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Omega-3 Fatty Acid Deficiency Selectively Up-Regulates Delta6-Desaturase Expression and Activity Indices in Rat Liver: Prevention by Normalization of Omega-3 Fatty Acid Status

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Abstract

This study investigated the effects of perinatal dietary omega-3 (*n*-3) fatty acid depletion and subsequent repletion on the expression of genes that regulate long-chain polyunsaturated fatty acid (PUFA) biosynthesis in rat liver and brain. It was hypothesized that chronic *n*-3 fatty acid deficiency would increase liver *Fads1* and *Fads2* mRNA expression/activity, and that *n*-3 fatty acid repletion would normalize this response. Adult rats fed the *n*-3-free diet during perinatal development exhibited significantly lower erythrocyte, liver, and frontal cortex LC*n*-3 fatty acid composition and reciprocal elevations in LC*n*-6 fatty acid composition compared with controls and repleted rats. Liver *Fads2*, but not *Fads1*, *Elovl2*, or *Elovl5*, mRNA expression was significantly greater in *n*-3 deficient rats compared with controls, and was partially normalized in repleted rats. The liver 18:3*n*-6/18:2*n*-6 ratio, an index of delta6-desaturase activity, was significantly greater in *n*-3 deficient rats compared with control and repleted rats, and was positively correlated with *Fads2* mRNA expression among all rats. The liver 18:3*n*-6/18:2*n*-6 ratio, but not *Fads2* mRNA expression, was also positively correlated with erythrocyte and frontal cortex LC*n*-6 fatty acid compositions. Neither *Fads1* or *Fads2* mRNA expression were altered in brain cortex of *n*-3 deficient rats. These results confirm previous findings that liver, but not brain, delta6-desaturase expression and activity indices are negatively regulated by dietary *n*-3 fatty acids.

Keywords

Omega-3 fatty acid; Delta5-desaturase (*Fads1*); Delta6-desaturase (*Fads2*); Elongase-2/5 (*Elovl2*, *Elovl5*); Erythrocyte; Liver; Frontal cortex; Rat

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1. Introduction

Polyunsaturated fatty acids (PUFA), a lipid family comprised of omega-3 (*n*-3) and omega-6 (*n*-6) fatty acids, are critical components of cellular membranes and regulate multiple physiological events. A body of preclinical evidence indicates that dietary-induced perturbations in PUFA homeostasis in response to *n*-3 fatty acid deficiency during development lead to abnormalities in inflammatory [1] and synaptic [2] signaling, alterations in central neurotransmitter systems [3], and neurocognitive deficits [4] in adulthood. Moreover, *n*-3 fatty acid deficiency has been implicated in the pathophysiology of several diseases, including rheumatoid arthritis [5], coronary heart disease [6], hepatic steatosis [7], and psychiatric disorders including major depression and schizophrenia [8]. Therefore, developing a better understanding of the impact of *n*-3 fatty acid deficiency on the mechanisms mediating PUFA homeostasis may provide important insight into pathophysiology processes.

The biosynthesis of long-chain (LC) *n*-3 fatty acids, including docosahexaenoic acid (22:6*n*-3), and LC*n*-6 fatty acids, including arachidonic acid (20:4*n*-6), from short-chain dietary precursors α -linolenic acid (ALA, 18:3*n*-3) and linoleic acid (LA, 18:2*n*-6), respectively, are mediated by delta6-desaturase (*Fads2*), delta5-desaturase (*Fads1*), and elongases-2/5 (*Elovl2*, *Elovl5*) enzymes [9–12]. Extant evidence from pharmacological [13], mutant mouse [14], and human genomic [15] studies support the critical role of delta5- and delta6-desaturase enzymes for maintaining tissue PUFA homeostasis. Furthermore, because delta6-desaturase is a common and rate-limiting step for both LC*n*-6 and LC*n*-3 fatty acid biosynthesis, selective dietary LA (18:2*n*-6) deficiency is associated with reciprocal elevations in peripheral and central membrane LC*n*-3 fatty acid composition [16], and selective dietary ALA (18:3*n*-3) deficiency is associated with reciprocal elevations in peripheral and central membrane LC*n*-6 fatty acid composition [17]. Therefore, tissue PUFA homeostasis is governed by gene-diet interactions.

