation with troglitazone at concentrations of 10 μ*M* or 30 μ*M* for 4 hrs. Viability of HepG2 cells was not affected by treatment with 10 μ*M* of troglitazone but was reduced by treatment with 30 μ*M* of troglitazone ($P < 0.05$, Fig. 1).

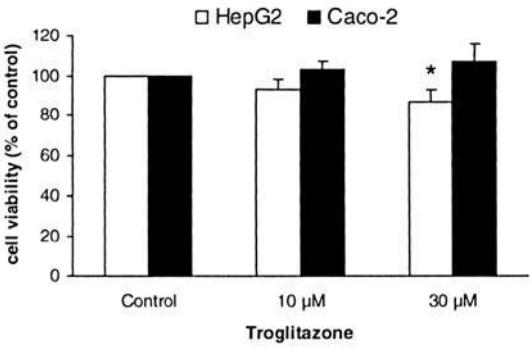

Figure 1. Effect of troglitazone on viability of HepG2 and Caco-2 cells. Cells were treated with buffer containing either 10 μM or 30 μM of troglitazone or with buffer containing vehicle alone (control = 100%) for 4 hrs. Viability of the cells was checked by MTT assay. Values are means ± SD ($n = 9$). *, significantly different from control cells; $P < 0.05$.

Effect of Troglitazone on Gene Expression of PPARγ, SREBP-2, HMG-CoA Reductase, LDL-Receptor, and Insigs in HepG2 and Caco-2 Cells. PPARγ expression was detected in both HepG2 and Caco-2 cells. The expression of PPARγ, however, was not influenced by incubation of the cells with troglitazone in concentrations of either 10 μM or 30 μM (Fig. 2). Incubation with 10 μM or 30 μM of troglitazone caused a significant reduction of the relative mRNA concentration of SREBP-2 in both HepG2 and Caco-2 cells ($P < 0.05$, Fig. 3). Relative mRNA concentrations of SREBP-2 target genes, HMG-CoA reductase, and LDL receptor remained unaffected in HepG2 and Caco-2 cells incubated with 10 μM of troglitazone compared to control cells. In contrast, incubation with 30 μM of troglitazone caused a significant reduction of the mRNA concentration of HMG-CoA reductase and LDL receptor in both types of cells ($P < 0.05$, Fig. 3). In HepG2 cells, Insig-1 mRNA was not detectable under our experimental conditions. In Caco-2 cells, Insig-1 mRNA was detected; its concentration, however, was not influenced by troglitazone (values

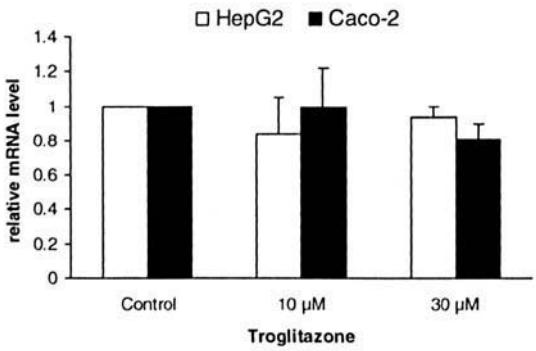

Figure 2. Effect of troglitazone on the relative mRNA concentration of PPARγ in HepG2 and Caco-2 cells. Cells were treated with buffer containing either 10 μM or 30 μM of troglitazone or with buffer containing vehicle alone (control = 1) for 4 hrs. Relative mRNA concentration was determined by RT-PCR with real-time detection using GAPDH mRNA for normalization. Values are means ± SD ($n = 4$).

