THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Diet induced altered fatty acid composition and the activation of their metabolizing enzymes in mice

by

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ABBREVIATIONS

AA –	Arachidonic Acid
DHA –	Docosahexaenoic Acid
DPA –	Docosapentaenoic Acid
EA -	Eicosaenoic acid
ELOVL –	Elongase
EPA –	Eicosapentaenoic Acid
ESI-MS/MS –	Electrospray Ionization Mass Spectroscopy
FA –	Fatty Acid
FADS –	Fatty acid desaturase
GC –	Gas Chromatography
GLA –	γ-Linoleic Acid
LA –	Linoleic Acid
LN –	Linolenic Acid
LPC –	Lysophosphatidylcholine
LXR –	Liver X Receptor
MUFA –	Monounsaturated Fatty Acid
NHR –	nuclear hormone receptor
OA –	Oleic Acid
PA –	Palmitic Acid
PC –	Phosphatidylcholine
PCG1α –	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1α
PE –	Phosphatidylethanolamine
PL –	Phospholipid
POA –	Palmitoleic Acid
PPAR –	Peroxisome Proliferator Activating Receptor
PPRE –	Peroxisome Proliferator Response Element
PUFA –	Polyunsaturated Fatty Acid
QRT-PCR –	Quantitative Real Time Polymerase Chain Reaction
RAR –	Retinoic Acid Receptor
RXR –	Retinoid X Receptor
SA –	Stearic Acid
SAFA –	Saturated Fatty Acid
SCD1 –	Stearoyl-CoA desaturase 1 (Δ-9-desaturase)
SREBP –	Sterol Regulatory Element-Binding Protein
TF –	Transcription Factor
VA –	Vitamin A
VC –	Vaccenic Acid

CONTENT

1. INTRODUCTION

Dietary fat is a macronutrient which delivers substrates to generate metabolic energy and mediators to influence biochemical pathways regulating lipid metabolism and inflammatory responses [1]. More importantly dietary fat provides fatty acids (FA) to build and maintain cellular membranes surrounding cells and cellular compartments [2]. The main FAs incorporated into cellular membranes belong to the family of saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA) [3]. Additionally polyunsaturated fatty acids (PUFAs) can be found in cellular membranes. In this thesis the content of MUFA, SAFA and PUFA in membrane phospholipids was investigated whether determined by gene expression of specific metabolizing enzymes (SCD1, ELOVL6, FADS1, FADS2 and ELOVL5) or by diet derived FAs. Stearoyl-CoA desaturase-1 (SCD1) and elongase 6 (ELOVL6) are major metabolizing enzymes of MUFA/SAFA metabolism [4]. The regulation of their gene expression is strictly associated with the MUFA and SAFA content in phospholipids (PL) of cellular membranes. PUFAs on the other hand are metabolized by fatty acid desaturase 1 (FADS1), fatty acid desaturase 2 (FADS2) and elongase 5 (ELOVL5) but their content in PL seems mainly determined by dietary fat. This thesis aims to investigate associations of FA composition incorporated into PL to gene expression of FA metabolizing enzymes and dietary fat intake.

2. THEORETICAL BACKGROUND

Dietary fat and fatty acids are primarily known to provide energy but more importantly they are basic structures of membrane building PLs and regulators of fat metabolism. For this purpose it is important to explain first the characteristics of intervened dietary fats, their classification, regulation and functions known until now.

2.1. Dietary Fat – Sources and Subclasses of Fatty Acids

Each dietary fat is characterized by its fatty acid (FA) profile. The specific FA composition is strongly dependent on its source, which can origin from animals or plants (Table 1). FAs are grouped into several subclasses. They can be distinguished according to the number of double bounds between carbon atoms into saturated fatty acids (SAFAs) without any double bound, monounsaturated fatty acids (MUFAs) containing one double bound and polyunsaturated fatty acids (PUFAs) containing two and more double bounds (Figure 1) [2]. FAs are further classified within each subgroup depending on the number of carbons of the carbon chain as

short-chain (SCFA) <8 carbons, middle-chain (MCFA) 8-12-carbons and long-chain (LCFA) >14 carbons FAs [5]. The location of the first double bound counted from the last carbon (acid group is considered first carbon C1) indicates the family of ω– or n3-, n6-, n7- or n9-fatty acids [6, 7] (Figure 1).

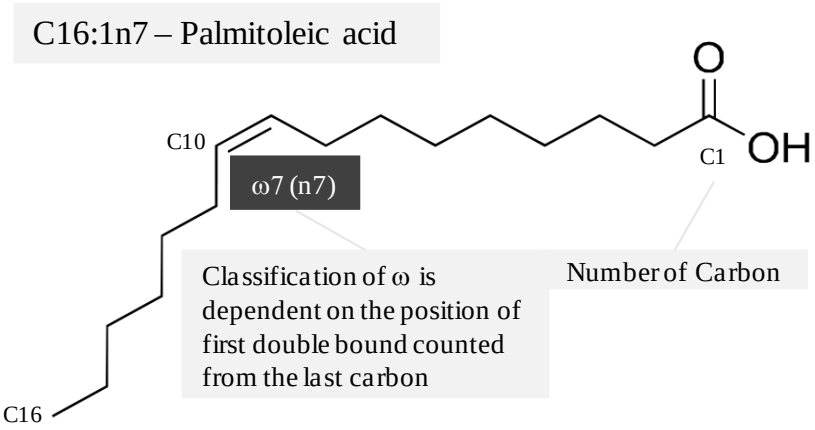

Figure 1. Classification of FAs by the example of MUFA palmitoleic acid 16:1n7 (16–number of carbons (C1-first position acid group, first carbon, C16-last position, last carbon), 1–number of double bounds, ω7 (n7)-position of double bound, 7 carbons counted from C16 [8].

fatty acid	**shorthand notation**	**natural resource**
n6-PUFAs		
linoleic acid (LA)	18:2n6	plant oils (corn oil, sunflower, soybean, safflower oil)
γ-linoleic acid (GLA)	18:3n6	evening primrose oil, borage oil, blackcurrant
arachidonic acid (AA)	20:4n6	mosses, liverwort, fern, fungus
n3-PUFAs		
α-linolenic acid (LN, ALA)	18:3n3	plants (canola, soybean, rapeseed, linseed, walnuts, flaxseed)
eicosapentaenoic acid (EPA)	20:5n3	algae, fish (salmon, white tuna, mackerel, rainbow trout, sardines), seal, higher plants
docosahexaenoic acid (DHA)	22:6n3	fish oil, brain, retina, algae, not higher plants
docosapentaenoic acid (DPA)	22:5n3	fish oil, seal oil
MUFA		
palmitoleic acid (POA)	16:1n7	major: plant oils (macadamia nut oil, sea buckthorn oil) minor: animal oils
oleic acid (OA)	18:1n9	major: olive oil, minor: animal fats, plant fats
vaccenic acid (VC)	18:1n7	major: as trans-fatty acid in dairy products (e.g. butter)
eicosenoic acid (EA)	20:1n9	plants, nuts
SAFA		
palmitic acid (PA)	16:0	major: palm oil, coconut fat, minor: olive oil
stearic acid (SA)	18:0	major: lard, milk derived fats, hydrogenated fats minor: plant fats, marine oils

Table 1. Selected FAs and their natural resources [6, 7, 9-11].

2.2. Monounsaturated (MUFA) and Saturated Fatty Acids (SAFA)

Monounsaturated fatty acids (MUFAs) – MUFAs contain one double bound which can appear in different positions. They are incorporated into cell membranes by up to 45% depending on type of tissue and cell compartment and they are therefore one of the most abundant FAs in cellular membranes [2, 12]. The most important members of the MUFA-family distributed in human cellular membranes are palmitoleic acid (POA, 16:1n7), oleic acid (OA, 18:1n9) and vaccenic acid (VC, 18:1n7) [2]. POA revealed regulatory influences on lipogenesis, desaturation and β-oxidation if added to bovine treated adipocytes [13]. Oleic acid (OA), as the main fatty acid in olive oil, contributes to health benefits such as reduction of accumulation of hepatic triglyceride and improved glucose response in insulin resistant subjects [14]. Further OA is suggested to modulate innate and adaptive immune response [15]. MUFAs are not essential. They can be synthesized endogenously by *de-novo* synthesis or obtained from diet (Table 1) [7, 10, 16]. The typical Mediterranean diet pattern contains high amounts of MUFAs due to usage of olive oil as the main dietary fat source combined with a high intake of PUFAs deriving foods like fish and vegetables [17]. Benefits for health and survival associated with this diet include all-cause mortality, the prevalence of certain chronic diseases, including cardiovascular disease (CVD), metabolic disorders and various types of cancer [18]. The fat composition of the Mediterranean diet is one of the major differences to other diet patterns such as Western diets (rich in SAFA and trans-FAs). [18].

Saturated fatty acids (SAFAs) – SAFAs are found in animal derived fats like lard and butter and in plant derived fats like coconut oil (Table 1) [19]. They are not essential and distributed in animal tissues by 30-40% in phospholipids [20]. The most common SAFAs are palmitic acid (PA, 16:0) contained in animal tissue between 15 and 25% and stearic acid (SA, 18:0) and contribute to animal tissue between 10 and 20% [20, 21]. PA (16:0) and SA (18:0) are also the most abundant SAFAs in human cellular membranes [2]. Consumption of diets high in SAFAs as in Western diet are not recommended because associations to development of metabolic and cognitive disorders, obesity and obesity related disorders were found [22-25]. Additionally diets high in SAFA are suggested to elevate blood cholesterol in humans [19] and to contribute therefore to the development of CVDs which is currently under discussion [26, 27]. Already after short term consumption of diets high in SAFA and simultaneously low in essential PUFAs a deficiency of essential fatty acid can cause skin impairments and reduced reproductive ability [28, 29]. However despite their negative health impact SAFAs are crucial for regular cell function. PA is a co-regulator of protein-membrane interactions

and subcellular trafficking of proteins by palmitoylation of proteins [20] and is an important precursor for the formation of ceramides and sphingomyelins in cellular membranes [30]. SA is a poor substrate for the cholesterol synthesis due to its preferred conversion to OA [21].

2.2.1. MUFA/SAFA metabolism

The d*e-novo* synthesis of SAFAs is strictly regulated by e.g. dietary influences [31]. However, MUFAs and SAFAs derived from diet can also directly enter into the endogenous MUFA/SAFA pool [20]. Dietary MUFAs and SAFAs are further indirectly involved in the regulation of gene expression of enzymes belonging to the *de-novo* synthesis [20, 32].

De-novo Synthesis - FAs can be endogenously synthesized from the basic C2-structure of acetyl-CoA. One carbon is added irreversibly to acetyl-CoA by acetyl-CoA-carboxylase to the product malonyl-CoA, a C3-structure. Malonyl-CoA is the precursor for FA-synthesis by the FA synthase multi-enzyme complex [21, 33]. PA (16:0, C16) is the product of this synthesis. Gene expression of enzymes involved in the *de-novo* synthesis is induced by insulin, the dietary uptake of SAFAs (until C16), high amounts of carbohydrates and cholesterol [21]. The dietary intake of PUFAs and the effect of glucagon on the other hand reduce the *de-novo* synthesis of FAs [21, 31]. Further 16:0 derived from *de-novo* synthesis or from diet can be elongated by ELOVL6 and/or desaturated by SCD1 to SA or POA (Figure 2) [21, 34]. SA is mainly further desaturated to the product OA performed by SCD1 (Figure 2) [35, 36]. Together with elongation and desaturation the function of the *de-novo* synthesis is discussed as more for maintenance of membranes and less for the refill of fat storage [33].

Dietary fat uptake – Dietary fat mainly in form of triglycerides is digested in the intestinal tract to free fatty acids, mono- and dipeptides by lipases and hydrolases [37]. Digesting fatty acids and bile salt form additionally an emulsion to enhance FA uptake into enterocyte by passive permeation or by active transport via fatty acid transport protein 4 (FATP4), fatty acid transporter (FAT/CD36) or fatty acid binding protein plasma membrane (FABPpm) [37, 38]. MUFA, SAFA and PUFA are following a similar permeation in the intestine uptake, however PUFAs require less bile salt emulsion compared to SAFAs [39]. The enterocyte forms chylomicrons containing triglycerides and cholesterol which are released into the lymph flow. Chylomicrons are then transported within the blood stream and pass peripheral organs to arrive as remnants at the liver where they are absorbed [40]. In the hepatocyte lipoproteins containing free fatty acids, cholesterol and phospholipids are formed and further released into blood stream to reach peripheral body cells. Lipoproteins such as (very low density lipoproteins (VLDL), intermediate density (IDL), low density (LDL, high density

(HDL)) are distinguished by their fat/protein ratio and differ additionally in their composition of the hydrophobic and protein parts [40]. The main function of circulating lipoproteins is the transport of fatty acids and cholesterol for distribution to peripheral cells and liver or for their removal [40].

2.2.2. MUFA/SAFA metabolizing enzymes

Stearoyl-CoA desaturase 1 (SCD1) – SCD1 (Δ-9-desaturase) belongs to the desaturase family, mainly expressed in white adipose tissue (WAT) and strongly inducible in liver [21]. SCD1 inserts an additional double bound into SAFAs and is the rate-limiting enzyme for endogenous MUFAs derived from SAFAs [3, 41]. PA and SA are main substrates which are desaturated into POA and OA respectively (Figure 2) [3, 21, 41]. SCD1-/- mice revealed a protection against high carbohydrate diet induced adiposity and hepatic steatosis, but remained insulin sensitive with high fat diet [42-44]. Furthermore FA composition of membranes in SCD1-/- mice revealed reduced SCD1 metabolic product 18:1n9 and increased SCD1 substrates 18:0 [3, 21, 33, 45]. SCD1-/- mice display further an altered not always beneficial cellular response to inflammation and stress [43]. As it was found that the resistance to obesity in SCD1-/- mice is associated with disruption of the epidermal lipid barrier causing a severe skin phenotype [46].

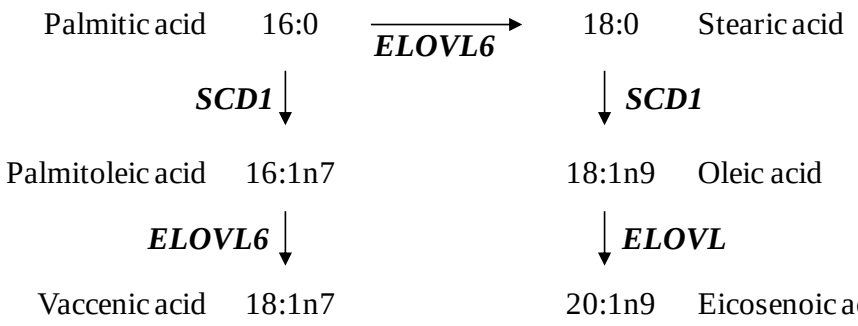

Figure 2. Metabolism of MUFAs and SAFAs by SCD1 and ELOVL6 starting from PA the product of *de-novo* FA synthesis. SCD1-Stearoyl-CoA desaturase1, ELOVL6-elongase6 [3, 21, 34, 47, 48]

Elongase 6 (ELOVL6) – ELOVL6 elongates SAFAs like PA and MUFAs such as POA and OA by two carbons [49]. The metabolic products of these reactions are SA, VC and eicosenoic acid (EA, 20:1n9) (Figure 2) [21, 49-51]. Overexpression of ELOVL6 proved a crucial function of this enzyme to provide SA which is further desaturated to OA by SCD1 [50]. On the other hand the knock-out model of ELOVL6 revealed the role of this enzyme in

MUFA/SAFA metabolism [51]. Obese ELOVL6–/– mice fed a high fat diet develop hepatosteatosis, but were protected against hyperinsulinemia, hyperglycemia, hyperleptimenia and non-alcoholic liver steatosis [34, 51, 52].

2.2.3. Specific MUFA/SAFA ratios of SCD1 and ELOVL6

Enzymatic activity of fat metabolizing enzymes was found to correlate with specific FA substrate/product ratio. The ratios of 18:1n9/18:0 and 16:1n7/16:0 describe the enzymatic activity of SCD1 known as "desaturation index" or "SCD1 index" [53]. Any alterations in gene expression pattern of SCD1 are proposed to correlate with these ratios. However, homozygote knock-out of SCD1 in mice reduced the 18:1n9/18:0 ratio only to 50% compared to heterozygotic wild type mice [45, 54]. The ratio of 16:1n7/16:0 was diminished by two third in the very same model [45, 54]. Further diet enriched by 18:1n9 increased the ratio of 18:1n9/18:0 phospholipids and ceramides while supplementation with 16:1n7 in bovine adipocyte cultures decreased 16:1n7/16:0 and 18:1n9/18:0 ratios [13, 55]. Chronic diseases like heart failure risk correlate positively with 18:1n9/18:0 while mortality cancer rates correlate positively with 16:1n7/16:0 [56, 57]. Negative correlation of 16:1n7/16:0 in PL, sterols and triglycerides was reported to the prevalence of atopic dermatitis [58].

ELOVL6 specific FA ratios 18:0/16:0 and 18:1n7/16:n7 decrease in ELOVL6-/- models due to less metabolic products of ELOVL6 18:0 and 18:1 [51, 59]. Reduction of these specific FA ratios was observed after an n3-PUFA enriched diet [34, 50]. Overexpression of ELOVL6 increased the elongation activity leading to an increase of its metabolic products [50]. This was measured for 16:0/18:0 in hepatocytes cultures in glucose rich medium [48].

Enzyme / FA ratio		Regulations and correlations
MUFA/SAFA		
SCD1	18:1n9/18:0	correlation with enzyme activity of SCD1 [45, 54, 60-62]/ reduction by supplementation with n3-PUFAs and diet rich in n3-PUFAs [55, 63, 64], by AA (n6-PUFA) [63], by 16:1n7 (MUFA) [13]/ increase by supplementation with glucose [48]/ positive correlation with BMI, waist circumference [65, 66]/ negative correlation with training, reduction with increased training [67]
	16:1n7/16:0	correlation with enzymatic hepatic activity of SCD1 [45, 54], in VLDL [61] and in SCD1-/- [68], high intake of SAFA diet [69]/ reduction by n3-PUFA enriched diet [55, 63], reduction by n6-PUFA (AA) [63]/ positive with prevalence of atopic dermatitis [58], with cancer mortality rate [57], risk for heart failure [56]
ELOVL6	18:0/16:0	correlation with gene expression and activity [48, 50, 51]/ no association in VLDL to enzyme activity [61]/ positive correlation with physical exercise [67]/ reduction by n3-PUFA rich diets [34, 50]/ increase by supplementation with glucose [48]
	18:1n7/16:1n7	correlation with enzymatic activity [50] / induction by PPARα ligand on lactate-medium in hepatocytes [48]

Table 2. Specific FA ratios and the enzymatic activity of metabolizing enzymes SCD1 and ELOVL6.

SAFAs and MUFAs are most abundant in cellular membranes. They are non-essential fatty acids and can be provided by diet or synthesized endogenously by *de-novo* synthesis. The main metabolizing enzymes of SAFA and MUFA are SCD1 and ELOVL6. The activity of these enzymes is suggested to be represented by specific fatty acid ratios which are known for SCD1 as “desaturation index”.

2.3. Polyunsaturated fatty acids (PUFAs)

PUFAs, mainly n3- and n6-PUFAs, are essential FAs which cannot be generated in humans and have to be provided by diet. A combination of several sources of dietary plant and animal fat is recommended to provide sufficient amounts of essential FAs (Table 1) [7, 9, 70-72]. Higher n3-PUFAs such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are characteristic for animal fat such as fish oil or seal fat while n6-PUFAs like linoleic acid (LA) are rather found in plant oils such as corn oil, safflower oil, soybean oil, sunflower oil and plant oil based dietary fats like margarine (Table 1) [7, 9, 10, 39]. Sunflower oil contains mainly linoleic acid (18:2n6), an n6-PUFA which is one of the main dietary fats consumed in Western diet [10, 72].

The intake of n3-PUFAs originating especially from fish oil is recommended because of their positive correlation to reduce the risk for cardiovascular diseases and their immunomodulatory function [7, 9, 10, 70, 73, 74]. However dietary oils high in n6-PUFAs like LA are preferably consumed. The imbalance of dietary n3-PUFAs and n6-PUFAs ratio is also in discussion to promote the development of chronic diseases like diabetes, cardio vascular diseases and obesity [9, 27, 75, 76]. This development can be reversed by increased intake of dietary n3-PUFAs [77]. n6-PUFA-rich dietary oils are in general considered a healthier substitute to dietary fats rich in SAFAs assuming to reduce possible health risks caused by SAFA-rich diets [6, 10, 27, 72]. Negative implications on health caused by high intake of n6-PUFAs are proposed to be related to their immunomodulatory function [76]. n3-PUFAs deliver precursors for anti-inflammatory mediators while mediators derived from n6-PUFAs are pro-inflammatory [78]. Due to the fact that n3- and n6-PUFAs compete for the same metabolic enzymes for the formation of inflammation mediators, a higher intake of n6-PUFAs leads to an increased generation of n6-PUFA derived pro-inflammatory mediators [27, 78, 79]. Therefore a general shift towards higher n6-PUFA derived inflammation responses can be observed [27, 79].

2.3.1. PUFA metabolism

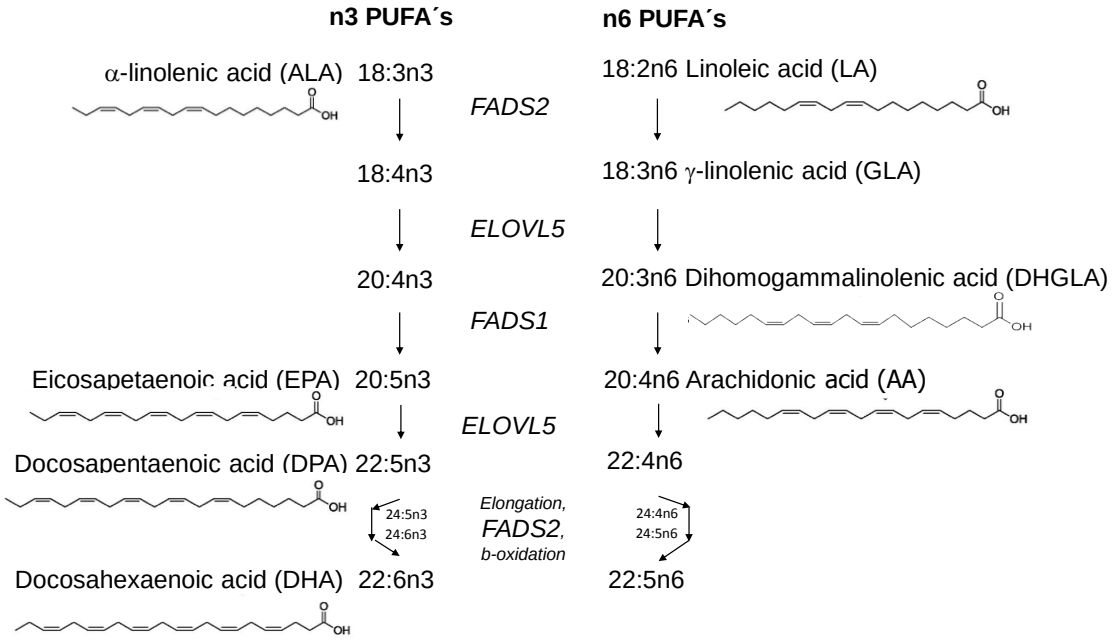

Figure 3. Metabolism of n3- and n6-PUFAs. FADS–fatty acid desaturase, ELOVL–elongase (adapted from [1, 10, 80, 81])

n3- and n6-PUFAs are mainly metabolized by fatty acid desaturase 1 (FADS1, Δ-5-desaturase), fatty acid desaturase 2 (FADS2, Δ-6-desaturase) and elongase 5 (ELOVL5) (Figure 3) [1]. The metabolism starts with the desaturation of n3-PUFA ALA and n6-PUFA LA by FADS2 (Figure 3). FADS2 desaturates ALA to 18:4n3 and LA to GLA. 18:4n3 and GLA are further elongated by ELOVL5 to 20:4n3 and DHGLA respectively followed by a desaturation by FADS1 to EPA and AA respectively. A further elongation by ELOVL5 leads to DPA in the n3-PUFA pathway and to 22:4n6 in the n6-PUFA pathway. FADS2 desaturates then DPA to DHA and 22:4n6 to 22:5n6 (Figure 3) [10, 78]. The available amounts of n3- and n6-PUFAs by dietary intake influence directly this metabolic competition [16, 76].

2.3.2. PUFA metabolizing enzymes

The enzymatic activity of FADS1, FADS2 and ELOVL5 is believed to determine partially the endogenous content of PUFAs. Also dietary supplementation with PUFAs can alter the endogenous PUFA pool. Dietary supplementation with n3-PUFA, for example, increases the content of n3-PUFAs EPA and DPA in humans derived directly from diet or by increased enzymatic activity of metabolizing enzymes [82]. On the contrary supplementation with dietary fat high in n6-PUFA LA, as typical in Western diet, did not increase the n6-PUFA metabolite AA in plasma/serum phospholipids [83, 84]. However, dietary AA supplementation did increase AA content in phospholipids in plasma of mice [85]. This

suggests a regulation of endogenous available AA derived from n6-PUFAs like LA by the metabolizing enzymes FADS1 or FADS2, which are rate limiting for the conversion of GLA to AA [83].

FADS1 – FADS1 has been found rate limiting for endogenous AA content [86]. A significant increase of 20:3n6 and reduction of 20:4n6 can be observed in phospholipids in FADS1-/- mice compared to their wild type counterparts [86]. FADS1-/- mice face additionally a short life span which can be extended comparable to wild type mice by supplementation of the FADS1 metabolic product AA [86]. In healthy subjects FADS1 activity was associated with increased EPA and DHA content in plasma. Further n3-PUFA rich diet increased FADS1 specific ratios in wild type mice compared to olive oil diet [55, 81].

