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Omega-3 Fatty Acid Deficiency Increases Stearoyl-CoA Desaturase Expression and Activity Indices in Rat Liver: Positive Association with Non-Fasting Plasma Triglyceride Levels

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Abstract

Although omega-3 (*n*-3) fatty acids negatively regulate triglyceride biosynthesis, the mechanisms mediating this effect are poorly understood, and emerging evidence suggests that stearoyl-CoA desaturase (Scd1) is required for *de novo* triglyceride biosynthesis. To investigate this mechanism, we determined the effects of perinatal *n*-3 deficiency and postnatal repletion on rat liver *Scd1* mRNA expression and activity indices (liver 16:1/16:0 & 18:1/18:0 ratios), and determined relationships with postprandial (non-fasting) plasma triglyceride levels. Rats were fed conventional diets with or without the *n*-3 fatty acid precursor α-linolenic acid (ALA, 18:3*n*-3) during perinatal development (E0-P100), and a subset of rats fed the ALA− diet were switched to the ALA+ diet post-weaning (P21-P100, repletion). Compared with controls, rats fed the ALA− diet exhibited significantly lower liver long-chain *n*-3 fatty acid compositions and elevations in monounsaturated fatty acid composition, both of which were normalized in repleted rats. Liver *Scd1* mRNA expression and activity indices (16:1/16:0 & 18:1/18:0 ratios) were significantly greater in *n*-3 deficient rats compared with controls and repleted rats. Among all rats, liver *Scd1* mRNA expression was positively correlated with liver 18:1/18:0 and 16:1/16:0 ratios. Plasma triglyceride levels, but not glucose or insulin levels, were significantly greater in *n*-3 deficient rats compared with controls and repleted rats. Liver *Scd1* mRNA expression and activity indices were positively correlated with plasma triglyceride levels. These preclinical findings demonstrate that *n*-3 fatty acid status is an important determinant of liver *Scd1* mRNA expression and activity, and suggest that down-regulation of *Scd1* is a mechanism by which *n*-3 fatty acids repress constitutive triglyceride biosynthesis.

Keywords

Omega-3 fatty acid; stearoyl-CoA desaturase-1 (Scd1); triglyceride; glucose; liver; rat

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1. Introduction

Elevated fasting plasma triglyceride (TG) levels are an independent risk factor for cardiovascular disease [1], and postprandial (non-fasting) TG levels are a strong predictor of future cardiovascular risk [2–4]. Supplementation with long-chain omega-3 (*n*-3) fatty acids, including eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3), dose-dependently decrease elevated TG levels in patients with hypertriglyceridemia [5,6], and lower levels of long-chain *n*-3 fatty acids are associated with increased cardiovascular disease risk [7,8]. Recurrent mood disorders, including major depressive disorder and bipolar disorder, are associated with long-chain *n*-3 fatty acid deficits [9], elevated TG levels [10,11], and excess mortality attributable in part to premature cardiovascular-related disease [12]. While these clinical observations suggest that *n*-3 fatty acid status is an important determinant of lipid homeostasis, the mechanisms mediating this relationship remain poorly understood.

An emerging body of evidence suggests that stearoyl-CoA desaturase (*Scd1*, delta9-desaturase) plays a central role in regulating hepatic TG biosynthesis. Scd1 is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (MUFA), palimitoleic acid (16:1*n*-7) and oleic acid (18:1*n*-9), from saturated fatty acids (SFA), palmitic acid (16:0) and stearic acid (18:0), respectively. Oleic acid is a required substrate for the synthesis of TG [13], and *Scd1* mutant mice exhibit deficits in TG biosynthesis [14–16]. Pharmacological inhibition of the Scd1 enzyme reduces elevated TG levels in rodent disease models [17]. In rodents [16] and human subjects [18,19], liver *Scd1* mRNA expression is correlated with activity indices (16:1/16:0 and 18:1/18:0 ratios) in liver and plasma triglyceride fractions, and elevations in the plasma 18:1/18:0 ratio (‘desaturation index’) are positively correlated with plasma TG levels [18]. This body of evidence suggests that Scd1 plays a central role in the regulation of hepatic TG biosynthesis.

