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ORIGINAL ARTICLE

Effect of Synthetic Ligands of PPAR α, β/δ, γ, RAR, RXR and LXR on the Fatty Acid Composition of Phospholipids in Mice

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Abstract Nuclear hormone receptors are transcription factors that can be activated by nutrition-derived ligands and alter the expression of various specific target genes. Stearoyl-Coenzyme A desaturase (SCD1) converts palmitic acid (16:0) to palmitoleic acid (16:1n-7) as well as stearic acid (18:0) to oleic acid (18:1n-9). At the same time, elongase 6 (ELOVL6) elongates 16:1n-7 and 18:1n-9 to vaccenic acid (18:1n-7) and eicosenoic acid (20:1n-9). We examined how synthetic selective ligands of nuclear hormone receptors alter the gene expression of hepatic enzymes in mice. In addition, we examined how the regulation of these two enzymes influences fatty acid composition of phospholipids in liver and plasma. Mice were gavaged daily for 1 week with synthetic ligands of peroxisome proliferator-activated receptor (PPAR) α, β/δ, γ, liver X receptor (LXR), retinoic acid receptor (RAR) and retinoid-X receptor (RXR) for 1 week. Phospholipids from liver and plasma were analysed using ESI-MS/MS and GC after saponification. Hepatic gene expression of SCD1 and ELOVL6 was measured using QRT-PCR. SCD1 and ELOVL6 expression increased after the gavage of LXR and RXR ligands. The analysis of fatty acid composition of total phospholipids in plasma and liver showed increased percentage contributions of the SCD1 and ELOVL6 products 18:1n-9, 18:1n-7 and 20:1n-9 after LXR and RXR ligand application. Analysis of total phospholipids from plasma and liver revealed a significant increase in monounsaturated fatty acids bound in phosphatidylcholine (PtdCho) and lysophosphatidylcholine (PtdEtn) after LXR and RXR ligand administration. Increased hepatic gene expression of SCD1 and ELOVL6 after gavage of selective RXR or LXR ligands to mice resulted in increased concentrations of their metabolic products in phospholipids of liver and plasma.

Keywords Nuclear receptor · Monounsaturated fatty acids · Polyunsaturated fatty acid · Phospholipids · SCD1 · ELOVL6

Abbreviations

PPAR	Peroxisome proliferator-activated receptor
LXR	Liver X receptor
RXR	Retinoid X receptor
RAR	Retinoic acid receptor
MUFA	Monounsaturated fatty acid(s)
PUFA	Polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)
SCD1	Steroyl-coenzyme A desaturase
ELOVL6	Elongase 6

Introduction

Nuclear hormone receptors which can be activated by nutritional derived ligands are transcription factors that

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modulate the expression of various specific target genes at the transcriptional level. Peroxisome proliferator-activated receptor α (PPARα) is mainly expressed in the liver, skeletal and heart muscle as well as in blood vessels and the pancreas. PPARα plays an important role in fatty acid uptake, liver steatosis, cardiac lipid accumulation, and is critical for the development of atherosclerosis [1, 2]. PPARβ/δ is ubiquitously expressed, and is involved in HDL-cholesterol metabolism in obese and non-obese mice [2]. PPARγ is mainly expressed in adipose tissue, in macrophages and in low levels in the liver. It is primarily involved in fatty acid uptake and inflammation control [3, 4]. Apart from the synthetic ligands used in our study for each PPAR isoform, mainly monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were also reported to be natural activators [5].

Liver X receptor (LXR) is highly expressed in the liver, and to a lesser extent in the adipose tissue and in macrophages. LXR is a key modulator of lipid metabolism, inflammatory signalling and regulation of cholesterol efflux in macrophages. Cholesterol and its metabolites are natural ligands that can activate LXR [6]. Retinoid-X receptor (RXR) and retinoic acid receptor (RAR) are activated by vitamin A derivates like all-*trans*-retinoic acid (ATRA) and its *cis*-isomer the 9-*cis*-retinoic acid (9CRA). This activation can also take place by specific synthetic ligands. Furthermore RXR forms heterodimers with various nuclear receptors including the LXR, RAR and PPARs [3, 7], which is crucial to induce a signal transduction. Alternatively besides natural ligands also synthetic, high specific ligands for LXR, RXR, RAR and PPARs have been developed.

