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RESEARCH ARTICLE

Monocarboxylate transporter 1 and CD147 are up-regulated by natural and synthetic peroxisome proliferator-activated receptor α agonists in livers of rodents and pigs

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Monocarboxylate transporter (MCT)-1 mediates the transport of ketone bodies and other monocarboxylic acids across the plasma membrane. MCT1 is up-regulated by peroxisome proliferator-activated receptor (PPAR)-α, a transcription factor that mediates the adaptive response to fasting by up-regulation of genes involved in fatty acid oxidation and ketogenesis. Here, we show for the first time that MCT1 is up-regulated by dietary natural PPAR-α agonists. Both, an oxidized fat and conjugated linoleic acids increased MCT1 mRNA concentration in the liver of rats. Also, in the liver of pigs as non-proliferating species MCT1 was up-regulated upon PPAR-α activation by clofibrate, oxidized fat and fasting. Concomitant with up-regulation of MCT1, mRNA level of CD147 was increased in livers of rats and pigs. CD147 is a plasma membrane glycoprotein that is required for translocation and transport activity of MCT1. CD147 mRNA increase upon PPAR-α activation could not be observed in mice lacking PPAR-α, which also fail in up-regulation of MCT1 indicating a co-regulation of MCT1 and CD147. Analysis of the 5′-flanking region of mouse MCT1 gene by reporter gene assay revealed that promoter activity of mouse MCT1 was not induced by PPAR-α, indicating that the 5′-flanking region is not involved in MCT1 regulation by PPAR-α.

Keywords:
CD147 / Conjugated linoleic acids / Monocarboxylate transporter-1 / Oxidized fat / Peroxisome proliferator-activated receptor-α

1 Introduction

Monocarboxylate transporter (MCT)-1 is a member of the proton-linked MCT family, which is known to comprise at least 14 isoforms [1]. MCT are involved in the transport of monocarboxylates such as lactate, pyruvate and ketone bodies as well as short chain fatty acids and thus are important to energy metabolism, homeostasis and pH control in various tissues [2, 3]. Regulation of MCT has been demonstrated to occur *via* transcriptional, translational and post-transcriptional mechanisms that appear to be age- and tissue-dependent [4–6]. In colonic epithelium, both MCT1 mRNA and protein are up-regulated by butyrate *via* NF-κB-dependent regulation of MCT1 promoter [7, 8]. MCT1 is up-regulated during diet-induced ketosis and by lactate in rat brain and L6 cells, respectively [9, 10]. Furthermore, MCT1 is regulated by its association with the cell surface protein CD147, a widely expressed glycoprotein of the immunoglobulin supergene family [11–13]. The initial association of CD147 and MCT1 is required for the translocation of MCT1 to the plasma membrane [11]. In addition, the continued presence and correct conformation of CD147 are critical for transporter function of MCT1 [14].

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Abbreviations: CLA, conjugated linoleic acids; **MCT,** monocarboxylate transporter; **mHCS,** mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase; **PPAR,** peroxisome proliferator-activated receptor; **PPRE,** PPAR response element; **TBARS,** thiobarbituric acid substance

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Recently we could show that MCT1 is up-regulated by peroxisome proliferator-activated receptor (PPAR)-α [15]. PPAR-α is a lipid-activated nuclear hormone receptor that acts as a nutritional state sensor in mammalian cells and mediates an adaptive response to fasting [16, 17]. Upon fasting, it stimulates transcription of a magnitude of genes involving those for synthesis of ketone bodies [18]. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mHCS) catalysing the first rate-limiting step in ketogenesis is regulated by direct binding of PPAR-α to a functional PPAR response element (PPRE) within its promoter [19].

We could demonstrate that MCT1 mRNA was up-regulated by both fasting and a synthetic PPAR-α agonist in rats and that this increase depends on the presence of PPAR-α since it was absent in mice lacking PPAR-α [15]. Thus, during fasting, up-regulation of MCT1 by PPAR-α may support the supply of cells with energy fuels. However, the mechanism of regulation of MCT1 by PPAR-α is still unknown.

Recently, we and others could demonstrate that PPAR-α is also activated by various naturally occurring lipids such as n-3 fatty acids, conjugated linoleic acids (CLAs) and oxidized fats [20–23]. It was shown that oxidized fats, which comprise a growing part of human nutrition in western countries [24], influence lipid metabolism in rats similar to synthetic PPAR-α agonists [25] and activate PPAR-α also in pigs as a member of non-proliferating species [26]. Non-proliferating species like human and pig have a lower expression of PPAR-α in liver and the response of many genes to PPAR-α activation is much weaker than in proliferating species like rodents [27, 28].

Thus, the aim of the present study was to investigate whether MCT1 is also up-regulated by natural PPAR-α agonists like oxidized fats and CLA. Therefore, we performed an experiment with rats that were orally administered a fresh fat, an oxidized fat, CLA or clofibrate as positive control. We determined expression of MCT1 in liver of rats by measurement of its mRNA concentration using RT-PCR. Taking into account the critical role of CD147 for MCT1 activity [14], we also determined CD147 mRNA concentration. To test whether MCT1 is also up-regulated in non-proliferating species, we conducted an experiment with pigs that were fed a fresh fat, an oxidized fat or clofibrate and analysed expression of MCT1 and CD147 in their livers. In a second experiment with pigs, we tested the effect of fasting on MCT1/CD147 expression in liver. To estimate PPAR-α activation, mRNA level of the PPAR-α target gene mHCS, the key enzyme of ketone body synthesis, was analysed in all experiments. In rat as well as pig experiments we observed an up-regulation of both MCT1 and CD147 upon PPAR-α activation. Treatment of wild-type mice and mice lacking PPAR-α (knockout) with the synthetic PPAR-α agonist WY 14 643 revealed that not only up-regulation of MCT1 but also that of CD147 is absent in PPAR-α knockout mice, indicating a co-regulation of these two genes. In an attempt to analyse the mechanism of PPAR-α-mediated up-regulation of MCT1, we performed promoter activation studies.

2 Materials and methods

2.1 Animals and diets

All experimental procedures described followed guidelines for the care and handling of laboratory animals and were approved by the regional council of Saxony-Anhalt.