Although the liver is thought to be the principal organ involved in LC*n*-3 and LC*n*-6 PUFA biosynthesis, delta5- and delta6-desaturase enzymes are also expressed in brain [18]. Prior studies have found that LC*n*-3 fatty acid deficiency is associated with an up-regulation of *Fads1* and *Fads2* mRNA expression and activity in rat liver but not brain [18,19]. These findings suggest that liver *Fads1* and *Fads2* mRNA expression are negatively regulated by dietary *n*-3 fatty acids. The present study sought to replicate these findings using the perinatal *n*-3 fatty acid deficiency model, which produces robust reductions in peripheral and central LC*n*-3 fatty acid levels in adulthood, and to additionally investigate whether these effects could be corrected by normalization of *n*-3 fatty acid status. Based on prior evidence, it was hypothesized that perinatal *n*-3 fatty acid deficiency would increase liver, but not brain, *Fads1* and *Fads2* mRNA expression/activity, and that normalization of *n*-3 fatty acid status would prevent these changes.

2. Methods and materials

2.1. Diets

The compositions of the α -linolenic acid (ALA, 18:3*n*-3)-fortified (control, ALA+, TD. 04285) and ALA- (TD.04286) diets are presented in Table 1 (Harlan-TEKLAD, Madison, WI). 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%). The control diet was fortified with flaxseed oil, which contains the short-chain *n*-3 fatty acid precursor ALA (18:3*n*-3). 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 short-chain *n*-6 fatty acid precursor linoleic acid (LA, 18:2*n*-6), and that

neither diet contained preformed long-chain *n*-3 or *n*-6 fatty acids including DHA and AA, respectively (Table 1).

2.2. Animals

Male offspring bred in-house to nulliparous female Long-Evans hooded rats were used. For perinatal ALA deficiency, dams were fed the ALA- 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, plasma isolated by centrifugation, and erythrocytes washed 3x with 4°C 0.9% NaCl. The brain was dissected on ice to isolate the frontal cortex (olfactory tubercle and residual striatal tissue were removed), and liver samples 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 NIH.

2.3. Analysis of fatty acid methyl esters

The gas chromatography procedure used to determine erythrocyte, liver, and brain cortex fatty acid composition has been described in detail previously [20]. The methods for saponification and methylation of fatty acids for gas chromatographic analysis follows that originally reported by Mecalfe et al [21]. 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). 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. For liver, we focused our primary analysis on product/precursor ratios as indices of delta6-desaturase (18:3*n*-6/18:2*n*-6), elongase-5 (20:3*n*-6/18:3*n*-6), delta5-desaturase (20:4*n*-6/20:3*n*-6), and elongase-2 (22:4*n*-6/20:4*n*-6) activities. We also determined principal LC*n*-3 fatty acids (20:5*n*-3, 22:5*n*-3, 22:6*n*-3) and principal LC*n*-6 fatty acids (20:2*n*-6, 20:4*n*-6, 22:4*n*-6, 22:5*n*-6). Fatty acid composition data are expressed as weight percent total fatty acid composition.