Several *in vitro* and *ex vivo* studies have found that short and long-chain *n*-3 and *n*-6 polyunsaturated fatty acids (PUFA), but not oleic acid (18:1*n*-9) or stearic acid (18:0), decrease *Scd1* expression at the level of transcription and mRNA stability [20–22]. Additionally, dietary supplementation with long-chain *n*-3 fatty acids (fish oil) decrease *Scd1* activity in liver microsomes *ex vivo* [23] and reduce liver TG synthesis and/or secretion in different rodent models [24–29]. Moreover, a prior study found that dietary supplementation with EPA (20:5*n*-3) decreased mouse liver *Scd1* mRNA expression and hepatic TG content [30]. While these findings implicate *n*-3 fatty acids as a negative regulator of liver *Scd1* mRNA expression, another study found that chronic dietary *n*-3 fatty acid deficiency resulting in a depletion of rat liver *n*-3 fatty acids did not significantly alter liver *Scd1* mRNA expression [31]. To further evaluate this mechanism, we investigated the effect of perinatal *n*-3 fatty acid deficiency, which produces robust reductions in peripheral *n*-3 fatty acid levels in adulthood, and repletion on liver *Scd1* mRNA expression and activity indices (liver 18:1/18:0 & 16:1/16:0 ratios), and investigated relationships with non-fasting plasma TG concentrations. In view of evidence also implicating *Scd1* expression/activity in insulin sensitivity [32,33], we additionally investigated relationships with plasma glucose and insulin concentrations. Our primary hypothesis was that *n*-3 fatty acid deficiency would increase liver *Scd1* expression/activity in association with plasma TG levels, and that this response would be corrected by normalization of *n*-3 fatty acid status.

2. Materials and methods

2.1. Diets

Diets were either α-linolenic acid (ALA, 18:3*n*-3)-fortified (ALA+, TD.04285) or ALA− free (ALA−, TD.04286)(Harlan-TEKLAD, Madison, WI). The compositions of ALA+ and

ALA− diets are presented in Table 1. Diets were vacuum packaged and stored at 4°C. Both diets provided 3.8 Kcal/g, and were matched for percent kcal from protein (19.2%), carbohydrate (64.4%), and fat (16.5%). Analysis of diet fatty acid composition by gas chromatography confirmed that both diets were closely matched in saturated fatty acids, monounsaturated fatty acids, and the *n*-6 fatty acid precursor linoleic acid (18:2*n*-6), and that neither diet contained preformed *n*-3 or *n*-6 fatty acids including DHA and AA, respectively (Table 1).

2.2. Animals

The experimental design used for generating the different treatment groups is illustrated in Figure 1. Male offspring bred in-house to nulliparous female Long-Evans hooded rats were used. For perinatal n-3 deficiency, dams were fed the n-3-deficient diet for 1 month prior to mating through weaning, and male offspring were maintained on the ALA− diet post-weaning (P21) to adulthood (P100)(n=10). Controls were born to dams maintained on the ALA+ diet, and received the ALA+ diet post-weaning (P21) to adulthood (P100)(n=10). Repleted rats were offspring of dams maintained on the ALA− diet, and switched to ALA+ diet post-weaning (P21) to adulthood (P100)(n=10). Rats were housed 2 per cage with food and water available *ad libitum*, and maintained under standard *vivarium* conditions on a 12:12 h light:dark cycle. Food (g/kg/d) and water (ml/kg/d) intake and body weight (kg) were recorded. Rats were sacrificed by decapitation on P100–101 between 9:00–12:00 am in a counter-balanced manner relative to the common removal of food hoppers at 9:00 am. Trunk blood was collected into EDTA-coated tubes, and plasma isolated by centrifugation at 4°C. Liver was harvested and flash frozen in liquid nitrogen. All samples were stored at −80°C. All experimental procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee, and adhere to the guidelines set by the National Institutes of Health.