2.1.1 Rat experiment

Male Sprague–Dawley rats supplied by Charles River (Sulzfeld, Germany) with an average initial body weight of 115 g (±25 g; SD), were randomly assigned to four groups of nine rats each. They were kept individually in Macrolon cages in a room controlled for temperature (22±2°C), relative humidity (50–60%) and light (12 h light/dark cycle). All rats were fed a commercial standard basal diet (''altromin 1324'', Altromin, Lage, Germany) containing 19% crude protein (herbal source, mainly soy), 4% crude fat, 6% crude fibre and 7% crude ash. To standardize food intake, the diets were fed daily in restricted amounts of 12 g *per* day, equivalent to an intake of 143 kJ metabolizable energy *per* day. Water was available *ad libitum* from nipple drinkers during the whole experiment. All rats were treated with 2 mL of the experimental fat by gavage once a day 2 h after the beginning of the light cycle. The first group (control group) received sunflower oil, the second group (oxidized fat group) oxidized sunflower oil, the third group (CLA group) CLA (BASF, Ludwigshafen Germany) and the fourth group (clofibrate group) 250 mg clofibrate (Fluka, Buchs, Switzerland) *per* kg body weight in 2 mL of sunflower oil.

The oxidized fat was prepared by heating sunflower oil for 25 days at 60°C. The extent of lipid peroxidation was determined by assaying the peroxide value, concentration of thiobarbituric acid substances (TBARS), concentration of conjugated dienes, concentration of total carbonyls, acid values and the percentage of total polar compounds as described earlier [25]. The concentrations of lipid peroxidation products were (oxidized *versus* fresh fat): peroxide value (379 *versus* 3 mEq O_2/kg), TBARS (13.1 *versus* 1.1 mmol/kg), conjugated dienes (274 *versus* <1 mmol/kg), total carbonyls (96.9 *versus* 2.9 mmol/kg), acid value (5.8 *versus* 0.4 g KOH/kg), and polar compounds (27.8 *versus* 5.1%). The CLA oil contained 60 g CLA isomers (29.3 g *c*9*t*11-CLA, 28.9 g *t*10*c*12-CLA, 1.4 g *c*10*c*12-CLA, 0.3 g *t*9*t*11-CLA) *per* 100 g CLA oil as analysed by Ag^+-HPLC-DAD [29]. At day 6 of treatment, rats received the last dose of the experimental fats and 9 g of the diet again 2 h after the beginning of the light cycle and were killed 4 h later by decapitation under light anaesthesia with diethyl ether.

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2.1.2 Pig experiment

Twenty-seven male 8-wk-old pigs (German Landrace × Large White × Pietrain), obtained from a local breeder, were kept in a room under controlled temperature of 23 ± 2°C and 50–60% relative humidity with lighting from 06.00 to 18.00. After 1 wk of adaptation the pigs were weighed, randomly assigned to three groups of nine pigs each with mean body weights of 12.0 ± 1.1 kg (control group), 12.2 ± 0.9 kg (oxidized fat group) and 11.9 ± 0.6 kg (clofibrate group) and then fed the experimental diets. The diets were nutritionally adequate for growing pigs [30] and contained 14.4 MJ/kg metabolizable energy. The composition of the experimental diet was (g/kg diet): wheat (400), soy bean meal (230), wheat bran (150), barley (98.1), fat (90) and mineral premix (30) including L-lysine, L-threonine and DL-methionine. The first (control) group obtained fresh fat, the second group (oxidized fat group) oxidized fat and the third group (clofibrate group) fresh fat with additionally 5 g clofibrate *per* kg diet, which was freshly added to the diet each day.

The oxidized fat was sunflower oil that was heated at 200°C for 24 h. The fresh fat was a mixture of sunflower oil and palm oil in a ratio of 93:7. This ratio was chosen to equalize the fatty acid composition of the fresh fat with that of the oxidized fat, since the heating process caused a loss of PUFA. The major fatty acids in the fresh fat and the oxidized fat were (g/100 g total fatty acids): palmitic acid (16:0), 9.0 *versus* 6.8; stearic acid (18:0), 4.0 *versus* 4.2; oleic acid [18:1(n-9)], 24.3 *versus* 24.1; linoleic acid [18:2(n-6)], 60.0 *versus* 60.6; and α-linolenic acid [18:3(n-3)], 0.1 *versus* 0.1. Other fatty acids, including transfatty acids, were present only in traces (< 0.5 g/100 g fatty acids). The extent of lipid peroxidation in the fats after inclusion into the diets was estimated after extraction from the diets with a mixture of hexane and isopropanol according to the method of Hara and Radin [31]. The concentrations of lipid peroxidation products were (oxidized *versus* fresh fat): peroxide value (10.0 *versus* 2.5 mEq O_2/kg), TBARS (271 *versus* 9 μmol/kg), conjugated dienes (89.1 *versus* 22.7 mmol/kg), total carbonyls (24.5 *versus* 2.5 mmol/kg) and acid value (8.0 *versus* 1.6 g KOH/kg). To standardize food intake, each pig received daily 700 g of diet throughout the whole experiment. Water was provided by nipple drinking systems *ad libitum*. The experimental diets were fed for 28 days. After completing of the feeding period the pigs were fed with 300 g of the respective diets 4 h before killing with a captive bolt pistol.

In a second experiment, 20 male, 10-wk-old pigs received a nutritionally adequate diet [30]. One day before the start of the experiment, at an average body weight of 25 kg, the pigs were divided into two groups of ten animals each. At the day of the experiment, the first group (control group) received the diet *ad libitum* for the next 24 h. The second group received their last meal at 8 a.m. and was then fasted for the next 24 h. Pigs were then killed at the morning of the next day either in the fed status (control group) or after a 24-h fasting period (treatment group).