ELOVL5 – ELOVL5 is crucial for the 18:4n3 content, an elongation product of 18:3n6. In ELOVL5-/- mice elongation of 18:3n6 and 18:4n3 by 88-90% was reduced [87]. 18:3n6 accumulates in liver of these mice and the elongation and desaturation product AA (20:4n6) was significantly lower. Additionally n3-PUFA downstream product DHA and EPA decreased while n3-PUFA substrates of ELOVL5 accumulated [87]. Supplementation with AA and DHA compensated the deactivated ELOVL5 enzymatic reaction in ELOVL5-/- [87]. On the other hand overexpression of ELOVL5 in mice lead to an altered SAFA and PUFA content in liver and plasma, an increase of 20:3n6 in liver and plasma and decrease of 20:4n6 and n3-PUFA pathway product 22:6n3 [59, 84]. In phenotype ELOVL5-/- mice develop hepatic steatosis [87].

FADS2 – The knock-out of FADS2 lead to a reduction of enzymatic products (n3-PUFA EPA and DHA) and higher level of substrates (n3-PUFA ALA) in phospholipids in mice receiving a canola or flaxseed diet both rich in LA [88]. Menhaden oil supplementation rich in n3-PUFAs or supplementation with DHA restored partially the liver lipid profile in auxotrophic FADS2-/- mice similar to wild type mice [88, 89]. FADS2-/- phenotype reveals further resistance to obesity and FADS2-/- male and female mice are sterile [89, 90].

2.3.3. Specific PUFA ratios for FADS1, FADS2 and ELOVL5

The most frequently reported FA ratio to reflect FADS1 enzymatic activity is 20:4n6/20:3n6 (Table 3). This ratio was reported to increase after n3-PUFA rich diet suggesting an increase of enzymatic activity in healthy subjects [55]. Inverse correlations were found for waist-hip-ratio (WHR) and body-mass-index (BMI) in healthy subjects and a positive correlation to risk cardio-vascular-diseases [65, 81].

The ratio 18:3n6/18:2n6 is frequently proposed to represent FADS2 enzymatic activity which increases with n3-PUFA enriched diet, but also reduces in healthy subjects after fish oil supplemented diet [55, 91]. Furthermore positive correlations were found for this ratio in AD patients, biomarker for diabetes and insulin resistance, biomarker indicating obesity, increased BMI and alcohol consumption but not for physical exercise [58, 66, 67, 92].

Several FA ratios are proposed to represent the enzymatic activity of ELOVL5 (Table 3). Increased ratios of 20:3n6/18:3n6, 22:4n6/20:4n6, 22:5n3/20:5n3, 20:5n3/18:3n3, 22:5n3/18:3n3, 22:5n3/20:5n3 were observed by ELOVL5 overexpression in transfection models [59, 93]. Diet related increase of FA ratios of 20:4n6/18:2n6 and 22:6n3/18:3n3 was achieved by high fat diet in liver of mice [59, 93].

Enzyme / FA ratio		Regulations and correlations
PUFAs		
FADS1	20:4n6/20:3n6	positive association with specific gene cluster for FADS1 [82], partially with SNPs of gene [94]/ decrease in triglyceride of trained rats [67]/ inverse association to BMI and WHR [65], obesity marker [66], triglyceride content and blood pressure in erythrocyte in middle-aged Korean adults [92]/ positive association with high risk for CHD [81]/ increase after n3-PUFA rich diet [55]
	20:4n6/18:2n6	higher in Caucasian females using hormone contraception [95]
	20:5n3/18:3n3	male and female similar level in Caucasians and East Asians, but higher in Caucasians female using anti-contraceptives [95]
FADS2	18:3n6/18:2n6	no correlation to training [67]/ no correlation to PUFA intake, significant increase by n3-PUFA supplementation in healthy subjects [55]/ positive correlation to BMI, waist circumference and alcohol consumption [65, 81]/ positive association in patients with atopic dermatitis [58], diabetic parameter and insulin resistance in erythrocyte in middle-aged Korean adults [92]/ significant correlation to obesity marker [66]
	20:3n6/18:2n6	positive association to FADS2 activity [82, 94]/ lower in triglycerides but higher in phospholipids of atopic dermatitis patients [58], increase in brain of patients with bipolar disorder [96]/ positive associated in hepatic PL [61]/ decrease in activity after h n3-PUFA diet [91], reduction by n3-PUFA diet [55], no change by SAFA and MUFA diet
	22:6n3/20:5n3	positive association with enzymatic activity [82, 95]
	20:5n3/18:3n3	no correlation to atopic dermatitis diseased patients [58]
	22:6n3/18:3n3	no correlation to atopic dermatitis diseased patients [58]
ELOVL5	20:3n6/18:3n6	Increased in liver of transfected mice [59]
	20:4n6/18:2n6	Increased after high fat diet [48, 93]
	22:4n6/20:4n6	increased after transfection [59]/ decreased after n3-PUFA supplementation [91]
	22:5n3/20:5n3	increased by transfection in rat primary hepatocytes [59]
	20:5n3/18:3n3	increased in liver by transfection in mice after high fat diet [93]
	22:5n3/18:3n3	increased in liver after transfection in mice after low fat and high fat diet [93]
	22:5n3/20:5n3	Increased after transfection [59]
	22:6n3/18:3n3	reverse association with obesity, increased by transfection, increases after low and high fat diet [93]

Table 3. Specific FA ratios and the enzymatic activity of the metabolizing enzymes FADS1, ELOVL5 and FADS2.

PUFAs are essential fatty acids and are in less amounts incorporated into cellular membranes compared to MUFAs and SAFAs depending on the type of tissue or organ. Endogenously they are metabolized mainly by FADS1, FADS2 and ELOVL5 to form long chain n3- and n6-PUFAS. Several fatty acid ratios are suggested to represent enzyme specific activity and are associated with different health implications.

2.4. Gene expression of fat metabolizing enzymes

MUFA and SAFA metabolizing enzymes SCD1 and ELOVL6 - Regulation of SCD1 and ELOVL6 gene expression is mediated through specific TFs like NHRs activated by dietary and synthetic ligands [3, 97]. Studies of the SCD1 mouse promoter revealed specific binding sites for SREBP-1c (Figure 5) [4]. Thus regulatory impacts on SREBP–1c alter also the gene expression of SCD1 [98, 99]. Dietary inducers of SCD1 gene expression are SAFAs, cholesterol, glucose and fasting [4, 32, 100]. A diet rich in PUFA or conjugated LA on the other hand suppresses the gene expression of SCD1 [101, 102]. This suppression is mediated through SREBP-1c shown in liver of wild type and obese mice [1, 85].

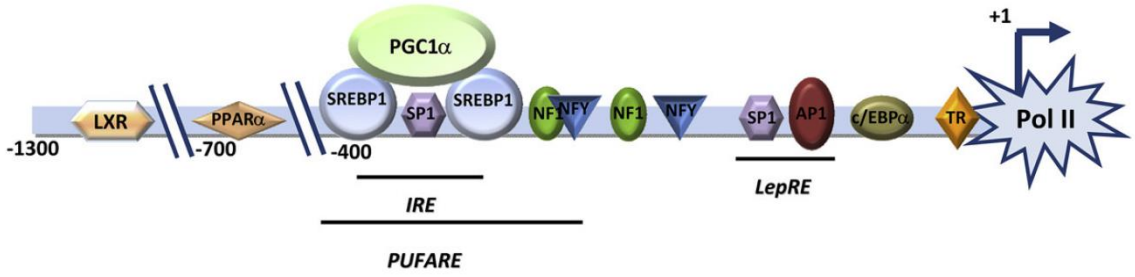

Figure 5. Promoter region of SCD1 displaying TF areas like SREBP-1c activated by PGC1α (SCD1 - Stearoyl-CoA desaturase 1, TF-transcription factor, SREBP-1c - Sterol Regulatory Element-Binding Protein-1c, PGC1α - Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1α), POL II – RNA polymerase II, PUFARE – PUFA response element, IRE – insulin response element, LepRE – leptin response element, LXR – liver X receptor, [4]

The ELOVL6 gene is also a SREBP-1c target gene [1, 42, 47, 48]. Promoter studies found SREBP-1c specific binding sequences within the ELOVL6 promoter (Figure 6) [103]. Furthermore the knock-out of ELOVL6 gene inhibits the SCD1 gene expression revealing their connection to each other [51].

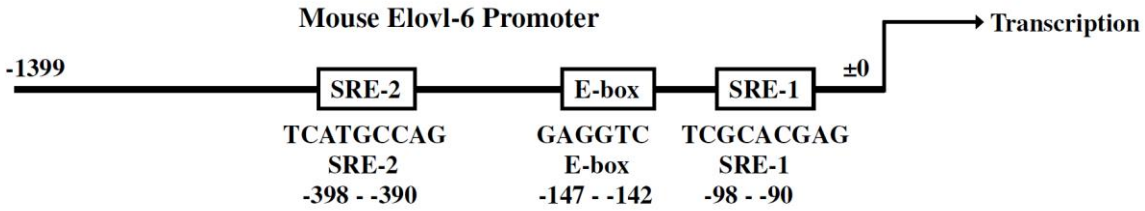

Figure 6. Scheme of the mouse ELOVL6 promoter. E-box – enhancer box, SRE - sterol response elements [103].

PUFA metabolizing enzymes ELOVL5, FADS1 and FADS2 – Genes of ELOVL5, FADS1 and FADS2 can be regulated by NHRs which act via SREBP-1c [85, 99]. FADS1 and FADS2 gene expression are induced by high carbohydrate diet or high carbohydrate diet combined with 18:1n9 and high SAFA [34, 85]. Also gene expression of ELOVL5 is reported to be induced by high carbohydrate diet [104]. Although FADS1 and FADS2 are mainly

target genes of SREBP-1c additional regulation by activation of LXRα for FADS1, FADS2 and ELOVL5 in secondary manner via the SCREBP-1a pathway has been reported [34, 104, 105]. Diets high in PUFAs like EPA, DHA and AA suppress the expression of FADS1 and FADS2 in liver assumed by the activation of PPARα [34, 106].

Sterol Regulatory Element-Binding Protein (SREBP-1c) is a common TF regulating the gene expression of MUFA/SAFA metabolizing enzymes SCD1, ELOVL6 and PUFA metabolizing enzymes FADS1, FADS2 and ELOVL5 [34, 48, 99]. SREBP-1c is mainly expressed in the liver and WAT and plays a major role in the regulation of FA, glycerolipid and cholesterol metabolism [98, 107, 108]. Induction of SREBP-1c has been shown by diets high in carbohydrates and high in SAFA while n3-PUFA supplemented diets suppress SREBP-1c and its function [98, 99, 109]. Further high cholesterol levels increase SREBP-1c activity while low intracellular cholesterol is associated with low SREBP-1c activity [108].

2.4.1. Nuclear Hormone Receptors regulated by Fatty Acids

Nuclear hormone receptors (NHRs) are transcription factors which after stimulation induce or suppress gene expression of their specific target genes [110]. Activation of NHRs can be achieved by specific synthetic ligands or by natural ligands derived from diet. FAs are potential activators for the group of NHRs peroxisome proliferator activated receptor (PPAR) (α, β/δ, γ), but also for the NHR liver-X-receptor (LXR) and several other TFs like the sterol regulatory element-binding protein (SREBPs) (Figure 4) [1, 11, 110-112]. LXR and SREBP1c can be activated by dietary SAFAs [32, 49, 113-115]. Activation of PPARα and PPARβ/δ can be achieved by SAFAs as well as by unsaturated FAs like AA (n6-PUFA) [111], while PPARγ was reported to be sensitive to PUFAs [111]. PPARα and PPARβ/δ regulate the gene expression of genes involved in fatty acid oxidation while PPARγ regulates the expression of genes important for adipogenesis and lipid storage [111, 112, 116, 117]. LXR activation alters the gene expression of genes involved in lipogenesis [118]. NHRs retinoic acid receptor (RAR) and retinoid X receptor (RXR) can be activated by FAs but remain more sensitive to forms of vitamin A [119, 120]. PUFAs like DHA, AA and DPA were found to bind to the ligand domain of the RXR [119]. RAR, PPARα, β/δ, γ and LXR are required to build heterodimers with RXR for their functionality (Figure 4) [120].

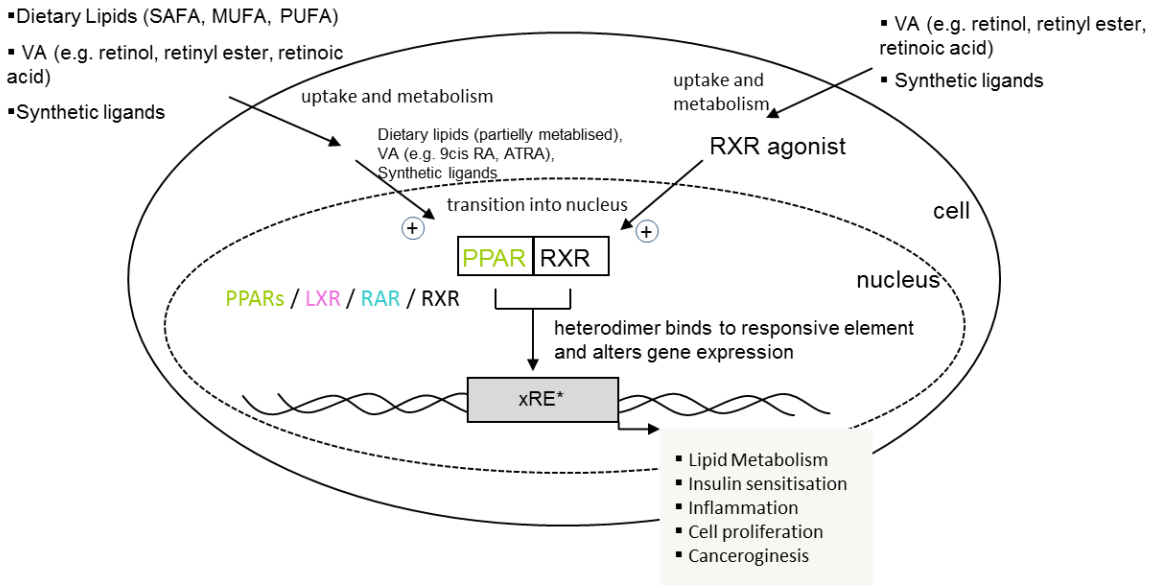

Figure 4. Signal transduction of NHRs after activation. PUFA - polyunsaturated fatty acid, PPAR - peroxisome proliferator-activated receptor, RXR – retinoid X receptor, RAR - retinoic acid receptor, RA – retinoic acid, LXR - liver X receptor, xRE – receptor specific response element, VA – vitamin A, SAFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid, (adapted from [101, 120-123])

2.4.3. NHRs and Vitamin A

Vitamin A is a fat soluble vitamin derived from plants as pro-vitamin β-carotene and from animals as retinyl-ester (RE) (Figure 7) [124]. β-carotene is cleaved in the intestine to retinal and transported as retinoic acid (RA) or retinol to the extrahepatic tissue [125]. Vitamin A is taken up in intestine as retinyl-ester and transported to periphery by chylomicrons where RE is converted to retinol [123, 126]. Bound to retinol binding protein (RBP) RE are delivered to all body cells [123]. Main metabolic active forms of vitamin A 9-cis retinoic acid (9-cis-RA) and all-*trans* retinoic acid (ATRA) are accounted for most of the physiological functions of vitamin A [110, 120, 124, 127, 128]. NHRs activated by active forms of vitamin A are retinoid X receptor (RXR) and retinoic acid receptor (RAR) [112, 117, 120, 129] (Figure 4). RAR is more sensitive to ATRA as a ligand while RXR responds to ATRA and 9-cis-RA [40, 117, 129, 130]. Recently 9-cis-13,14-dihydroretinoic acid has been presented as a more potential metabolic forms of vitamin A [131, 132]. RXR fulfils the special role of forming heterodimers with other NHRs in order to enable and support their activity (Figure 4) [120]. The regulation pathways of vitamin A-activated NHRs includes lipid metabolism and thermogenesis [40, 127]. RA content is crucial for the activity of RXR and RAR. RA is catabolized to inactive metabolites all-*trans*-4-oxo-retinoic acid and all-*trans*-4-hydroxy-retinoic acid by the enzyme cytochrome Cyp26A1. CYP26A1 plays a major role in the clearance of RA content and therefor controls the availability of active RXR ligands [133, 134].

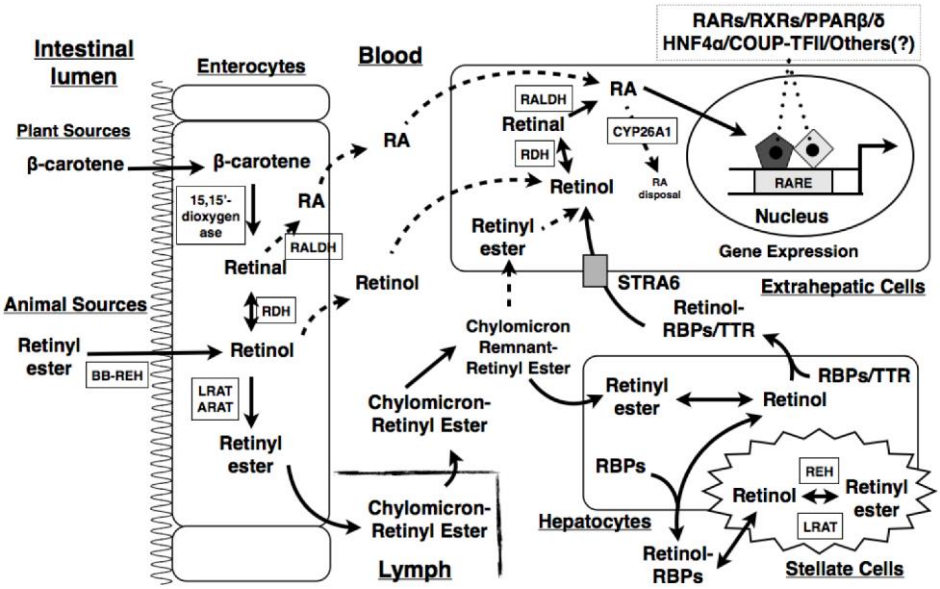

Figure 7. Scheme of Vitamin A metabolism. Uptake, distribution and secretion of vitamin A in human. RA – retinoic acid, STRA6 – stimulated by retinoic acid gene 6 protein, RDH – retinol dehydrogenase, RALDH – retinal dehydrogenase, LRAT – lecithin retinoic acyltransferase, ARAT – retinol acyltranferase, REH – retinyl ester hydrolase, RBP – retinol binding protein, TTR – transthyretin, RARE – retinoic acid response element [125].

Connecting vitamin A and fatty acid regulatory pathways by CYP26A1 - Regulatory pathways of vitamin A and FAs are crosslinked to each other by the activation of RXR (Figure 4, Figure 7). As mentioned in the previous chapter RXR is crucial to form heterodimers with other NHRs regulating fatty acid controlled target genes (Figure 4; 2.4.1.). An activation of RXR can be achieved by several forms of RA; e.g. as recently published by 9-cis-13,14-dihydroretinoic acid [131]. The clearance of RA is dependent on enzymatic activity of CYP26A1 (2.4.3.). This enzyme is induced by vitamin A but also by n3-PUFAs like DHA [135] which could lead to an increased clearance of RA. Less available RA could lead to a reduced activation of RXR which could affect the gene expression of its target genes including fat metabolizing enzymes [135].

This chapter described fatty acid controlled gene regulation of fatty acid metabolizing enzymes. The physiologic function of fatty acids to alter gene expression via activation of NHRs was explained including the important role of the SREBP as transcription factor for target genes including fat metabolizing enzymes. Crucial for this function is the heterodimerisation of NHRs with RXR. A link between vitamin A metabolism, the natural ligand for RXR, and fatty acid controlled gene expression regulation could be assumed.

2.5. Fatty Acids determine Phospholipids (PLs) and Membrane Composition

FAs fulfil one of their major roles in providing the basic structures for the construction of cellular membranes. Cellular membranes are assemblies of PLs, cholesterol and glycoproteins forming a lipid-bilayer that compartments cells and endogenous cell bodies like endoplasmic reticulum or lipoproteins [16, 35]. PLs are triglycerides esterified with FAs on two hydroxyl positions and a phosphate residue on position one, which classifies as e.g. PL-choline (Figure 8) [2, 16]. The esterified FAs represent the hydrophobic part of the PL while the phosphate residue is hydrophilic. In cellular membranes PLs form double layers consisting of two PL-layers. Hydrophobic parts of these two PLs connect leading to hydrophobic inner part while the hydrophilic part of the PL forms the outer layer of the membrane (Figure 8) [16]. PLs are grouped into several subclasses according to the structure of the phosphate-residue [2]. Phosphatidylcholine (PC), sphingomyelins and phosphatidylethanolamine (PE) are the most abundant PLs in membranes depending on the cell type [16]. Lyso-phosphatidylcholine (LPC) a derivate of PC is generated by cleavage of one FA from PC by phospholipases. Therefore LPCs contain only one esterified FA, while in other PL two FAs are found [2].

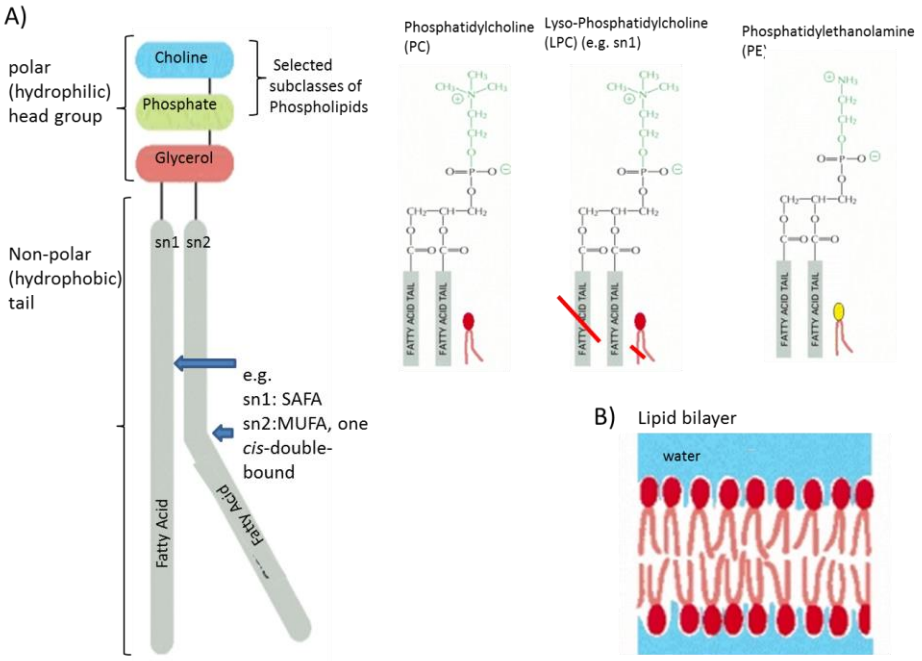

Figure 8. Structure of phospholipids A) Structure of PLs represented by phosphatidylcholine (PC), Lyso-phosphatidylcholine (LPC), phosphatidylethanolamine (PE), SAFA -saturated fatty acid, MUFA - monounsaturated fatty acid. B) Lipid bilayers the basic structure of cellular membranes and lipid micelles (modified from [2, 136])

Main FAs incorporated into PLs are MUFAs and SAFAs but also PUFAs can be found in less amounts [2, 35, 136]. The constant turnover of cellular membranes allows adaptation of their composition depending on the type of dietary fat available [70, 137, 138]. This adaption demands up to 20% of available energy for example in the brain [139]. Clinical investigations of human blood revealed that alterations of dietary fat intake are detectable in cell membranes of erythrocytes leading to an alteration of their phenotype [140]. Diets high in sunflower oil increase the content of 18:2 in PLs of cell membranes of erythrocytes whereas fish oil enriched diet increase the content of n3-PUFAs like DHA and EPA [140]. This observation are reported further in liver, muscle and brain cells [16, 77, 138]. Alteration of composition of membranes by e.g. dietary fat influence their fluidity, signal transduction, functionality of membrane bound enzymes, cellular metabolic rate and transport proteins leading to altered cellular signals but is also of further interest as biomarker for dietary fat intake and for association with development of chronic diseases [2, 10, 16, 141-144].

3. AIM OF CONDUCTED STUDIES

All conducted studies aimed to investigate the FA content in PLs depending on the fact whether they come from the diet or from hepatic gene expression of FA metabolizing enzymes. In our studies different dietary fats, dietary vitamin A but also synthetic NHR ligands were applied. To investigate the role of metabolizing enzymes on FA composition in PLs specific FA ratios for each enzyme were calculated and compared to the respective gene expression pattern.

Study 1 Influence of dietary fat – The aim of the study was to find out whether the content of MUFA, SAFA and PUFA in PLs is regulated by alterations of gene expression of FA metabolizing enzymes SCD1, ELOVL6, FADS1, FADS2 and ELOVL5. Our questions was whether do the different dietary fats affect the hepatic gene expression according to their contained FAs? Further it was investigated whether the suppression of gene expression of fat metabolizing enzymes by dietary PUFAs could be caused by vitamin A metabolism.

Study 2 Synergism of dietary fat and vitamin A – In order to answer the question if nutrients interfere with each other on similar target gene expression pattern, different amounts of dietary vitamin A capable to induce gene expression of SCD1 and different amounts of sunflower oil suppressing gene expression of SCD1 were applied. Furthermore the impact of the combined diet containing these nutrients on gene expression of SCD1 and ELOVL6 was investigated in a dose dependent manner.

