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n-3 Fatty acids ameliorate hepatic steatosis and dysfunction after LXR agonist ingestion in mice

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Abstract

Liver X receptor (LXR) agonists slow atherogenesis, but cause hepatic steatosis and dysfunction in part by increasing expression of sterol regulatory element binding protein 1-c (SREBP1-c), a transcription factor that upregulates fatty acid (FA) synthesis. n-3 FAs decrease hepatic FA synthesis by down-regulating SREBP1-c. To test the hypothesis that n-3 FAs decrease hepatic steatosis in mice given LXR agonist, C57BL/6 mice received daily gavage of an LXR agonist T0901317 (LXR_T) or vehicle for 4 weeks with concomitant intakes chow or high-fat diets enriched in saturated fat (SAT) or n-3 fat (n-3). Mice on LXR_T and SAT developed hepatomegaly with a large increase in size and number of hepatic lipid droplets; an n-3 diet reduced liver weight/body weight with decreased hepatic steatosis and triglyceride levels. Effects of n-3 diet on hepatic lipogenesis were linked to a blunting of LXR_T upregulation of hepatic SREBP1-c and FA synthase mRNA. n-3 diets also normalized LXR_T-mediated increases of plasma ALT and AST levels, whereas SAT diet increased these markers.

Conclusion—These studies suggest that n-3 FA when given together with LXR agonists have the potential to improve both hepatic steatosis and hepatotoxicity in humans that might receive LXR agonists to decrease risk of atherosclerosis.

Keywords

n-3 diet; LXR agonist; hepatic steatosis; hepatic dysfunction; sterol regulatory element binding protein 1-c

1. Introduction

Hepatic steatosis, the excessive lipids accumulation in the liver, is part of the spectrum of nonalcoholic fatty liver diseases (NAFLD), which include the consequences of steatohepatitis and cirrhosis [1], and it can be triggered with various reasons. The most common factor associated with hepatic steatosis is insulin resistance. Insulin resistant states

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such as leptin-deficient ob/ob mice, lipodystrophic mice, and mice fed a high-fat or sucrose diet develop hepatic steatosis [2]. Liver X receptor (LXR) activation is also associated with hepatic steatosis.

LXRs, LXR α and LXR β , are members of the nuclear receptor superfamily and are involved in regulation of cholesterol and lipid metabolism. LXR α is mainly expressed in the liver and intestine, whereas LXR β expression is ubiquitous but with low levels in the liver. Activation of LXR reduces hepatic cholesterol synthesis [3] and intestinal cholesterol absorption [4], whereas it increases expression of several genes implicated in reverse cholesterol transport and mobilization of cholesterol [5], bile acid synthesis from cholesterol [6] and cholesterol excretion into bile [7]. Treatment with LXR agonists, such as T0901317, protect against atherosclerosis in apolipoprotein E or low-density lipoprotein receptor deficient mice [8,9]. Thus LXR agonists have been considered as potential therapeutic anti-atherosclerotic agents. However, activation of LXR also induces expression of the sterol regulatory binding protein-1c (SREBP-1c) [3] which subsequently activates various genes involved in lipogenesis and triglyceride metabolism. Severe hepatic steatosis develops in mice after ingestion of LXR agonists [3,10]. In LXR α knockout (KO) mice, hepatic steatosis did not occur after treatment with high doses of an LXR agonist [3].

High saturated fat (SAT) intakes have been suggested to induce hepatic steatosis [11–13]. C57BL/6 mice fed a high fat diet based on lard developed features of both non-alcoholic fatty liver disease and non-alcoholic fatty pancreatic disease, a condition that is related to metabolic syndrome [13]. In contrast, n-3 fatty acids (FA) from fish oil have been shown to lower elevated serum triglycerides [14] and have been suggested to inhibit hepatic lipogenesis [15] via downregulation of SREBP-1c gene as competitive inhibitors of LXR ligands [16], and stimulate FA oxidation in liver [17]. We have reported that long chain unsaturated FA such as n-3 DHA are potent inhibitors of processing of the inactive precursor form of SREBP-1c to its active form that acts in the mature form to activate lipogenesis [18]. Although recent studies suggest that n-3 FA improve hepatic steatosis in human and animals [19–22], there is little information on the effects of n-3 FA when given together in the presence of LXR agonists.