2.4. Liver and brain mRNA expression

A detailed description of the RT-PCR procedure has been published previously [22]. Frozen liver and frontal cortex were 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 delta-6 desaturase (TaqMan Gene Expression Assay ID: *Fads2*, Rn00580220_m1), delta-5 desaturase (*Fads1*, Rn00584915_m1), elongase-2 (*Elovl2*, Rn01450661_m1), elongase-5 (*Elovl5*, Rn00592812_m1), and sterol regulatory element-binding protein-1c (*SREBP1c*, Rn01495769_m1) were measured by real-time quantitative PCR using an ABI 7900HT500 Real Time PCR System (Applied Biosystems, Foster City, CA). Frontal cortex mRNA levels of delta-6 desaturase (*Fads2*, Rn00580220_m1) and delta-5 desaturase (*Fads1*, Rn00584915_m1) were also determined in a subset (n=5) of rats from each group. The nucleotide sequences of primer/probe sets can be obtained from www.appliedbiosystems.com. Sample were run in triplicate in Microamp Fast 96 well reaction plates using 20 µl reaction volumes consisting of 10 µl of 2X TaqMan Fast Universal PCR Mastermix (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 [23].

2.5. Statistical analyses

Group (control, deficient, repleted) differences in fatty acid composition and gene expression were evaluated with a one-way ANOVA, and individual group differences were evaluated with unpaired *t*-tests (2-tail, $\alpha=0.05$). Parametric (Pearson) correlation analyses were performed to determine relationships between fatty acid and gene expression data (2-tail, $\alpha=0.05$). 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, $F(2,44)=0.6$, $p=0.58$ (CON: 60.8 ± 2.6 ; DEF: 58 ± 2.5 ; REP: 60.0 ± 2.1 g/kg/d), water intake, $F(2,44)=2.4$, $p=0.1$ (CON: 45.9 ± 5.2 ; DEF: 45.7 ± 6.2 ; REP: 42.1 ± 2.9 ml/kg/d), or endpoint (P100) body weight, $F(2,29)=1.1$, $p=0.34$ (CON: 461 ± 12 ; DEF: 431 ± 14 ; REP: 441 ± 14 kg).

3.2. Peripheral and central long-chain PUFA composition

There was a significant main effect of treatment (diet) on LCN-3 fatty acid composition in erythrocytes, $F(2,29)=905$, $p \leq 0.0001$ (Fig. 1A), liver $F(2,29)=380$, $p \leq 0.0001$ (Fig. 1B), and frontal cortex, $F(2,29)=1481$, $p \leq 0.0001$ (Fig. 1C). In all cases, LCN-3 fatty acid composition was significantly lower in rats maintained in the ALA- diet compared with controls and repleted rats, and did not differ between control and repleted rats. There was a significant main effect of treatment on LCN-6 fatty acid composition in erythrocytes, $F(2,29)=470$, $p \leq 0.0001$ (Fig. 1D), liver $F(2,29)=2.9$, $p=0.04$ (Fig. 1E), and frontal cortex, $F(2,29)=472$, $p \leq 0.0001$ (Fig. 1F). In all cases, LCN-6 fatty acid composition was significantly greater in rats maintained in the ALA- diet compared with controls and repleted rats, and did not differ between control and repleted rats. Accordingly, the LCN-6/LCN-3 fatty acid ratio was significantly greater erythrocytes, $F(2,29)=3447$, $p \leq 0.0001$ (Fig. 1G), liver $F(2,29)=1748$, $p=0.04$ (Fig. 1H), and frontal cortex, $F(2,29)=413$, $p \leq 0.0001$ (Fig. 1I) of rats maintained in the ALA- diet compared with controls and repleted rats, and did not differ between control and repleted rats.

3.3. Liver and brain mRNA expression

The main effect of treatment was not significant for *GAPDH* mRNA expression in liver, $F(2,29)=2.1$, $p=0.14$, or frontal cortex, $F(2,14)=0.19$, $p=0.83$. There was a significant main effect of treatment for liver *Fads2/GAPDH* mRNA expression, $F(2,29)=3.4$, $p=0.04$ (Fig. 2A). *Fads2* mRNA expression was significantly greater in rats maintained in the ALA- diet compared with controls ($p=0.02$) but not repleted rats ($p=0.1$), and did not differ between control and repleted rats ($p=0.47$). The main effect of treatment was not significant for liver *Elovl5* mRNA expression, $F(2,29)=1.2$, $p=0.33$ (Fig. 2B), *Fads1* mRNA expression, $F(2,29)=0.1$, $p=0.89$ (Fig. 2C), *Elovl2* mRNA expression, $F(2,29)=0.6$, $p=0.55$ (Fig. 2D), or *SREBP1c* mRNA expression, $F(2,29)=0.06$, $p=0.94$ (Fig. 2E). In frontal cortex, the main effect of treatment was not significant for *Fads2* mRNA expression, $F(2,14)=0.21$, $p=0.82$, or *Fads1* mRNA expression, $F(2,14)=0.1$, $p=0.89$ (data not shown).