2.3. Fatty acid composition

The gas chromatography procedure used to determine liver fatty acid composition has been described in detail previously [34]. Briefly, total fatty acid composition was determined with a Shimadzu GC-2010 (Shimadzu Scientific Instruments Inc., Columbia MD). The column is a DB-23 (123-2332): 30m (length), I.D. (mm) 0.32 wide bore, film thickness of 0.25 μM (J&W Scientific, Folsom CA). The carrier gas is helium with a column flow rate of 2.5 ml/min. Analysis of fatty acid methyl esters was based on area under the curve calculated with Shimadzu Class VP 7.4 software. Fatty acid identification was based on retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap PA). The limit of detection was 7 μg of an individual fatty acid in a 100 mg sample of liver tissue. Data are expressed as weight percent of total fatty acids (mg fatty acid/100 mg fatty acids). All analyses were performed by a technician blinded to treatment. We focused our primary analysis on the principle substrates (16:0, 18:0) and products (16:1*n*-7, 18:1*n*-9) of Scd1, and liver 16:1/16:0 and 18:1/18:0 ratios were calculated as indices of liver *Scd1* activity.

2.4. Plasma TG, glucose, and insulin levels

Plasma TG (GPO-PAP, RANDOX Laboratories Ltd., Antrim UK), glucose (GOD-POD, Genzyme Diagnostics P.E.I. Inc., Charlottetown, PE, Canada), and insulin (ELISA, Linco Research, St. Charles MI, USA) concentrations were determined using commercially available kits according to the manufacturer's instructions. All analyses were performed by a technician blinded to treatment.

2.5. Liver Scd1 mRNA expression

Frozen liver was homogenized (BioLogics Model 300 V/T ultrasonic homogenizer, Manassas, VA) in Tri Reagent (MRC Inc., Cincinnati, OH), and total RNA isolated and purified using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Total RNA was treated to remove potential DNA contamination using RNase-free DNase (Qiagen, Valencia, CA), and RNA quantified using a Nanodrop instrument (Nanodrop Instruments, Wilmington, DE). RNA quality was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). cDNA was prepared from 2 μg total RNA using a high-capacity RT cDNA Archive Kit (Applied Biosystems, Foster City, CA) along with no RT controls to confirm lack of genomic DNA contamination. Liver mRNA levels of stearoyl-CoA desaturase (*Scd1*, Rn00594894_g1) were measured by real-time quantitative PCR using an ABI 7900HT500 Real Time PCR System (Applied Biosystems, Foster City, CA). The nucleotide sequences of primer/probe sets can be obtained from www.appliedbiosystems.com. Sample were run in Microamp Fast 96 well reaction plates using 20 μl reaction volumes consisting of 10 μl of 2X TaqMan Fast Universal PCR Matermix (Applied Biosystems, Foster City, CA), 1 μl of TaqMan gene expression assay, and 9 μl of cDNA. No template controls (NTC) substituting DEPC-treated water for cDNA were run with each plate to verify lack of cross contamination. Thermal cycling conditions were: 95°C for 10 min followed by 95°C for 1 sec denaturing step and 60°C for 20 sec, annealing step for 40 cycles. Data were analyzed by comparing the difference between target gene and endogenous control (*GAPDH*, Rn99999916_s1) cycle thresholds for each sample using the comparative Ct method [35].

2.6. Statistical analysis

Group (control, deficient, repleted) differences in fatty acid composition, plasma TG, glucose, and insulin levels, and liver gene expression were evaluated with a one-way ANOVA, and individual group differences compared with unpaired *t*-tests (2-tail, α=0.05). Parametric (Pearson) correlation analyses were performed to determine relationships between liver fatty acid data, plasma analytes, and gene expression data (2-tail, α=0.05). Analyses were performed with GB-STAT (V.10, Dynamic Microsystems, Inc., Silver Springs MD).

3. Results

3.1. Food/water intake and body weight

There were no significant group differences in food intake, $F(2,29)=0.6$, $p=0.58$ (CON: 60.8±2.6; DEF: 58±2.5; REP: 60.0±2.1 g/kg/d) or water intake, $F(2,29)=2.4$, $p=0.1$ (CON: 45.9±5.2; DEF: 45.7±6.2; REP: 42.1±2.9 ml/kg/d). There were no significant group differences in baseline (P60) body weight, $F(2,29)=1.0$, $p=0.38$ (CON: 349.9±8.6; DEF: 345±10; REP: 351±9.2 kg) or endpoint body weight, $F(2,29)=1.1$, $p=0.34$ (CON: 461±12; DEF: 431±14; REP: 441±14 kg).