SCD1 is an enzyme mainly expressed in adipogenic tissues including hepatic and adipose tissue [8, 9]. SCD1 desaturates 16:0 and 18:0 to 16:1n-7 and 18:1n-9, respectively. The regulation of SCD1 and its consequences on metabolic disease has been studied in several models. The depletion of SCD1 in SCD1-knock-out mouse models reduces triglyceride synthesis in the liver [10] and further protects against induced hepatic steatosis and adiposity [11]. Elongases extend fatty acids by two additional carbon atoms. The enzyme ELOVL6 extends 16:0, 16:1n-7 and 18:1n-9 to 18:0, 18:1n-7 and 20:1n-9, respectively [12, 13]. It is mainly expressed in brown and white adipose tissue, hepatic tissue and brain [12, 14]. The gene expression of both enzymes is suggested to be regulated by the SREBP1, a transcription factor inducible by the activated nuclear hormone receptors LXR and RXR but not by other hormone receptors like PPARα and PPARγ [15–17].

This study investigated how the nuclear hormone receptors RAR, RXR, PPARs and LXR alter the gene expression of the enzymes SCD1 and ELOVL6 by the application of their specific selective ligands. Due to this alteration of the gene expression the impact on the composition of various phospholipids and the contents of the metabolic products of SCD1 and ELOVL6 had been studied in plasma and liver of mice. The activation of the nuclear hormone receptors RAR, RXR, PPARs and LXR can also occur by various semi-stable and multi-potent endogenous/nutritional-relevant ligands. An induction of these receptors regulates various nutritional relevant pathways like in the present study - the metabolism of monounsaturated and saturated fatty acids. We postulate that an alteration of gene expression of SCD1 and ELOVL6 caused by the application of different nuclear hormone receptor ligands influences the content of MUFAs/saturated fatty acids (SFAs) and therefore indirectly effects the fatty acid composition of phospholipids and membranes.

Materials and Methods

Experimental Design

Animal experiments were performed at the Laboratory Animal Core Facility at the University of Debrecen in Hungary in accordance with Hungarian ethical guidelines. Six to eight week old female C57BL6 mice, purchased from Charles River (Budapest, H), were fed for 2 weeks with chow (VRF1, Altromin, D). After the acclimatization period, animals were gavaged daily for one week with specific synthetic ligands dissolved in 25% Cremophor EL (Sigma-Aldrich, Budapest, H)/water (v/v) (Table 1). The vehicle (cremophor EL) was applied at 5 ml/kg body weight (b.w.). Rosiglitazone a PPARγ ligand was bought at Biomol (Butler Pike, USA) [18] and LG268 an RXR ligand [19] was a gift from Ligand Pharmaceuticals (San Diego, CA, USA). AM580 (RAR ligand) [20], GW7647 (PPARα ligand) [21] and GW0742 (PPARβ/δ ligand) [22] were purchased from Biotrend Chem. GmbH (Cologne, D) and T0901317 (LXR ligand) [23] from the Cayman Chemical Company (Tallinn, EST).

Table 1 Amount of ligands applied to mice by oral gavage

Receptor	Specific ligand	Daily dose
Vehicle	Cremophor/EL	5 ml/kg b.w.
PPARα	GW7647	3 mg/kg b.w.
PPARβ/δ	GW0742	5 mg/kg b.w.
PPARγ	Rosiglitazone	3 mg/kg b.w.
LXR	T0901317	20 mg/kg b.w.
RAR	AM580	10 mg/kg b.w.
RXR	LG268	30 mg/kg b.w.

Ligands: GW7647, GW0742, Rosiglitazone, T0901317, AM580 and LG268 were dissolved in 25% cremophor/water (v/v) and gavaged once a day

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Animal Study

Mice had free access to water and food over the duration of the experiment. They were kept at 22 °C room temperature with a 12 h day/night cycle. All animals were killed by anaesthesia with halothane. Blood collection was carried out by cardiac puncture. The blood was centrifuged for 10 min and plasma was stored at −80 °C. The mice were dissected, and liver samples were weighed and immediately frozen in liquid nitrogen and later stored at −80 °C.