2.1.3 Mouse experiment

Female PPAR-α knockout mice (129S4/SvJae-*Ppara*^{tm1Gonz}/J) and corresponding wild-type control mice (129S1/SvImJ) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Mice from both genotypes with an average initial body weight of 24.3 g (± 3.2; SD) were randomly assigned to two groups and kept individually in Macrolon cages in a room controlled for temperature (22 ± 2°C), relative humidity (50–60%) and light (12-h light/dark cycle). Mice of the treatment groups (wild-type mice $n = 8$ and PPAR-α^{--}mice $n = 8$) obtained 40 mg/kg body weight WY 14 643 once a day 2 h after beginning of the light cycle by gavage for 4 days. WY 14 643 was dissolved in DMSO/sun flower oil (50/50 v/v) at a final concentration of 8 mg/mL as described earlier [32]. Control animals (wild-type mice $n = 8$ and PPAR-α^{--}mice $n = 8$) obtained the appropriate volume of the vehicle DMSO/sun flower oil. All mice were fed with a commercial standard basal diet (''altromin 1324''). To standardize food intake, the diets were fed daily in restricted amounts of 4 g *per* day. Water was available *ad libitum* from nipple drinkers during the whole experiment. At day 4 of treatment, mice received the last dose of WY 14 643 or vehicle alone and 1 g of the diet and were killed 4 h later by decapitation under light anaesthesia with diethyl ether.

2.2 Sample collection

After killing, the livers were excised and samples for RNA isolation were snap-frozen in liquid nitrogen and stored at −80°C.

2.3 RT-PCR analysis

Total RNA isolation from liver samples and cDNA synthesis were carried out as described earlier [20]. The mRNA expression of genes was measured by real-time detection PCR using SYBR® Green I and the Rotor Gene 2000 system (Corbett Research, Mortlake, Australia) as described earlier [15]. Primer pairs were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/) and are listed in Table 1. For determination of mRNA concentration a threshold cycle and amplification efficiency were obtained from each amplification curve using the software RotorGene 4.6 (Corbett Research). Calculation of the relative mRNA concentration was made using the amplification efficiencies and the threshold cycle values [33]. The housekeeping gene β-actin was used for normalization.

2.4 Promoter reporter gene constructs

To identify putative PPRE in the mouse *MCT1* promoter, approximately 2.2 kb of the 5′-flanking region including

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Table 1. Characteristics of the specific primers used for RT-PCR analysis

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	PCR product size (bp)	T_m (°C)	NCBI GenBank
β-Actin (rat)	ATCGTGCGTGACATTAAAGAGAAG	GGACAGTGAGGCCAGGATAGAG	429	60	BC063166
CD147 (rat)	GGCACCATCGTAACCTCTGT	TCTTTCCCACCTTGATCCTG	265	60	BC059145
MCT1 (rat)	AAGCGGAGGAAAAGAAGAGG	TAGACTAGGGGGCCAGCAGAA	217	60	NM_012716
mHMG-CoA synthase (rat)	GGCCTTGGACCGATGCTATGC	GGGAGGCCTTGGTTTTCTTGTTG	323	58	BC083543
β-Actin (pig)	GACATCCGCAAGGACCTCTA	ACATCTGCTGGAAGGTGGAC	205	60	BC142413
CD147 (pig)	CCTCGGAGACCAAGACAGAG	TCATTCACGTGGTGTCCACT	289	60	EU404087
MCT1 (pig)	GGTGGAGGTCCTATCAGCAG	TCCTGCACGGTGTTACAGAA	122	60	NM_001128445
mHMG-CoA synthase (pig)	GGACCAAACAGACCTGGAGA	ATGGTCTCAGTGCCCACTTC	198	62	NM_214380
β-Actin (mouse)	ACGGCCAGGTCATCACTATTG	CACAGGATTCCATACCCAAGAA	87	66	NM_007393
CD147 (mouse)	ACTGGGGAAGAAGAGGCAAT	AACCAACACCAGGACCTCAG	246	60	BC010270
mHMG-CoA synthase (mouse)	CCTCTGTGAATCCTGGGTGT	CTGTGGGGAAAGATCTGCAT	141	60	NM_008256
MCT1 (mouse)	GTGACCATTGTGGAATGCTG	CTCCGCTTTCTGTTCTTTGG	186	60	AF058055

positions −2142 to +56 relative to the transcription start site (NCBI GenBank NT_039240) were analysed using NUBIScan [34]. Furthermore, the sequence of the first intron starting at position +169 relative to the transcription start site and including about 10 kb was analysed.

The mouse *MCT1* promoter/intron construct containing at least two putative PPRE was generated by PCR amplification of the mouse *MCT1* sequence from positions −1656 to +868 using mouse BAC clone RP23-208N5 (imaGene, Berlin, Germany). The promoter construct pGL4.10-m*MCT1*_−1654/+56 was generated by subcloning of the generated PCR product containing two adapters of SacI and XhoI site into the SacI and XhoI sites of the pGL4.10[luc2] basic reporter vector (encoding the synthetic Firefly luciferase reporter gene; Promega, Mannheim, Germany). The PCR primer sequences were as follows: 5′-atcgagctccgaactcagaaatctgcctg-3′ and 5′-tagctcgaggctccttggctgctgcac-3′. The reporter gene construct pGL4.23-m*MCT1*_+151/+868 containing sequences from the first intron including at least one putative PPRE was generated by subcloning of the generated PCR product containing two adapters of XhoI and HindIII site into the XhoI and HindIII sites of the pGL4.23[luc2/minP] reporter vector (containing a minimal promoter sequence and the synthetic Firefly luciferase reporter gene; Promega). The PCR primer sequences were as follows: 5′-atcctcgagcacacataacggttag-3′ and 5′-tagaagcttatgtactcggcaaagggatg-3′. After cloning, the integrity and fidelity of all m*MCT1* constructs were verified by DNA sequencing.

2.5 Transient transfection and dual luciferase assay

For promoter activation studies, HepG2 cells were used, which are an accepted model for studying the regulation of genes by PPAR-α [35–37]. HepG2 cells (about 70% confluent) were transiently transfected with either the generated MCT1 promoter constructs, negative control vector pGL4.10-m*MCT1*_0 and pGL4.23-m*MCT1*_0, respectively, or positive control vector 3 × ACO PPRE (containing three copies of consensus PPRE from the ACO promoter in front of a luciferase reporter gene; this vector was a generous gift from Dr. Sander Kersten, Nutrigenomics Consortium, Top Institute Food and Nutrition, Wageningen, The Netherlands), and pGL4.74[hRluc/TK] (encoding the Renilla luciferase reporter gene; Promega), which was used as an internal control reporter vector to normalize for differences in transfection efficiency, using FuGENE 6 transfection reagent (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol. In addition, co-transfections with mouse *PPAR*-α expression plasmid pCMX-m*PPAR*-α and mouse *RXR*-α expression plasmid pCMX-m*RXR*-α (both, generous gifts from R. M. Evans, Salk Institute for Biological Studies, San Diego, CA, USA) were carried out. Subsequently, cells were treated with either 100 μM WY 14 643 or vehicle (DMSO = control) for 24 h.