In light of these collective data, we hypothesized that an n-3 FA diet, in contrast to SAT rich diet, would decrease hepatic steatosis in mice given LXR agonist T0901317 (LXR_T), a potent and selective agonist for both LXR α and LXR β . We show that an n-3 FA-rich diet markedly decreases triglyceride content and lipid droplets accumulation relative to a SAT diet in the livers of C57BL/6 mice given LXR_T. Also, the n-3 diet reduced liver dysfunction related plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels associated with LXR activation and these effects were accompanied by blunting of the LXR_T upregulation of SREBP1-c and FA synthase (FAS) mRNA expression in liver.

2. Materials and Methods

Feeding protocols

Male C57BL/6 mice were obtained from Jackson Laboratory and maintained on a 12-hour light/dark cycle. They were fed a normal chow diet for acclimation for 1 week after delivery. At 12 weeks of age, they were randomized to receive either an LXR agonist, T0901317 (LXR_T) (Cayman, Ann Arbor, MI), or vehicle with segregation to dietary groups (n=8–9 for each group) of normal chow (4.5% fat, w/w), or high-fat diets (21% fat, w/w) enriched in either n-3 or saturated fat (SAT) for 4 weeks, as previously detailed [23]. T0901317 (20 mg/kg body weight), a potent and selective agonist for both LXR α and LXR β , dissolved in ethanol was administered daily by oral gavage in corn oil. Control mice received ethanol plus corn oil. The fat content of the SAT diet consisted of 71% SAT from coconut oil, 19%

monounsaturated fat from olive oil, and 9% polyunsaturated fats from safflower and corn oil (TD 97108, Harlan Teklad, Madison, WI). In the n-3 diet, 95% of total fat was from menhaden oil enriched in EPA and DHA and 5% from corn oil, w/w (960195, MP Biomedicals, Irvine, CA). The SAT and n-3 diets contained 0.2% cholesterol (w/w) and the chow diet 0.02% cholesterol (w/w). The FA composition of each diet was detailed previously [23]. In our previous report [23] these diets changed plasma fatty acyl compositions to reflect the dietary fatty acid intakes. All animal procedures were in compliance and approved by the Institutional Animal Care and Use Committee of Columbia University.

Blood and hepatic lipids

Mice were fasted for 4 hours, and blood samples were collected by retro-orbital blood sampling. Plasma was separated by centrifugation and stored at -70°C until analyzed. Plasma free fatty acid (FFA) levels were measured using the Wako NEFA C kit (Wako, Neuss, Germany) and triglyceride and total cholesterol levels were determined using enzymatic kits (Roche Diagnostics Corp., Indianapolis, IN) according to the manufacturer's procedures as previously detailed [24].

For hepatic lipid determination, livers obtained after mice were fasted for 4 hours and sacrificed. Total liver lipids were extracted according to a modified method from Folch et al. [25]. Briefly, liver tissues (~200 mg) were homogenized in 2mL of PBS and extracted twice with 2mL of a chloroform/methanol (v/v = 2:1) solution. The organic layer was dried under nitrogen gas and resolubilized in 200 μL of chloroform containing 2% Triton X-100. This extract was dried again and resuspended in 200 μL of water to a final concentration of 2% Triton X-100 [26] and then assayed for triglyceride and cholesterol concentrations using commercial kits as described above.

Blood transaminase activities

Blood was obtained by retro-orbital plexus and sent to a Columbia University animal analytical laboratory for analysis of plasma ALT (alanine aminotransferase) and AST (aspartate aminotransferase) as an index of hepatocellular injury. Samples were measured by an automatic chemistry analyzer (Analyst III; Hemagen Diagnostics, Inc., Columbia, MD).

Histological analyses

We used liquid nitrogen frozen livers for Oil Red O staining to evaluate hepatic lipid content. Sections were photographed at $\times 10$ magnification.