3.2. Liver PUFA composition

Group differences in liver fatty acid composition are presented in Table 2. There was a significant main effect of treatment (diet) for liver DHA (22:6*n*-3) composition, $F(2,29)=221$, $p\leq0.0001$, arachidonic acid (AA, 20:4*n*-6) composition, $F(2,29)=4.7$, $p=0.01$, and linoleic acid (18:2*n*-6) composition, $F(2,29)=110$, $p\leq0.0001$. In all cases, these fatty acids were significantly lower in *n*-3 deficient rats compared with both controls and repleted rats, and did not differ between control and repleted rats. The short-chain ALA (18:3*n*-3) and long-chain *n*-3 fatty acids EPA (20:5*n*-3) and docosapentaenoic acid (22:5*n*-3) were depleted (not detectable) in *n*-3 deficient rat liver.

3.3. Liver Scd1 mRNA expression and product/precursor ratios

The main effect of treatment was not significant for *GAPDH* mRNA expression, $F(2,26)=2.1$, $p=0.14$. There was a significant main effect of treatment for liver *Scd1/GAPDH* mRNA expression, $F(2,26)=41.5$, $p\leq0.0001$ (Fig. 2A). *Scd1* mRNA expression was significantly greater in *n*-3-deficient rats compared with both controls ($p\leq0.0001$) and repleted rats ($p\leq0.0001$), and was lower in repleted rats compared with controls ($p=0.008$). The main effect of treatment was significant for the liver 16:1/16:0 ratio $F(2,29)=119$, $p\leq0.0001$ (Fig. 2B). The 16:1/16:0 ratio was significantly greater in *n*-3-deficient rats compared with both controls ($p\leq0.0001$) and repleted rats ($p\leq0.0001$), and did not differ between control and repleted rats ($p=0.96$). The main effect of treatment was significant for the liver 18:1/18:0 ratio $F(2,29)=29$, $p\leq0.0001$ (Fig. 2C). The 18:1/18:0 ratio was significantly greater in *n*-3-deficient rats compared with both controls ($p\leq0.0001$) and repleted rats ($p\leq0.0001$), and did not differ between control and repleted rats ($p=0.74$).

3.4. Plasma TG, glucose, and insulin levels

There was a significant main effect of treatment for plasma TG concentrations, $F(2,29)=4.1$, $p=0.03$ (Fig. 3A). TG concentrations were significantly higher in *n*-3-deficient rats compared with both controls ($p=0.04$) and repleted rats ($p=0.03$), and did not differ between control and repleted rats ($p=0.67$). The main effect of treatment was not significant for plasma glucose concentrations, $F(2,29)=0.6$, $p=0.56$ (Fig. 3B) or plasma insulin concentrations, $F(2,29)=0.05$, $p=0.95$ (Fig. 3C).

3.5. Linear regression analyses

Among all rats (n=30), liver *Scd1* mRNA expression was positively correlated with the liver 18:1/18:0 ($r = +0.91$, $p\leq0.0001$)(Fig. 4A) and 16:1/16:0 ($r = +0.94$, $p\leq0.0001$)(Fig. 4B) ratios, and inversely correlated with liver DHA ($r = -0.84$, $p\leq0.0001$), AA ($r = -0.69$, $p\leq0.0001$), and LA ($r = -0.89$, $p\leq0.0001$) compositions. The 18:1/18:0 ratio was inversely correlated with liver DHA ($r = -0.86$, $p\leq0.0001$), AA ($r = -0.85$, $p\leq0.0001$), and LA ($r = -0.86$, $p\leq0.0001$) compositions. Similarly, the 16:1/16:0 ratio was inversely correlated with liver DHA ($r = -0.91$, $p\leq0.0001$), AA ($r = -0.63$, $p=0.0002$), and LA ($r = -0.93$, $p\leq0.0001$) compositions. Plasma TG concentrations were positively correlated with *Scd1* mRNA expression ($r = +0.50$, $p=0.004$), as well as 18:1/18:0 ($r = +0.66$, $p=0.0001$)(Fig. 4C) and 16:1/16:0 ($r = +0.49$, $p=0.007$)(Fig. 4D) ratios. Plasma TG concentrations were inversely correlated with liver DHA ($r = -0.33$, $p=0.04$), AA ($r = -0.63$, $p=0.0002$), and LA ($r = -0.36$, $p=0.04$) compositions. No significant correlations were observed for plasma glucose or insulin concentrations.