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Afterwards the cells were washed with phosphate-buffered saline and lysed with passive lysis buffer (Promega). Luciferase activities were determined with the Dual-Luciferase Reporter Assay System from Promega according to the manufacturer's instructions using a Mithras LB940 luminometer (Berthold Technologies, Bad Wildbad, Germany) as described earlier [38].

2.6 Statistics

Data of all experiments were analysed using the Minitab Statistical Software (Minitab, State College, PA, USA). Treatment effects of rat and pig experiments were evaluated by one-way ANOVA. For significant F values ($p<0.05$), means of the treatments (fasting, clofibrate, oxidized fat, CLA) were compared pairwise with the control group by Student's t test. Treatment effects of mice experiment were analysed by two-way ANOVA with classification factors being treatment (WY 14 643), genotype and the interaction of treatment (WY 14 643) and genotype. In all experiments, means were considered significantly different for $p<0.05$.

3 Results

3.1 Effect of PPAR-α agonists on MCT1/CD147 expression in rat liver

mRNA concentration of the PPAR-α target gene mHCS was higher in rats fed oxidized fat, CLA or clofibrate compared with control (fresh fat) rats ($p<0.05$; Fig. 1), indicating activation of PPAR-α by all agonists tested. Furthermore, both MCT1 and CD147 mRNA concentrations were higher in the liver of rats fed oxidized fat, CLA or clofibrate compared with control rats ($p<0.05$; Fig. 1).

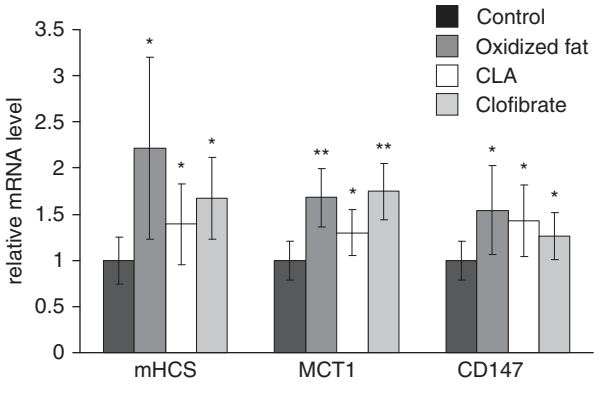

Figure 1. Effect of oxidized fat, CLA and clofibrate on the mRNA concentrations of mHCS, MCT1 and CD147 in rat liver. Rats were treated orally with fresh fat (control group), oxidized fat, CLA or 250 mg/kg of clofibrate for 6 days. Total RNA was extracted from rat livers and mRNA concentrations were determined by real-time detection RT-PCR analysis using β-actin mRNA concentration for normalization. Values are means ± SD ($n=9$). Symbols indicate significant differences from control rats (*$p<0.05$, **$p<0.001$).

3.2 Effect of PPAR-α agonists and fasting on MCT1/CD147 expression in pig liver

mHCS was up-regulated by both oxidized fat and clofibrate in the liver of pigs compared with pigs fed a fresh fat (control group), indicating PPAR-α activation ($p<0.05$; Fig. 2A). mRNA concentration of MCT1 was higher in the liver of pigs fed the oxidized fat or clofibrate than in the livers of control pigs ($p<0.05$; Fig. 2). CD147 mRNA level was higher in the liver of clofibrate-treated than in that of control pigs ($p<0.05$) but was not influenced by oxidized fat treatment (Fig. 2A). Fasting of pigs for 24 h led to a potent activation of PPAR-α in liver as indicated by a strong up-regulation of mHCS compared with non-fasted (control) pigs ($p<0.001$, Fig. 2B).

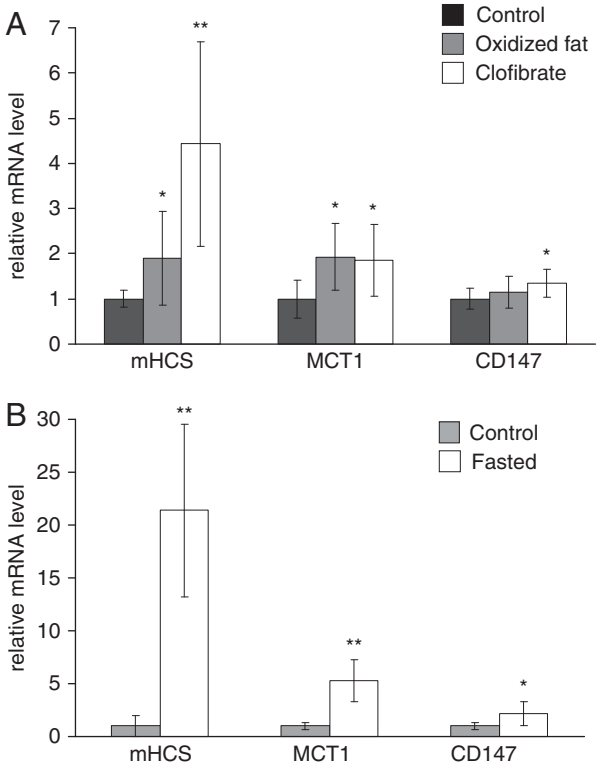

Figure 2. Effect of oxidized fat, clofibrate (A) and fasting (B) on the mRNA concentrations of mHCS, MCT1 and CD147 in pig liver. (A) Pigs were fed a diet containing fresh fat (control group), oxidized fat or 5 g/kg diet of clofibrate for 28 days. (B) Pigs were fasted for 24 h before killing; control animals received standard diet *ad libitum*. Total RNA was extracted from pig livers and mRNA concentrations were determined by real-time detection RT-PCR analysis using β-actin mRNA concentration for normalization. Values are means ± SD ($n=9$ (oxidized fat/clofibrate); $n=10$ (fasting)). Symbols indicate significant differences from control rats (*$p<0.05$, **$p<0.001$).

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Furthermore, both, MCT1 ($p<0.001$) and CD147 mRNA ($p<0.05$) concentrations were higher in the liver of fasted than that of control pigs (Fig. 2B).