Analysis of Composition of Fatty Acid in Total Phospholipids in Plasma by Gas Chromatography (GC)

For the analysis of plasma fatty acids, frozen plasma samples were thawed and the dipentadecanoylphosphatidylcholine (Phosphatidylcholine Dipentadecanoyl, Sigma-Aldrich, Budapest, Hungary) internal standard was added. Lipids were extracted by the addition of 3 ml chloroform and 1 ml methanol according to the method of [24]. The mixture was vortexed at 3,000 rpm for 15 min. The lower layer was then aspirated into vials and evaporated under an N2 stream. Lipid extracts were reconstituted in 70 µl chloroform and lipid classes were separated by thin layer chromatography (TLC). The solvent-mix for TLC of plasma lipids was as follows: hexane:diethyl ether:chloroform:acetic acid (21:6:3:1, v/v). The bands were stained with dichlorofluorescein, removed by scraping and transesterified in 1 ml of 3 N-HCl-methanol solution (Methanolic HCl, 3 N, Supelco, Budapest, Hungary) at 84 °C for 45 min [25]. Fatty acids were analysed by high-resolution capillary GC using a Finnigan 9001 gas chromatograph (Finnigan/Tremetrics Inc., Austin, TX, USA) with split injection (ratio 1:25), automatic sampler (A200SE; CTC Analytic, Zwingen, CH, USA) and flame ionisation detector with a DB-23 cyanopropyl column of 40 m length (J & W Scientific, Folsom, CA, USA). The temperature program was set to the following parameters: temperature of injector at 80 °C/min up to 280 °C, temperature of detector at 280 °C, temperature of column area at 60 °C for 0.2 min, temperature increase by 40 °C/min up to 180 °C, 5 min isothermal period, temperature increase by 1.5 °C/min up to 200 °C, 8.5 min isothermal period, temperature increase by 40 °C/min up to 240 °C and 13 min isothermal period. The constant linear velocity was 0.3 m/s (referred to 100 °C). Peak identification was confirmed by comparison with authentic mixtures of weighed fatty acid (FA) methyl esters (GLC-463: Nu-Chek Prep, Elysian, MN, USA; and Supelco 37 FAME Mix: Supelco, Bellefonte, PA, USA). Individual FA response factors determined from these weighed standards were used to calculate the percentage by weight for individual FA between 12 and 24 carbon atoms from the percentage of area under the curve.

RNA Isolation from Liver and QRT-PCR

Total RNA was isolated from liver and quantified by QRT-PCR (quantitative real time-PCR). In brief, samples of liver tissue (50 mg) were homogenized in Trizol (10 mg tissue/100 µl Trizol, Sigma-Aldrich, Budapest, Hungary) and extracted with chloroform (20 µl/100 µl Trizol). The aqueous phase was mixed with 700 µl of ethanol (70% v/v) and loaded on the RNA isolation column (GenElute Mammalian Total RNA Miniprep Kit, Sigma-Aldrich, Budapest, Hungary). RNA was isolated from tissues according to the given protocol of Sigma-Aldrich and eluted in nuclease free water. Concentration and purity were measured by Nanodrop (Thermo, Budapest, Hungary), while the RNA quality was examined by agarose-gel-electrophoresis (1%, Sigma-Aldrich, Hungary). cDNA was obtained by reverse transcription (10 min 25 °C, 120 min 42 °C, 5 min 72 °C) and amplified via QRT-PCR (40 cycles: 12 s 94 °C, 45 s 60 °C, 60 s 94 °C). Primer and probe for expression analysis (Taq-Man-Gene Expression Assay) as well as quantitative real-time PCR detection system (ABI-PRISM, 7900HT Sequence Detection System) were purchased from Applied Biosystems (Budapest, Hungary). The expression of genes was normalized to cyclophilin A (house-keeping gene): primer 77"+" 5′-CGA TGACGAGCCCTTGG-3′, primer 142 "−" 5′-TCTGCTG TCTTTGGAACTTTGTC-3′, probe (69+, 96+): FAM-CG CGTCTCCTTCGAGCTGTTTGCA. The amplification signal was detected and analysed by the SDS2.1 program from Applied Biosystems, Budapest, Hungary. The expression of SCD1, ELOVL6 and cyclophilin A was determined in the liver.