4. Discussion

This study demonstrates that chronic dietary *n*-3 fatty acid deficiency robustly up-regulates *Scd1* mRNA expression in rat liver, and that this response is prevented by normalization of liver *n*-3 fatty acid composition. Greater liver *Scd1* mRNA expression was associated with greater liver Scd1 activity indices (16:1/16:0 & 18:1/18:0), and liver *Scd1* mRNA expression was positively correlated with both 16:1/16:0 and 18:1/18:0 ratios. We additionally demonstrate that *n*-3 fatty acid deficiency significantly increases non-fasting plasma TG concentrations, and that this response is positively correlated with liver *Scd1* mRNA expression and activity indices. These effects could not be attributed to group differences in dietary fat intake, and were not associated with greater body weight gain or plasma glucose of insulin concentrations. Together, these findings demonstrate that *n*-3 fatty acids negatively regulate liver Scd1 expression/activity *in vivo*, and support prior evidence that this mechanism is associated with elevated TG biosynthesis.

The finding of greater liver *Scd1* mRNA expression in *n*-3 deficient rats is not consistent with a prior study finding that chronic dietary *n*-3 fatty acid deficiency did not significantly alter rat liver *Scd1* mRNA expression [31]. Potentially relevant differences between the present and this prior study include when *n*-3 fatty acid deficiency was initiated (perinatal vs. post-weaning), rat strain (Long-Evans hooded vs. Fisher-344), and diet composition. Despite similar 97–99% depletions of long-chain *n*-3 fatty acids in the livers of *n*-3 deficient rats in both studies, the Igarshi et al. [31] study found liver AA composition was increased in *n*-3 deficient rats, whereas it was reduced in the present study. Moreover, reductions in liver LA (18:2*n*-6) composition were more robust in the present study. Importantly, AA and LA are both potent inhibitors of *Scd1* transcription *in vitro* and *in vivo* [21,22,36], and liver AA and LA compositions were both inversely correlated with *Scd1* mRNA expression in the present study. Taken together, these data suggest that up-regulation of liver Scd1 mRNA expression in response to *n*-3 fatty acid deficiency may require concomitant reductions liver AA and LA composition.

The observation that liver *Scd1* mRNA expression was positively correlated with liver 16:1/16:0 and 18:1/18:0 ratios is consistent with elevated liver Scd1 enzyme activity in *n*-3 deficient rats [16,18,19]. This finding is also consistent with the prior finding that *n*-3 deficient rats exhibit elevated indices of Scd1 activity in adipocytes [37], and the finding that feeding long-chain *n*-3 fatty acids significantly decrease Scd1 enzyme activity in liver microsomes *ex vivo* [23] and *in vivo* mouse liver [30]. Furthermore, the present finding that liver 16:1/16:0 and 18:1/18:0 ratios were both positively correlated with plasma TG levels is consistent with prior *in vivo* studies demonstrating that liver Scd1 expression and activity indices are required for hepatic TG biosynthesis [14,15]. Together, these data suggest that up-regulation of liver *Scd1* expression/activity is a mechanism contributing to elevated TG concentrations in response to *n*-3 fatty acid deficiency.

Prior preclinical and clinical studies have found that Scd1 activity indices are positively associated with excess adiposity and obesity [38]. For example, *Scd1* mutant mice exhibit reduced adiposity independent of body weight gain, and are resistance to diet-induced obesity [39]. In the present study, elevations in liver Scd1 expression and activity in *n*-3 deficient rats were not associated with significantly greater body weight gain. Indeed, *n*-3 deficient rats exhibited a trend towards lower endpoint body weight compared with controls. Interestingly, a prior study also found that *n*-3 deficient mice exhibited a significant decrease in body weight despite greater liver TG content [40]. Although we did not investigate visceral adiposity in the present study, these findings suggest that elevated liver Scd1 expression/activity and TG synthesis resulting from *n*-3 fatty acid deficiency do not lead to excessive weight gain under the current dietary conditions. Additional studies are warranted to further evaluate the relationship liver Scd1 expression/activity and visceral adiposity in the *n*-3 fatty acid deficient rat model.