3.4. Liver product/precursor ratios

There was a significant main effect of treatment for liver 18:2n-6 composition, $F(2,29)=110$, $p\leq 0.0001$, which was significantly lower in rats maintained in the ALA- diet compared with both controls ($p\leq 0.0001$) and repleted rats ($p\leq 0.0001$), and did not differ between control and repleted rats ($p=0.55$). There was a significant main effect of treatment for the liver 18:3n-6/18:2n-6 ratio, an index of delta6-desaturase activity, $F(2,29)=81$, $p\leq 0.0001$ (Fig. 3A). The 18:3/18:2 ratio was significantly greater in rats maintained in the ALA- diet compared with both controls ($p\leq 0.0001$) and repleted rats ($p\leq 0.0001$), and did not differ between control and repleted rats ($p=0.49$). The main effect of treatment was not significant for the liver 20:3n-6/18:3n-6 ratio (elongase-5), $F(2,29)=0.3$, $p=0.77$ (Fig. 3B) or the 20:4n-6/20:3n-6 ratio (delta5-desaturase), $F(2,29)=0.1$, $p=0.86$ (Fig. 3C). There was a significant main effect of treatment for the liver 22:4n-6/20:4n-6 ratio (elongase-2), $F(2,29)=5.9$, $p=0.007$ (Fig. 3D). The 22:4n-6/20:4n-6 ratio was significantly greater in rats maintained in the ALA- diet compared with both controls ($p=0.005$) and repleted rats ($p=0.02$), and did not differ between control and repleted rats ($p=0.22$).

3.5. Relationships between liver mRNA expression and activity indices

Among all rats ($n=30$), liver *Fads2/GAPDH* mRNA expression was positively correlated with liver 18:3n-6/18:2n-6 ($r = +0.63$, $p=0.001$), 20:3n-6/18:2n-6 ($r = +0.61$, $p=0.0008$), and 20:4n-6/18:2n-6 ($r = +0.52$, $p=0.005$) ratios. Liver *Fads1/GAPDH* mRNA expression was not significantly correlated with the liver 20:4n-6/20:3n-6 ratio ($r = +0.12$, $p=0.56$) or the 20:4n-6/18:2n-6 ratio ($r = -0.02$, $p=0.92$). Liver *Elovl2/GAPDH* mRNA expression was not significantly correlated with the liver 22:4n-6/20:4n-6 ratio ($r = -0.16$, $p=0.42$) or the 20:4n-6/18:2n-6 ratio ($r = +0.07$, $p=0.71$). Liver *Elovl5/GAPDH* mRNA expression was not significantly correlated with the liver 20:3n-6/18:3n-6 ratio ($r = +0.11$, $p=0.62$) or the 20:4n-6/18:2n-6 ratio ($r = -0.19$, $p=0.34$).

3.6. Relationships between liver mRNA expression and LCn-6 fatty acids

Liver *Fads2/GAPDH* mRNA expression was not correlated with 20:4n-6 composition in erythrocytes ($r = +0.16$, $p=0.42$) or frontal cortex ($r = +0.20$, $p=0.31$), 22:4n-6 composition in erythrocytes ($r = +0.29$, $p=0.54$) or frontal cortex ($r = +0.37$, $p=0.06$), and was moderately correlated with 22:5n-6 composition in erythrocytes ($r = +0.45$, $p=0.02$) and frontal cortex ($r = +0.34$, $p=0.09$). Liver *Fads1/GAPDH*, *Elovl2/GAPDH*, and *Elovl5/GAPDH* mRNA expressions, and associated activity indices, were not significantly correlated with erythrocyte or frontal cortex 20:4n-6, 22:4n-6, or 22:5n-6 compositions ($p>0.05$).