Analysis of Lipid Species in Plasma and Liver by ESI-MS/MS

Liver homogenate and plasma were extracted according to the procedure described by Bligh and Dyer et al. [26] in the presence of non-naturally occurring lipid species as internal standards. Lipids were quantified by electrospray ionization tandem mass spectrometry (ESI-MS/MS) in positive ion mode as described previously by Brugger et al. and Liebisch et al. [27–29]. Samples were quantified by direct flow injection analysis using the analytical setup described by Liebisch et al. [28, 29]. A precursor ion scan of *m/z* 184 specific for phosphocholine containing lipids was used for phosphatidylcholine (PtdCho), sphingomyelin (CerP_Cho) [28] and lysophosphatidylcholine (LysoPtdCho) [30]. Neutral loss scans of 141 and 185 were used for phosphatidylethanolamine (PtdEtn) and phosphatidylserine

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(PtdSer), respectively [27]. Ceramide was analyzed similarly to a previously described methodology [31] using *N*-heptadecanoyl-sphingosine as the internal standard. Quantification was achieved by standard addition calibration to liver homogenates or plasma using a number of naturally occurring lipid species for each lipid class. The following non-naturally occurring lipid species were used as internal standards: PtdCho 28:0, 44:0, LysoPtdCho 13:0, 19:0, PtdEtn 28:0, 40:0, PtdSer 28:0, 40:0. Quantification was performed by standard addition calibration to plasma and liver homogenates using several naturally occurring lipid species for each lipid class (PtdCho 34:1, 36:2, 38:4, 40:0; Sphingomyelin 16:0, 18:1, 18:0; LysoPtdCho 16:0, 18:1, 18:0; PtdEtn 34:1, 36:2, 38:4, 40:6 and PtdEtnp16:0/20:4; PtdSer 34:1, 36:2, 38:4, 40:6). All standards were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Isotopic overlap corrections of lipid species as well as data analysis by self programmed Excel Macros were performed for all lipid classes according to the principles described previously [28].

Statistical Analysis

Results were displayed as mean with standard error and statistically analysed by ANOVA followed by the Bonferroni post hoc test using the program SPSS (15.0) (SPSS Inc., Chicago, USA). Statistically significant differences were displayed at a value of $P < 0.05$.

Results

Analysis of Fatty Acids Composition in Plasma Phospholipids

Plasma phospholipids fatty acids for substrates, products and product/substrate ratios of SCD1 and ELOVL6 enzymes in the treatment groups are compared to the vehicle group in Table 2. Palmitic acid (16:0) decreased significantly in treatment groups of LXR, RAR and RXR, whereas significantly increased in the PPARα group. Stearic acid (18:0) percentages decreased significantly in the RXR group and increased significantly in the RAR group. Percentages of palmitoleic acid (16:1n-7) did not differ, whereas vaccenic acid (18:1n-7), oleic acid (18:1n-9) and eicosenoic acid (20:1n-9) percentages increased significantly in the LXR and RXR groups. The product/substrate ratios exhibited significant increases following treatment (with the exception of the 20:1n-9/18:1n-9 ratio). Specifically, a significant increase of the 16:1n-7/16:0 ratio has been observed in the LXR group, of the 18:1n-9/18:0 ratio in the PPARα, LXR and RXR groups and of the 18:1n-7/16:1n-7 ratio in PPARγ and RXR groups.