Study 3 Regulation by NHRs – The aim of this study was to investigate if the synthetic ligands are capable to activate NHRs which alters gene expression of target genes like SCD1 and ELOVL6. The different regulatory impact of several NHRs, like PPARγ, PPARα, PPARβ/δ, RXR, LXR and RAR was investigated on gene expression of SCD1 and ELOVL6.

Contributions to studies conducted in this thesis

The author of this thesis designed, fully planned and performed all included studies. Analysis of fat metabolizing enzymes by QRT-PCR was conducted by the author as well as data analysis and final publication. FA analyses in these studies have been performed by co-authors of the included publication. Diet preparations as well as performance of the animal studies were supported by Dr. Johanna Mihály and Dr. Gamze Aydemir.

4. MATERIALS AND METHODS

4.1. Diet preparation

Study 1 – Influence of dietary fat - The experimental diets (Table 4) were formulated according to Bonilla et al. [145] and contained 415 g/kg diet wheat starch, 280 g/kg diet sucrose, 180 g/kg diet casein from bovine milk, 20 g/kg diet cellulose VIVAPUR. The content of minerals was 45 g/kg diet (Mineral-Spurenelemente-Vormischung C1000). The vitamin content was 10 g/kg diet (Vitamin-Vormischung C1000). The source of dietary fat was different in each experimental diet but the content was the same by adding 5 g/kg diet which is considered a normal fat content [145]. The fat sources were sunflower oil, fish oil, coconut fat and olive oil and margarine.

Ingredients	**g/kg diet**
Wheat starch	415
Sucrose	280
Casein	180
Cellulose	20
Minerals	45
Vitamins	10
Dietary fat (sunflower oil/fish oil/coconut fat/olive oil/margarine	5

Table 4. Composition of diet containing different types of dietary fat

Study 2 - Synergism of dietary fat and vitamin A - Experimental diets were prepared according to the protocol of Bonilla et al. [145]. Sunflower oil was used as dietary fat which contained 67.9% of the n6-fatty acid linoleic acid (18:2n6). Vitamin mix (Vitamin-Vormischung C1000) containing either 2,500 RE (retinol equivalents)/kg diet or no vitamin A (deficient diet) was added to diets. The high vitamin A amounts was achieved by adding 324,000 RE/kg diet of retinyl-palmitate supplement (RETPAL) per kg diet. The final vitamin A amount in diets was 0 RE in vitamin A deficient diet, 2,500 RE/kg diet vitamin A normal diet and 326,500 RE/kg diet for high vitamin A diet. The content of macronutrients in these diets was not based on an isocaloric distribution. According to the aim of the study diets containing high, normal and low fat contents of carbohydrate (sucrose) content were altered to compensate amount differences per kg diet (Table 5).

Ingredients (g/kg diet)	Diet groups				
	Low Fat (LF, 2%) VA normal	Normal Fat (NF, 5%) VA deficient	Normal Fat (NF, 5%) VA normal	Normal Fat (NF, 5%) VA high	High Fat (HF, 25%) VA normal
Starch	430	415	415	415	325
Sucrose	295	280	280	280	170
Sunflower oil	20	50	50	50	250
Casein	180	180	180	180	180
Mineral mix	45	45	45	45	45
Vitamin Mix*	10	10	10	10	10
Total VA content (RE/kg diet)	2,500	0	2,500	326,500	2,500
Caloric value (kcal /kg diet)	3800	3950	3950	3950	4950

Table 5. Diet and feeding scheme of different dietary combinations of fat (sunflower oil) and of vitamin A. *- vitamin mix in the normal fat diet group provided different amounts of vitamin A: vitamin mix without vitamin A (VA-deficient diet), vitamin mix with vitamin A containing 2,500 RE/kg retinyl-palmitate (RETPAL) (VA-normal diet), vitamin mix with high vitamin A containing in total 326,500 RE/kg (VA high diet). Sunflower oil was added as dietary fat which contained either 2% (low fat diet), 5% (normal fat diet) or 25% (high fat diet).

Study 3 - Regulation by NHRs - Mice were fed for two weeks with chow diet and had water ad libitum (VRF1, Altromin, D). After the acclimatization period, animals were gavaged daily for one week with specific synthetic ligands dissolved in 25% Cremophor EL. The vehicle (Cremophor EL) was applied at 5 ml/kg body weight (b.w.). Dosage of each synthetic ligand is listed in table 6. For activation of PPARγ the ligand rosiglitazone was used, for the activation of RXR the ligand LG268, for the activation of RAR the synthetic ligand AM580, for the activation of PPARα the synthetic ligand GW7647, for the activation of PPAR β/δ the synthetic ligand GW0742 and for the activation of LXR the synthetic ligand T0901317.

Receptor	Specific ligand	Daily dosage	Reference
Vehicle	Cremophore / EL	5 ml / kg b.w.	
PPARγ	Rosiglitazone	3 mg / kg b.w.	[146]
PPARβ/δ	GW0742	5 mg / kg b.w.	[147]
PPARα	GW7647	3 mg / kg b.w.	[148]
RAR	AM580	10 mg / kg b.w.	[149]
LXRα	T090117	20 mg / kg b.w.	[150]
RXR	LG268	30 mg / kg b.w.	[151]

Table 6. Application of specific ligands for activation of nuclear hormone receptors. Specific ligands were dissolved in 25% Cremophore / water (v/v) and gavaged to 6 female mice in each treatment group.

4.2. Animal Studies

All studies were performed *in vivo* on a mouse model. In the phase of acclimatization 6-8 weeks old C57BL6 female mice were fed for two weeks with general animal chow diet (VRF1). 8 animals were housed per cage at an average temperature of 22°C with a daily 12h light and 12h dark cycle. The animals had access to water and food ad libitum. After the two weeks of acclimatization the specific treatment or diets were applied.

Animal experiments were performed at the Laboratory Animal Core Facility of the University of Debrecen (Debrecen, H) in accordance to the ethical guidelines of Hungary.

Study 1 – Influence of Dietary Fat - Six to eight-week-old female C57BL6 mice, purchased from Charles River (Budapest, H), were fed first for two weeks with regular chow (VRF1, Altromin GmbH, Lage, D). After the acclimatization period of two weeks, the animals were divided into different feeding groups (n = 6 each group) and received for 4 weeks diets containing each a different kind of a dietary fat.

Study 2 – Synergism of dietary fat and Vitamin A - After the acclimatization period animals received a vitamin A deficient (0 RE/kg diet) diet containing 5% sunflower oil as dietary lipid for 10 weeks, which represented a diet with a normal fat content (Table 4)[145]. Animals were divided into different feeding groups (n = 6 per group) and were fed for 4 weeks with specific diets containing different amounts of dietary fat and equal amounts of vitamin A (2,500 RE/kg diet, normal vitamin A). Additionally the normal fat/normal vitamin A diet group was augmented by two other normal fat diet groups receiving different amounts of vitamin A, 0 RE/kg diet (vitamin A deficient) and 326,500 RE/kg diet (vitamin A high) (Table 5).

Study 3 - Regulation by NHRs - After the acclimatization period, animals were gavaged daily for one week with specific synthetic ligands dissolved in 25% Cremophor EL/water (v/v).

4.3. Sample Preparation and Analysis

Sample Preparation - Animals were anaesthetized by halothane and the blood was taken by cardiac puncture. Blood and organs were obtained. Organs were frozen immediately in liquid nitrogen and plasma was immediately removed and frozen after centrifuging of the blood samples at max. speed for 10 minutes. Organs and plasma were stored at -80°C.

Analysis of Gene Expression by QRT-PCR - Hepatic gene expression was measured by expression level of mRNA of the desired target gene. RNA was extracted from tissue, transcribed into cDNA and amplified by QRT-PCR.

RNA-isolation from Tissue - RNA-isolation from frozen tissue samples was performed via a combined protocol using Tri reagent and the Sigma RNA isolation kit to receive pure RNA samples free from RNAses. The frozen tissue samples weighted approximately 20 mg - 50 mg.

Homogenization: Frozen tissue samples were homogenized in 10mg/100µl of Trizol with a mechanical mill (Qiagen Tissue Lyser) for 3 minutes. After incubation for 15 minutes at RT the homogenate was centrifuged at 13000 x g for 5 minutes to remove disturbing structures of the organ. Supernatants were transferred into a 1.5 ml new reaction tube.

Extraction: RNA was extracted from the homogenate with chloroform (20µl/100µl Trizol), which was added to the homogenate, then thoroughly mixed and incubated for 3 minutes at RT. The samples were centrifuged at 13000 x g for 15 minutes and the aqueous phase was removed into a new reaction tube and mixed with 700 µl ethanol (70%).

Loading: The RNA containing ethanol mixture was loaded onto the binding column (Gen Elute Binding column) of the Sigma RNA isolation kit which was then centrifuged for 15 seconds at 13000 x g. The supernatant was discarded after the centrifugation.

Washing: For the washing of the binding membrane 500 µl of wash buffer (solution Nr. 1) was added onto the column, centrifuged at 13000 x g for 15 seconds and then the column was transferred to a new 2 ml collection tube. The column was further washed two times with 500 µl of the wash-solution (solution Nr. 2 containing 96% of ethanol), centrifuged at 13000 x g for 15 seconds.

Elution of RNA: The column was transferred to a new 2 ml collection tube, 50 µl nuclease free water was added onto the binding column. The column was then centrifuged at 13.000 x g for 1 min. The eluted RNA was stored at -70°C.

Concentration and purity of the eluted RNA was measured by NanoDrop spectrophotometer and the quality was verified by 1% agarose gel electrophoresis. Visibility of RNA under UV-light was achieved by adding ethidiumbromid to the solution.

Synthesis of cDNA - RNA was transcribed to single stranded cDNA by reverse transcriptase reaction. 3µg of RNA per sample were used to achieve an equal amount of cDNA for the QRT-PCR (Table 7). The reaction was performed in a 2720 Thermal Cycler from Applied Biosystems. The reaction was performed according to the following protocol: 10 minutes at 25°C followed by 120 minutes at 42°C and 5 minutes at 72°C. Finally the reaction was cooled down to 4°C. cDNA samples were diluted 1:10 with nuclease free water and stored at -80°C.

Compound	used concentration	volume in µl for 1 reaction
5x SSII buffer		8
DTT	0.1 M	4
dNTP	2.5 mM	8
random hexamer primer	3 µg/µl	0.2
SSIIMnlv	200U/µl	0.2
volume of Mastermix		20
RNA	3µg (final volume + water = 20)	20
final volume of sample		40

Table 7. Protocol for the synthesis of cDNA from RNA

Amplification by QRT-PCR using FAM-TAMRA. - The expressions of the house keeping gene Cyclophilin A and the target genes SCD1, ELOVL6, FADS1, FADS2 and ELOVL5 were amplified and detected with the TAMRA amplification system. Each reaction contained 5 µl Mastermix and 5 µl cDNA (Table 8). Primer and probe for expression analysis (TaqMan Gene Expression Assay) as well as quantitative real-time PCR detection system (ABI-PRISM, 7900HT Sequence Detection System) were purchased from Applied Biosystems. The QRT-PCR reaction was performed according to the following protocol: 1 min at 94°C for denaturation of double strand, 40 cycles of 12 sec at 94°C for annealing and 30 sec at 60°C for amplification. The expression of target genes was normalized to the house keeping gene cyclophilin A (house-keeping gene): +primer 77"+"5´-CGATGACGAGCCCTTGG-3´, -primer 142"-"5´-TCTGCTGTCTTTGGAACTTTGTC-3´, probe (69+, 96+): FAM-CGCGTCTCCTTCGAGCTGTTTGCA, quencher tetramethylrhodamine (TAMRA). The amplification signal was detected and analyzed via SDS2.1. Sequence Detection System Software. The quantification of the specific mRNA expression compared to the housekeeping gene was calculated by the comparative C_T method. Sequences for primer and probe of the target genes ELOVL6, SCD1, FADS1, FADS2 and ELOVL5 were purchased from Applied Biosystems, Budapest, Hungary.

Compound	used concentration	volume in µl for 1 reaction
Water		2.1
MgCl2	25 mM	1.2
10xTaq buffer		1
dNTP	2.5 mM	0.5
Taq-Polymerase	5U/µl	0.0625
primer-	100 µM	0.0375
primer+	100 µM	0.0375
Probe	20 µM	0.0625
final volume of Mastermix		5

Table 8. Protocol for the analysis via QRT-PCR

Analysis of PLs in plasma and FA composition in diet by GC – The analysis of frozen plasma (50 µl) and diet samples via GC was performed by Prof. Dr. Tamas Decsi and Dr. Tamas Marosvölgyi (University of Pecs, H).

Diet and plasma samples were analyzed by gas chromatography. For the analysis frozen samples were thawed and the pentadecanoylphosphatidylcholine as internal standard was added. Lipids were extracted by the addition of 3 ml chloroform and 1 ml methanol according to the method of Folch [152]. 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, § N, Supelco, Budapest, H) at 84°C for 45 min [153]. Fatty acids were analyzed 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 ionization 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. In study 1 all values were normalized to coconut fat (=1) to compare diet groups with each other and using coconut fat as a reference. The effect of diet on final FA profile in phospholipids was expressed as A-C and are shown in each enzyme.

Analysis of liver and plasma samples by ESI-MS/MS – PLs were analyzed in plasma and liver. Frozen tissue samples (20-50 mg) and frozen plasma samples (70 µl) were sent for the analysis of FA composition in specific PLs via ESI-MS/MS to Prof. Dr. Gerd Schmitz and PD Dr. Gerhard Liebisch (University of Regensburg, D).

Liver homogenates and plasma were extracted according to the procedure described by Bligh and Dyer et al. [154] 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. [155-157]. Samples were quantified by direct flow injection analysis using the analytical protocol described by Liebisch et al. [156, 157]. A precursor ion scan of m/z 184 specific for phosphocholine containing lipids was used for phosphatidylcholine (PC), sphingomyelin (SM) [157] and lysophosphatidylcholine (LPC) [156]. Neutral loss scans of 141 and 185 were used for phosphatidylethanolamine (PE) and phosphatidylserine (PS), respectively [157]. Ceramide was analyzed similarly to a previously described methodology [158] using N-heptadecanoyl-sphingosine as the internal standard. The following non-naturally occurring lipid species were used as internal standards: PC 28:0, 44:0, LPC 13:0, 19:0 PE 28:0, 40:0, PS 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 (PC 34:1, 36:2, 38:4, 40:0; SM d18:1/16:0, d18:1/18:1, d18:1/18:0; LPC 16:0, 18:1, 18:0; PE 34:1, 36:2, 38:4, 40:6 and PE 16:0/20:4; PS 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 [147]. Lipid species were annotated according to the recently published proposal for shorthand notation of lipid structures that are derived from mass spectrometry [159]. Glycerophospholipid annotation is based on the assumption of even numbered carbon chains only. The amounts of incorporated FAs were calculated as percentages.

4.4. Statistics

Results were displayed as mean and standard error mean. Statistical analysis was performed by Kruskal-Wallis- test followed by paired analysis in study 1 and by ANOVA followed by the Bonferroni post hoc test for study 2 and study 3 using the program SPSS (15.0) (SPSS Inc., Chicago, USA). Statistically significant differences were accepted and displayed from a value of $p < 0.05$ and $p<0.01$.

5. RESULTS

In three *in vivo* studies on mice main enzymes of the SAFA, MUFA and PUFA metabolism were studied and the consequences on fatty acid (FA) contents in phospholipids (PLs). PLs in plasma were analyzed in total in their composition by gas chromatography (GC) and for selected PLs subgroups measured in plasma in liver by electrospray ionization tandem mass spectrometry (ESI-MS/MS). The first study investigated regarding the altered gene expression of enzymes of the MUFA/SAFA and PUFA metabolism by several dietary fats. The study of PUFA rich dietary fat -sunflower oil- in combination with the micronutrient vitamin A examined the synergism of inducing and repressing signals on the gene expression of MUFA and SAFA metabolizing enzymes SCD1 and ELOVL6. Furthermore altered hepatic gene expression of SCD1 and ELOVL6 by activation of selected NHRs was studied.

5.1. Study 1 – Influence of Dietary Fat

5.1.1. MUFA/SAFA content in PLs is determined mainly by enzymatic activity of SCD1 and ELOVL6 regulated by dietary fatty acids

Fatty acid composition of diets

The composition of selected FAs in diets applied in this study was analyzed by GC (Table 9). Coconut fat diet displayed more than 90% SAFAs of all measured fatty acids, especially 16:0 (18.2%) and 18:0 (20.5%). Margarine contained a mixture of SAFA (31.3%), MUFA (44.6%) and PUFA (n6-PUFAs 19.0%, n3-PUFAs 5.03%). The characteristic FA in olive oil was the MUFA 18:1n9 (70.4%), while n6-PUFAs (10.6%) and SAFAs (15.2%) were found in less amounts. Sunflower oil mainly contained the n6-PUFA 18:2n6 (67.2%), further MUFA (20.0%) and SAFAs (11.6%). Fish oil displayed highest amounts of n3-PUFAs (37.1%) among the investigated dietary fats, mainly EPA (16.1%) and DHA (13.7%). Further 31.6% SAFA and 24.8% MUFA were measured in fish oil diet.

	COC	MAR	OLI	SUN	FIS
SAFA	93.35	31.34	15.24	11.59	31.58
16:0	18.21	21.24	11.9	6.47	20.56
18:0	20.47	3.51	2.7	4.07	4.11
MUFA	2.43	44.58	73.56	19.98	24.77
16:1n7	0.06	0.24	1.21	0.02	8.3
18:1n7	0.1	1.92	1.79	0.47	3.22
18:1n9	2.16	40.89	70.4	19.44	10.51
20:1n9	0.08	0.85	0.17	0.05	1.82
n6-PUFA	3.7	18.97	10.63	68.17	5.94
18:2n6	3.23	18.69	10.52	67.86	3.51
18:3n6	<0.001	0.01	<0.001	<0.001	0.26
20:3n6	<0.001	<0.001	<0.001	0.04	0.19
20:4n6	<0.001	0.02	<0.001	0.06	1.02
22:4n6	0.02	0.02	<0.001	<0.001	0.11
22:5n6	<0.001	0.03	<0.001	<0.001	0.46
n3-PUFA	0.32	5.03	0.49	0.20	37.14
18:3n3	0.29	4.79	0.45	0.17	1.29
18:4n3	<0.001	0.14	0.02	<0.001	3.09
20:5n3	<0.001	0.03	0.02	0.03	16.11
22:5n3	0.02	<0.001	<0.001	<0.001	2.77
22:6n3	0.02	0.02	<0.001	0	13.72

Table 9. Composition of selected fatty acids of experimental diets analyzed by GC and displayed as weight %. COC – coconut fat; MAR – margarine; OLI – olive oil; SUN – sunflower oil; FIS – fish oil

Role of SCD1 on MUFA/SAFA content

Amounts of FAs and FA ratios in diet - In all diet groups FAs of SCD1 metabolism (16:0, 16:1n7, 18:0, 18:1n9) were analyzed and normalized to the values found in coconut fat supplemented diet. Comparisons between all diet groups showed that the amount of contained 16:0 in diet was the highest in margarine (21.24%) supplemented diet and lowest in sunflower oil (6.47%) supplemented diet (Table 9, Figure 9A). Highest values of 16:1n7 were measured in fish oil (8.3%) supplemented diet and lowest in sunflower oil (0.02) supplemented diet (Table 9, Figure 9A). 18:0 was measured highest in coconut fat (20.47%) supplemented diet and lowest in olive oil (2.7%) supplemented diet (Table 9, Figure 9A). The 18:1n9 was the highest in olive oil (70.4%) supplemented diet and lowest in coconut fat (2.19%) supplemented diet (Figure 9A). The ratio of substrates and products of SCD1 was measured to be the highest in olive oil supplemented diet and lowest in coconut fat supplemented diet (Figure 9B). The ratio of 16:1n7/16:0 was calculated the highest in fish oil

supplemented diet and lowest in coconut fat supplemented diet. The ratio of 18:1n9/18:0 was highest in olive oil supplemented diet and lowest in coconut fat supplemented diet (Figure 9C).

Gene expression in liver of supplemented mice **-** The hepatic gene expression of SCD1 was highest after margarine supplemented diet and lowest after fish oil supplemented diet (Table 10, Figure 9D). Significant differences could be shown between gene expression after fish oil diet and the coconut, sunflower oil ($p<0.05$), as well as between fish oil and margarine and olive oil supplemented diet groups ($p<0.01$). Furthermore hepatic gene expression of SCD1 was significantly higher after coconut fat supplemented diet compared to olive oil supplemented diet ($p < 0.05$).

Amounts of FAs and FA ratios in phospholipids (PLs) of supplemented animals **-** In plasma PLs, 16:0 and 18:0 were found highest in PLs of animals fed fish oil (37.67%±3.89, 25.45%±7.99) supplemented diet while lowest amounts were found in animals fed a margarine (22.47%±2.37, 16.14%±4.92) supplemented diet (Table 10, Figure 9E). The highest content of 18:1n9 and 16:1n7 was measured in those fed with margarine (21.62%±6.21, 2.27%±1.73) and lowest in those following a sunflower oil (7.72%±1.14, 0.48%±0.07) supplemented diet (Table 10, Figure 9E). In PLs the ratio of substrates and products of SCD1 was highest in margarine supplemented diet fed mice and lowest after the consumption of fish oil supplemented diet (Figure 9F). The highest ratio in PLs of margarine supplemented diet group was significantly different to olive oil, sunflower oil ($p<0.05$) and fish oil supplemented diet ($p<0.01$). The lowest ratio after fish oil supplemented diet was further significantly different to coconut fat, and olive oil supplemented diet groups ($p<0.01$). Specific ratios of 16:1n7/16:0 and 18:1n9/18:0 were highest after margarine supplemented diet (Figure 9G) which was significantly different for 18:1n9/18:0 in sunflower and fish oil supplemented diet groups. Lowest ratio of 16:1n7/16:0 was calculated after consumption of sunflower oil supplemented diet which was significantly different in coconut fat ($p<0.01$) and fish oil supplemented diet group ($p<0.05$). The lowest value of 18:1n9/18:0 calculated in fish oil supplemented feeding group was significantly different in coconut fat, olive oil ($p<0.01$) and margarine supplemented diet groups (Figure 9G).

In summary, the amounts of SAFAs 16:0 and 18:0 measured in diet differed from those measured in PLs, which was true for all experimental diets. The only agreement observed between diet and PLs was for the MUFA 16:1n7 which was lowest in both diet and PLs after

sunflower oil supplemented diet. No other agreement between PLs and diet of the MUFA and SAFA composition could be found. A comparison between the hepatic gene expression to specific SCD1 ratios in diet and in PLs was only reflected in the ratio of 18:1n9/18:0 in PLs.

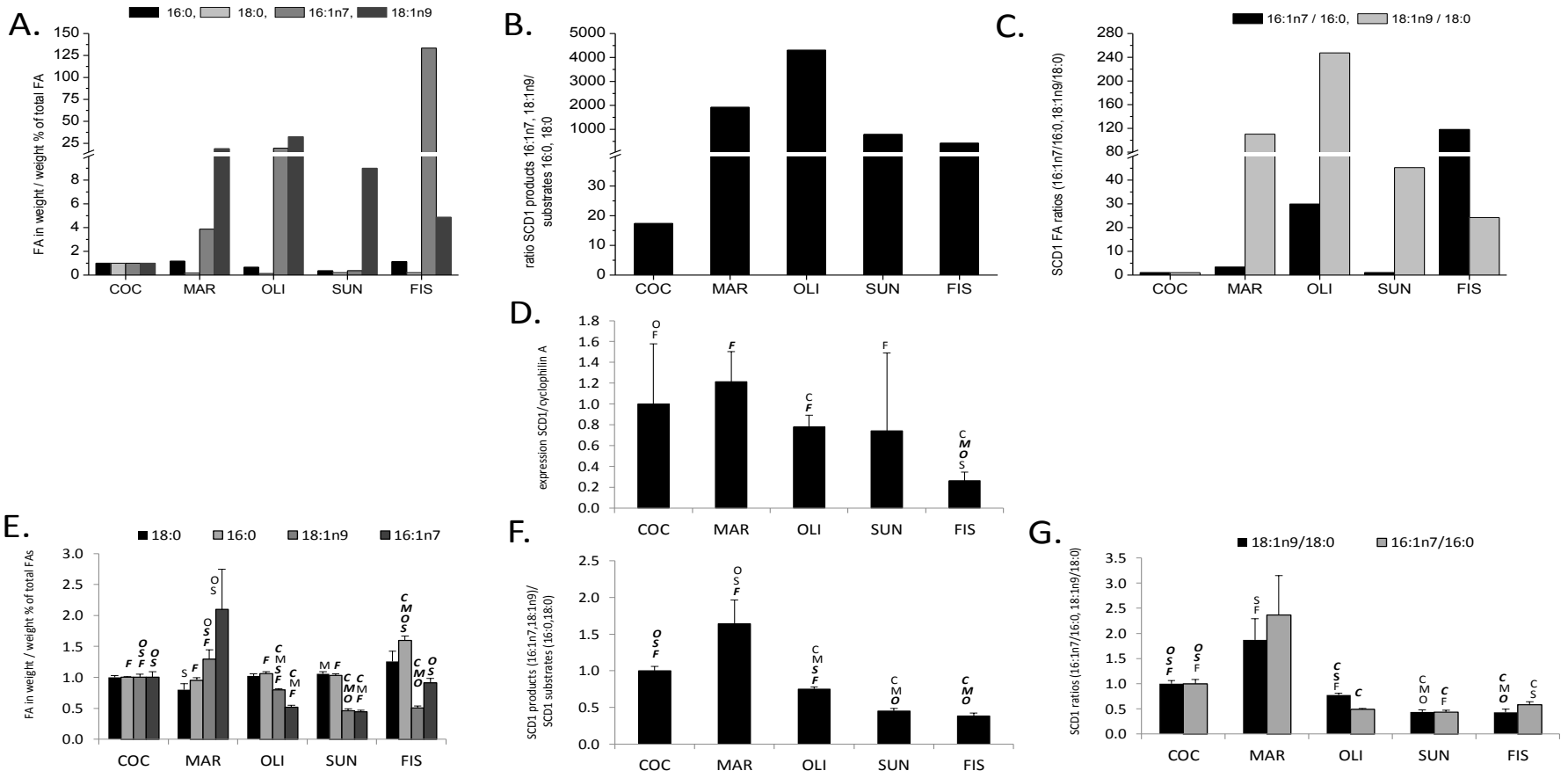

Figure 9. SCD1. A) SCD1 relevant FAs in diets, in weight/weight %, B) FA ratios (substrates and products of SCD1) in experimental diets, C) Specific SCD1 ratios of FAs in diet groups, D) Hepatic gene expression of SCD1, E) SCD1 relevant FAs in PLs, in weight/weight %, F) FA ratios (substrates and products of SCD1) in PLs of several feeding groups, G) Specific SCD1 ratios of FAs in Pls. FA composition of diet and PLs in plasma was analyzed by GC. Hepatic gene expression of SCD1 was analyzed by QRT-PCR. All values were normalized to 1 which is the coconut diet. Statistic differences were accepted for $p<0.05$ (regular font) and for $p<0.01$ (italic/bold). Each diet group was compared to all the other diet groups. Statistical differences are marked with the letter of the diet group to which it was compared. COC, C – coconut fat; MAR, M – margarine; OLI, O – olive oil; SUN, S – sunflower oil; FIS, F – fish oil

Role of ELOVL6 on MUFA/SAFA content

Amounts of FAs and FA ratios in diet - 18:1n7 (ELOVL6 product) was highest in fish oil (3.22%) supplemented diet and lowest in coconut (0.1%) supplemented diet (Table 9, Figure 10A). The ratio of substrates and products of ELOVL6 was highest in coconut fat supplemented diet and lowest in margarine supplemented diet (Figure 10B). Ratio of 18:0/16:0 was highest in coconut fat supplemented diet and lowest in margarine supplemented diet (Figure 10C). 18:1n7/16:1n7 was highest in sunflower oil supplemented diet and lowest in fish oil supplemented diet (Figure 10C).