3.3 Effect of WY 14 643 on MCT1/CD147 expression in PPAR-α knockout mice

As already observed [15], both mHCS and MCT1 mRNA concentrations increased in wild-type mice treated with WY 14 643 but were unchanged in PPAR-α knockout mice ($p<0.05$; Fig. 3). Also, CD147 mRNA was higher in livers of wild-type mice treated with WY 14 643 compared with control mice, whereas this up-regulation was not observed in PPAR-α knockout mice ($p<0.05$; Fig. 3).

3.4 Analysis of MCT1 promoter activity

Sequence analysis of the 5′-flanking region and the first intron of mouse MCT1 using NUBIScan revealed putative PPRE at positions −622 to −615 and +437 to +449 relative to the transcription start site. To test whether mouse MCT1 promoter is activated by PPAR-α, we cloned the 5′-regulatory region of mouse MCT1 (sequence spanning from −1654 to +56) into a luciferase reporter vector, and transiently transfected HepG2 cells with the promoter reporter construct, which was named pGL4.10-m*MCT1*_−1654/+56. As shown in Fig. 4, luciferase activity of HepG2 cells transiently transfected with the pGL4.10-m*MCT1*_−1654/+56 construct was much higher than that of cells transfected with negative control vector pGL4.10-m*MCT1*_0, indicating strong transcriptional activity of mouse *MCT1* promoter. However, the PPAR-α ligand WY 14 643 did not increase the luciferase activity in HepG2 cells transiently transfected with the pGL4.10-m*MCT1*_−1654/+56 construct but that of HepG2 cells transiently transfected with the positive control vector 3 × ACO PPRE (Fig. 4). In addition, co-transfection of mouse *PPAR*-α expression plasmid pCMX-m*PPAR*-α and mouse *RXR*-α expression plasmid pCMX-m*RXR*-α did not increase transcriptional activity of mouse *MCT1* promoter in the absence or presence of WY 14 643. In contrast, luciferase activity of HepG2 cells transfected with the positive control vector 3 × ACO PPRE was strongly increased upon co-transfection of pCMX-m*PPAR*-α and pCMX-m*RXR*-α in the absence and presence of WY 14 643, indicating the functionality of the assay (Fig. 4). In a second trial, we cloned the first 700 bp of the first intron of mouse *MCT1* (sequence spanning from +151 to +868) into a luciferase reporter vector with minimal promoter. Transfection of HepG2 cells with the resulting plasmid pGL4.23-m*MCT1*_+151/+868 did not increase luciferase activity of the cells both, in the presence of WY 14 643 and upon co-transfection with expression plasmids for mouse *PPAR*-α and mouse *RXR*-α (data not shown).

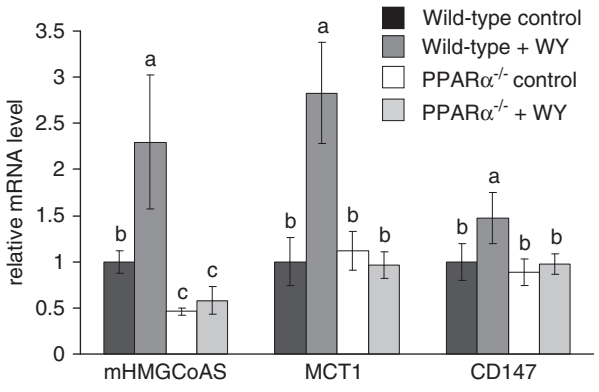

Figure 3. Effect of WY 14 643 on the mRNA concentrations of mHCS, MCT1 and CD147 in the liver of wild-type and PPAR-$\alpha^{-/-}$ (knockout) mice. Mice were treated orally with 40 mg/kg of WY 14 643 for 4 days. Control mice obtained the appropriate volume of vehicle sunflower oil/DMSO. Total RNA was extracted from livers and mRNA concentrations were determined by real-time detection RT-PCR analysis using β-actin mRNA concentration for normalization. Values are means ± SD ($n=8$). Means with unlike letters differ significantly ($p<0.05$). Significant effects ($p<0.05$) from two-way ANOVA: mHCS: genotype, WY 14 643, WY 14 643 × genotype; MCT1: genotype, WY 14 643, WY 14 643 × genotype; CD147: genotype, WY 14 643, WY 14 643 × genotype.

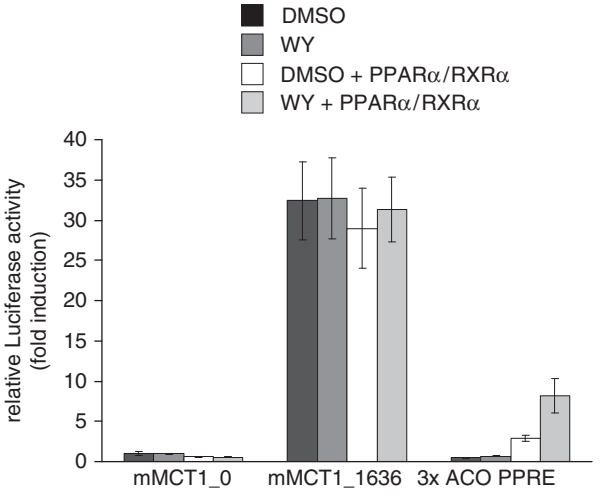

Figure 4. Effect of the PPAR-α agonist WY 14 643 on the promoter activity of mouse MCT1 in HepG2 cells. HepG2 cells were transiently transfected with either pGL4.10-m*MCT1*_−1654/+56 construct containing one putative PPRE of mouse *MCT1* promoter, negative control vector pGL4.10-m*MCT1*_0 or positive control vector 3 × ACO PPRE, and internal control vector pGL4.74. In addition, co-transfections with mouse *PPAR*-α expression plasmid pCMX-m*PPAR*-α and mouse *RXR*-α expression plasmid pCMX-m*RXR*-α were carried out. Subsequently, cells were treated with 100 μM WY 14 643 or vehicle (DMSO = control) for 24 h. Afterwards cells were lysed and luciferase activities were determined. Relative luciferase activity is shown as the ratio of *Firefly*/*Renilla* luciferase. One representative result from three independent experiments is presented; values are means ± SD from three replicates.