Table 2 Analysis of fatty acid composition of phospholipids in plasma of mice

	Vehicle Mean ± SD	PPAR α Mean ± SD	PPAR δ/β Mean ± SD	PPAR γ Mean ± SD	LXR Mean ± SD	RAR Mean ± SD	RXR Mean ± SD
16:0	27.129 ± 0.722	31.030 ± 0.503*	28.233 ± 0.238	26.637 ± 0.463	20.793 ± 0.424*	23.278 ± 0.247*	24.394 ± 0.126*
16:1n-7	0.430 ± 0.051	0.505 ± 0.026	0.419 ± 0.048	0.381 ± 0.032	0.647 ± 0.120	0.206 ± 0.018	0.627 ± 0.049
18:0	22.376 ± 0.591	16.728 ± 0.395	20.603 ± 0.836	21.856 ± 0.458	20.638 ± 0.590	24.762 ± 0.330*	18.644 ± 0.972*
18:1n-7	1.509 ± 0.136	1.751 ± 0.131	1.829 ± 0.168	1.697 ± 0.141	4.576 ± 0.374*	1.231 ± 0.059	5.112 ± 0.239*
18:1n-9	5.209 ± 0.207	6.221 ± 0.120	6.173 ± 0.301	4.847 ± 0.072	8.356 ± 0.281*	4.812 ± 0.208	9.515 ± 0.295*
20:1n-9	0.209 ± 0.023	0.158 ± 0.010	0.206 ± 0.010	0.158 ± 0.028	0.351 ± 0.021*	0.205 ± 0.016	0.371 ± 0.020*
18:0/16:0	0.830 ± 0.043	0.539 ± 0.012*	0.730 ± 0.029	0.821 ± 0.013	1.065 ± 0.022*	0.993 ± 0.021*	0.765 ± 0.041
18:1n-9/18:0	0.234 ± 0.014	0.373 ± 0.013*	0.305 ± 0.025	0.222 ± 0.005	0.409 ± 0.027*	0.195 ± 0.011	0.518 ± 0.034*
16:1n-7/16:0	0.016 ± 0.002	0.016 ± 0.001	0.015 ± 0.002	0.014 ± 0.001	0.032 ± 0.007*	0.009 ± 0.001	0.026 ± 0.002
18:1n-7/16:1n-7	3.607 ± 0.285	3.460 ± 0.148	4.471 ± 0.213	4.500 ± 0.261	7.894 ± 1.098*	6.168 ± 0.519	8.377 ± 0.685*
20:1n-9/18:1n-9	0.041 ± 0.005	0.025 ± 0.002	0.034 ± 0.002	0.032 ± 0.005	0.042 ± 0.002	0.043 ± 0.004	0.039 ± 0.001

Substrates, products and product/substrate ratios for steroyl-coenzyme A desaturase and elongase 6 enzymes in plasma phospholipids in mice ($n = 6$). Data are given as means ± standard error of percentage of total fatty acid and statistically compared to vehicle, *$P < 0.05$

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Hepatic Gene Expression of SCD1 and ELOVL6

Hepatic SCD1 and ELOVL6 expression significantly increased in animals gavaged with LXR and RXR ligands, while treatment with PPARα, PPARβ/δ and PPARγ ligand did not show any significant alteration. Application of an RAR ligand leads to a slight, non-significant suppression of hepatic SCD1 expression, while the expression of ELOVL6 was not effected (Fig. 1).

Analysis of Composition of Fatty Acids of PtdEtn, PtdCho and LysoPtdCho by ESI-MS/MS

Saturated and monounsaturated fatty acids of selected phospholipid classes phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn) and lysophosphatidylcholine (LysoPtdCho) were determined in plasma and liver by ESI-MS/MS and displayed in mean of % of specific lipid class related to total lipid class (Fig. 2). The composition of phosphatidylethanolamine (PtdEtn) species containing one monounsaturated fatty acid (36:1 and 34:1) was mainly influenced by the application of RXR and LXR ligand leading to an increase of bound monounsaturated fatty acids in liver tissue and plasma (Fig. 2). The oral gavage of the RAR ligand, by contrast, decreased monounsaturated fatty acids (displayed by: 32:1, 34:1) in liver and plasma. As with PtdEtn, an increase of monounsaturated fatty acids (displayed by: 32:1, 34:1 and 36:1) in PtdCho was determined in the liver and plasma after the application of RXR and LXR agonists, while the activation of RAR, PPARα and PPARγ lead to a decrease of phosphatidylcholine (PtdCho) 36:1 in the liver and plasma. Furthermore, RAR activation reduced the content of PtdCho 32:1, 34:1 and 36:1 in liver and plasma. The activation of RXR and LXR by their synthetic ligands altered the composition of lysophosphatidylcholine (LysoPtdCho) in liver and plasma in which monounsaturated fatty acids (16:1 and 18:1) were increased. 16:1 in plasma and 18:1 in liver were additionally increased by application of ligands for RXR and PPARα, respectively. A reduction of 18:1 in liver tissue and 16:1 in plasma and liver was determined after the gavage of the RAR agonist. Additionally, the composition of ceramides and sphingomyelins were investigated and remained unchanged in 16:0, 18:0, 16:1 and 18:1 species. In summary, activation of RXR and LXR by their specific ligands was found to lead to an increase of bound MUFAs.