4. Discussion

The principal finding of the present study is that chronic dietary ALA (18:3 n -3) depletion, resulting in robust elevations in peripheral and central LC n -6/LC n -3 fatty acid ratios, significantly and selectively increased *Fads2* mRNA expression and delta6-desaturase activity (18:3 n -6/18:2 n -6 ratio) in rat liver. *Fads1*, *Elovl2*, and *Elovl5* mRNA expression were not significantly altered in rat liver, and greater liver *Fads2* mRNA expression in n -3-deficient rats was not accompanied by elevations in the transcription factor *SREBP1c*. Elevations in liver *Fads2* mRNA expression and the 18:3 n -6/18:2 n -6 ratio could not be attributed to group differences in dietary intake of 18:2 n -6. Furthermore, elevations in liver *Fads2* mRNA expression and the 18:3 n -6/18:2 n -6 ratio in n -3-deficient rat liver were corrected by prior normalization of n -3 fatty acid status. Neither *Fads1* or *Fads2* mRNA expression were altered in the frontal cortex n -3 fatty acid deficient rats. These findings demonstrate that rat liver delta6-desaturase expression and activity are selectively up-regulated in rat liver, but not brain, in response to n -3 fatty acid deficiency, and indicate that this response is correctable by normalization of n -3 fatty acid status.

This study has three notable limitations. First, we did not directly evaluate enzyme activity, and used liver product/precursor ratios as estimates. However, the pattern of changes in liver fatty acids observed in n -3 deficient rats, reductions in the short-chain precursor (18:2 n -6) and reciprocal elevations in long-chain products, is consistent with elevated delta-6 desaturase activity [13,14]. Moreover, the liver 18:3/18:2 ratio was positively correlated with liver *Fads2* mRNA expression. Second, only male rats were investigated, precluding evaluation of gender differences. However, male rats were selected to obviate potential interactions with ovarian hormones previously found to influence primary outcome measures [20,24]. Third, because we investigated one strain of rat, the results may not be generalizable to other rat strains.

In the present study we found that only *Fads2* mRNA expression was up-regulated in the liver of n -3-deficient rats, and that *Fads1*, *Elovl2*, and *Elovl5* mRNA expression were not significantly altered. This finding differs from a prior study finding that dietary ALA depletion initiated post-weaning resulted in a significant elevations in *Fads2*, *Fads1*, *Elovl2* and *Elovl5* mRNA expression in rat liver [18]. We did however observe a significant increase in elongase-2 activity indices, and there was a trend for greater *Elovl2* mRNA expression. Potentially relevant differences between these studies include dietary PUFA composition and when n -3 fatty acid deficiency was initiated (perinatal vs. post-weaning). It is notable, however, that both studies observed similar reductions in liver LC n -3 fatty acid levels. It may also be relevant that these studies used different rat strains (Long-Evans hooded vs. Fisher-344), and a prior study found that liver delta-6 and delta-5 desaturase activities differ significantly between rat strains maintained on the same diet [25]. Additional studies will be required to determine whether these methodological differences can account for these discrepant findings.