A role for *n*-3 fatty acids in the regulation of glucose metabolic homeostasis is supported by a number of findings [41], and several findings suggest that elevations in liver Scd1 activity and TG biosynthesis are associated with insulin resistance. For example, *Scd1* mutant mice exhibit both impaired TG biosynthesis [14,15] and increased insulin sensitivity [33]. Pharmacological inhibition of the Scd1 enzyme reduces elevated TG and glucose levels in rodent disease models [17,32]. In human subjects, elevations in the plasma 18:1/18:0 ratio, an index of SCD1 enzyme activity (‘desaturation index’), are associated with greater plasma TG levels and insulin resistance [18,42]. In the present study, elevations in liver *Scd1* expression/activity and plasma TG levels were not accompanied by significant alterations in plasma glucose or insulin levels. Indeed, *n*-3 deficient rats exhibited a trend towards lower glucose levels. Although additional studies will be required to further characterize this relationship, this finding suggest that elevated Scd1 expression/activity and plasma TG

levels are not sufficient to induce a dysregulation in glucose homeostasis under the current dietary conditions. In agreement with a prior study [43], these findings also suggest that elevated liver Scd1 expression/activity in response to *n*-3 fatty acid deficiency is not mediated through an insulin-dependent mechanism.

In summary, the present data demonstrate that *n*-3 fatty acid deficiency up-regulates liver *Scd1* mRNA expression and activity indices and increase plasma TG concentrations *in vivo*. The present data also demonstrate that normalization of liver *n*-3 fatty acid status normalizes liver *Scd1* mRNA expression/activity and plasma TG levels. Taken in conjunction with prior findings, these data suggest that up-regulation of liver *Scd1* expression/activity may represent a mechanism contributing to elevated plasma TG concentrations in response to *n*-3 fatty acid deficiency. These and previous data encourage future clinical studies to investigate the relationship between *Scd1* mRNA expression/activity and plasma TG levels in disorders associated with *n*-3 fatty acid deficiency to evaluate pathophysiological relevance.

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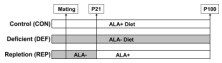

Figure 1.

Diagram illustrating the experimental design used to generate control (CON), *n*-3-deficient (DEF), and *n*-3-repleted (REP) rats. Controls were born to nulliparous dams maintained on diet fortified with α-linolenic acid (ALA+ diet), and received the ALA+ diet post-weaning (P21) to adulthood (P100). For perinatal *n*-3 deficiency, dams were fed the ALA− diet for 1 month prior to mating through weaning, and offspring were maintained on the ALA− diet post-weaning (P21) to adulthood (P100). Repleted rats were offspring of dams maintained on the ALA− diet for 1 month prior to mating, and switched to ALA+ diet post-weaning (P21) to adulthood (P100).

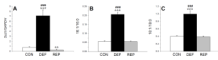

Figure 2.
Liver *Scd1/GAPDH* mRNA expression (**A**), and liver 16:1/16:0 (**B**) and 18:1/18:0 (**C**) ratios in control (CON, *n*=10), *n*-3-deficient (DEF, *n*=10), and *n*-3-repleted (REP, *n*=10) rats. Values are group mean ± S.E.M. $^{**}p \leq 0.01$, $^{***}p \leq 0.0001$ *vs.* controls, $^{\#\#\#}p \leq 0.0001$ *vs.* DEF rats.

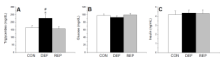

Figure 3.
Plasma triglyceride (**A**), glucose (**B**), and insulin (**C**) concentrations in control (CON, *n*=10), *n*-3-deficient (DEF, *n*=10), and *n*-3-repleted (REP, *n*=10) rats. Values are group mean ± S.E.M. **p*≤0.05 *vs.* controls, $^{\#}p$≤0.05 *vs.* DEF rats.