Gene expression in liver of supplemented mice - The hepatic gene expression of ELOVL6 was significantly decreased after fish oil supplemented diet compared to margarine ($p<0.01$) and olive oil supplemented diet group ($p<0.05$). Furthermore there was a significant difference of the higher gene expression value of margarine diet group compared to the lower gene expression measured in olive oil diet group ($p<0.05$, Table 10, Figure 10D).

Amounts of FAs and FA ratios in phospholipids (PLs) of supplemented animals - Amounts of 18:0, 16:0 and 16:n7 in PLs were described previously in SCD1. 18:1n7 was highest in PLs in coconut fat (4.95%±0.33) supplemented diet group which was significantly different to margarine ($p<0.05$) and other diet groups ($p<0.01$). 18:1n7 was lowest in fish oil feeding group (1.61%±0.22) and was significantly different to coconut fat, margarine and olive oil diet group ($p<0.01$) (Table 10, Figure 10E). Ratio of substrates and products of ELOVL6 in PLs was highest in coconut fat supplemented diet group (significantly different in margarine and fish oil supplemented diet group, $p<0.05$) and lowest in PLs of fish oil supplemented diet group (significant different to coconut and sunflower oil supplemented diet group, $p<0.05$) (Figure 10F). Ratio of 18:0/16:0 was highest in PLs in sunflower oil feeding group and lowest in PLs in fish oil diet group (Figure 10G). 18:1n7/16:1n7 in PLs was highest after olive oil supplemented diet which was significant different to margarine ($p<0.05$) and fish oil ($p<0.01$) diet group and lowest in fish oil supplemented diet group which was significantly different in coconut, olive oil and sunflower oil diet group ($p<0.01$, Figure 10G).

In summary, single fatty acids (16:0, 18:0, 16:1n7, 18:1n7) involved in the ELOVL6 metabolic pathway measured in diet did not reflect the values in PLs. The enzyme specific ratios of fatty acids in PLs did not reflect the hepatic gene expression of ELOVL6 except for the fish oil fed group which showed lowest values of these ratios together with hepatic gene expression.

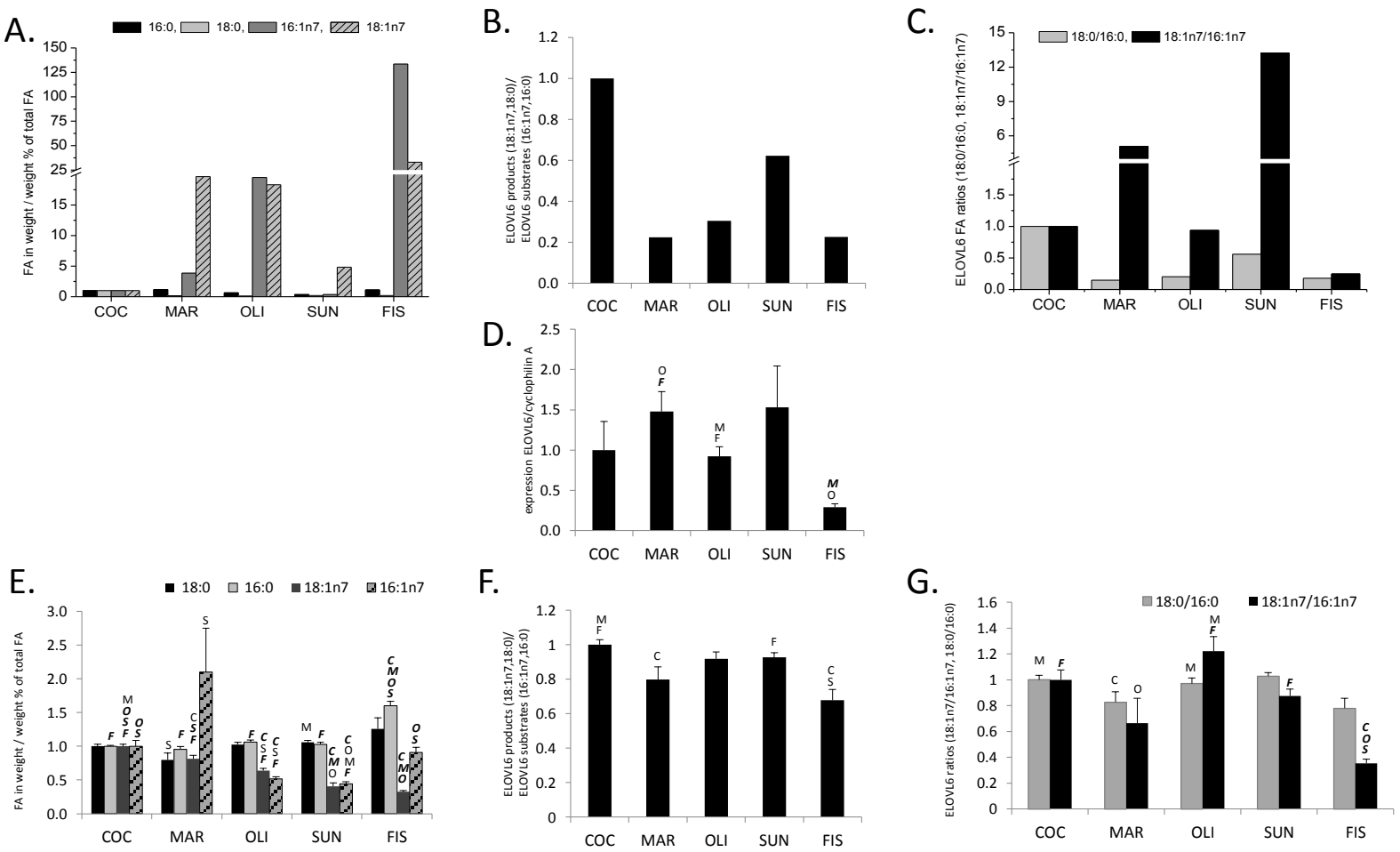

Figure 10. ELOVL6. A) ELOVL6 relevant fatty acids in diets, in weight/weight %, B) FA ratios (substrates and products of ELOVL6) in diets of several feeding groups, C) Specific ELOVL6 ratios of FAs in diet groups, D) Hepatic gene expression of ELOVL6, E) ELOVL6 relevant FAs in PLs, in weight/weight %, F) FA ratios (substrates and products of ELOVL6) in PLs of several feeding groups, G) Specific ELOVL6 ratios of FAs in PLs FA composition of diet and PLs in plasma was analyzed by GC. Hepatic gene expression of ELOVL6 was analyzed by QRT-PCR. All values were normalized to 1 which is the coconut diet. Statistic differences were accepted for $p<0.05$ (regular font) and for $p<0.01$ (italic/bold). Each diet group was compared to all the other diet groups. Statistical differences are marked with the letter of the diet group to which it was compared. COC, C – coconut fat; MAR, M – margarine; OLI, O – olive oil; SUN, S – sunflower oil; FIS, F – fish oil

5.1.2. FADS1, FADS2 and ELOVL5 influence PUFA content only marginal, mainly dietary PUFAs determine PUFA content in PLs

Role of FADS1 and FADS2 on PUFA content

Amounts of FAs and FA ratios in diet. The highest amounts of 18:2n6 measured 67.86% were found in sunflower oil supplemented diet while 18:3n6 (0.26%) and 20:4n6 (1.02%) were highest in fish oil (0.26%, 1.02%) supplemented diet (Table 9, Figure 11A). The FAs 20:3n6 (0.19%), 20:5n3 (16.11%), 22:5n3 (2.77%) and 22:6n3 (13.72%) were found to be the highest in fish oil supplemented diet (Table 9, Figure 11A). The margarine supplemented diet displayed 18:3n3 (4.79%) in the highest amount. Contrary, the lowest amounts of 18:3n3 (0.17%) were measured in sunflower oil supplemented diet while 20:5n3 (<0.001%) was lowest in coconut fat supplemented diet (Table 9, Figure 11A). The specific FADS1 ratio 20:4n6/20:3n6 was highest in fish oil supplemented diet (Figure 11B) and lowest in olive oil supplemented diet (Figure 11B). FADS2 specific FA ratios 18:3n6/18:2n6 and 22:6n3/22:5n3 were highest in fish oil supplemented diet (Figure 11B) and lowest in sunflower oil diet. Ratios of 20:4n6/18:2n6 and 20:5n3/18:3n3 were highest in fish oil supplemented diet. 20:4n6/18:2n6 was lowest in sunflower oil supplemented diet and 20:5n3/18:3n3 was lowest in margarine diet (Figure 11B).

***Gene expression in liver of supplemented mice*.** The hepatic gene expression of FADS1 was significantly reduced in animals fed fish oil supplemented diet compared to other diets ($p<0.01$, Table 10, Figure 11C). Hepatic gene expression of FADS2 was significantly reduced in animals fed fish oil supplemented diet compared to other diets ($p<0.01$) (Table 10, Figure 11C). Further hepatic gene expression of FADS2 in coconut fat supplemented diet was also significantly increased compared to olive oil supplemented diet group ($p<0.01$) (Table 10, Figure 11C).

Amounts of FAs and FA ratios in phospholipids (PLs) of supplemented animals. The FAs 18:2n6 (19.07%±0.61), 18:3n6 (0.17%±0.01) and 20:4n6 (14.69%±0.34) were found in highest amounts in PLs of the sunflower oil supplemented animals which were significantly different to other diet groups for 18:2n6 and 20:4n6 ($p<0.01$, Table 10, Figure 11D). Highest 20:5n3 (6.83%±1.71), 22:5n3 (0.60%±0.16) and 22:6n3 (5.63%±1.44) were measured in PLs of fish oil supplemented diet group (20:5n3 significantly different to coconut, margarine and olive oil supplemented diet group ($p<0.05$, Table 10, Figure 11D). 20:3n6 (2.88%±0.10) was

highest in olive oil supplemented diet group significantly different to sunflower and fish oil supplemented diet group ($p<0.01$), while 18:3n3 (0.23%±0.05) was significant to other diet groups highest in PLs of the margarine feeding group ($p<0.05$, Table 10, Figure 11D). Lowest amounts of 18:2n6 (4.06%±0.58), 20:3n6 (0.46%±0.08) and 20:4n6 (3.22%±0.65) in PLs of fish oil diet group significantly different to other diet groups ($p<0.01$, Table 10, Figure 11D). 18:3n6 (0.05%±0.02) lowest in PLs of fish oil diet group was significant different to margarine a sunflower oil diet group ($p<0.05$). 20:5n3 (0.02%±<0.01) and 22:6n3 (2.76%±0.11) were lowest in PLs after sunflower oil supplemented diet which was significantly different to other diet groups ($p<0.05$). 22:5n3 (0.09%±0.10) was lowest in sunflower oil diet group significantly different to coconut fat and olive oil diet group ($p<0.05$) and further to margarine ($p<0.01$). 18:3n3 (0.05%±<0.01) was measured lowest in PLs in the olive oil supplemented diet group significant to coconut and margarine supplemented diet group ($p<0.05$). A reduced FADS1 specific ratio of 20:4n6/20:3n6 was observed in PLs of animals fed a coconut supplemented diet significant to sunflower and fish oil diet ($p<0.01$). FADS2 specific ratio 18:3n6/18:2n6 was non-significantly lowest in olive oil supplemented diet group and highest in fish oil diet group. 22:6n3/22:5n3 was lowest in PLs after fish oil supplemented diet significant to all other diet groups ($p<0.01$) (Figure 11E). Highest 22:6n3/22:5n3 was observed in coconut fat diet group which was significant to fish oil diet group ($p<0.01$) (Figure 11E). 20:4n6/18:2n6 was non-significantly lowest in margarine supplemented diet group and highest in coconut diet group (Figure 11E). 20:5n3/18:3n3 was lowest in PLs in sunflower oil supplemented diet group significantly different to margarine ($p<0.01$) and the other diet groups ($p<0.05$) and highest in fish oil diet group significantly different to sunflower oil diet group ($p<0.05$) and the other investigated diet groups ($p<0.01$) (Figure 11E).

In summary, 18:2n6 was measured highest in sunflower oil supplemented diet as well as in PLs of this diet group. 20:5n3, 22:5n3 and 22:6n3 were highest in diet and in PLs of the fish diet supplemented group. In similar fashion, 18:3n3 was highest in diet and in PLs of margarine supplemented diet group. FADS1 specific FA ratio calculated in diet did not reflect a similar pattern in PLs. Ratio of 20:4n6/20:3n6 is comparable in diet and PLs in fish oil diet group. FADS2 specific FA ratios showed that 18:2n6/18:3n6 were highest in fish oil supplemented diet also calculated highest in PLs in the fish oil supplemented diet group. Further 20:5n3/18:3n3 highest in fish oil diet group was also highest in PLs. Comparing the hepatic gene expression with specific enzyme ratios in PLs there was an agreement to FADS2

specific FA ratio of 22:6n3/22:5n3. This was lowest in PLs of fish diet group in agreement with lowest gene expression of FADS2 in this diet group.

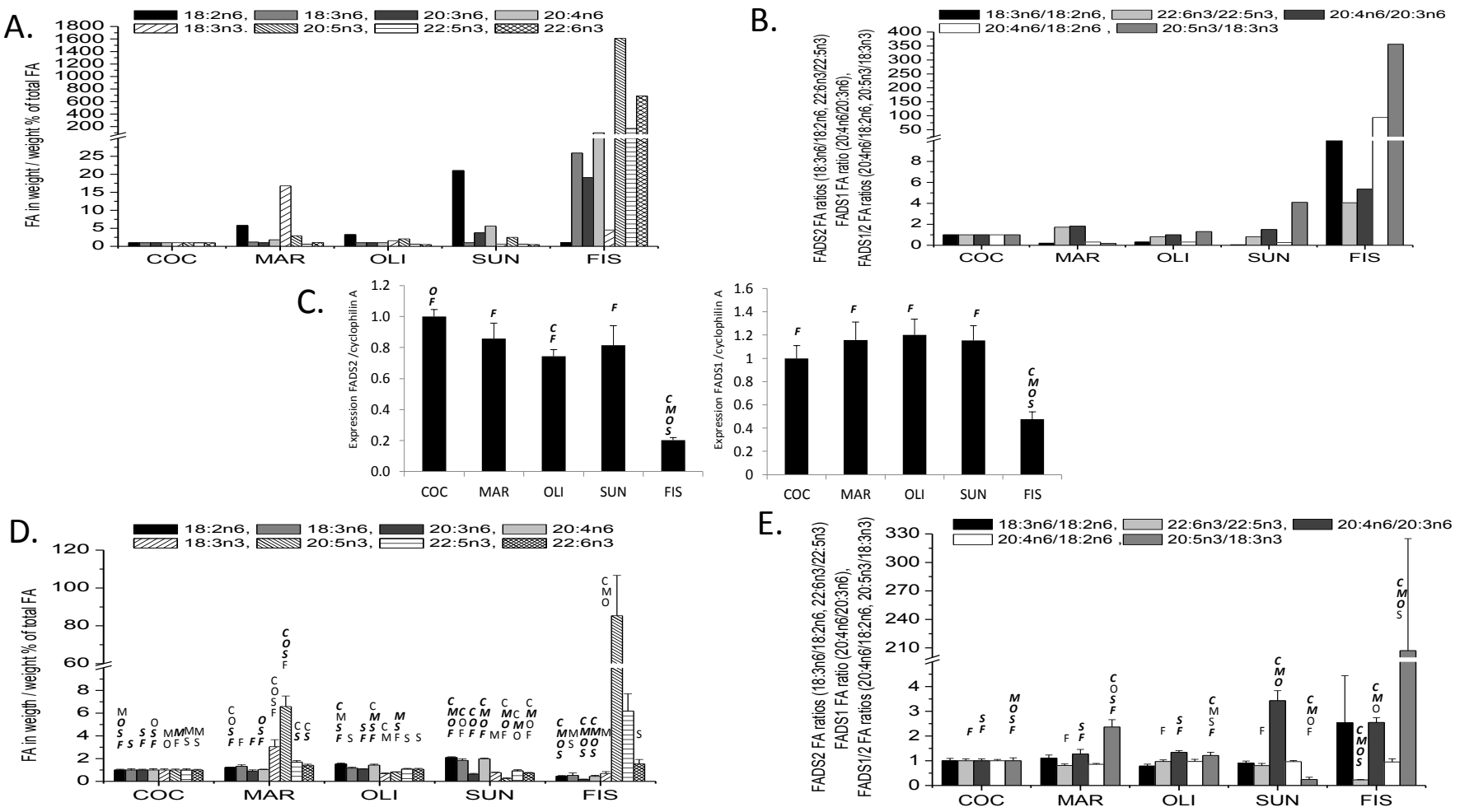

Figure 11. FADS1 and FADS2. A) Relevant FAs in diets, in weight/weight %, B) Specific FADS1 and FADS2 FA ratios in diet groups, C) Hepatic gene expression of FADS1 and FADS2, D) Relevant FAs in PLs, in weight/weight %, E) Specific FADS1 and FADS2nFA ratios in PLs. FA composition of diet and PLs in plasma was analyzed by GC. Hepatic gene expression of FADS1 and FADS2 was analyzed by QRT-PCR. All values were normalized to 1 which is the coconut diet. Statistic differences were accepted for $p<0.05$ (regular font) and for $p<0.01$ (italic/bold). Each diet group was compared to all the other diet groups. Statistical differences are marked with the letter of the diet group to which it was compared. COC, C – coconut fat; MAR, M – margarine; OLI, O – olive oil; SUN, S – sunflower oil; FIS, F – fish oil

Role of ELOVL5 on PUFA content

Amounts of FAs and FA ratios in diet - 18:3n6, 20:4n6, 20:5n3 and 22:5n3 were previously described. 22:4n6 (0.11%) were highest in fish oil supplemented diet (Table 9, Figure 12A). Combined calculated ratio of ELOVL5-specific products vs substrate ratios was lowest in fish oil supplementation diet and highest in coconut fat supplementation diet (Figure 12B). Specific ratio of 20:3n6/18:3n6, 22:4n6/20:4n6 and 22:5n3/20:5n3 were lowest in fish oil supplemented diet (Figure 12C). 20:3n6/18:3n6 was highest in sunflower oil supplemented diet while 22:4n6/20:4n6 and 22:5n3/20:5n3 were highest in coconut fat supplemented diet (Figure 12C).

Gene expression in liver of supplemented mice - The hepatic gene expression of ELOVL5 was significantly decreased after fish oil supplemented diet compared to other diets ($p<0.05$) (Table 10, Figure 12D). A significant decrease of hepatic gene expression was also found after coconut fat supplemented diet compared to olive oil diet ($p<0.05$) (Table 10, Figure 12D).

Amounts of FAs and FA ratios in phospholipids (PLs) of supplemented animals - 18:2n6, 18:3n6, 20:4n6, 20:5n3 and 22:5n3 were previously described. 22:4n6 (0.28%±0.02) was highest in PLs in sunflower oil supplemented diet group (significant to other diet groups, $p<0.01$) (Table 10, Figure 12E). The lowest value of 22:4n6 (0.11%±0.02) in PLs was measured in margarine supplemented diet group significantly different to coconut, olive oil and sunflower oil supplemented diet group ($p<0.01$) (Table 10, Figure 12E). Ratio of substrates and products was lowest in PLs of the sunflower oil supplemented diet group which was significantly different to margarine, coconut and olive oil supplemented diet group ($p<0.05$) and highest in the coconut fat supplemented diet group significantly different to sunflower ($p<0.01$) and olive and fish oil supplemented diet group ($p<0.01$) (Figure 12F). Specific ratios of 20:3n6/18:3n6, 22:4n6/20:4n6 and 22:5n3/20:5n3 were calculated in PLs (Figure 12G). 20:3n6/18:3n6 was lowest after sunflower oil supplemented diet (significantly different to coconut and olive oil supplemented diet group, $p<0.01$) and highest after coconut fat supplemented diet (significant different to sunflower oil supplemented diet, $p<0.01$ and fish oil diet group, $p<0.01$). 22:4n6/20:4n6 was lowest after margarine (significant different to olive oil supplemented diet, $p<0.01$) and highest after fish oil supplemented diet. 22:5n3/20:5n3 was lowest after fish oil supplemented diet which was significantly different

to coconut fat, margarine and olive oil supplemented diet group ($p<0.01$) and highest after sunflower oil supplemented diet (Figure 12G).

Substrate and product ratio of ELOVL5 was highest in diet and in PLs of coconut fat supplemented diet group. Only the enzyme specific ratio of ELOVL5 22:5n3/20:5n3 was lowest in fish oil supplemented diet and in PLs of animals fed a fish oil supplemented diet. Comparing the hepatic gene expression to enzyme specific ratios in PLs only the lowest amount of 22:5n3/20:5n3 in fish supplemented diet group agreed with the lowest gene expression of ELOVL5 in this feeding group.