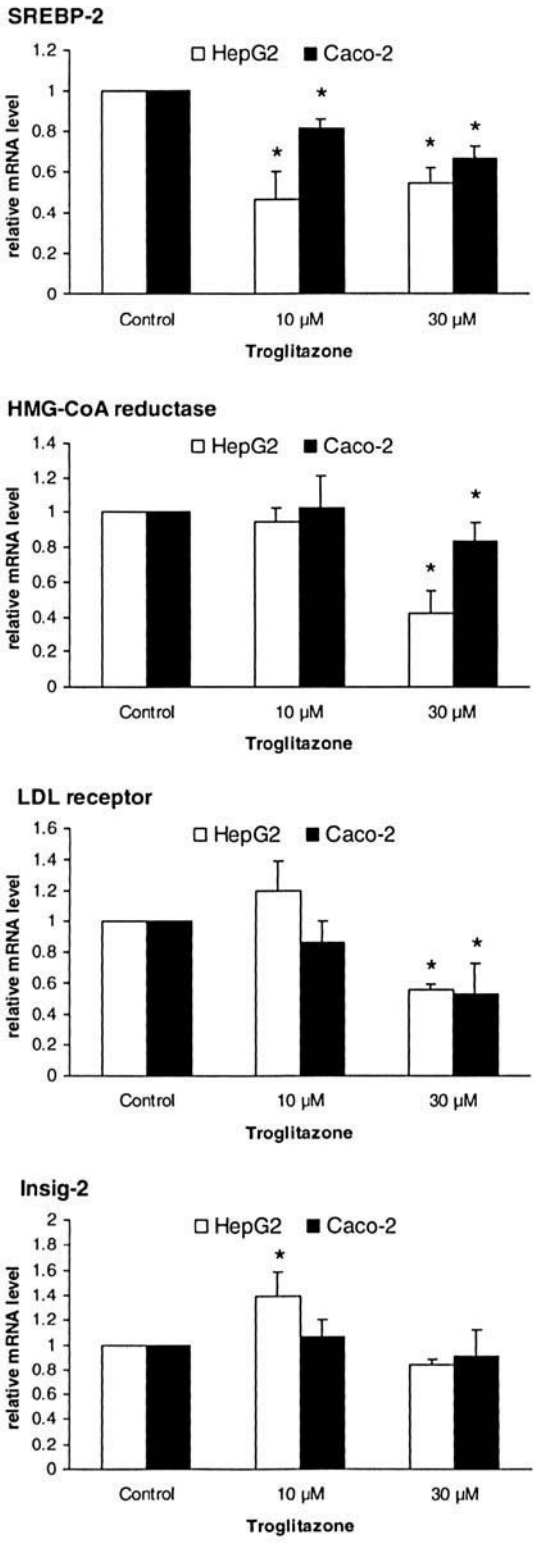

Figure 3. Effect of troglitazone on the relative mRNA concentrations of SREBP-2, HMG-CoA reductase, LDL receptor and Insig-2 in HepG2 and Caco-2 cells. Cells were treated with buffer containing either 10 μM or 30 μM of troglitazone or with buffer containing vehicle alone (control = 1) for 4 hrs. Relative mRNA concentrations were determined by RT-PCR with real-time detection using GAPDH mRNA for normalization. Values are means ± SD ($n = 4$). *, significantly different from control cells; $P < 0.05$.

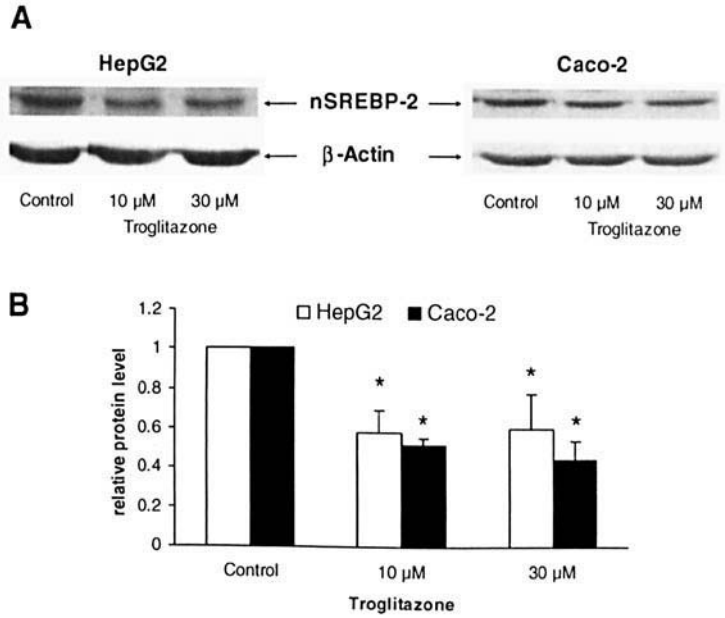

Figure 4. Effect of troglitazone on the relative protein concentrations of nuclear SREBP-2 in HepG2 and Caco-2 cells. Cells were treated with buffer containing either 10 μ*M* or 30 μ*M* of troglitazone or with buffer containing vehicle alone (control = 1) for 4 hrs. Equal amounts of cell proteins were separated by SDS-PAGE and analyzed by Western immunoblotting. β-Actin was used as loading control. (A) Representative immunoblots of mature SREBP-2 and β-actin. (B) Relative intensity of the bands in A was quantified by densitometry. Values are means ± SD ($n = 3$). *, significantly different from control cells; $P < 0.05$.

compared to control [=1]: 10 μ*M*, 0.96 ± 0.32; 30 μ*M*, 0.80 ± 0.24). Insig-2 mRNA was detected in both types of cells. In HepG2 cells treated with 10 μ*M* of troglitazone, mRNA concentration of Insig-2 was higher than in control cells ($P < 0.05$); HepG2 cells treated with 30 μ*M* of troglitazone did not differ in their Insig-2 mRNA concentration from control cells (Fig. 3). In Caco-2 cells, the mRNA concentration of Insig-2 was not influenced by treatment with troglitazone.