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4 Discussion

Recently, we could demonstrate that MCT1, which is responsible for transport of ketone bodies and other monocarboxylic acids in and out of cells, is up-regulated by PPAR-α, which was activated by synthetic ligands and fasting, respectively, in rats and mice [15]. The present study was conducted to examine whether MCT1 expression is also influenced by dietary natural PPAR-α agonists. Here, we show that feeding of rats with both, an oxidized fat and CLA, increased MCT1 mRNA concentration in liver compared with control rats fed a fresh fat, in a comparable extent as observed for the synthetic PPAR-α agonist clofibrate. Furthermore, also mRNA concentration of mHCS was up-regulated in liver of rats upon treatment with oxidized fat and CLA, respectively, indicating PPAR-α activation. mHCS catalyses the first rate-limiting step in ketogenesis and has a functional PPRE within its promoter [19]. It is known that oxidized fatty acids and CLA are also able to activate PPAR-γ [39, 40], indicating that also PPAR-γ activation by oxidized fat and CLA may contribute to the observed induction of MCT1. Nevertheless, in our previous study we could show that the synthetic and selective PPAR-γ agonist troglitazone did not increase MCT1 mRNA in Fao rat hepatoma cells [15]. Thus, we conclude that up-regulation of MCT1 by CLA and oxidized fat is rather mediated by activation of PPAR-α than PPAR-γ. Therefore, the data of the present study indicate that the dietary PPAR-α agonists oxidized fat and CLA have similar effects on ketone body synthesis and transport than synthetic PPAR-α agonists in rats. This is in accordance with previous studies showing that dietary oxidized fats exert similar effects on cholesterol metabolism and carnitine homeostasis as synthetic PPAR-α agonists in rats [25, 41].

Since pigs as well as humans belong to the so-called non-proliferating species that response differently upon PPAR-α activation compared with proliferating species as rats and mice [27, 28], pigs represent a suitable model for humans to study the effects of PPAR-α activation. It has been already shown that clofibrate, oxidized fat as well as fasting activate PPAR-α in liver and other tissues of pigs [26, 27, 42]. This is consistent with the up-regulation of mHCS in the liver of pigs treated with clofibrate or oxidized fat as well as fasted pigs compared with control groups observed in this study. Concomitant with the raise in mHCS mRNA, also up-regulation of MCT1 was observed in the liver of pigs of the treatment groups compared with control pigs. Thus, not only in proliferating species like rats and mice but also in pigs as a model for non-proliferating species up-regulation of ketogenesis upon PPAR-α activation is accompanied by an up-regulation of ketone body transporter MCT1. Since MCT1 is also involved in the transport of pharmaceutical agents like γ-hydroxybutyrate, salicylic acid and statins [43] up-regulation of MCT1 upon dietary intake of oxidized fats or CLA may also have implications for drug delivery in humans.

The chaperone and transmembrane protein CD147 is essential for the correct membrane assembly and transporter function of MCT1 [11, 14]. Several studies demonstrated that up-regulation of MCT1 by different stimuli was accompanied by an up-regulation of its chaperone CD147 [44–46]. Furthermore, silencing studies showed that maturation and cell surface expression of CD147 depend on MCT1 expression [47, 48]. Also in our study, up-regulation of MCT1 was generally accompanied by an increase of CD147 mRNA level supporting that CD147 activity is regulated by MCT1. In accordance with that, CD147 up-regulation upon PPAR-α activation could not be observed in PPAR-α knockout mice, which also failed in up-regulation of MCT1. However, in other studies, maturation and surface expression but not mRNA level of CD147 were affected [45, 47], indicating differences in the regulatory mechanisms.

Transcriptional regulation of genes by PPAR is mediated by binding of activated PPAR/retinoid X receptor heterodimers to specific DNA sequences, called PPRE present in and around the promoter of PPAR target genes [49, 50]. Furthermore, several studies demonstrate functionally active PPRE in intronic sequences of PPAR-α target genes [51–53]. Whether or not the up-regulation of MCT1 is mediated by binding of PPAR-α to PPRE sequences within the *MCT1* gene is not known. *In silico* analysis of mouse MCT1 promoter and the first intron revealed at least to putative PPRE at positions −622 to −615 and +437 to +449 relative to the transcription start site with high homology to the consensus PPRE (AGGTCAAAGGTCA). To determine whether these putative PPRE are fuctional, we cloned the proximal promoter region and the respective part of the first intron, respectively, in luciferase reporter vectors. However, for both reporter gene constructs, no induction of promoter activity by the PPAR-α agonist WY 14 643, with or without co-expression of m*PPAR*-α/m*RXR*-α, could be observed. This indicates that mouse *MCT1* gene is probably not regulated by direct binding of PPAR-α/RXR-α to sequences in the 5′-regulatory region. Also, the putative PPRE in the analysed first intron seems not to be responsible for regulation of mouse *MCT1* by PPAR-α. We cannot exclude that other than the analysed sequences of mouse *MCT1* gene are responsible for its regulation by PPAR-α. On the other hand, an indirect regulation of MCT1 by PPAR-α by interference with other regulatory pathways or proteins that regulate MCT1 expression is possible. Substrate-induced up-regulation of MCT1 was demonstrated [4, 10]. However, since incubation of Fao cells with β-hydroxybutyrate and acetoacetate did only slightly increase MCT1 mRNA concentration (data not shown) other mechanisms than substrate induced activation of MCT1 by increased ketone body concentrations may account for the observed up-regulation of MCT1 by PPAR-α.

In conclusion, this study shows that natural PPAR-α agonists like constituents of oxidized fats and CLA up-regulate MCT1 in liver. Also, in pigs that belong as humans to the non-proliferating species, MCT1 was up-regulated upon PPAR-α activation by clofibrate, oxidized fat and fasting. Since MCT1 is involved in the transport of pharmaceutical agents, up-regulation of MCT1 by dietary PPAR-α

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agonists may also have implications for drug delivery. We could also show that the chaperone CD147, which is essential for translocation and activity of MCT1, is co-induced with MCT1 also in a PPAR-α-dependent manner. However, the mechanism of up-regulation of MCT1 by PPAR-α seems not to involve a transcriptional action of PPAR-α on the 5′-flanking region of mouse *MCT1*.

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5 References

[1] Halestrap, A. P., Meredith, D., The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflugers Arch.* 2004, *447*, 619–628.