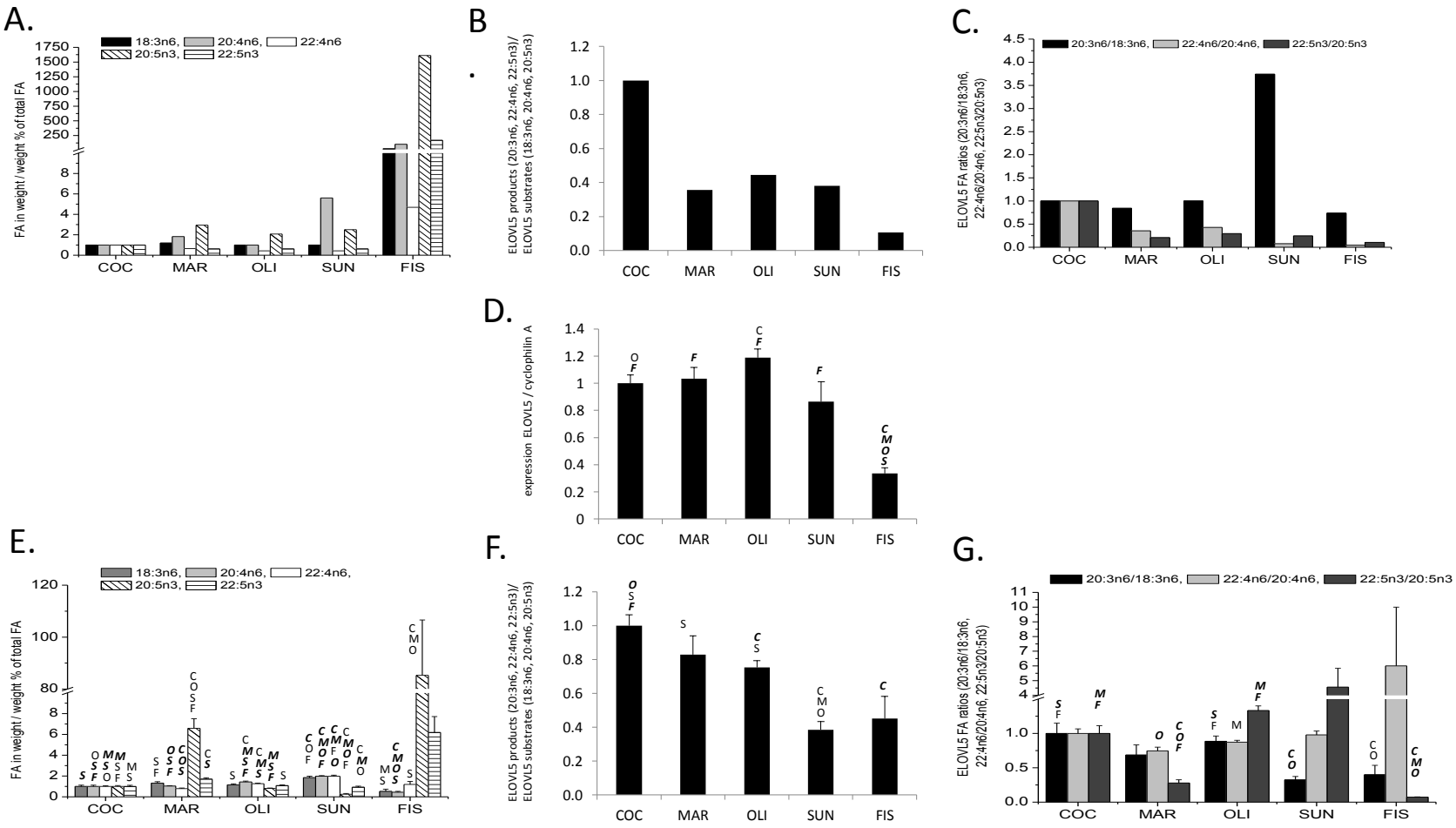

Figure 12. ELOVL5. A) ELOVL5 relevant FAs in diets, in weight/weight %, B) FA ratios (substrates and products of ELOVL5) in diets of several feeding groups, C) Specific ELOVL5 ratios of FAs in diet groups, D) Hepatic gene expression of ELOVL5, E) ELOVL5 relevant FAs in PLs, in weight/weight %, F) FA ratios (substrates and products of ELOVL5) in PLs of several feeding groups, G) Specific ELOVL5 ratios of FAs in Pls. FA composition of diet and PLs in plasma was analyzed by GC. Hepatic gene expression of ELOVL5 was analyzed by QRT-PCR. All values were normalized to 1 which is the coconut diet. Statistic differences were accepted for $p<0.05$ (regular font) and for $p<0.01$ (italic/bold). Each diet group was compared to all the other diet groups. Statistical differences are marked with the letter of the diet group to which it was compared. COC, C – coconut fat; MAR, M – margarine; OLI, O – olive oil; SUN, S - sunflower oil; FIS, F– fish oil

	COC		MAR		OLI		SUN		FIS	
	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.
SAFAs										
16:0	23.56	± 0.71(*F*)	22.47	± 2.37(***F***)	24.98	± 1.85(***F***)	24.24	± 1.72(***F***)	37.67	± 3.89(***C,M,S,O***)
18:0	20.21	± 1.43	16.14	± 4.92	20.72	± 1.64	21.38	± 1.57	25.45	± 7.99
MUFAs										
16:1n7	1.08	± 0.24(***S,O***)	2.27	± 1.73(O,S)	0.56	± 0.08(***C***,M,***F***)	0.48	± 0.07(***C***,M,***F***)	0.98	± 0.20(***S,O***)
18:1n7	4.95	± 0.33(M,***F,S,O***)	4.02	± 0.64(C,***F,S***)	3.16	± 0.42(***C,F***,S)	2.02	± 0.57(***C,M***,O)	1.61	± 0.22(***C,M,O***)
18:1n9	16.71	± 2.16(***F,S,O***)	21.62	± 6.21(***F,S,O***)	13.34	± 0.83(***C,M,F,S***)	7.72	± 1.14(***C,M,O***)	8.48	± 1.25(***C,M,O***)
n3-PUFAs										
18:3n3	0.08	± 0.01(M,O)	0.23	± 0.05(C,F,S,O)	0.05	± <0.01(C,M)	0.06	± <0.01(M)	0.05	± 0.01(M)
20:5n3	0.08	± 0.01(***M***,F,S)	0.53	± 0.07(***C,***F,***S,O***)	0.06	± <0.01(***M,F,S***)	0.02	± <0.01(C,***M,O***,F)	6.83	± 1.71(C,M,O)
22:6n3	3.67	± 0.21(M,S)	5.18	± 0.39(C,***S***)	3.95	± 0.25(S)	2.76	± 0.11(C,***M***,O,F)	5.63	± 1.44(S)
22:5n3	0.10	± 0.02(M,S)	0.17	± 0.03(C,***S***)	0.11	± 0.01(S)	0.09	± 0.10(C,***M***,O)	0.60	± 0.16
n6-PUFAs										
18:2n6	9.08	± 0.69(M,***F,S,O***)	11.15	± 0.28(C,***F,S,***O)	13.81	± 0.76(***C***,M,***F,S***)	19.07	± 0.61(***C,M,F,O***)	4.06	± 0.58(***C,M,S,O***)
18:3n6	0.09	± 0.01(***S***)	0.13	± 0.01(F,S)	0.11	± 0.01(S)	0.17	± 0.01(***C***,F,O)	0.05	± 0.02(M,S)
20:3n6	2.67	± 0.18(***F,S***)	2.40	± 0.29(***F***)	2.88	± 0.10(***F,S***)	1.67	± 0.20(***C,F,O***)	0.46	± 0.08(***C,M,S,O***)
20:4n6	7.38	± 0.83(***F,S***,O)	7.66	± 0.21(***F,S,O***)	10.57	± 0.57(C,***M,F,S***)	14.69	± 0.34(***C,M,F,O***)	3.22	± 0.65(***C,M,S,O***)
22:4n6	0.14	± 0.02(***M,S***,O)	0.11	± 0.02(***C,S,O***)	0.18	± 0.02(C,***M,S***)	0.28	± 0.02(***C,M***,F,***O***)	0.17	± 0.14(S)
Gene Expression										
SCD1	4.52	± 1.06(O,F)	5.49	± 0.54(***F***)	3.52	± 0.21(C,***F***)	3.35	± 1.38(F)	1.19	± 0.15(C***MO***S)
ELOVL6	0.005	± 0.002(F)	0.007	± 0.001(F)	0.004	± 0.001(F)	0.007	± 0.002(F)	0.001	± 0.000(CMOS)
FADS1	0.07	± 0.01(***F***)	0.08	± 0.01(***F***)	0.09	± 0.01(***F***)	0.08	± 0.01(***F***)	0.03	± 0.00(***CMOS***)
FADS2	0.18	± 0.01(***O,F***)	0.16	± 0.02(***F***)	0.14	± 0.01(***C,F***)	0.15	± 0.02(***F***)	0.04	± 0.00(***CMOS***)
ELOVL5	0.25	± 0.02(O,*F*)	0.26	± 0.02(***F***)	0.30	± 0.02(C,***F***)	0.22	± 0.04(***F***)	0.08	± 0.01(***CMOS***)

Table 10. Summarizing data of FAs in PLs. FAs were analyzed by GC in plasma and displayed by weight/weight %. SAFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid, COC, C – coconut fat, MAR, M – margarine, OLI, O – olive oil, SUN, S – sunflower oil, FIS, F – fish oil. Statistic differences were accepted for $p<0.05$ (regular font) and for $p<0.01$ (italic/bold). Each diet group was compared to all the other diet groups. Statistical differences are marked with the letter of the diet group to which it was compared.

5.1.3. CYP26A1 is induced by Fish Oil Diet

Hepatic gene expression of CYP26A1 was significantly increased in fish oil supplemented diet group compared to margarine supplemented diet (Figure 13A). A higher gene expression level was also measured in coconut fat supplemented diet group which was significant compared to margarine diet group ($p<0.05$). Further in the highest hepatic gene expression of CYP26A1 was measured after activation of RAR which was significant compared to control ($p<0.05$, Figure 13B).

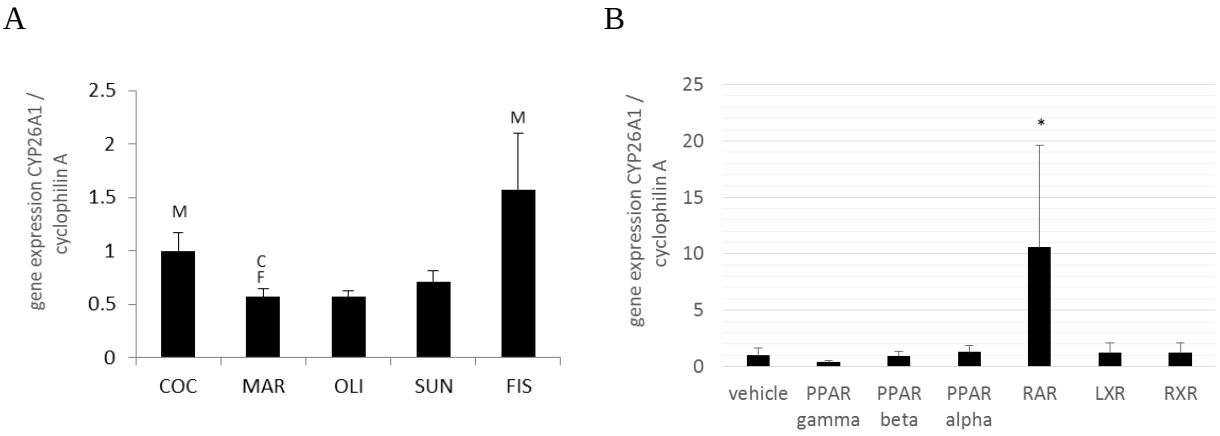

Figure 13. Hepatic gene expression of CYP26A1. A) Hepatic gene expression of CYP26A1 was analyzed by QRT-PCR. All values were normalized to 1 which is the coconut diet. Statistic differences were accepted for $p<0.05$. Each diet group was compared to all the other diet groups. Statistical differences are marked with the letter of the diet group to which it was compared. COC, C – coconut fat; MAR, M – margarine; OLI – olive oil; SUN – sunflower oil; FIS, F – fish oil. B) Hepatic gene expression of CYP26A1 after administration of synthetic and specific ligands of the nuclear hormone receptor family like PPARα, β/δ or γ, RXR and LXR. Statistic difference was accepted for $p<0.05$.

5.2. Study 2 - Synergism of dietary fat and vitamin A - Inductive effect of vitamin A abolished in combination with sunflower oil

In this study diets were combined with different amounts of sunflower oil and different amounts of vitamin A to investigate their influence on FA metabolism of SCD1 and ELOVL6.

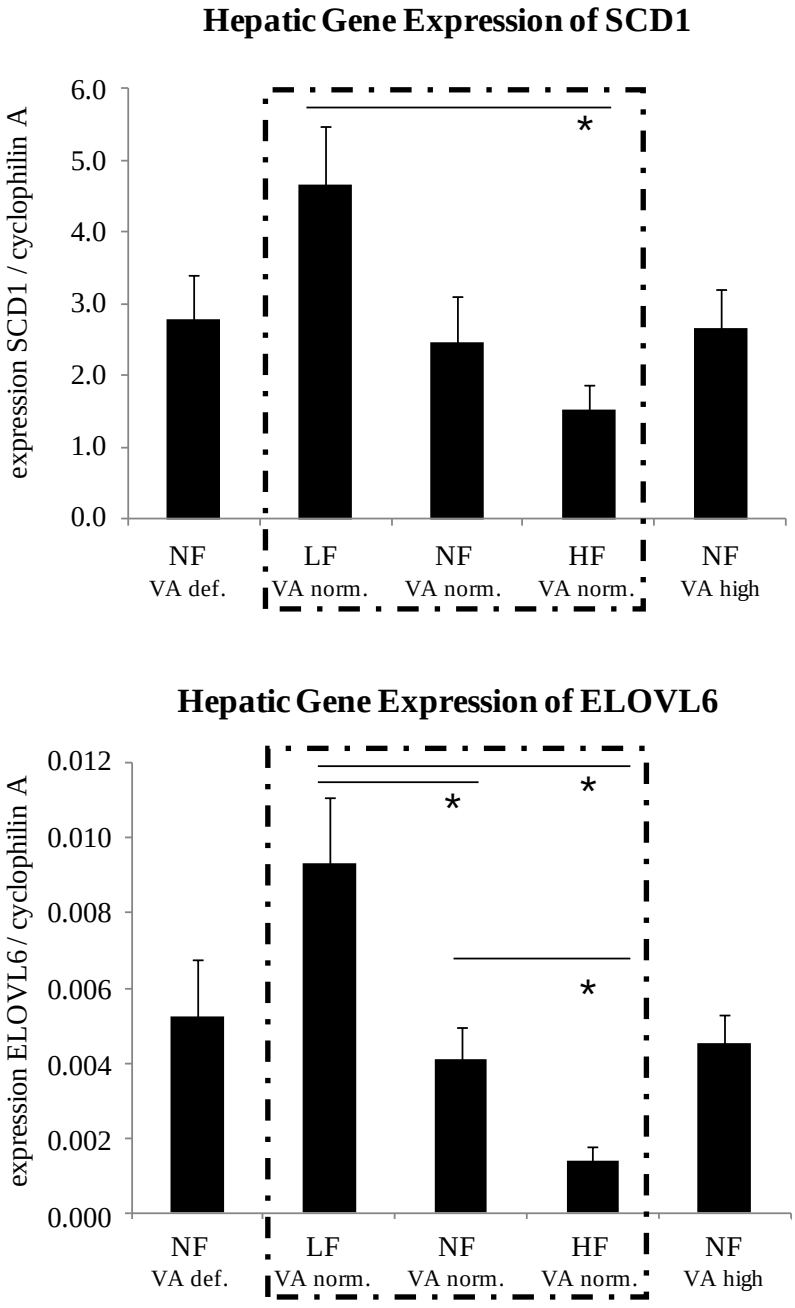

Figure 14. Hepatic expression of SCD1 and ELOVL6 determined by QRT-PCR after diets low and high in dietary fat or higher in vitamin A. LF-low fat diet, NF-normal fat diet, HF-high fat diet, vitamin A – VA, def. - deficient, norm.– normal. Statistical analysis was displayed as *$p<0.05$

Influence of dietary fat on hepatic gene expression of SCD1 and ELOVL6 – The highest hepatic gene expression of SCD1 and ELOVL6 was observed in mice fed a low fat (LF) compared to mice fed a high fat (HF) diet (Figure 14). The hepatic gene expression of ELOVL6 was significantly higher in mice fed LF diet compared to the other diets. The alteration of gene expression of these two enzymes in liver displayed a tendency of decrease with increasing amount of dietary fat.

Influence of different amounts of dietary vitamin A on hepatic gene expression of SCD1 and ELOVL6 - NF diets were supplemented with different amounts of vitamin A to determine the effect of vitamin A on gene expression and FA composition. Different amounts of dietary vitamin A had no effect on the hepatic expression of SCD1 and ELOVL6 (Figure 14).

Influence of dietary fat on PL composition analyzed by GC - Dietary fat did not affect the total contribution of SAFAs (Table 11) in PLs. However, the content of 16:0 (palmitic acid) bound in PLs decreased after a HF diet compared to a LF diet. In contrast, mice fed a HF diet showed higher plasma values of 18:0 (stearic acid) bound in PLs compared to NF and LF diet.

The contribution of total MUFAs bound to PLs was higher in the LF diet vs. the HF diet. The contributions of 18:1n9 and 18:1n7 were the highest in the LF diet. Significant lower values of the sum of all MUFAs as well as of 18:1n9, 18:1n7 and 20:1n9 incorporated into PLs were observed in plasma of mice fed NF diet compared to the HF diet. Mice fed a LF diet showed significantly lower amounts of 16:1n7, 18:1n9 and 18:1n7 than those of mice fed a HF diet. Ratios of selected SAFAs and MUFAs are shown in table 6. The ratio of total SAFAs divided by total MUFAs as well as the ratios of 18:0/16:0, 18:1n7/16:1n7 and 20:1n9/18:1n9 significantly increased after the HF diet compared to the LF diet. The ratios of 16:1n7/16:0 and 18:1n9/18:0 significantly decreased in the HF diet compared to the LF diet. Comparisons between ratios of NF diet to HF diet showed a decrease in 18:0/16:0, 18:1n7/16:1n7 and 20:1n9/18:1n9 ratios, while 18:1n9/18:0 ratio was increased. The ratio of 18:1n7/16:1n7 was significantly decreased comparing LF diet to HF diet.

Influence of different amounts of dietary vitamin A on PL composition analyzed by GC - The analysis via GC revealed that the total amount of MUFA was not influenced by dietary vitamin A (Table 11). However, the ratio of 18:1n9/18:0 decreased significantly in mice fed VA deficient diet compared to those fed the high vitamin A diet. Other ratios were not influenced by dietary vitamin A.

	Effect of Vitamin A				Effect of Dietary Fat					
	NF VA deficient (n=6)		NF VA high (n=6)		LF VA normal (n=6)		NF VA normal (n=6)		HF VA normal (n=6)	
	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.
sum SAFA	48.760	± 1.282	47.770	± 0.600	51.422	± 0.907	47.377	± 1.335	49.843	± 2.013
16:0	30.202	± 0.485*	20.602	± 0.368*§	28.413	± 0.704	26.355	± 0.694	21.571	± 0.831#*
18:0	17.343	± 1.015	25.776	± 0.591*§	21.381	± 0.852	20.017	± 1.095	26.819	± 1.500#*
sum MUFA	12.387	± 0.489	10.401	± 0.261	14.267	± 1.155*	10.619	± 0.523	5.719	± 0.322#*
16:1n7	0.520	± 0.031	0.345	± 0.029	0.875	± 0.202	0.530	± 0.052	0.182	± 0.032#
18:1n9	8.887	± 0.433*	7.034	± 0.165	10.342	± 0.456*	7.294	± 0.441	4.007	± 0.172#*
18:1n7	1.853	± 0.112	1.408	± 0.067	1.842	± 0.293	1.753	± 0.094	0.801	± 0.106#*
20:1n9	0.215	± 0.023	0.252	± 0.014	0.274	± 0.076	0.234	± 0.026	0.216	± 0.009*
sum SAFA / sum MUFA	3.265	± 0.107	4.128	± 0.606	3.366	± 0.291	5.185	± 0.332	10.107	± 1.275#*
18:0/16:0	0.575	± 0.034	1.255	± 0.045*§	0.753	± 0.029	0.763	± 0.047	1.248	± 0.066#*
16:1n7/16:0	0.017	± 0.001	0.017	± 0.001	0.032	± 0.008	0.020	± 0.002	0.008	± 0.001#
18:1n9/18:0	0.526	± 0.052	0.274	± 0.011§	0.491	± 0.040	0.374	± 0.037	0.152	± 0.011#*
18:1n7/16:1n7	3.576	± 0.130	4.196	± 0.277	2.233	± 0.214*	3.378	± 0.163	4.642	± 0.325#*
20:1n9/18:1n9	0.025	± 0.003	0.036	± 0.002	0.025	± 0.006	0.032	± 0.003	0.054	± 0.002#*

Table 11. Analysis of FA composition by GC of plasma PLs in mice fed a diet containing different amounts of fat (sunflower oil) and vitamin A (VA). Amounts are displayed in mean in weight %, s.e.m. – standard error of mean, SAFA – saturated fatty acid, MUFA – monounsaturated fatty acid, LF – low fat, NF – normal fat, HF – high fat. Statistical analysis compared to control *p<0.05 different to NF VA normal, #-p<0.05 different HF VA normal vs LF VA normal, § p<0.05 different of NF VA deficient to NF VA high.

Influence of dietary fat on PE, PC and LPC composition analyzed by ESI-MS/MS- In liver PLs, mice fed LF diets showed increased incorporation of PE 34:1, PC 32:1, 34:1, 36:1 and LPC 16:1, 18:1, 18:0 compared to NF diet (Figure 15). Lower percentages of incorporated FAs were observed in PC 32:0, 36:0 and LPC 16:0. Furthermore, ratios of PE 34:1/34:0, PC 32:1/32:0, 34:1/34:0, 36:1/36:0 and LPC 16:1/16:0, 18:1/18:0 also decreased. HF diet compared to NF diet displayed a decrease in PE 34:1, PC 32:1, 34:1, 36:1 and LPC 16:1, 18:1, 16:0. An increase was measured in PE 34:0 and PC 36:0.

In plasma PLs, mice fed LF diet had higher incorporation of PE 34:1, PC 32:1, 34:1, 36:0, 36:1 and LPC 16:1, 18:1 compared to NF diet and the ratios of PC 32:1/32:0, 34:1/34:0, 36:1/36:0 and LPC 16:1/16:0, 18:1/18:0 were decreased. An increase was measured for PC 32:0, 34:0, 36:0 and LPC 18:0. Comparing HF diet to NF diet amounts of PE 34:1, PC 32:1, 34:1, 36:1 and LPC 16:0, 16:1, 18:1 decreased. Ratios of PC 32:1/32:0, 34:1/34:0, 36:1/36:0 and LPC 16:1/16:0, 18:1/18:0 increased as well.

In summary, LF diets resulted in more MUFAs than SAFAs being incorporated in PLs in both liver and plasma. In contrast, mice fed HF diets showed more SAFAs and less MUFAs being incorporated into PLs.

Influence of different amounts of dietary vitamin A on PE, PC and LPC composition analyzed by ESI-MS/MS - The total amount of plasma SAFAs was not influenced by different amounts of vitamin A. However, when looking at individual SAFAs and specific PL (Figure 15), there was a significant increase in plasma amounts of LPC 18:0 in mice fed with the high VA diet compared to the VA deficient diet. In contrast, plasma amount of LPC 16:0 were higher in animals receiving a deficient vitamin A diet compared to animals receiving a high vitamin A diet. This result was also observed in plasma PLs analyzed by GC incorporating lower amounts of 16:0 and higher amounts of 18:0 after a high vitamin A diet.

The analysis of PE, PC and LPC species in liver and plasma showed an increase of LPC 18:1 and LPC 16:1 amounts in liver and plasma of mice fed VA deficient diets compared to high vitamin A diet (Figure 15). This increase has also been observed in liver and plasma for PC 32:1 and 34:1 as well as for PE 34:1.

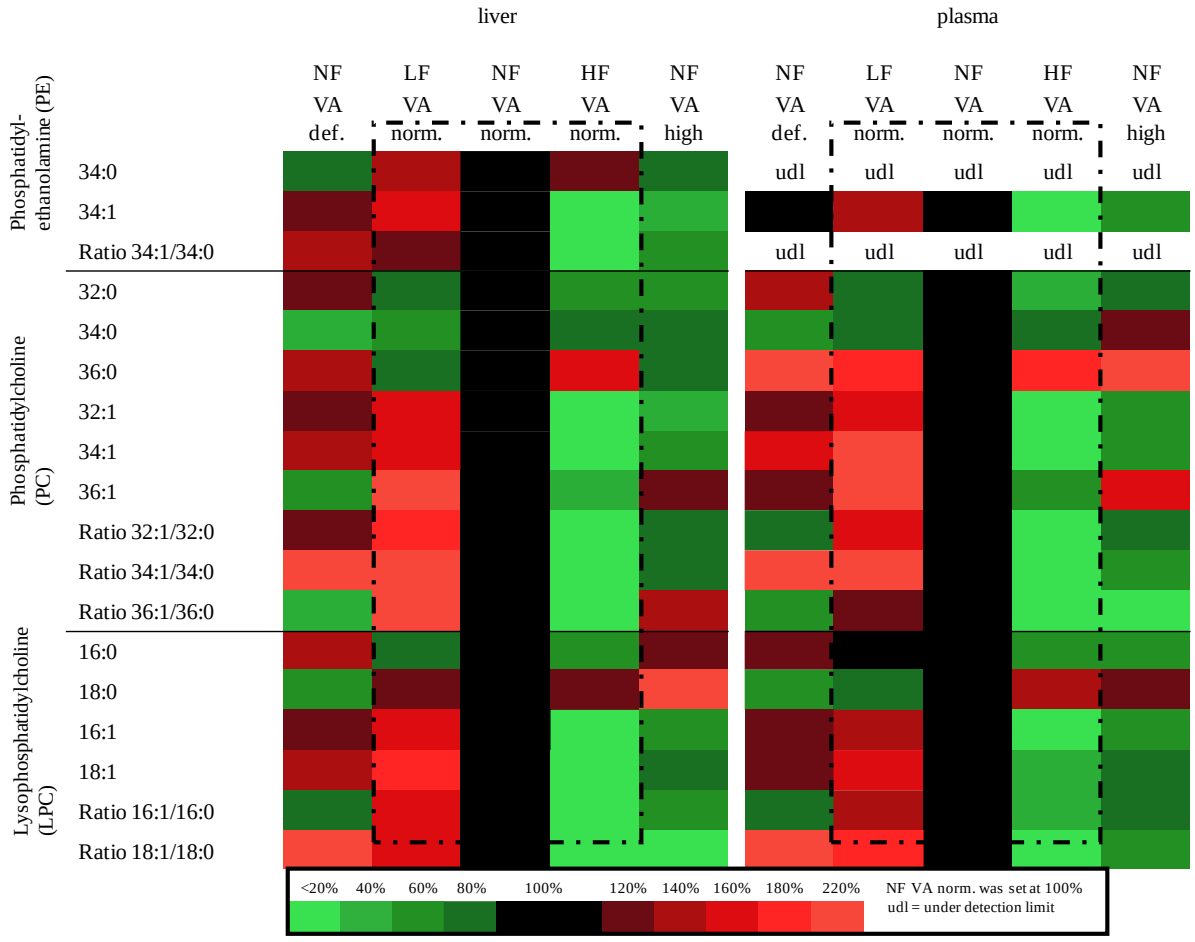

Figure 15. Analysis of composition of PC, LPC and PE determined by ESI-MS/MS in liver and plasma after diets low and high in dietary fat or higher in vitamin A. LF-low fat diet, NF-normal fat diet, HF-high fat diet, vitamin A- VA, def.-deficient, norm.-normal. The amount of each incorporated fatty acid was calculated in percent compared to the normal fat, normal vitamin A (NF VA norm) diet, feeding group, which represents 100% (black). Red colors display an increased amount of this particular FA, while green means a decrease compared to the normal fat, normal vitamin A (NF VA norm) feeding group.