Consistent with prior studies [17,26], chronic dietary ALA depletion was associated with a reciprocal increase in peripheral and central LC n -6 fatty acid composition. In general, however, peripheral and central LC n -6 fatty acid compositions were not correlated with liver *Fads2* mRNA expression. This finding suggests the observed increase in LC n -6 fatty acid composition is mediated predominantly by a reduction in substrate competition for the delta-6 desaturase enzyme and/or reduced competition for incorporation of LC n -6 fatty acids into phospholipids. Furthermore, because n -3 deficiency did not increase *Fads2* or *Fads1* mRNA expression in frontal cortex, elevations in central membrane LC n -6 fatty acid composition in response to n -3 deficiency may also be mediated by reduced competition for incorporation into phospholipids. Nevertheless, the robust increase in LC n -6 fatty acid

biosynthesis and peripheral and central membrane composition in response to *n*-3 deficiency has implications for cellular functioning [1–4], and may contribute to the pathophysiology of several diseases associated with *n*-3 fatty acid deficiency [5–8].

In summary, the present data demonstrate that chronic dietary *n*-3 fatty acid deficiency during perinatal development, resulting in robust deficits in peripheral and central LC*n*-3 fatty acid composition, is associated with a selective up-regulation in delta6-desaturase expression and activity indices in rat liver. These findings confirm prior reports that *n*-3 deficiency is associated with elevated delta6-desaturase expression and activity in rat liver [18,19], and provide partial support for our hypothesis that chronic ALA deficiency would increase both *Fads1* and *Fads2* mRNA expression/activity in rat liver. In support of our hypothesis, the present study additionally demonstrates that elevations in liver delta6-desaturase expression and activity are preventable by prior normalization of *n*-3 fatty acid status. These data therefore demonstrate that maintaining adequate dietary *n*-3 fatty acid intake is necessary to prevent dysregulation in liver delta6-desaturase expression/activity and associated perturbations in peripheral and central PUFA homeostasis.

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Figure 1.

Effects of dietary ALA depletion and repletion on LCN-3 fatty acid composition in erythrocytes (20:5+22:5+22:6*n*-3)(**A**), liver (20:5+22:5+22:6*n*-3)(**B**), and frontal cortex (22:6*n*-3)(**C**), LCN-6 fatty acid composition in erythrocytes (20:3+20:4+22:4+22:5*n*-6) (**D**), liver (20:2+20:3+20:4+22:4+22:5*n*-6)(**E**), and frontal cortex (20:4+22:4+22:5*n*-6)(**F**), and the LCN-6/LCN-3 ratio in erythrocytes (**G**), liver (**H**), and frontal cortex (**I**) of control (CON, *n*=10), *n*-3-deficient (DEF, *n*=10), and *n*-3-repleted (REP, *n*=10) rats. Values are group mean $\pm$ S.E.M. **p*≤0.05, ****p*≤0.0001 vs. controls, #*p*≤0.05, ###*p*≤0.0001 vs. DEF rats.

Figure 2.

Liver expression of *Fads2*/GAPDH mRNA (**A**), *Elovl5*/GAPDH mRNA (**B**), *Fads1*/GAPDH mRNA (**C**), *Elovl2*/GAPDH mRNA (**D**), and *SREBP1c*/GAPDH mRNA (**E**) in control (CON, $n=10$), n -3-deficient (DEF, $n=10$), and n -3-repleted (REP, $n=10$) rats. Values are group mean $\pm$ S.E.M. * $p \leq 0.05$ vs. controls.

Figure 3. Liver indices of delta6-desaturase (18:3n-6/18:2n-6)(**A**), elongase-5 (20:3n-6/18:3n-6) (**B**), delta5-desaturase (20:4n-6/20:3n-6) (**C**), and elongase-2 (22:4n-6/20:4n-6) (**D**) activities in control (CON, $n=10$), n -3-deficient (DEF, $n=10$), and n -3-repleted (REP, $n=10$) rats. Values are group mean $\pm$ S.E.M. ** $p \leq 0.01$, *** $p \leq 0.0001$ vs. controls, # $p \leq 0.05$, ### $p \leq 0.0001$ vs. DEF rats.

Table 1

Ingredient and nutrient composition of the experimental diets

Ingredient ¹	ALA+	ALA-
Casein, vitamin 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 diet²
wt % of total fatty acids

nd = not detected