Effect of Troglitazone on Protein Concentrations of Nuclear SREBP-2 in HepG2 and Caco-2 Cells. Both HepG2 and Caco-2 cells treated with either 10 μ*M* or 30 μ*M* of troglitazone had significantly lower concentrations of nuclear SREBP-2 protein than the respective untreated control cells ($P < 0.05$, Fig. 4).

Effect of Troglitazone on Cholesterol Synthesis in HepG2 and Caco-2 Cells. Incubation with 10 μ*M* or 30 μ*M* of troglitazone caused a significant dose-dependent reduction of the cholesterol synthesis in both, HepG2 and Caco-2 cells compared with the untreated control cells ($P < 0.05$, Fig. 5).

Discussion

Liver and small intestine are the major sites of cholesterol synthesis in the human body. This study aimed to investigate whether activation of PPARγ by the synthetic agonist troglitazone influences expression of genes involved in cholesterol synthesis and the rate of cholesterol synthesis in cells of liver and intestine. As models of liver and intestine cells we used HepG2 and Caco-2 cells, which are commonly used for studies of the cholesterol metabolism (16–19). We found that both cell types express PPARγ mRNA. This agrees with several other studies which also reported expression of PPARγ on both mRNA and protein levels in these cells (26–32). In some studies, PPARγ mRNA expression was increased by troglitazone in cells or animal tissues (33, 34). In this study, we did not observe an increase of PPARγ mRNA in either cell type used by troglitazone treatment. We assume that troglitazone concentrations used in this study were too low to induce an increased gene expression of PPARγ. In a study with primary hepatocytes, treatment with 25 μ*M* of troglitazone

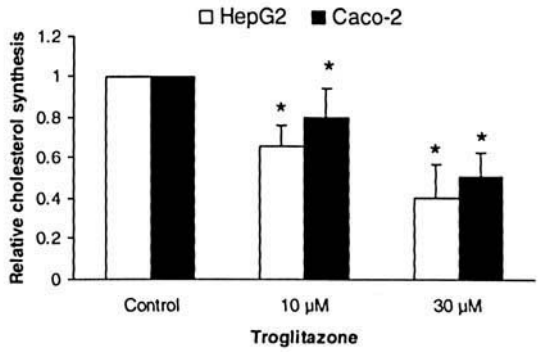

Figure 5. Effect of troglitazone on the relative cholesterol synthesis in HepG2 and Caco-2 cells. Cells were preincubated for 2 hrs with buffer containing either 10 μ*M* or 30 μ*M* of troglitazone or with buffer containing vehicle alone (control = 1). Thereafter, cells were incubated for further 2 hrs with or without the indicated concentrations of troglitazone with addition of [1,2-^{14}C] acetate in order to measure the newly synthesized cholesterol. Cell lipids were extracted with a mixture of hexane and isopropanol. Lipids were separated by thin-layer chromatography and lipid-bound radioactivity was detected and quantified by autoradiography. Values are means ± SD ($n = 9$). *, significantly different from control cells; $P < 0.05$.

did not upregulate PPARγ mRNA expression, and even 50 μ*M* of troglitazone caused only a 1.5-fold increase of PPARγ mRNA expression, although this concentration caused a 25-fold increase of PPARγ activity (35). That study also showed that several other PPARγ agonists caused a strong activation of PPARγ without having an effect on its mRNA expression. This means that activation of PPARγ is not necessarily associated with an upregulation of its mRNA expression. Several studies have already demonstrated PPARγ activation in both HepG2 and Caco-2 cells by troglitazone (36–39). Therefore, we assume that treatment with troglitazone caused PPARγ activation in these cells under the conditions used in our study.

Several studies demonstrated that treatment of HepG2 or colon cancer cells with troglitazone reduced cell viability in a dose-dependent way as a result of apoptosis (36–40). In order to avoid a strong reduction of cell viability, we treated cells with moderate concentrations of troglitazone over a relatively short time of 4 hrs. Nevertheless, under these conditions activity of HepG2 cells in MTT test was slightly reduced. This may be attributed to the apoptosis-inducing properties of troglitazone.