5.3. Study 3 - Regulation by Nuclear Hormone Receptors - RXR and LXR but not PPARs induce Gene Expression of SCD1 and ELOVL6 and alter the PL composition accordingly

In this experiment synthetic ligands activating PPARα, β/δ, γ, RAR, RXR and LXR were gavaged into mice and the effect of the activated NHRs on hepatic gene expression of SCD1 and ELOVL6 was investigated.

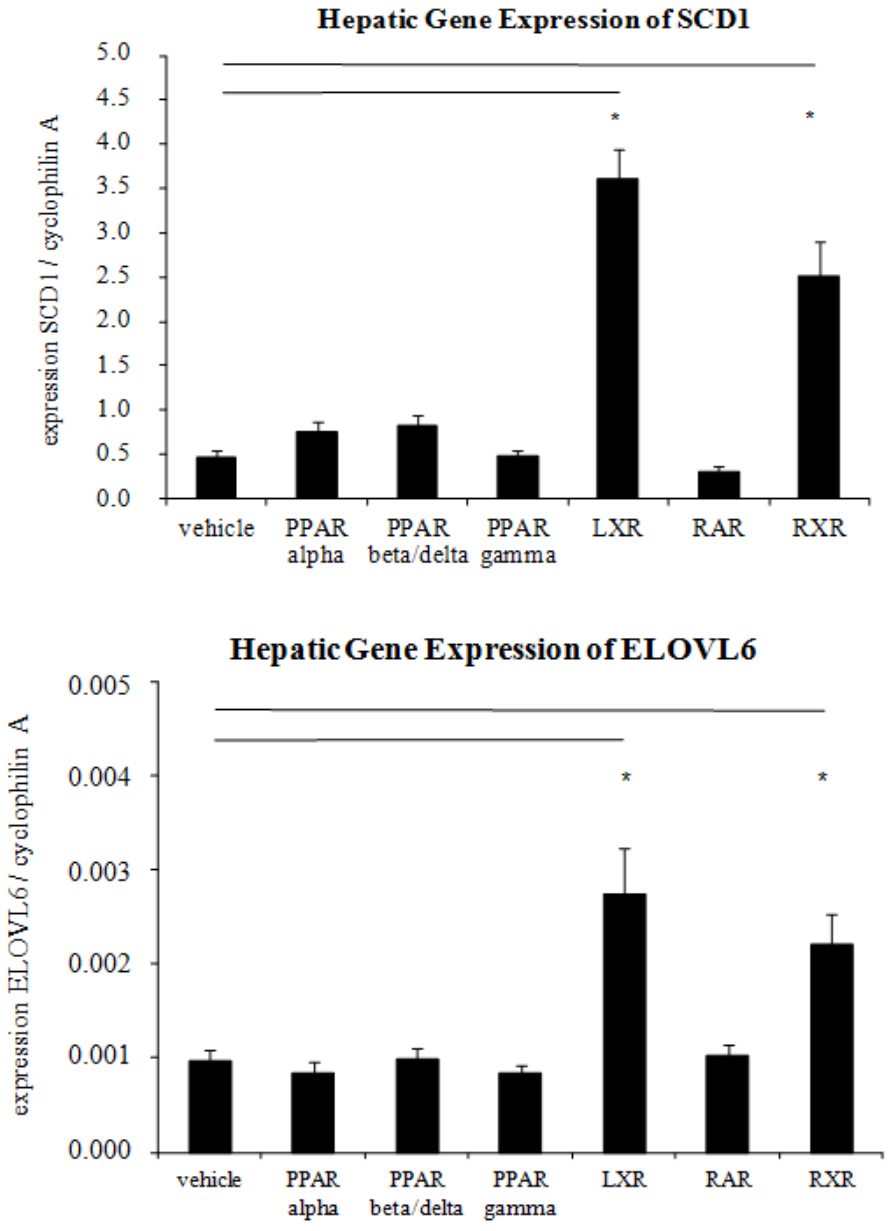

Figure 16. Gene expression of SCD1 and ELOVL6 in liver of mice (n = 6) Gene expression of SCD1 and ELOVL6 were normalized to cyclophilin A, mean ± standard error mean of gene expression were displayed and statistically analyzed compared to the vehicle, $p<0.05$ (*).

NHRs regulation of SCD1 and ELOVL6 hepatic gene expression - Hepatic SCD1 and ELOVL6 gene expression significantly increased in animals gavaged with LXR and RXR ligands, while treatment with PPARα, PPARβ/δ and PPARγ ligand resulted in no significant alteration. Application of an RAR ligand led to a slight, non-significant suppression of hepatic SCD1 expression (Figure 16). The ELOVL6 expression was not influenced by the application of the RAR synthetic ligand.

PL Analysis in Plasma by GC – FA composition plasma PL for substrates, products and product/substrate ratios of SCD1 and ELOVL6 enzymes in the treatment groups are compared to the vehicle group (Table 12). PA (16:0) percentages decreased significantly in the LXR, RAR and RXR groups, whereas significantly increased in the PPARα group. SA (18:0) percentages decreased significantly in the RXR, whereas increased significantly in the RAR group. Percentages of POA (16:1n7) did not differ, whereas VC (18:1n7), OA (18:1n9) and EA (20:1n9) percentages increased significantly in the LXR and RXR groups. The product/substrate ratios exhibited significant increases following treatment (with the exception of the 20:1n9/18:1n9 ratio). Specifically, significant increases of the 16:1n7/16:0 ratio were observed in the LXR group, of the 18:1n9/18:0 ratio in the PPARα, LXR and RXR groups and of the 18:1n7/16:1n7 ratio in PPARγ and RXR treatment groups.

	Vehicle		PPARα		PPARβ/δ		PPARγ		LXR		RAR		RXR	
	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.
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*
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*
16:1n7	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
18:1n7	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*
18:1n9	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*
20:1n9	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
18:1n9/18:0	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*
16:1n7/16:0	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
18:1n7/16:1n7	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*
20:1n9/18:1n9	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

Table 12. Analysis of FA composition of PLs in plasma of mice. Substrates, products and product/substrate ratios for SCD1 and ELOVL6 enzymes in plasma PLs in mice (n = 6). Data are given as mean ± s.e.m. (standard error of mean) of percentage of total FA and statistically compared to vehicle, $p < 0.05$ = *

PE, PC and LPC composition analyzed by ESI-MS/MS - SAFAs and MUFAs of selected PL classes PC, PE and LPC were determined in plasma and liver by ESI-MS/MS. Amounts of FAs were displayed in mean of % of the specific lipid class related to total lipid classes (Figure 17). The composition of PE species containing one MUFA (36:1 and 34:1) was mainly influenced by the application of RXR and LXR ligand leading to an increase of bound MUFAs in liver tissue and plasma (Figure 17). The oral gavage of RAR ligand, by contrast, decreased MUFAs (displayed by: 32:1, 34:1) in liver and plasma. As in case of PE, an increase of MUFAs (displayed by: 32:1, 34:1 and 36:1) in PC was determined in the liver and plasma after application of agonists for RXR and LXR, while the activation of RAR, PPARα and PPARγ lead to a decrease of PC 36:1 in the liver and plasma. Furthermore, RAR activation reduced the content of PC 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 LPC in liver and plasma in which MUFAs (16:1 and 18:1) were increased. 16:1 in plasma and 18:1 in liver were additionally increased by RXR and PPARα. A reduction of 18:1 in liver and 16:1 in plasma and liver was determined after the gavage of 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.

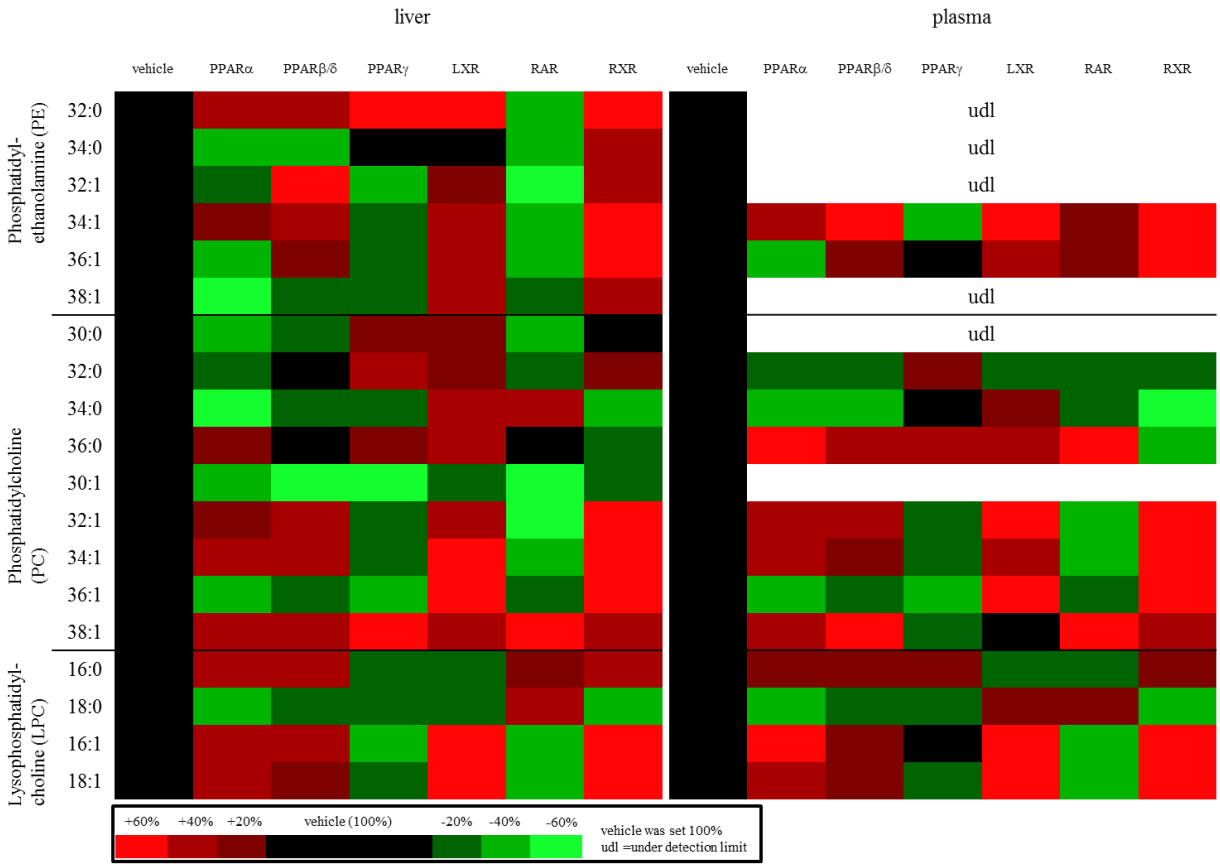

Figure 17. Analysis of the FAs in phosphatidylethanolamine (PE), phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) in liver and plasma of mice, n = 6. Species composition was determined by ESI-MS/MS and calculated as a percentage of total lipid class and fold change from the vehicle (= 100%). udl - under detection limit.

6. DISCUSSION

Cellular membranes fulfil the most crucial functions of maintenance of cellular homeostasis, reactivity to metabolic challenges and injuries affecting the integrity of cells and organs [70]. The assembling of PLs and incorporated FAs are determining factors for the biophysical and physiologic character of a cellular membrane [43, 70]. In this thesis different influences on FA composition of PLs and the possibility to refer specific FA ratios to their metabolizing enzymes were investigated.

6.1. MUFA and SAFAs content in PLs

In this studies the MUFA (18:1n9, 16:1n7) and SAFA (18:0, 16:0) content in PLs was mainly influenced by alterations of gene expression of rate limiting enzymes SCD1 and ELOVL6. MUFAs and SAFAs derived from diet revealed less impact on PL fatty acid composition. Comparing diet interventions MUFAs, in particular OA, were not found to alter gene expression of SCD1 [102, 160]. However a significantly lower hepatic gene expression of ELOVL6 and SCD1 after fish oil diet compared to olive oil was seen in this studies (Figure 9,10) which has been observed for SCD1 in adipocytes comparing OA to n3-PUFA treatment [102]. N3-PUFAs like found in fish oil or n6-PUFAs contained in sunflower oil were reported in several studies to be responsible for this suppression [34, 161]. For sunflower oil diet the suppression could be confirmed only in dose dependent manner (study2) and only partially in comparison to other diets (study1) [162]. Further n3-PUFAs have a higher potential to suppress gene expression of SCD1 compared to n6-PUFAs which increases with length of the carbon chain [102]. As it has been found that n6-PUFA AA supplementation suppresses SCD1 gene expression stronger than supplementation with n6-PUFA LA [102]. On the contrary, hepatic gene expression of SCD1 and ELOVL6 were increased by SAFA enriched coconut fat diet (Table 13, Figure 9,10) as found in this but also reported in previous studies [85, 162]. Surprisingly the highest induction of SCD1 gene expression in liver could be detected after margarine diet (Figure 10). The FA profile of margarine revealed a mixture of SAFA, PUFA and MUFA which would expect to suppress hepatic gene expression of SCD1 compared to coconut fat diet due to the high content of PUFAs. However hepatic gene expression of SCD1 as well as metabolic products of this enzyme incorporated into PLs were highest in margarine diet group. Margarine is a highly processed dietary fat which has not been investigated before for its potential to regulate the gene expression of SCD1. Margarine could be interesting new target for further studies.

Regulatory Influence	Regulation SCD1	Regulation ELOVL6
N6-PUFA rich dietary oils/ specific n6-PUFAs		
Sunflower oil	▲ (compared to fish oil) Study 1; ▼ high fat - dose dependent Study 2, [163]	▲ (compared to fish oil) Study 1; [162] ▼ high fat - dose dependent Study 2
Safflower oil	▼ [164]	
Corn oil	▼ [97, 165]	
PUFA enriched (AA)	▼ [31, 166] [85] [63] [102]	
LA	▼ [102]	▼ [34]
N3-PUFA rich dietary oils/ specific n3-PUFAs		
Fish oil	▼ (compared to other diets) Study 1, [31, 36, 63, 85]	▼ (compared to other diets) Study 1, [34, 162]
LN	▼ [102]	
DHA	▼ [167]	
EPA	▼ [85]	▼ [34]
Pikasol (n3-PUFAs)		▼ [55]
SAFA rich dietary oils/ specific SAFAs		
Coconut fat	▲ (compared to fish oil and olive oil) Study 1; [97, 165]	▲ (compared to fish oil) Study 1; [162]
PA	▲ [168] (in skeletal muscle); ▼ [169] (in skeletal muscle)	
SA		▲ [34]
MUFA rich dietary oils/ specific MUFAs		
Olive oil	▲ (compared to fish oil) Study 1; ▼ (compared to coconut fat) Study 1, ▲ (compared to fish oil) [160]	▲ (compared to fish oil) Study 1
OA	▬ [102] [160]	
POA	▼ [13]	
Lard		▲ [162]
Other dietary oils		
Margarine	▲ (compared to fish oil) Study 1	▲ (compared to fish oil) Study 1
cholesterol and cholesterol ester	▲ [32, 114]	

Table 13. Regulation of SCD1 and ELOLV6 hepatic gene expression by dietary fat. ▲ up regulated, ▼ down regulated

Other regulators of SCD1 and ELOVL6 gene expressions are dietary and non-dietary activators of NHRs (Table 14). Vitamin A, a natural ligand for NHRs, was reported to induce the hepatic gene expression of SCD1 (Table 14) [170, 171]. However, no induction by dietary vitamin A for hepatic gene expression of SCD1 and additionally for ELOVL6 could be proven within studies of this thesis if diet was synergistically applied with sunflower oil (Figure 14). Possibly the dose dependent suppressive effect of sunflower oil which has been shown also in previous studies abolished the inductive effect of vitamin A [163]. Therefore the role of vitamin A in the regulation of SCD1 expression remains controversial and reveals further the question of how several different nutrients interact with each other on gene expression regulation. Dietary supplement studies investigated so far mainly single isolated nutrients while interaction of nutrients have been studied rarely so far [172].

Further in this thesis in study3 an induction of gene expression of SCD1 and ELOVL6 was found by application of synthetic ligands to activate LXR and RXR. The induction of SCD1

by RXR and LXR has been previously reported [114, 173]. For ELOVL6 only the induction by LXR was reported so far [34, 103]. Other NHRs like PPAR α,β/δ,γ did not show any regulatory tendencies towards the hepatic gene expression of SCD1 or ELOVL6 although the induction of hepatic gene expression of SCD1 and ELOVL6 by PPARα and for SCD1 also by PPARγ in obese Zucker fa/fa rats and in HepG2-cells was proposed (Table 14) [171, 173-176].

Regulatory Influence	Regulation SCD1	Regulation ELOVL6
Nutritional		
vitamin A	▲ [171, 173]; ▬ Study 2	▬ Study 2
carbohydrates	▲ [3, 48, 177]	▲ [34, 48, 50]
fasting/refeeding	▲ [36]	▲ [36, 100]
NHRs		
PPARα	▬ Study 3; ▲ in liver [36, 48]	▬ Study 3;[103] ▲ [48]
PPARβ/δ	▬ Study 3	▬ Study 3
PPARγ (co-activation of PGC1α via SREBP-1c)	▬ Study 3; ▼in liver [116, 175]; ▲ [174], HepG2-cells [176]; WAT[175]	▬ Study 3
LXR	▲ Study 3;[60, 114, 178]	▲ [34]; Study 3; [103]
RAR	▲ [173]; ▼ Study 3	▬ Study 3
RXR	▲ [173], Study 3	▲ Study 3; ▬ [103]

Table 14. Nutritional and NHRs derived effect on SCD1 and ELOVL6 gene expression. ▲ up regulated, ▼ down regulated

SCD1 specific FAs ratios 18:1/18:0 and 16:1/16:0 are proposed as "SCD1" or "desaturation" index reflecting SCD1 enzymatic activity (Table 2) [45, 53, 61, 62]. However, comparing dietary FAs to FAs incorporated into PLs the ratio of 18:1n9/18:0 reflected well SCD1 gene expression in all studies conducted within this thesis. Suppression of SCD1 by fish oil diet for example lead to a reduction of this FA ratio in PLs. This could be observed in PL subgroups of PE, PC and LPC (study1). The suppressive effect of n3-PUFA supplementation was shown by others in reducing the ratio of 16:1n7/16:0 in ceramides and 18:1n9/18:0 in PLs which was also seen after a cod diet rich in n3-PUFAs in healthy subjects corresponding to lower triglycerides [55, 64]. Dose dependent suppression of SCD1 by sunflower oil was also followed by reduction of these specific fatty acid ratios in PLs, in PE, PC and in LPC accordingly similar to suppressed hepatic gene expression (study1). On the other hand SAFA rich diet induced the SCD1 gene expression which confirmed the higher ratio of substrate and products in PLs (study1) [32]. Similar, the induction of SCD1 by LXR and RXR activation increased SCD1 specific FA ratios in PLs (study3). Dietary influences on SCD1 specific FA ratios in PLs remained marginal. Dietary 18:1n9 like derived from olive oil diet was not associated with an increased 18:1n9/18:0 in PLs although reported by others in skeletal muscle in humans [55]. 16:1n7, however, high in fish oil diet led to an increase of

16:1n7/16:0 ratio in our study. Therefore the ratio of 16:1n7/16:0 could be considered to be less reliable to represent SCD1 gene expression.

The rate limiting enzyme of elongation of MUFA and SAFA ELOVL6 showed a repressed hepatic gene expression by n3-PUFAs containing fish oil diet which confirmed other previous studies [48, 50]. Further this repression led to reduced incorporation of metabolic products into PLs in liver (Figure 10). An increased hepatic gene expression of ELOVL6 was revealed in this thesis after consumption of coconut fat supplemented diet as well as by activation of RXR and LXR by synthetic ligands elevating ELOVL6 specific FA ratios in PLs in liver and in plasma (Figure 10, 16). According to results in this thesis the FA ratio of 18:1n7/16:1n7 is more reliable to represent ELOVL6 hepatic gene expression than the suggested ratio of 18:0/16:0 [51].

6.2. PUFA content in PLs

PUFAs in PLs are derived more likely from diet. For example, n6-PUFA 18:2n6 was measured highest for sunflower oil feeding group in diet and in PLs and n3-PUFAs of 20:5n3 (EPA), 22:5n3 (DPA) and 22:6n3 (DHA) highest in fish oil diet were measured also highest in PLs (Table 9, 10). However, also hepatic gene expression of PUFA metabolizing enzymes FADS1, FADS2 and ELOVL5 displayed partially influence on FA composition in PLs (study1). An increase of FADS1, FADS2 and ELOVL5 gene expression could be observed in margarine diet group compared to fish oil, which would explain the elevated level of downstream metabolic products of these enzymes incorporated into PLs (Figure 11, 12). These PUFA downstream products might not derive necessarily from diet but could derive from metabolism by desaturation and elongation by PUFA metabolizing enzymes. This result suggests that only 18:3n3 and 18:2n6 are absolutely essential to be provided by diet and can be converted to gain the desired higher n3-PUFAs like 20:5n3, 22:5n3 and 20:4n6, respectively. Supplementation with 18:3n3 enriched diets instate of consumption of fish is therefore recommended to avoid n3-PUFA shortage [179]. However, investigation of health benefits associated to non-fish oil n3-PUFA enriched substitutes revealed controversial results and are not fully proven [179, 180]. Further hepatic gene expression of PUFA metabolizing enzymes FADS1, FADS2 and ELOVL5 was suppressed in liver and other tissues by fish oil supplemented diets or diets high n3-PUFAs (Table 15) [55, 181]. Therefore PUFAs like 20:5n3, 22:5n3 and 22:6n3 incorporated in PLs of fish oil diet group derived more likely from diet than from enzymatic metabolism (Table 10). This suppression diminishes the generation further downstream products derived from 18:2n6 and is suggested

to reduce the pro-inflammatory metabolites derived from n6-PUFAs like 20:4n6 [182]. The results of studies in this thesis and previous studies in healthy humans suggest that the endogenous AA (20:4n6) content is limited by the metabolizing enzymes FADS1 and 2, the rate limiting enzyme in GLA (20:3n6) conversion to AA (20:4n6) [83]. Differently than previously reported studies results of study1 of this thesis revealed that availability of metabolic n6-PUFA substrates 18:2n6 like from margarine and from sunflower oil increased AA levels in plasma PLs (Figure 11). A high hepatic gene expression of FADS1 might contribute to an increased conversion of the main substrate 18:2n6 in sunflower oil to the metabolic product 20:4n6.

Regulator	Regulation FADS1	Regulation FADS2	Regulation ELOVL5
N6-PUFA rich dietary oils/ specific n6-PUFAs			
Sunflower oil	▲ versus fish oil **Study 1**	▲ versus fish oil **Study 1**	▲ versus fish oil **Study 1**
LA		▲ versus AA, EPA, ALA[181]	
AA		▼ compared to LA[181]	
N3-PUFA rich dietary oils/ specific n3-PUFAs			
Fish oil	▼ versus other diets **Study 1**	▼ versus other diets **Study 1**	▼ versus other diets **Study 1**
EPA	▼ versus LA[181], versus high carbohydrate diet[85]	▼ [181], versus high carbohydrate diet **[85]**	
ALA		▼ versus LA[181]	
Piksol (n3-PUFAs)	▼ [55]	▼ [55]	
SAFA rich dietary oils/ specific SAFAs			
Coconut oil	▲ versus fish oil **Study 1**	▲ versus fish oil and olive oil **Study 1**	▲ versus fish oil **Study 1**
MUFA rich dietary oils/ specific MUFAs			
Olive oil	▲ versus fish oil **Study 1**	▲ versus fish oil **Study 1**, ▼ versus coconut fat **Study 1**	▲ versus fish oil **Study 1**
Other dietary oils			
Margarine	▲ versus fish oil **Study 1**	▲ versus fish oil **Study 1**	▲ versus fish oil **Study 1**
Other regulatory influences			
PPARa	▲ [48]	▲ [48]	▲ [48]
LXR	▲ [48]	▲ [48]	
Insulin	▲ [48]	▲ [48]	

Table 15: Regulation of FADS1, FADS2, ELOVL5 hepatic gene expression by dietary fat. Study 1 – Influence of dietary fat; Study 2- Synergism of dietary fat and vitamin A; Study 3 – Regulation by NHRs. ▲ up regulated, ▼ down regulated

Discussing specific fatty acid ratios for PUFA metabolizing enzyme FADS1, FADS2 and ELOVL5 remains controversial because their reliability is not fully proven (Table 3). Enzymatic activity of FADS1 was investigated by specific FA ratio of 20:4n6/20:3n6 (study1). This FA ratio was found lowest in PLs and diet of fish oil supplemented diet group assuming a minor influence by hepatic gene expression of FADS1 (Figure 11). On the contrary however, in human this particular ratio was reported to increase with consumption of

n3-PUFA enriched diet [55]. FADS2 ratio of 22:6n3/22:5n3 in PLs reflects the hepatic gene expression of FADS2 (Figure 11). ELOVL5 ratio of 22:5n3/20:5n3 lowest in fish oil diet group in PLs reflected the reduced hepatic gene expression (Figure 12). Other proposed enzyme specific FA ratios followed the dietary pattern in PLs rather than the hepatic gene expression of the metabolic enzyme. The specific ratio of 18:3n6/18:2n6 is mainly proposed to indicate the enzymatic activity of FADS2 and was reported to be increased in PLs with n3-PUFA enriched diet in healthy subjects [55]. This result could be confirmed by study1 although the hepatic gene expression of FASD2 was significantly reduced in fish oil supplemented diet group (Figure 11).