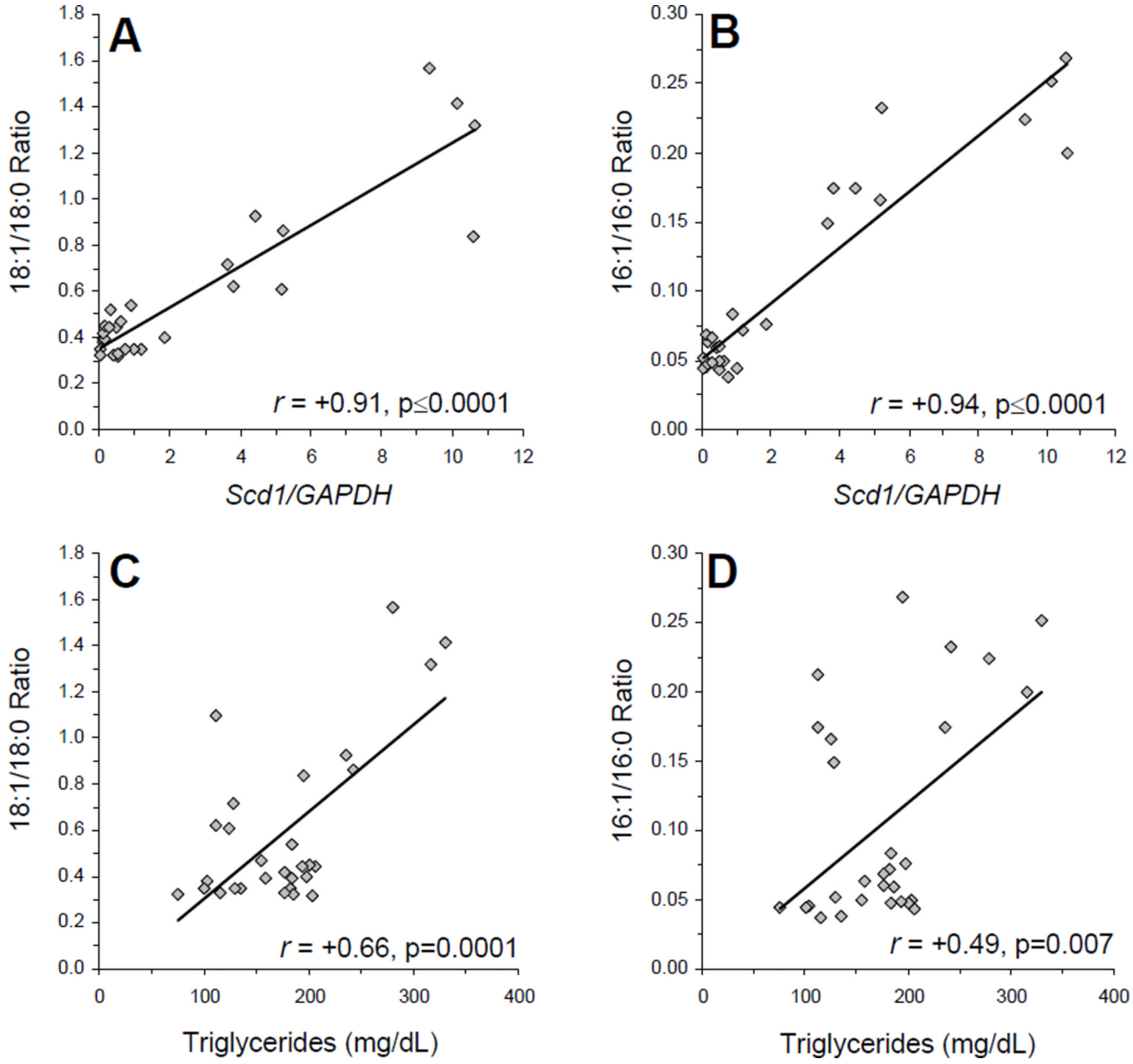

Figure 4.

Relationships between liver *Scd1* mRNA expression the liver 16:1/16:0 (**A**) and 18:1/18:0 (**B**) ratios, and relationship between plasma TG concentrations and liver 16:1/16:0 (**C**) and 18:1/18:0 (**D**) ratios among all rats (*n*=30). Pearson correlation coefficients and associated *p*-values (two-tailed) are presented.