In the present study, treatment of HepG2 or Caco-2 cells with troglitazone lowered the protein concentration of mature SREBP-2 in the nucleus, and in turn reduced the relative mRNA concentration of its target genes, HMG-CoA reductase and LDL receptor. We assume that downregulation of the HMG-CoA reductase expression was associated with a reduced protein concentration of that enzyme, and this might have been responsible for the reduced cholesterol synthesis observed in HepG2 and Caco-2 cells treated with troglitazone. The reduced concentration of the mature SREBP-2 in the nucleus in the cells treated with troglitazone could have two distinctive reasons. Troglitazone either inhibited the processing of the immature SREBP-2 in the Golgi or inhibited gene expression of SREBP and thus the biosynthesis of the immature SREBP-2. Insig-1 and -2 play a key role in the proteolytic procession of the immature SREBP-2. Both Insig-1 and Insig-2 are able to prevent the translocation of the SREBP-SCAP complex from ER to Golgi and the proteolytic activation of SREBP (4, 5). In a recent study, activation of PPARγ by rosiglitazone reduced the concentration of mature SREBP in white adipose tissue by an upregulation of Insig-1 (6). In that study PPARγ activation also led to a transactivation of the Insig-1 promoter. In Caco-2 cells, expression levels of both Insig-1 and -2 were not influenced by troglitazone. In HepG2 cells, we, like Janowski (16), did not detect Insig-1 mRNA. The expression of Insig-2 was increased by 10 μ*M* of troglitazone, but this effect did not emerge by incubation with 30 μ*M* of troglitazone. Thus, the study does not indicate that the reduction of the protein concentration of mature SREBP-2 in Caco-2 cells was primarily due to an increased expression of Insig-1 or -2. However, because we measured mRNA concentrations of Insigs only after 4 hrs of incubation, we cannot exclude the possibility that Insigs could have been upregulated previously, which in turn could have inhibited proteolytic procession of the immature SREBP-2. However, the finding that gene expression of SREBP-2 was lowered in cells treated with troglitazone suggests that the reduced concentration of mature SREBP-2 in nuclei might have been due at least in part to reduced formation of the immature SREBP-2.

The finding that troglitazone reduces cholesterol synthesis is in agreement with a recent study in which incubation with 20 μ*M* of troglitazone for 2 hrs caused a strong reduction of cholesterol biosynthesis in HepG2 cells (41). In contrast, in another study, incubation of HepG2 cells with relatively small concentrations (0.05–5 μ*M*) of troglitazone over a period of 24 hrs increased gene expression and activity of LDL receptor (42). The disagreement between this study and our study could be due to the longer incubation period used in that study. It may be that treatment of cells with troglitazone caused initially a reduction of cholesterol synthesis and uptake by LDL receptor, leading to a cellular cholesterol depletion which may then have caused an upregulation of SREBP-2 processing and LDL receptor expression *via* downregulation of Insig-1. Also in disagreement with our study, PPARγ activation in THP-1 macrophages by treatment with troglitazone or rosiglitazone caused an upregulation of HMG-CoA reductase mRNA expression (12). The difference between that study and our study may be due to the cell type used. Macrophages are able to excrete free cholesterol *via* ABCA1 onto apoA1, and this efflux is stimulated by PPARγ (43, 44). We suspect that an increased efflux of cholesterol from macrophages by PPARγ could lead to an activation of the SREBP-2–dependent cholesterol synthesis in this cell type.

In conclusion, our study shows that PPARγ activation by troglitazone downregulates cholesterol synthesis in HepG2 and Caco-2 cells by a reduced concentration of nuclear SREBP-2. It is suggested that similar effects of PPARγ activation could play a role in liver and small intestine *in vivo*. Cholesterol biosynthesis in liver and intestine is of physiologic relevance because it is closely related to the cholesterol concentration in plasma and LDL, which is strongly associated with the risk of coronary heart disease (45). Some naturally occurring compounds which are known to reduce the cholesterol concentration in plasma and/or liver, such as n-3 PUFA, oxidized fatty acids, and conjugated linoleic acids, are ligands of PPARγ (46–48). Our study suggests that these compounds could exert their hypocholesterolemic effects at least partially through a downregulation of cholesterol synthesis by PPARγ activation. For instance, we have observed that dietary oxidized fats led to reduced concentrations of cholesterol in liver and plasma of rats (49, 50). Moreover, it has been shown that n-3 PUFA lowered the concentration of mature SREBP-2 in HepG2 cells (51) and rat liver (52). The molecular mechanisms underlying the effects of oxidized fatty acids or n-3 PUFA on the cholesterol metabolism have not yet

been fully elucidated. It cannot be excluded that these fatty acids inhibit cholesterol synthesis *via* PPARγ activation. Therefore, further studies should focus on the effects of PPARγ activation on SREBP-2 mediated cholesterol synthesis *in vivo*.

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