6.3. Regulatory link between fat and vitamin A metabolism

Although research progresses on the regulatory potentials of several dietary fats, it remains unclear for almost 40 years why PUFAs and especially n3-PUFAs suppress so efficiently SREBP-1c target genes like SCD1and ELOVL6. One explanation suggested the ability of n3-PUFAs especially DHA to reduce trans-activation of LXRα, a SREBP1c regulator [183]. This question was followed studying if the suppressive effect could be connected to the RXR activation and vitamin A metabolism. The administration of fish oil supplemented diet induced the hepatic gene expression of CYP26A1, a target gene of RAR and therefore of natural dietary vitamin A. CYP26A1 is the rate limiting enzyme for the availability of RXR natural ligands derived from dietary vitamin A [135]. RXR is activated by vitamin A derived metabolites 9-cis RA which is found to be catabolized by Cyp26a1. Less available natural ligands capable to activate RXR could be an explanation for a reduced expression level of RXR target genes. RXR target genes involve the TF SREBP1c which controls targets like fat metabolizing enzymes such as SCD1 and ELOVL6 as well as FADS1, FADS2 and ELOVL5 [85]. Gene expressions of all of these enzymes were suppressed in liver after fish oil diet leading to reduced metabolic products of these enzymes incorporated into PLs. However, this hypothesis requires further investigation.

7. SUMMARY

Dietary fat provides FAs incorporated into PLs forming cellular membranes. These membranes are constantly renewed and the composition of FAs aligns according to the provided dietary fat. Influences on membrane PL FA composition were investigated in this thesis. *In vivo* studies in mice have been performed investigating the effect of different types of dietary fat, different amounts of sunflower oil combined with vitamin A and regulation by activated NHRs on PLs and cellular membrane FA profile.

Most important findings of this thesis were that MUFA and SAFA content in PLs is strictly controlled by their metabolizing enzymes SCD1 and ELOVL6. Especially alteration of SCD1 hepatic gene expression by dietary fat altered the PLs profile of its metabolic products accordingly. The SCD1 FA ratio 18:1n9/18:0 was found to be more reliable as "desaturase index" within all conducted studies. Most surprisingly margarine diet was the strongest inducer of SCD1, it was even more effective than coconut fat diet high in SAFA. Furthermore the inductive effect of dietary vitamin A on hepatic gene expression of SCD1 and ELOVL6 was abolished if diet was combined with n6-PUFA rich sunflower oil. Sunflower oil supplemented diet suppressed SCD1 and ELOVL6 gene expression dose dependently. This assumes that nutrients might interfere with each other with unexpected outcome. ELOVL6 activity was most reliable represented by the FA ratio 18:0/16:0. On the contrary to MUFA and SAFA profile in PLs PUFAs in PLs were more but not totally dependent on available dietary PUFAs. Fish oil enriched diet suppresses the hepatic gene expression of main PUFA metabolizing enzymes FADS1, FADS2 and ELOVL5 which was reflected in reduced metabolic activity. This resulted partially in less metabolic products measured in FA profile of PLs. Repressed gene expression could be associated with reliable FA ratios representing enzymatic activity for 22:6n3/22:5n3 FADS2. PUFAs in PLs profiles are more likely to derive directly from diet, but might be adjustable by PUFA enzyme metabolism. The results of this thesis suggest further that inhibition of hepatic gene expression of fat metabolizing enzymes by n3-PUFA enriched diet is caused by reduces activity of RXR. RXR is a heterodimer forming NHR which can be activated by isoforms of vitamin A. CYP26A1 catabolizes active vitamin A derived RXR ligands into inactive metabolites. Induction of CYP26a1 by fish oil supplemented diet decreases the availability of vitamin A and less RXR is activated.

8. REFERENCES

8.1. References

1. Jump DB: **Dietary polyunsaturated fatty acids and regulation of gene transcription**. *Curr Opin Lipidol* 2002, **13**(2):155-164.
2. Hodson L, Skeaff CM, Fielding BA: **Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake**. *Prog Lipid Res* 2008, **47**(5):348-380.
3. Ntambi JM, Miyazaki M: **Regulation of stearoyl-CoA desaturases and role in metabolism**. *Prog Lipid Res* 2004, **43**(2):91-104.
4. Mauvoisin D, Mounier C: **Hormonal and nutritional regulation of SCD1 gene expression**. *Biochimie* 2011, **93**(1):78-86.
5. Papamandjaris AA, MacDougall DE, Jones PJ: **Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications**. *Life Sci* 1998, **62**(14):1203-1215.
6. Din JN, Newby DE, Flapan AD: **Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment**. *Bmj* 2004, **328**(7430):30-35.
7. Shaikh SR, Edidin M: **Polyunsaturated fatty acids, membrane organization, T cells, and antigen presentation**. *Am J Clin Nutr* 2006, **84**(6):1277-1289.
8. Cordomi A, Prades J, Frau J, Vogler O, Funari SS, Perez JJ, Escriba PV, Barcelo F: **Interactions of fatty acids with phosphatidylethanolamine membranes: X-ray diffraction and molecular dynamics studies**. *J Lipid Res* 2010, **51**(5):1113-1124.
9. Fares H, Lavie CJ, DiNicolantonio JJ, O'Keefe JH, Milani RV: **Omega-3 fatty acids: a growing ocean of choices**. *Current atherosclerosis reports* 2014, **16**(2):389.
10. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C: **Health implications of high dietary omega-6 polyunsaturated Fatty acids**. *Journal of nutrition and metabolism* 2012, **2012**:539426.
11. Sauma L, Stenkula KG, Kjolhede P, Stralfors P, Soderstrom M, Nystrom FH: **PPAR-gamma response element activity in intact primary human adipocytes: effects of fatty acids**. *Nutrition* 2006, **22**(1):60-68.
12. Dobrzyn A, Dobrzyn P: **Stearoyl-CoA desaturase--a new player in skeletal muscle metabolism regulation**. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society* 2006, **57 Suppl 10**:31-42.
13. Burns TA, Duckett SK, Pratt SL, Jenkins TC: **Supplemental palmitoleic (C16:1 cis-9) acid reduces lipogenesis and desaturation in bovine adipocyte cultures**. *Journal of animal science* 2012, **90**(10):3433-3441.
14. Elias S, Wisam S, Luai A, Massad B, Nimer A: **Lipotoxicity in Obesity: Benefit of Olive Oil**. *Advances in experimental medicine and biology* 2017, **960**:607-617.
15. Carrillo C, Cavia Mdel M, Alonso-Torre S: **Role of oleic acid in immune system; mechanism of action; a review**. *Nutricion hospitalaria* 2012, **27**(4):978-990.
16. Hulbert AJ: **Metabolism and longevity: is there a role for membrane fatty acids?** *Integrative and comparative biology* 2010, **50**(5):808-817.
17. Serra-Majem L, de la Cruz JN, Ribas L, Salleras L: **Mediterranean diet and health: is all the secret in olive oil?** *Pathophysiology of haemostasis and thrombosis* 2003, **33**(5-6):461-465.
18. Ogce F, Ceber E, Ekti R, Oran NT: **Comparison of mediterranean, Western and Japanese diets and some recommendations**. *Asian Pacific journal of cancer prevention : APJCP* 2008, **9**(2):351-356.
19. Gattereau A, Delisle HF: **The unsettled question: butter or margarine?** *Canadian Medical Association journal* 1970, **103**(3):268-271.

20. Legrand P, Rioux V: **The complex and important cellular and metabolic functions of saturated fatty acids**. *Lipids* 2010, **45**(10):941-946.
21. Sampath H, Ntambi JM: **The fate and intermediary metabolism of stearic acid**. *Lipids* 2005, **40**(12):1187-1191.
22. Kanoski SE, Davidson TL: **Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity**. *Physiology & behavior* 2011, **103**(1):59-68.
23. Riccardi G, Giacco R, Rivellese AA: **Dietary fat, insulin sensitivity and the metabolic syndrome**. *Clinical nutrition* 2004, **23**(4):447-456.
24. Sampath H, Miyazaki M, Dobrzyn A, Ntambi JM: **Stearoyl-CoA desaturase-1 mediates the pro-lipogenic effects of dietary saturated fat**. *J Biol Chem* 2007, **282**(4):2483-2493.
25. van Dijk SJ, Feskens EJ, Bos MB, Hoelen DW, Heijligenberg R, Bromhaar MG, de Groot LC, de Vries JH, Muller M, Afman LA: **A saturated fatty acid-rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome**. *Am J Clin Nutr* 2009, **90**(6):1656-1664.
26. Mozaffarian D, Clarke R: **Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils**. *Eur J Clin Nutr* 2009, **63 Suppl 2**:S22-33.
27. Simopoulos AP: **Essential fatty acids in health and chronic disease**. *Am J Clin Nutr* 1999, **70**(3 Suppl):560S-569S.
28. Hluwalia B, Pincus G, Holman RT: **Essential fatty acid deficiency and its effects upon reproductive organs of male rabbits**. *J Nutr* 1967, **92**(2):205-214.
29. Menton DN: **The effects of essential fatty acid deficiency on the skin of the mouse**. *The American journal of anatomy* 1968, **122**(2):337-355.
30. Ridgway N. MR: **Biochemistry of lipids, lipoproteins and membranes**. *Elsevier academic press* 2016, **6th Edition**:600 pages.
31. Berger A, Mutch DM, German JB, Roberts MA: **Dietary effects of arachidonate-rich fungal oil and fish oil on murine hepatic and hippocampal gene expression**. *Lipids in health and disease* 2002, **1**:2.
32. Lin J, Yang R, Tarr PT, Wu PH, Handschin C, Li S, Yang W, Pei L, Uldry M, Tontonoz P *et al*: **Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP**. *Cell* 2005, **120**(2):261-273.
33. Collins JM, Neville MJ, Pinnick KE, Hodson L, Ruyter B, van Dijk TH, Reijngoud DJ, Fielding MD, Frayn KN: **De novo lipogenesis in the differentiating human adipocyte can provide all fatty acids necessary for maturation**. *J Lipid Res* 2011, **52**(9):1683-1692.
34. Matsuzaka T, Shimano H, Yahagi N, Yoshikawa T, Amemiya-Kudo M, Hasty AH, Okazaki H, Tamura Y, Iizuka Y, Ohashi K *et al*: **Cloning and characterization of a mammalian fatty acyl-CoA elongase as a lipogenic enzyme regulated by SREBPs**. *J Lipid Res* 2002, **43**(6):911-920.
35. Rhee SK, Kayani AJ, Ciszek A, Brenna JT: **Desaturation and interconversion of dietary stearic and palmitic acids in human plasma and lipoproteins**. *Am J Clin Nutr* 1997, **65**(2):451-458.
36. Wang Y, Botolin D, Christian B, Busik J, Xu J, Jump DB: **Tissue-specific, nutritional, and developmental regulation of rat fatty acid elongases**. *J Lipid Res* 2005, **46**(4):706-715.
37. Carlier H, Bernard A, Caselli C: **Digestion and absorption of polyunsaturated fatty acids**. *Reproduction, nutrition, development* 1991, **31**(5):475-500.

38. Masson CJ, Plat J, Mensink RP, Namiot A, Kisielewski W, Namiot Z, Fullekrug J, Ehehalt R, Glatz JF, Pelsers MM: **Fatty acid- and cholesterol transporter protein expression along the human intestinal tract**. *PloS one* 2010, **5**(4):e10380.
39. Liu JJ, Green P, John Mann J, Rapoport SI, Sublette ME: **Pathways of polyunsaturated fatty acid utilization: implications for brain function in neuropsychiatric health and disease**. *Brain research* 2015, **1597**:220-246.
40. Bonet ML, Ribot J, Palou A: **Lipid metabolism in mammalian tissues and its control by retinoic acid**. *Biochim Biophys Acta* 2012, **1821**(1):177-189.
41. Dobrzyn A, Dobrzyn P, Miyazaki M, Sampath H, Chu K, Ntambi JM: **Stearoyl-CoA desaturase 1 deficiency increases CTP:choline cytidylyltransferase translocation into the membrane and enhances phosphatidylcholine synthesis in liver**. *J Biol Chem* 2005, **280**(24):23356-23362.
42. Miyazaki M, Flowers MT, Sampath H, Chu K, Otzelberger C, Liu X, Ntambi JM: **Hepatic stearoyl-CoA desaturase-1 deficiency protects mice from carbohydrate-induced adiposity and hepatic steatosis**. *Cell Metab* 2007, **6**(6):484-496.
43. Liu X, Strable MS, Ntambi JM: **Stearoyl CoA desaturase 1: role in cellular inflammation and stress**. *Advances in nutrition* 2011, **2**(1):15-22.
44. Gutierrez-Juarez R, Pocai A, Mulas C, Ono H, Bhanot S, Monia BP, Rossetti L: **Critical role of stearoyl-CoA desaturase-1 (SCD1) in the onset of diet-induced hepatic insulin resistance**. *The Journal of clinical investigation* 2006, **116**(6):1686-1695.
45. Attie AD, Krauss RM, Gray-Keller MP, Brownlie A, Miyazaki M, Kastelein JJ, Lusis AJ, Stalenhoef AF, Stoehr JP, Hayden MR *et al*: **Relationship between stearoyl-CoA desaturase activity and plasma triglycerides in human and mouse hypertriglyceridemia**. *J Lipid Res* 2002, **43**(11):1899-1907.
46. Binczek E, Jenke B, Holz B, Gunter RH, Thevis M, Stoffel W: **Obesity resistance of the stearoyl-CoA desaturase-deficient (scd1-/-) mouse results from disruption of the epidermal lipid barrier and adaptive thermoregulation**. *Biological chemistry* 2007, **388**(4):405-418.
47. Moon YA, Shah NA, Mohapatra S, Warrington JA, Horton JD: **Identification of a mammalian long chain fatty acyl elongase regulated by sterol regulatory element-binding proteins**. *J Biol Chem* 2001, **276**(48):45358-45366.
48. Wang Y, Botolin D, Xu J, Christian B, Mitchell E, Jayaprakasam B, Nair MG, Peters JM, Busik JV, Olson LK *et al*: **Regulation of hepatic fatty acid elongase and desaturase expression in diabetes and obesity**. *J Lipid Res* 2006, **47**(9):2028-2041.
49. Strable MS, Ntambi JM: **Genetic control of de novo lipogenesis: role in diet-induced obesity**. *Critical reviews in biochemistry and molecular biology* 2010, **45**(3):199-214.
50. Green CD, Ozguden-Akkoc CG, Wang Y, Jump DB, Olson LK: **Role of fatty acid elongases in determination of de novo synthesized monounsaturated fatty acid species**. *J Lipid Res* 2010, **51**(7):1871-1877.
51. Matsuzaka T, Shimano H, Yahagi N, Kato T, Atsumi A, Yamamoto T, Inoue N, Ishikawa M, Okada S, Ishigaki N *et al*: **Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance**. *Nature medicine* 2007, **13**(10):1193-1202.
52. Morcillo S, Martin-Nunez GM, Rojo-Martinez G, Almaraz MC, Garcia-Escobar E, Mansego ML, de Marco G, Chaves FJ, Soriguer F: **ELOVL6 genetic variation is related to insulin sensitivity: a new candidate gene in energy metabolism**. *PloS one* 2011, **6**(6):e21198.

53. Hofacer R, Magrisso IJ, Jandacek R, Rider T, Tso P, Benoit SC, McNamara RK: **Omega-3 fatty acid deficiency increases stearoyl-CoA desaturase expression and activity indices in rat liver: positive association with non-fasting plasma triglyceride levels**. *Prostaglandins Leukot Essent Fatty Acids* 2012, **86**(1-2):71-77.
54. Brown JM, Chung S, Sawyer JK, Degirolamo C, Alger HM, Nguyen TM, Zhu X, Duong MN, Brown AL, Lord C *et al*: **Combined therapy of dietary fish oil and stearoyl-CoA desaturase 1 inhibition prevents the metabolic syndrome and atherosclerosis**. *Arteriosclerosis, thrombosis, and vascular biology* 2010, **30**(1):24-30.
55. Vessby B, Gustafsson IB, Tengblad S, Berglund L: **Indices of fatty acid desaturase activity in healthy human subjects: effects of different types of dietary fat**. *Br J Nutr* 2013, **110**(5):871-879.
56. Djousse L, Weir NL, Hanson NQ, Tsai MY, Gaziano JM: **Plasma phospholipid concentration of cis-palmitoleic acid and risk of heart failure**. *Circulation Heart failure* 2012, **5**(6):703-709.
57. Byberg L, Kilander L, Warensjo Lemming E, Michaelsson K, Vessby B: **Cancer death is related to high palmitoleic acid in serum and to polymorphisms in the SCD-1 gene in healthy Swedish men**. *Am J Clin Nutr* 2014, **99**(3):551-558.
58. Mihaly J, Marosvolgyi T, Szegedi A, Koroskenyi K, Lucas R, Torocsik D, Garcia AL, Decsi T, Ruhl R: **Increased FADS2-derived n-6 PUFAs and reduced n-3 PUFAs in plasma of atopic dermatitis patients**. *Skin pharmacology and physiology* 2014, **27**(5):242-248.
59. Wang Y, Torres-Gonzalez M, Tripathy S, Botolin D, Christian B, Jump DB: **Elevated hepatic fatty acid elongase-5 activity affects multiple pathways controlling hepatic lipid and carbohydrate composition**. *J Lipid Res* 2008, **49**(7):1538-1552.
60. Chu K, Miyazaki M, Man WC, Ntambi JM: **Stearoyl-coenzyme A desaturase 1 deficiency protects against hypertriglyceridemia and increases plasma high-density lipoprotein cholesterol induced by liver X receptor activation**. 2006.
61. Peter A, Cegan A, Wagner S, Lehmann R, Stefan N, Konigsrainer A, Konigsrainer I, Haring HU, Schleicher E: **Hepatic lipid composition and stearoyl-coenzyme A desaturase 1 mRNA expression can be estimated from plasma VLDL fatty acid ratios**. *Clinical chemistry* 2009, **55**(12):2113-2120.
62. Scaglia N, Igal RA: **Stearoyl-CoA desaturase is involved in the control of proliferation, anchorage-independent growth, and survival in human transformed cells**. *J Biol Chem* 2005, **280**(27):25339-25349.
63. Mutch DM, Grigorov M, Berger A, Fay LB, Roberts MA, Watkins SM, Williamson G, German JB: **An integrative metabolism approach identifies stearoyl-CoA desaturase as a target for an arachidonate-enriched diet**. *FASEB J* 2005, **19**(6):599-601.
64. Telle-Hansen VH, Larsen LN, Hostmark AT, Molin M, Dahl L, Almendingen K, Ulven SM: **Daily intake of cod or salmon for 2 weeks decreases the 18:1n-9/18:0 ratio and serum triacylglycerols in healthy subjects**. *Lipids* 2012, **47**(2):151-160.
65. Schiller K, Jacobs S, Jansen E, Weikert C, di Giuseppe R, Boeing H, Schulze MB, Kroger J: **Associated factors of estimated desaturase activity in the EPIC-Potsdam study**. *Nutr Metab Cardiovasc Dis* 2014, **24**(5):503-510.
66. Warensjo E, Ohrvall M, Vessby B: **Fatty acid composition and estimated desaturase activities are associated with obesity and lifestyle variables in men and women**. *Nutr Metab Cardiovasc Dis* 2006, **16**(2):128-136.
67. Petridou A, Nikolaidis MG, Matsakas A, Schulz T, Michna H, Mougios V: **Effect of exercise training on the fatty acid composition of lipid classes in rat liver, skeletal**

muscle, and adipose tissue. *European journal of applied physiology* 2005, **94**(1-2):84-92.
68. Scaglia N, Caviglia JM, Igal RA: **High stearoyl-CoA desaturase protein and activity levels in simian virus 40 transformed-human lung fibroblasts**. *Biochim Biophys Acta* 2005, **1687**(1-3):141-151.
69. Warensjo E, Riserus U, Gustafsson IB, Mohsen R, Cederholm T, Vessby B: **Effects of saturated and unsaturated fatty acids on estimated desaturase activities during a controlled dietary intervention**. *Nutr Metab Cardiovasc Dis* 2008, **18**(10):683-690.
70. Hulbert AJ, Kelly MA, Abbott SK: **Polyunsaturated fats, membrane lipids and animal longevity**. *Journal of comparative physiology B, Biochemical, systemic, and environmental physiology* 2014, **184**(2):149-166.
71. Phinney SD, Odin RS, Johnson SB, Holman RT: **Reduced arachidonate in serum phospholipids and cholesteryl esters associated with vegetarian diets in humans**. *Am J Clin Nutr* 1990, **51**(3):385-392.
72. Simopoulos AP: **Nutrigenetics/Nutrigenomics**. *Annual review of public health* 2010, **31**:53-68.
73. Yamagishi K, Iso H, Date C, Fukui M, Wakai K, Kikuchi S, Inaba Y, Tanabe N, Tamakoshi A, Japan Collaborative Cohort Study for Evaluation of Cancer Risk Study G: **Fish, omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nationwide community-based cohort of Japanese men and women the JACC (Japan Collaborative Cohort Study for Evaluation of Cancer Risk) Study**. *Journal of the American College of Cardiology* 2008, **52**(12):988-996.
74. Fenton JI, Hord NG, Ghosh S, Gurzell EA: **Immunomodulation by dietary long chain omega-3 fatty acids and the potential for adverse health outcomes**. *Prostaglandins Leukot Essent Fatty Acids* 2013, **89**(6):379-390.
75. Jump DB, Depner CM, Tripathy S: **Omega-3 fatty acid supplementation and cardiovascular disease**. *J Lipid Res* 2012, **53**(12):2525-2545.
76. Simopoulos AP: **The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease**. *Asia Pacific journal of clinical nutrition* 2008, **17 Suppl 1**:131-134.
77. de Wilde MC, Hogyes E, Kiliaan AJ, Farkas T, Luiten PG, Farkas E: **Dietary fatty acids alter blood pressure, behavior and brain membrane composition of hypertensive rats**. *Brain research* 2003, **988**(1-2):9-19.
78. Das UN: **A defect in the activity of Delta6 and Delta5 desaturases may be a factor in the initiation and progression of atherosclerosis**. *Prostaglandins Leukot Essent Fatty Acids* 2007, **76**(5):251-268.
79. Bosma-den Boer MM, van Wetten ML, Pruimboom L: **Chronic inflammatory diseases are stimulated by current lifestyle: how diet, stress levels and medication prevent our body from recovering**. *Nutrition & metabolism* 2012, **9**(1):32.
80. Beaudoin F, Michaelson LV, Hey SJ, Lewis MJ, Shewry PR, Sayanova O, Napier JA: **Heterologous reconstitution in yeast of the polyunsaturated fatty acid biosynthetic pathway**. *Proc Natl Acad Sci U S A* 2000, **97**(12):6421-6426.
81. Lu Y, Vaarhorst A, Merry AH, Dolle ME, Hovenier R, Imholz S, Schouten LJ, Heijmans BT, Muller M, Slagboom PE *et al*: **Markers of endogenous desaturase activity and risk of coronary heart disease in the CAREMA cohort study**. *PloS one* 2012, **7**(7):e41681.
82. Morales E, Bustamante M, Gonzalez JR, Guxens M, Torrent M, Mendez M, Garcia-Esteban R, Julvez J, Forns J, Vrijheid M *et al*: **Genetic variants of the FADS gene**

cluster and ELOVL gene family, colostrums LC-PUFA levels, breastfeeding, and child cognition. *PloS one* 2011, **6**(2):e17181.
83. Rett BS, Whelan J: **Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults consuming Western-type diets: a systematic review**. *Nutrition & metabolism* 2011, **8**:36.
84. Tripathy S, Torres-Gonzalez M, Jump DB: **Elevated hepatic fatty acid elongase-5 activity corrects dietary fat-induced hyperglycemia in obese C57BL/6J mice**. *J Lipid Res* 2010, **51**(9):2642-2654.
85. Sekiya M, Yahagi N, Matsuzaka T, Najima Y, Nakakuki M, Nagai R, Ishibashi S, Osuga J, Yamada N, Shimano H: **Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression**. *Hepatology* 2003, **38**(6):1529-1539.
86. Fan YY, Monk JM, Hou TY, Callway E, Vincent L, Weeks B, Yang P, Chapkin RS: **Characterization of an arachidonic acid-deficient (Fads1 knockout) mouse model**. *J Lipid Res* 2012, **53**(7):1287-1295.
87. Moon YA, Hammer RE, Horton JD: **Deletion of ELOVL5 leads to fatty liver through activation of SREBP-1c in mice**. *J Lipid Res* 2009, **50**(3):412-423.
88. Monteiro J, Li FJ, Maclennan M, Rabalski A, Moghadasian MH, Nakamura MT, Ma DW: **Menhaden oil, but not safflower or soybean oil, aids in restoring the polyunsaturated fatty acid profile in the novel delta-6-desaturase null mouse**. *Lipids in health and disease* 2012, **11**:60.
89. Stoffel W, Hammels I, Jenke B, Binczek E, Schmidt-Soltau I, Brodesser S, Odenthal M, Thevis M: **Obesity resistance and deregulation of lipogenesis in Delta6-fatty acid desaturase (FADS2) deficiency**. *EMBO reports* 2014, **15**(1):110-120.
90. Stoffel W, Holz B, Jenke B, Binczek E, Gunter RH, Kiss C, Karakesisoglou I, Thevis M, Weber AA, Arnhold S *et al*: **Delta6-desaturase (FADS2) deficiency unveils the role of omega3- and omega6-polyunsaturated fatty acids**. *The EMBO journal* 2008, **27**(17):2281-2292.
91. Cormier H, Rudkowska I, Lemieux S, Couture P, Julien P, Vohl MC: **Effects of FADS and ELOVL polymorphisms on indexes of desaturase and elongase activities: results from a pre-post fish oil supplementation**. *Genes & nutrition* 2014, **9**(6):437.
92. Kim SR, Jeon SY, Lee SM: **The association of cardiovascular risk factors with saturated fatty acids and fatty acid desaturase indices in erythrocyte in middle-aged Korean adults**. *Lipids in health and disease* 2015, **14**:133.
93. Tripathy S, Lytle KA, Stevens RD, Bain JR, Newgard CB, Greenberg AS, Huang LS, Jump DB: **Fatty acid elongase-5 (Elovl5) regulates hepatic triglyceride catabolism in obese C57BL/6J mice**. *J Lipid Res* 2014, **55**(7):1448-1464.
94. Bokor S, Dumont J, Spinneker A, Gonzalez-Gross M, Nova E, Widhalm K, Moschonis G, Stehle P, Amouyel P, De Henauw S *et al*: **Single nucleotide polymorphisms in the FADS gene cluster are associated with delta-5 and delta-6 desaturase activities estimated by serum fatty acid ratios**. *J Lipid Res* 2010, **51**(8):2325-2333.
95. Abdelmagid SA, Clarke SE, Roke K, Nielsen DE, Badawi A, El-Sohemy A, Mutch DM, Ma DW: **Ethnicity, sex, FADS genetic variation, and hormonal contraceptive use influence delta-5- and delta-6-desaturase indices and plasma docosahexaenoic acid concentration in young Canadian adults: a cross-sectional study**. *Nutrition & metabolism* 2015, **12**:14.