Table 1

Diet Compositions

Ingredient[1]	**ALA+**	**ALA−**
Casein, vitamine free	20	20
Carbohydrate		
Cornstarch	20	20
Sucrose	27	27
Dextrose	9.9	9.9
Maltose-dextrin	6	6
Cellulose	5	5
Mineral mix (AIMN-93G-MX)	3.5	3.5
Vitamin mix (AIN-93-VX)	1	1
L-Cystine	0.3	0.3
Choline bitartrate	0.25	0.25
TBHQ	0.002	0.002
Fat		
Hydrogenated coconut oil	4.5	5.1
Safflower	1.9	1.9
Flaxseed	0.6	0
Fatty acid composition[2]		
C8:0	4.3	5.0
C10:0	3.8	4.2
C12:0	29	32.8
C14:0	11	12.5
C16:0	8.3	8.7
C18:0	9.4	10.2
18:1n-9	6.7	4.7
18:2n-6	22.7	21.9
20:4n-6	nd	nd
18:3n-3	4.6	nd
22:6n-3	nd	nd

[1] g/100 g diet

[2] wt % of total fatty acids

nd = not detected

Table 2

Liver fatty acid composition

Fatty Acid[1]	CON (n=10)	DEF (n=10)	REP (n=10)	P-value[2]
C14:0	0.46 ± 0.03	1.63 ± 0.07 ***	0.38 ± 0.01 ###	*P<0.0001*
C16:0	18.33 ± 0.24	22.67 ± 0.84 ***	18.20 ± 0.21 ###	*P<0.0001*
C18:0	18.09 ± 0.37	15.50 ± 0.52 ***	18.32 ± 0.29 ###	*P<0.0001*
Total SFA	36.88 ± 0.20	39.80 ± 0.62 ***	36.91 ± 0.18 ###	*P<0.0001*
16:1n-7	1.01 ± 0.10	4.71 ± 0.41 ***	1.00 ± 0.07 ###	*P<0.0001*
18:1n-9	7.14 ± 0.25	15.01 ± 1.18 ***	7.06 ± 0.29 ###	*P<0.0001*
18:1n-7	3.06 ± 0.14	4.66 ± 0.24 ***	2.67 ± 0.05 ###	*P<0.0001*
Total MUFA	11.21 ± 0.42	24.38 ± 1.44 ***	10.72 ± 0.36 ###	*P<0.0001*
18:2n-6	18.07 ± 0.78	7.30 ± 0.60 ***	18.60 ± 0.37 ###	*P<0.0001*
18:3n-6	0.21 ± 0.01	0.17 ± 0.02	0.20 ± 0.01	*P=0.17*
20:3n-6	0.71 ± 0.04	0.61 ± 0.05	0.68 ± 0.02	*P=0.21*
20:4n-6	23.11 ± 0.51	20.19 ± 1.14 *	22.96 ± 0.39 #	*P=0.017*
22:4n-6	0.41 ± 0.03	0.48 ± 0.04	0.46 ± 0.02	*P=0.25*
22:5n-6	0.19 ± 0.02	4.70 ± 0.23 ***	0.22 ± 0.02 ###	*P<0.0001*
Total n-6	43.08 ± 0.52	33.76 ± 1.84 ***	43.49 ± 0.46 ###	*P<0.0001*
18:3n-3	0.54 ± 0.05	nd ***	0.61 ± 0.02 ###	*P<0.0001*
20:5n-3	0.29 ± 0.02	nd ***	0.29 ± 0.01 ###	*P<0.0001*
22:5n-3	0.93 ± 0.03	nd ***	0.95 ± 0.02 ###	*P<0.0001*
22:6n-3	6.63 ± 0.36	0.47 ± 0.03 ***	6.80 ± 0.21 ###	*P<0.0001*
Total n-3	8.39 ± 0.28	0.47 ± 0.03 ***	8.65 ± 0.21 ###	*P<0.0001*

[1] Data are mean fatty acid wt % of total fatty acids ± SEM (nd = not detected)

[2] One-way ANOVA (2-tailed),

* $P \leq 0.05$,

*** $P \leq 0.001$ versus CON,

\# $P \leq 0.05$,

$P \leq 0.001$ versus DEF.