[2] Enerson, B. E., Drewes, L. R., Molecular features, regulation, and function of monocarboxylate transporters: implications for drug delivery. *J. Pharm. Sci.* 2003, *92*, 1531–1544.

[3] Halestrap, A. P., Price, N. T., The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. *Biochem. J.* 1999, *343*, 281–299.

[4] Cuff, M. A., Shirazi-Beechey, S. P., The human monocarboxylate transporter, MCT1: genomic organization and promoter analysis. *Biochem. Biophys. Res. Commun.* 2002, *292*, 1048–1056.

[5] Enoki, T., Yoshida, Y., Lally, J., Hatta, H., Bonen, A., Testosterone increases lactate transport, monocarboxylate transporter (MCT) 1 and MCT4 in rat skeletal muscle. *J. Physiol.* 2006, *577*, 433–443.

[6] Chenal, J., Pellerin, L., Noradrenaline enhances the expression of the neuronal monocarboxylate transporter MCT2 by translational activation *via* stimulation of PI3K/Akt and the mTOR/S6K pathway. *J. Neurochem.* 2007, *102*, 389–397.

[7] Cuff, M. A., Lambert, D. W., Shirazi-Beechey, S. P., Substrate-induced regulation of the human colonic monocarboxylate transporter, MCT1. *J. Physiol.* 2002, *539*, 361–371.

[8] Borthakur, A., Saksena, S., Gill, R. K., Alrefai, W. A. *et al.*, Regulation of monocarboxylate transporter 1 (MCT1) promoter by butyrate in human intestinal epithelial cells: involvement of NF-kappaB pathway. *J. Cell. Biochem.* 2008, *103*, 1452–1463.

[9] Leino, R. L., Gerhart, D. Z., Duelli, R., Enerson, B. E., Drewes, L. R., Diet-induced ketosis increases monocarboxylate transporter (MCT1) levels in rat brain. *Neurochem. Int.* 2001, *38*, 519–527.

[10] Hashimoto, T., Hussien, R., Oommen, S., Gohil, K., Brooks, G. A., Lactate sensitive transcription factor network in L6 cells: activation of MCT1 and mitochondrial biogenesis. *FASEB J.* 2007, *21*, 2602–2612.

[11] Kirk, P., Wilson, M. C., Heddle, C., Brown, M. H. *et al.*, CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. *EMBO J.* 2000, *19*, 3896–3904.

[12] Wilson, M. C., Meredith, D., Halestrap, A. P., Fluorescence resonance energy transfer studies on the interaction between the lactate transporter MCT1 and CD147 provide information on the topology and stoichiometry of the complex in situ. *J. Biol. Chem.* 2002, *277*, 3666–3672.

[13] Nehme, C. L., Fayos, B. E., Bartles, J. R., Distribution of the integral plasma membrane glycoprotein CE9 (MRC OX-47) among rat tissues and its induction by diverse stimuli of metabolic activation. *Biochem. J.* 1995, *310*, 693–698.

[14] Wilson, M. C., Meredith, D., Fox, J. E., Manoharan, C. *et al.*, Basigin (CD147) is the target for organomercurial inhibition of monocarboxylate transporter isoforms 1 and 4: the ancillary protein for the insensitive MCT2 is EMBIGIN (gp70). *J. Biol. Chem.* 2005, *280*, 27213–27221.

[15] König, B., Koch, A., Giggel, K., Dordschbal, B. *et al.*, Monocarboxylate transporter (MCT)-1 is up-regulated by PPARalpha. *Biochim. Biophys. Acta* 2008, *1780*, 899–904.

[16] Desvergne, B., Wahli, W., Peroxisome proliferator-activated receptors: Nuclear control of metabolism [Review]. *Endocr. Rev.* 1999, *20*, 649–688.

[17] Hihi, A. K., Michalik, L., Wahli, W., PPARs: transcriptional effectors of fatty acids and their derivatives. *Cell. Mol. Life Sci.* 2002, *59*, 790–798.

[18] Mandard, S., Muller, M., Kersten, S., Peroxisome proliferator-activated receptor alpha target genes. *Cell. Mol. Life Sci.* 2004, *61*, 393–416.

[19] Rodriguez, J. C., Gil-Gomez, G., Hegardt, F. G., Haro, D., Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. *J. Biol. Chem.* 1994, *269*, 18767–18772.

[20] König, B., Eder, K., Differential action of 13-HPODE on PPAR-alpha downstream genes in rat Fao and human HepG2 hepatoma cell lines. *J. Nutr. Biochem.* 2006, *17*, 410–418.

[21] Moya-Camarena, S. Y., Van den Heuvel, J. P., Belury, M. A., Conjugated linoleic acid activates peroxisome proliferator-activated receptor alpha and beta subtypes but does not induce hepatic peroxisome proliferation in Sprague-Dawley rats. *Biochim. Biophys. Acta* 1999, *1436*, 331–342.

[22] Moya-Camarena, S. Y., Vanden Heuvel, J. P., Blanchard, S. G., Leesnitzer, L. A., Belury, M. A., Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARalpha. *J. Lipid Res.* 1999, *40*, 1426–1433.

[23] Clarke, S. D., Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. *J. Nutr.* 2001, *131*, 1129–1132.

[24] Cohn, J. S., Oxidized fat in the diet, postprandial lipaemia and cardiovascular disease. *Curr. Opin. Lipidol.* 2002, *13*, 19–24.

[25] Koch, A., König, B., Spielmann, J., Leitner, A. *et al.*, Thermally oxidized oil increases the expression of insulin-induced genes and inhibits activation of sterol regulatory element-binding protein-2 in rat liver. *J. Nutr.* 2007, *137*, 2018–2023.

[26] Luci, S., König, B., Giemsa, B., Huber, S. *et al.*, Feeding of a deep-fried fat causes PPARalpha activation in the liver of

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pigs as a non-proliferating species. *Br. J. Nutr.* 2007, *97*, 872–882.

[27] Luci, S., Giemsa, B., Kluge, H., Eder, K., Clofibrate causes an upregulation of PPAR-{alpha} target genes but does not alter expression of SREBP target genes in liver and adipose tissue of pigs. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2007, *293*, R70–R77.

[28] Holden, P. R., Tugwood, J. D., Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences. *J. Mol. Endocrinol.* 1999, *22*, 1–8.