96. Liu Y, McNamara RK: **Elevated Delta-6 desaturase (FADS2) gene expression in the prefrontal cortex of patients with bipolar disorder**. *Journal of psychiatric research* 2011, **45**(2):269-272.
97. Enser M, Roberts JL: **The regulation of hepatic stearoyl-coenzyme A desaturase in obese-hyperglycaemic (ob/ob) mice by food intake and the fatty acid composition of the diet**. *The Biochemical journal* 1982, **206**(3):561-570.
98. Horton JD: **Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis**. *Biochem Soc Trans* 2002, **30**(Pt 6):1091-1095.
99. Nakamura MT, Nara TY: **Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases**. *Annual review of nutrition* 2004, **24**:345-376.
100. Turyn J, Stojek M, Swierczynski J: **Up-regulation of stearoyl-CoA desaturase 1 and elongase 6 genes expression in rat lipogenic tissues by chronic food restriction and chronic food restriction/refeeding**. *Molecular and cellular biochemistry* 2010, **345**(1-2):181-188.
101. Jump DB, Botolin D, Wang Y, Xu J, Christian B, Demeure O: **Fatty acid regulation of hepatic gene transcription**. *J Nutr* 2005, **135**(11):2503-2506.
102. Sessler AM, Kaur N, Palta JP, Ntambi JM: **Regulation of stearoyl-CoA desaturase 1 mRNA stability by polyunsaturated fatty acids in 3T3-L1 adipocytes**. *J Biol Chem* 1996, **271**(47):29854-29858.
103. Kumadaki S, Matsuzaka T, Kato T, Yahagi N, Yamamoto T, Okada S, Kobayashi K, Takahashi A, Yatoh S, Suzuki H *et al*: **Mouse Elovl-6 promoter is an SREBP target**. *Biochem Biophys Res Commun* 2008, **368**(2):261-266.
104. Qin Y, Dalen KT, Gustafsson JA, Nebb HI: **Regulation of hepatic fatty acid elongase 5 by LXRalpha-SREBP-1c**. *Biochim Biophys Acta* 2009, **1791**(2):140-147.
105. Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K *et al*: **Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes**. *J Biol Chem* 1999, **274**(50):35832-35839.
106. Cho HP, Nakamura M, Clarke SD: **Cloning, expression, and fatty acid regulation of the human delta-5 desaturase**. *J Biol Chem* 1999, **274**(52):37335-37339.
107. Desvergne B, Michalik L, Wahli W: **Be fit or be sick: peroxisome proliferator-activated receptors are down the road**. *Mol Endocrinol* 2004, **18**(6):1321-1332.
108. Martini C, Pallottini V: **Cholesterol: from feeding to gene regulation**. *Genes & nutrition* 2007, **2**(2):181-193.
109. Yoshikawa T, Shimano H, Yahagi N, Ide T, Amemiya-Kudo M, Matsuzaka T, Nakakuki M, Tomita S, Okazaki H, Tamura Y *et al*: **Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements**. *J Biol Chem* 2002, **277**(3):1705-1711.
110. Chawla A, Repa JJ, Evans RM, Mangelsdorf DJ: **Nuclear receptors and lipid physiology: opening the X-files**. *Science* 2001, **294**(5548):1866-1870.
111. Kliewer SA, Xu HE, Lambert MH, Willson TM: **Peroxisome proliferator-activated receptors: from genes to physiology**. *Recent progress in hormone research* 2001, **56**:239-263.
112. Shulman AI, Mangelsdorf DJ: **Retinoid x receptor heterodimers in the metabolic syndrome**. *The New England journal of medicine* 2005, **353**(6):604-615.
113. Pawar A, Botolin D, Mangelsdorf DJ, Jump DB: **The role of liver X receptor-alpha in the fatty acid regulation of hepatic gene expression**. *J Biol Chem* 2003, **278**(42):40736-40743.

114. Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, Mangelsdorf DJ: **Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta**. *Genes Dev* 2000, **14**(22):2819-2830.
115. Zelcer N, Tontonoz P: **Liver X receptors as integrators of metabolic and inflammatory signaling**. *The Journal of clinical investigation* 2006, **116**(3):607-614.
116. Gavrilova O, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, Nicol CJ, Vinson C, Gonzalez FJ, Reitman ML: **Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass**. *J Biol Chem* 2003, **278**(36):34268-34276.
117. Szanto A, Narkar V, Shen Q, Uray IP, Davies PJ, Nagy L: **Retinoid X receptors: X-ploring their (patho)physiological functions**. *Cell Death Differ* 2004, **11 Suppl 2**:S126-143.
118. Kalaany NY, Gauthier KC, Zavacki AM, Mammen PP, Kitazume T, Peterson JA, Horton JD, Garry DJ, Bianco AC, Mangelsdorf DJ: **LXRs regulate the balance between fat storage and oxidation**. *Cell Metab* 2005, **1**(4):231-244.
119. Lengqvist J, Mata De Urquiza A, Bergman AC, Willson TM, Sjovall J, Perlmann T, Griffiths WJ: **Polyunsaturated fatty acids including docosahexaenoic and arachidonic acid bind to the retinoid X receptor alpha ligand-binding domain**. *Molecular & cellular proteomics : MCP* 2004, **3**(7):692-703.
120. Mangelsdorf DJ, Evans RM: **The RXR heterodimers and orphan receptors**. *Cell* 1995, **83**(6):841-850.
121. Bordoni A, Di Nunzio M, Danesi F, Biagi PL: **Polyunsaturated fatty acids: From diet to binding to ppars and other nuclear receptors**. *Genes & nutrition* 2006, **1**(2):95-106.
122. Lee JM, Kim SS, Cho YS: **The Role of PPARgamma in Helicobacter pylori Infection and Gastric Carcinogenesis**. *PPAR Res* 2012, **2012**:687570.
123. Paik J, Vogel S, Quadro L, Piantedosi R, Gottesman M, Lai K, Hamberger L, Vieira Mde M, Blaner WS: **Vitamin A: overlapping delivery pathways to tissues from the circulation**. *J Nutr* 2004, **134**(1):276S-280S.
124. von Lintig J, Vogt K: **Vitamin A formation in animals: molecular identification and functional characterization of carotene cleaving enzymes**. *J Nutr* 2004, **134**(1):251S-256S.
125. Chen W, Chen G: **The Roles of Vitamin A in the Regulation of Carbohydrate, Lipid, and Protein Metabolism**. *Journal of clinical medicine* 2014, **3**(2):453-479.
126. Schreiber R, Taschler U, Preiss-Landl K, Wongsiriroj N, Zimmermann R, Lass A: **Retinyl ester hydrolases and their roles in vitamin A homeostasis**. *Biochim Biophys Acta* 2012, **1821**(1):113-123.
127. Takase S, Suruga K, Goda T: **Regulation of vitamin A metabolism-related gene expression**. *Br J Nutr* 2000, **84 Suppl 2**:S217-221.
128. Zhang R, Wang Y, Li R, Chen G: **Transcriptional Factors Mediating Retinoic Acid Signals in the Control of Energy Metabolism**. *International journal of molecular sciences* 2015, **16**(6):14210-14244.
129. Ross DA: **Vitamin A and public health: challenges for the next decade**. *The Proceedings of the Nutrition Society* 1998, **57**(1):159-165.
130. Michalik L, Wahli W: **Guiding ligands to nuclear receptors**. *Cell* 2007, **129**(4):649-651.
131. de Lera AR, Krezel W, Ruhl R: **An Endogenous Mammalian Retinoid X Receptor Ligand, At Last!** *ChemMedChem* 2016, **11**(10):1027-1037.

132. Ruhl R, Krzyzosiak A, Niewiadomska-Cimicka A, Rochel N, Szeles L, Vaz B, Wietrzych-Schindler M, Alvarez S, Szklenar M, Nagy L *et al*: **9-cis-13,14-Dihydroretinoic Acid Is an Endogenous Retinoid Acting as RXR Ligand in Mice**. *PLoS genetics* 2015, **11**(6):e1005213.
133. Lampen A, Meyer S, Nau H: **Effects of receptor-selective retinoids on CYP26 gene expression and metabolism of all-trans-retinoic acid in intestinal cells**. *Drug Metab Dispos* 2001, **29**(5):742-747.
134. Ross AC, Zolfaghari R: **Cytochrome P450s in the regulation of cellular retinoic acid metabolism**. *Annual review of nutrition* 2011, **31**:65-87.
135. Lampen A, Meyer S, Nau H: **Phytanic acid and docosahexaenoic acid increase the metabolism of all-trans-retinoic acid and CYP26 gene expression in intestinal cells**. *Biochim Biophys Acta* 2001, **1521**(1-3):97-106.
136. **Molecular biology of the cell**, 4. edn. New York, NY u.a.: Garland; 2002.
137. Abbott SK, Else PL, Atkins TA, Hulbert AJ: **Fatty acid composition of membrane bilayers: importance of diet polyunsaturated fat balance**. *Biochim Biophys Acta* 2012, **1818**(5):1309-1317.
138. Yehuda S, Rabinovitz S, Carasso RL, Mostofsky DI: **The role of polyunsaturated fatty acids in restoring the aging neuronal membrane**. *Neurobiology of aging* 2002, **23**(5):843-853.
139. Purdon AD, Rosenberger TA, Shetty HU, Rapoport SI: **Energy consumption by phospholipid metabolism in mammalian brain**. *Neurochemical research* 2002, **27**(12):1641-1647.
140. Escudero A, Montilla JC, Garcia JM, Sanchez-Quevedo MC, Periago JL, Hortelano P, Suarez MD: **Effect of dietary (n-9), (n-6) and (n-3) fatty acids on membrane lipid composition and morphology of rat erythrocytes**. *Biochim Biophys Acta* 1998, **1394**(1):65-73.
141. Schachter D: **Fluidity and function of hepatocyte plasma membranes**. *Hepatology* 1984, **4**(1):140-151.
142. Dainese E, De Fabritiis G, Sabatucci A, Oddi S, Angelucci CB, Di Pancrazio C, Giorgino T, Stanley N, Del Carlo M, Cravatt BF *et al*: **Membrane lipids are key modulators of the endocannabinoid-hydrolase FAAH**. *The Biochemical journal* 2014, **457**(3):463-472.
143. Van Voorst F, De Kruijff B: **Role of lipids in the translocation of proteins across membranes**. *The Biochemical journal* 2000, **347 Pt 3**:601-612.
144. Jacobsson L, Lindgarde F, Manthorpe R, Akesson B: **Correlation of fatty acid composition of adipose tissue lipids and serum phosphatidylcholine and serum concentrations of micronutrients with disease duration in rheumatoid arthritis**. *Annals of the rheumatic diseases* 1990, **49**(11):901-905.
145. Bonilla S, Redonnet A, Noel-Suberville C, Pallet V, Garcin H, Higueret P: **High-fat diets affect the expression of nuclear retinoic acid receptor in rat liver**. *Br J Nutr* 2000, **83**(6):665-671.
146. Muurling M, Mensink RP, Pijl H, Romijn JA, Havekes LM, Voshol PJ: **Rosiglitazone improves muscle insulin sensitivity, irrespective of increased triglyceride content, in ob/ob mice**. *Metabolism: clinical and experimental* 2003, **52**(8):1078-1083.
147. Li AC, Binder CJ, Gutierrez A, Brown KK, Plotkin CR, Pattison JW, Valledor AF, Davis RA, Willson TM, Witztum JL *et al*: **Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPARalpha, beta/delta, and gamma**. *The Journal of clinical investigation* 2004, **114**(11):1564-1576.

148. Yue TL, Bao W, Jucker BM, Gu JL, Romanic AM, Brown PJ, Cui J, Thudium DT, Boyce R, Burns-Kurtis CL *et al*: **Activation of peroxisome proliferator-activated receptor-alpha protects the heart from ischemia/reperfusion injury**. *Circulation* 2003, **108**(19):2393-2399.
149. Elmazar MM, Ruhl R, Reichert U, Shroot B, Nau H: **RARalpha-mediated teratogenicity in mice is potentiated by an RXR agonist and reduced by an RAR antagonist: dissection of retinoid receptor-induced pathways**. *Toxicology and applied pharmacology* 1997, **146**(1):21-28.
150. Jakel H, Nowak M, Moitrot E, Dehondt H, Hum DW, Pennacchio LA, Fruchart-Najib J, Fruchart JC: **The liver X receptor ligand T0901317 down-regulates APOA5 gene expression through activation of SREBP-1c**. *J Biol Chem* 2004, **279**(44):45462-45469.
151. Zhang Y, Repa JJ, Gauthier K, Mangelsdorf DJ: **Regulation of lipoprotein lipase by the oxysterol receptors, LXRalpha and LXRbeta**. *J Biol Chem* 2001, **276**(46):43018-43024.
152. Folch J, Lees M, Sloane Stanley GH: **A simple method for the isolation and purification of total lipides from animal tissues**. *J Biol Chem* 1957, **226**(1):497-509.
153. Decsi T, Szabo E, Burus I, Marosvolgyi T, Kozari A, Erhardt E, Soltesz G: **Low contribution of n-3 polyunsaturated fatty acids to plasma and erythrocyte membrane lipids in diabetic young adults**. *Prostaglandins Leukot Essent Fatty Acids* 2007, **76**(3):159-164.
154. Bligh EG, Dyer WJ: **A rapid method of total lipid extraction and purification**. *Canadian journal of biochemistry and physiology* 1959, **37**(8):911-917.
155. Brugger B, Erben G, Sandhoff R, Wieland FT, Lehmann WD: **Quantitative analysis of biological membrane lipids at the low picomole level by nano-electrospray ionization tandem mass spectrometry**. *Proc Natl Acad Sci U S A* 1997, **94**(6):2339-2344.
156. Liebisch G, Binder M, Schifferer R, Langmann T, Schulz B, Schmitz G: **High throughput quantification of cholesterol and cholesteryl ester by electrospray ionization tandem mass spectrometry (ESI-MS/MS)**. *Biochim Biophys Acta* 2006, **1761**(1):121-128.
157. Liebisch G, Lieser B, Rathenberg J, Drobnik W, Schmitz G: **High-throughput quantification of phosphatidylcholine and sphingomyelin by electrospray ionization tandem mass spectrometry coupled with isotope correction algorithm**. *Biochim Biophys Acta* 2004, **1686**(1-2):108-117.
158. Liebisch G, Drobnik W, Reil M, Trumbach B, Arnecke R, Olgemoller B, Roscher A, Schmitz G: **Quantitative measurement of different ceramide species from crude cellular extracts by electrospray ionization tandem mass spectrometry (ESI-MS/MS)**. *J Lipid Res* 1999, **40**(8):1539-1546.
159. Liebisch G, Vizcaino JA, Kofeler H, Trotzmuller M, Griffiths WJ, Schmitz G, Spener F, Wakelam MJ: **Shorthand notation for lipid structures derived from mass spectrometry**. *J Lipid Res* 2013, **54**(6):1523-1530.
160. Deng X, Cagen LM, Wilcox HG, Park EA, Raghow R, Elam MB: **Regulation of the rat SREBP-1c promoter in primary rat hepatocytes**. *Biochem Biophys Res Commun* 2002, **290**(1):256-262.
161. Ntambi JM: **Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol**. *J Lipid Res* 1999, **40**(9):1549-1558.
162. Sun H, Jiang T, Wang S, He B, Zhang Y, Piao D, Yu C, Wu N, Han P: **The effect of LXRalpha, ChREBP and Elovl6 in liver and white adipose tissue on medium-**

and long-chain fatty acid diet-induced insulin resistance. *Diabetes research and clinical practice* 2013, **102**(3):183-192.
163. Jeffcoat R, James AT: **The control of stearoyl-CoA desaturase by dietary linoleic acid**. *FEBS Lett* 1978, **85**(1):114-118.
164. Shearn CT, Smathers RL, Jiang H, Orlicky DJ, Maclean KN, Petersen DR: **Increased dietary fat contributes to dysregulation of the LKB1/AMPK pathway and increased damage in a mouse model of early-stage ethanol-mediated steatosis**. *J Nutr Biochem* 2013, **24**(8):1436-1445.
165. Park EI, Paisley EA, Mangian HJ, Swartz DA, Wu MX, O'Morchoe PJ, Behr SR, Visek WJ, Kaput J: **Lipid level and type alter stearoyl CoA desaturase mRNA abundance differently in mice with distinct susceptibilities to diet-influenced diseases**. *J Nutr* 1997, **127**(4):566-573.
166. Tebbey PW, Buttke TM: **Stearoyl-CoA desaturase gene expression in lymphocytes**. *Biochem Biophys Res Commun* 1992, **186**(1):531-536.
167. Huang LL, Wan JB, Wang B, He CW, Ma H, Li TW, Kang JX: **Suppression of acute ethanol-induced hepatic steatosis by docosahexaenoic acid is associated with downregulation of stearoyl-CoA desaturase 1 and inflammatory cytokines**. *Prostaglandins Leukot Essent Fatty Acids* 2013, **88**(5):347-353.
168. Peter A, Weigert C, Staiger H, Machicao F, Schick F, Machann J, Stefan N, Thamer C, Haring HU, Schleicher E: **Individual stearoyl-coa desaturase 1 expression modulates endoplasmic reticulum stress and inflammation in human myotubes and is associated with skeletal muscle lipid storage and insulin sensitivity in vivo**. *Diabetes* 2009, **58**(8):1757-1765.
169. de Wilde J, Mohren R, van den Berg S, Boekschoten M, Dijk KW, de Groot P, Muller M, Mariman E, Smit E: **Short-term high fat-feeding results in morphological and metabolic adaptations in the skeletal muscle of C57BL/6J mice**. *Physiological genomics* 2008, **32**(3):360-369.
170. McClintick JN, Crabb DW, Tian H, Pinaire J, Smith JR, Jerome RE, Edenberg HJ: **Global effects of vitamin A deficiency on gene expression in rat liver: evidence for hypoandrogenism**. *J Nutr Biochem* 2006, **17**(5):345-355.
171. Miller CW, Waters KM, Ntambi JM: **Regulation of hepatic stearoyl-CoA desaturase gene 1 by vitamin A**. *Biochem Biophys Res Commun* 1997, **231**(1):206-210.
172. Letondor A, Buaud B, Vaysse C, Richard E, Laye S, Pallet V, Alfos S: **EPA/DHA and Vitamin A Supplementation Improves Spatial Memory and Alleviates the Age-related Decrease in Hippocampal RXRgamma and Kinase Expression in Rats**. *Frontiers in aging neuroscience* 2016, **8**:103.
173. Samuel W, Kutty RK, Nagineni S, Gordon JS, Prouty SM, Chandraratna RA, Wiggert B: **Regulation of stearoyl coenzyme A desaturase expression in human retinal pigment epithelial cells by retinoic acid**. *J Biol Chem* 2001, **276**(31):28744-28750.
174. Saliani N, Darabi M, Yousefi B, Baradaran B, Khaniani MS, Darabi M, Shaaker M, Mehdizadeh A, Naji T, Hashemi M: **PPARgamma agonist-induced alterations in Delta6-desaturase and stearoyl-CoA desaturase 1: Role of MEK/ERK1/2 pathway**. *World journal of hepatology* 2013, **5**(4):220-225.
175. Singh Ahuja H, Liu S, Crombie DL, Boehm M, Leibowitz MD, Heyman RA, Depre C, Nagy L, Tontonoz P, Davies PJ: **Differential effects of rexinoids and thiazolidinediones on metabolic gene expression in diabetic rodents**. *Molecular pharmacology* 2001, **59**(4):765-773.

176. Toyama T, Kudo N, Hibino Y, Mitsumoto A, Nishikawa M, Kawashima Y: **Effects of pioglitazone on stearoyl-CoA desaturase in obese Zucker fa/fa rats**. *Journal of pharmacological sciences* 2007, **104**(2):137-145.
177. Miyazaki M, Dobrzyn A, Man WC, Chu K, Sampath H, Kim HJ, Ntambi JM: **Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and -independent mechanisms**. *J Biol Chem* 2004, **279**(24):25164-25171.
178. Zhang X, Liu J, Su W, Wu J, Wang C, Kong X, Gustafsson JA, Ding J, Ma X, Guan Y: **Liver X receptor activation increases hepatic fatty acid desaturation by the induction of SCD1 expression through an LXRalpha-SREBP1c-dependent mechanism**. *Journal of diabetes* 2014, **6**(3):212-220.
179. Velliquette RA, Gillies PJ, Kris-Etherton PM, Green JW, Zhao G, Vanden Heuvel JP: **Regulation of human stearoyl-CoA desaturase by omega-3 and omega-6 fatty acids: Implications for the dietary management of elevated serum triglycerides**. *Journal of clinical lipidology* 2009, **3**(4):281-288.
180. Brostow DP, Odegaard AO, Koh WP, Duval S, Gross MD, Yuan JM, Pereira MA: **Omega-3 fatty acids and incident type 2 diabetes: the Singapore Chinese Health Study**. *Am J Clin Nutr* 2011, **94**(2):520-526.
181. Ralston JC, Matravadia S, Gaudio N, Holloway GP, Mutch DM: **Polyunsaturated fatty acid regulation of adipocyte FADS1 and FADS2 expression and function**. *Obesity* 2015, **23**(4):725-728.
182. Simopoulos AP: **An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity**. *Nutrients* 2016, **8**(3):128.
183. Howell G, 3rd, Deng X, Yellaturu C, Park EA, Wilcox HG, Raghow R, Elam MB: **N-3 polyunsaturated fatty acids suppress insulin-induced SREBP-1c transcription via reduced trans-activating capacity of LXRalpha**. *Biochim Biophys Acta* 2009, **1791**(12):1190-1196.

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List of publications related to the dissertation

1. **Weiss, K.**, Mihály, J., Liebisch, G., Marosvölgyi, T., Garcia, A. L., Schmitz, G., Decsi, T., Rühl, R.: Effect of high versus low doses of fat and vitamin A dietary supplementation on fatty acid composition of phospholipids in mice.
   Genes Nutr. 9 (1), 368, 2014.
   DOI: http://dx.doi.org/10.1007/s12263-013-0368-0
   IF: 2.794

2. **Weiss, K.**, Mihály, J., Liebisch, G., Marosvölgyi, T., Schmitz, G., Decsi, T., Rühl, R.: Effect of synthetic ligands of PPAR alfa, béta/delta, gamma, RAR, RXR and LXR on the fatty acid composition of phospholipids in mice.
   Lipids. 46 (11), 1013-1020, 2011.
   DOI: http://dx.doi.org/10.1007/s11745-011-3593-6
   IF: 2.129

List of other publications

3. Landrier, J. F., Kasiri, E., Karkeni, E., Mihály, J., Béke, G., **Weiss, K.**, Lucas, R., Aydemir, G., Salles, J., Walrand, S., de Lera, Á. R., Rühl, R.: Reduced adiponectin expression after high-fat diet is associated with selective up-regulation of ALDH1A1 and further retinoic acid receptor signaling in adipose tissue.
   Faseb J. [Epub ahead of print], 2016.
   IF: 5.299 (2015)

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4. Mihály, J., Gericke, J., Aydemir, G., **Weiss, K.**, Carlsen, H., Blomhoff, R., Garcia, J., Rühl, R.: Reduced retinoid signaling in the skin after systemic retinoid-X receptor ligand treatment in mice with potential relevance for skin disorders.
Dermatology. *225* (4), 304-311, 2012.
DOI: http://dx.doi.org/10.1159/000345496
IF: 2.024

Total IF of journals (all publications): 12,246
Total IF of journals (publications related to the dissertation): 4,923

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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9. KEYWORDS

Stearoyl-CoA desaturase, Elongase, Fatty acid desaturase, Nuclear Hormone Receptors, Phospholipids, Phosphatidylethanolamine, Phosphatidylcholine, Lysophosphatidylcholine, ESI-MS/MS, monounsaturated fatty acid, saturated fatty acid, polyunsaturated fatty acid

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