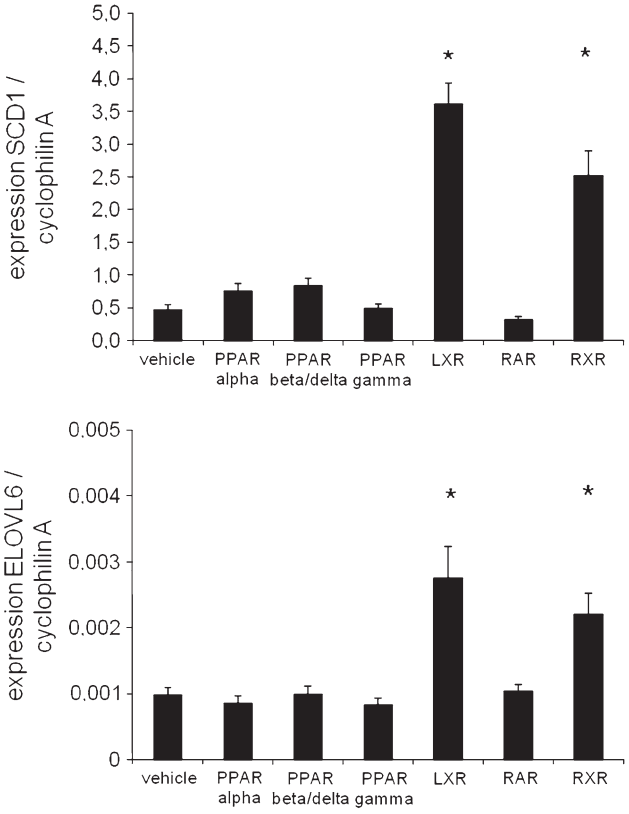

Fig. 1 Expression of SCD1 and ELOVL6 in liver of mice, $n = 6$, measured by QRT-PCR. Expression of SCD1 and ELOVL6 were normalized to cyclophilin A, mean ± standard error of gene expression were displayed and statistically analysed compared to the vehicle, $P < 0.05$(*)

Discussion

In this study, we investigated the influence of nuclear hormone receptor ligand treatment on hepatic SCD1 and ELOVL6 gene expression and the composition of SFA/MUFA bound to phospholipids in liver and plasma. We gavaged specific synthetic ligands of nuclear hormone receptors in concentrations that are able to activate these receptors regarding to previous published studies [18–23]. Our results revealed that the hepatic gene expression of SCD1 and ELOVL6 was significantly increased by LXR and RXR ligand treatment, while RAR and PPARα, β/δ and γ ligands did not significantly alter SCD1 and ELOVL6 gene expression. In addition, metabolic products of SCD1 and ELOVL6 such as 18:1n-7, 18:1n-9 and 20:1n-9 as well as product / substrate ratios 18:1n-9 / 18:0 and 18:1n-7 / 16:1n-7 was significantly increased by RXR and LXR ligand treatments, but not by PPARα, PPARβ/δ or PPARγ ligand treatment.

The nutritional impact of this study is how these nutrient-activated nuclear hormone receptors RXR, RAR, PPARs and LXR are regulating fatty acid metabolism and thereby membrane composition. The connection of food intake and nuclear hormone activations is not very deeply investigated [32, 33], so instead of semi-stable and multipotent natural ligands we used the synthetic ligands specific for these nuclear hormone receptors.