[29] Sehat, N., Rickert, R., Mossoba, M. M., Kramer, J. K. *et al.*, Improved separation of conjugated fatty acid methyl esters by silver ion-high-performance liquid chromatography. *Lipids* 1999, *34*, 407–413.

[30] National Research Council. *Nutrient Requirements of Swine*, 10th Revised Edn., National Academy of Sciences, Washington DC 1998.

[31] Hara, A., Radin, N. S., Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.* 1978, *90*, 420–426.

[32] Senekeo-Effenberger, K., Chen, S., Brace-Sinnokrak, E., Bonzo, J. A. *et al.*, Expression of the human UGT1 locus in transgenic mice by 4-chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid (WY-14643) and implications on drug metabolism through peroxisome proliferator-activated receptor alpha activation. *Drug Metab. Dispos.* 2007, *35*, 419–427.

[33] Pfaffl, M. W., A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 2001, *29*, e45.

[34] Podvinec, M., Kaufmann, M. R., Handschin, C., Meyer, U. A., NUBIScan, an in silico approach for prediction of nuclear receptor response elements. *Mol. Endocrinol.* 2002, *16*, 1269–1279.

[35] Nagasawa, M., Hara, T., Kashino, A., Akasaka, Y. *et al.*, Identification of a functional peroxisome proliferator-activated receptor (PPAR) response element (PPRE) in the human apolipoprotein A-IV gene. *Biochem. Pharmacol.* 2009, *78*, 523–530.

[36] Napal, L., Marrero, P. F., Haro, D., An intronic peroxisome proliferator-activated receptor-binding sequence mediates fatty acid induction of the human carnitine palmitoyltransferase 1A. *J. Mol. Biol.* 2005, *354*, 751–759.

[37] Gutgesell, A., Wen, G., Konig, B., Koch, A. *et al.*, Mouse carnitine-acylcarnitine translocase (CACT) is transcriptionally regulated by PPARalpha and PPARdelta in liver cells. *Biochim. Biophys. Acta* 2009, *1790*, 1206–1216.

[38] Ringseis, R., König, B., Leuner, B., Schubert, S. *et al.*, LDL receptor gene transcription is selectively induced by t10c12-CLA but not by c9t11-CLA in the human hepatoma cell line HepG2. *Biochim. Biophys. Acta* 2006, *1761*, 1235–1243.

[39] Krey, G., Braissant, O., L'Horset, F., Kalkhoven, E. *et al.*, Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* 1997, *11*, 779–791.

[40] Yu, Y., Correll, P. H., Vanden Heuvel, J. P., Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPAR gamma-dependent mechanism. *Biochim. Biophys. Acta* 2002, *1581*, 89–99.

[41] Koch, A., Konig, B., Luci, S., Stangl, G. I., Eder, K., Dietary oxidised fat up regulates the expression of organic cation transporters in liver and small intestine and alters carnitine concentrations in liver, muscle and plasma of rats. *Br. J. Nutr.* 2007, *98*, 882–889.

[42] Ringseis, R., Wege, N., Wen, G., Rauer, C. *et al.*, Carnitine synthesis and uptake into cells are stimulated by fasting in pigs as a model of nonproliferating species. *J. Nutr. Biochem.* 2009, *20*, 840–847.

[43] Morris, M. E., Felmlee, M. A., Overview of the proton-coupled MCT (SLC16A) family of transporters: characterization, function and role in the transport of the drug of abuse gamma-hydroxybutyric acid. *AAPS J.* 2008, *10*, 311–321.

[44] Benton, C. R., Yoshida, Y., Lally, J., Han, X. X. *et al.*, PGC-1alpha increases skeletal muscle lactate uptake by increasing the expression of MCT1 but not MCT2 or MCT4. *Physiol. Genomics* 2008, *35*, 45–54.

[45] Fanelli, A., Grollman, E. F., Wang, D., Philp, N. J., MCT1 and its accessory protein CD147 are differentially regulated by TSH in rat thyroid cells. *Am. J. Physiol. Endocrinol. Metab.* 2003, *285*, E1223–E1229.

[46] Kirat, D., Kondo, K., Shimada, R., Kato, S., Dietary pectin up-regulates monocaboxylate transporter 1 in the rat gastrointestinal tract. *Exp. Physiol.* 2009, *94*, 422–433.

[47] Gallagher, S. M., Castorino, J. J., Wang, D., Philp, N. J., Monocarboxylate transporter 4 regulates maturation and trafficking of CD147 to the plasma membrane in the metastatic breast cancer cell line MDA-MB-231. *Cancer Res.* 2007, *67*, 4182–4189.

[48] Deora, A. A., Philp, N., Hu, J., Bok, D., Rodriguez-Boulan, E., Mechanisms regulating tissue-specific polarity of monocarboxylate transporters and their chaperone CD147 in kidney and retinal epithelia. *Proc. Natl. Acad. Sci. USA* 2005, *102*, 16245–16250.

[49] Tan, N. S., Michalik, L., Desvergne, B., Wahli, W., Multiple expression control mechanisms of peroxisome proliferator-activated receptors and their target genes. *J. Steroid Biochem. Mol. Biol.* 2005, *93*, 99–105.

[50] Qi, C., Zhu, Y., Reddy, J. K., Peroxisome proliferator-activated receptors, coactivators, and downstream targets. *Cell. Biochem. Biophys.* 2000, *32*, 187–204.

[51] Sun, Y., Ng, L., Lam, W., Lo, C. K. *et al.*, Identification and characterization of a novel mouse peroxisome proliferator-activated receptor alpha-regulated and starvation-induced gene, Ppsig. *Int. J. Biochem. Cell. Biol.* 2008, *40*, 1775–1791.

[52] Mandard, S., Stienstra, R., Escher, P., Tan, N. S. *et al.*, Glycogen synthase 2 is a novel target gene of peroxisome proliferator-activated receptors. *Cell. Mol. Life Sci.* 2007, *64*, 1145–1157.

[53] Hansmannel, F., Clemencet, M. C., Le Jossic-Corcos, C., Osumi, T. *et al.*, Functional characterization of a peroxisome proliferator response-element located in the intron 3 of rat peroxisomal thiolase B gene. *Biochem. Biophys. Res. Commun.* 2003, *311*, 149–155.

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