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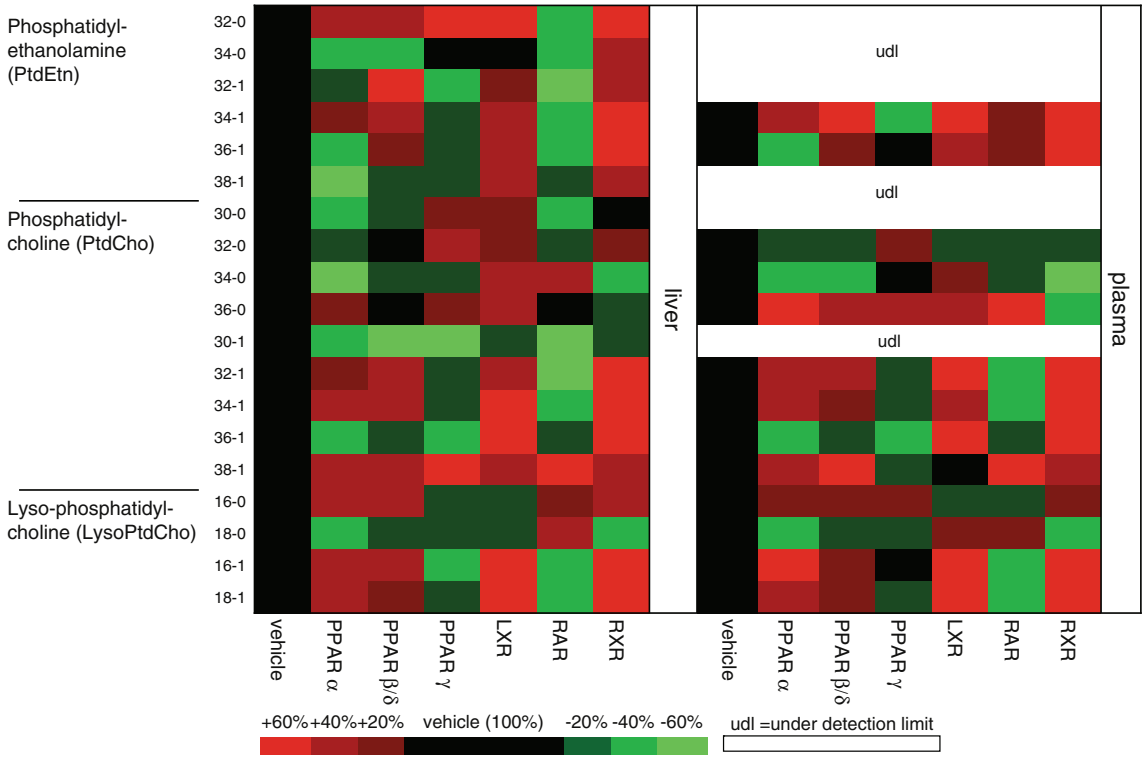

Fig. 2 Analysis of the fatty acids in PtdEtn (phosphatidylethanolamine), PtdCho (phosphatidylcholine) and LysoPtdCho (lysophosphatidylcholine) in liver and plasma of mice, $n = 6$. Percentage of total lipid class and fold change from the vehicle (=100%). *udl* under detection limit

Hepatic SCD1 expression has been extensively investigated and known to be an LXR target gene [10]. Our studies corroborate previous results in mice, namely, that the application of LXR and RXR ligands induces hepatic SCD1 expression. In these same studies, the gavage of PPARα ligands also resulted in an increased SCD1 gene expression, thereby increasing the plasma and hepatic ratios of 16:1n-7/16:0 and 18:1n-9/18:0 [34–37]. Both SCD1 and ELOVL6 are target genes of sterol regulatory element-binding protein 1 (SREBP1). It is a transcription factor inducible by the activation of LXR in hepatic tissue, adipose tissue and intestine [15, 16] and by a synthetic RXR ligand, but not by PPARα or PPARγ ligands [17]. An activation of this transcription factor pathways results in an induction of SCD1 and ELOVL6 [38]. As expected the PPARγ ligand rosiglitazone did not alter hepatic expression of ELOVL6 or SCD1 or phospholipid composition [37–39].

The role of vitamin A in the regulation of SCD1 expression remains controversial. Bioactive vitamin A metabolites like all-*trans*-retinoic acid and *9-cis* retinoic acid are natural ligands for the activation of RAR, RXR as well as PPARβ/δ mediated pathways [40]. RXR induces SCD1 gene expression, while RAR displayed tendencies to reduce it. The previously reported induction of gene expression of SCD1 by vitamin A has not been specified regarding the responsible nuclear hormone receptor RXR and RAR. RXR can form heterodimers with several nuclear hormone receptors like LXR, which are activated by metabolites of cholesterol and specific synthetic ligands like T091317 [41]. This LXR-RXR heterodimer can also be activated by the synthetic RXR ligand LG268, the potential natural ligand of RXR 9-*cis* retinoic acid or the selective ligands for LXR and result in an induced expression of LXR specific target genes [42, 43]. In summary, in our study, the activation of LXR by a synthetic LXR-ligand as well as by a synthetic RXR-ligand maybe responsible for the strong induction of the hepatic expression of SCD1 and we postulate that the RXR ligands induced effects are mediated via LXR–RXR pathways. This activation can be inhibited if other nuclear hormone receptors are activated by their ligands. PPARα and LXR are competitors to bind to RXR. This competition leads to a suppression of the SREBP-1c pathway (LXR activated) by the increased formation of PPARα/RXR and decreased availability of LXR/RXR [44].

Following LXR and RXR ligand application 18:1n-7 and 20:1n-9, metabolic products of ELOVL6, as well as product/substrate ratios of 18:1n-7/16:1n-7 but not 20:1n-9/18:1n-9 was significantly increased in plasma phospholipids. In contrast the ratio of 16:1/18:1 in liver of ELOVL6 knock-out mice increased, by reduced

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conversion of 16:1 to 18:1 compared to wild-type mice [13]. PtdCho, PtdEtn and LysoPtdCho were analysed separately, and fatty acid composition was determined in order to investigate, which specific phospholipids are influenced by nuclear receptor ligand treatments. PtdEtn and PtdCho contain two esterified fatty acids, while in LysoPtdCho only one fatty acid is contained. In our analysis of the specific phospholipid classes PtdCho and PtdEtn the two bound fatty acids were detected and resulted in an increase of monounsaturated fatty acids incorporated in PtdEtn and PtdCho after the application of LXR and RXR ligands and a tendency to decrease in liver and in plasma by the treatment with an RAR ligand. The increase could be explained by the induction of SCD1 and ELOVL6 in the liver, while the tendency of decrease may be the result of the tendency of suppressed expression of SCD1 by RAR ligand treatment.

In summary, hepatic SCD1 and ELOVL6 expression and product formation was found to be strongly influenced by the activation of LXR–RXR pathways, while RAR and PPARα, PPARγ, and PPARβ/δ pathways exerted minor influence.

Studies directly connecting the activation of nuclear hormone receptors with the formation of phospholipids, reported that the activation of LXR reduced the biosynthesis of PtdEtn by inhibiting the phosphoethanolamine cytidylyltransferase [45]. However, in our study, LXR resulted in an increased total content of PtdCho and PtdEtn in liver and in plasma. Additionally, with the administration of rosiglitazone the synthetic PPARγ ligand is thought to inhibit the formation of PtdCho and PtdEtn in a dose-dependent manner [46]. This could not be confirmed in our studies.

In conclusion, hepatic expression of SCD1 and ELOVL6 was significantly induced by LXR and RXR ligand application, while RAR, PPARα, β/δ and γ ligands did not significantly alter their gene expression. The nutritional relevance of these findings must be examined using various nutritional supplementations [47, 48], which have been shown to activate specific nuclear hormone receptor pathways to elucidate nutritional activation of gene expression pathways. Unfortunately selective activations by nutrients have been reported as difficult [49]. The MUFA incorporated into phospholipids were increased suggesting that the phospholipid MUFA and SFA compositions in plasma and liver are mainly under the control of SCD1 and ELOVL6 pathways.

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