RNA extraction and real time RT-PCR analysis

Total RNA was isolated from livers with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. RNA was reverse transcribed using the iScriptTM cDNA synthesis kit (BioRad, Hercules, CA). The expression of SREBP-1c and FAS mRNA was measured by real-time quantitative PCR with TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA). Detection of specific PCR products was performed in triplicate using the Mx4000 Multiplex Quantitative PCR System (Stratagene, La Jolla, CA), with 1 cycle at 50°C for 2 min and 1 cycle at 95°C for 10 min followed by 45 cycles at 95°C for 15 sec and 60°C for 1 min. Transcripts of the housekeeping gene β -actin in the same incubations were used for normalization. The primer sequences were as follows: SREBP-1c (sense, 5'-AGG TAA TAA CCC CGT ATA TCC -3'; antisense, 5'-GAT ACC ACG ATT GTT TTG G-3'), FAS (sense, 5'-TGC TCC CAG CTG CAG GC -3'; antisense, 5'-GCC CGG TAG CTC TGG GTG TA -3') and β -actin (sense, 5'-TGA AGT GTG ACG TTG ACA-3'; antisense, 5'-TAG AAG CAC TTG CGG TGC ACG ATG GAG-3').

Statistical analysis

All data are presented as the mean $\pm$ S.E. Statistical analyses were performed using the SPSS program (SPSS, Inc., Chicago, IL). One-way analysis of variance (ANOVA) followed by the Tukey's post-hoc test or two-way ANOVA test (factors: LXR_T, diets) were used to determine statistical significance at $p < 0.05$.

3. Results

Effect of LXR agonist and SAT vs n-3 diets on plasma and hepatic lipid levels

To determine how SAT vs n-3 diets and LXR activation influence plasma and hepatic lipids levels and liver weight, C57BL/6 mice received daily gavages of vehicle or LXR agonist, T0901317 (LXR_T), for 4 weeks and were fed a normal chow or high-fat diets enriched in either SAT or n-3. There were no significant differences in plasma triglyceride and FFA levels between mice gavaged with vehicle (control) or LXR_T (Table 1). SAT diet increased plasma triglyceride and FFA levels compared to a chow or n-3 diet in control mice but these lipids levels were changed little after treatment with LXR_T on the SAT or n-3 diet (Table 1). The plasma total cholesterol level was significantly increased by LXR_T treatment and the SAT diet led to a marked increase in plasma total cholesterol levels compared to chow in both control and LXR_T mice (Table 1). The n-3 diet, however, resulted in much lower plasma total cholesterol levels in both groups (Table 1).

Mice in each group had similar body weights at the end of the feeding periods (Fig. 1A). However, livers from mice that had received LXR_T weighed more than those in control groups on all diets (Fig. 1B). SAT diet led to a significant increase in the ratios of liver weight/body weight compared to a chow diet in LXR_T-given mice, whereas there was little effect on liver weight/body weight in mice fed an n-3 diet compared to chow diet (Fig. 1B). Hepatic triglyceride content also increased ~3-fold after LXR_T in C57BL/6 mice as previously observed by others [27] (Fig. 1C). The SAT diet increased hepatic triglyceride content 1.7-fold compared to chow feeding in control mice but LXR_T did not further increase hepatic triglyceride content in SAT-fed mice (Fig. 1C). In contrast, the n-3 diet reduced hepatic triglyceride content by 32% and 46%, respectively, compared to chow diet in both control and LXR_T mice (Fig. 1C). The SAT diet also increased hepatic cholesterol by 54% compared to a chow diet in control mice, whereas mice fed an n-3 diet had a 23% decrease of hepatic cholesterol compared to chow. As expected, hepatic cholesterol level decreased by 47% in chow-fed mice after treatment with LXR_T and there were no significant dietary differences in hepatic cholesterol among LXR_T fed mice (Fig. 1D).

Effect of LXR agonist and diets on hepatic histology and transaminases

Consistent with hepatic triglyceride content, Oil Red O staining of liver sections for neutral lipids showed no hepatic steatosis in vehicle-given control mice on chow or n-3 diet, whereas SAT diet feeding markedly increased lipid accumulation as shown by increases in both the number and size of liver fat droplets (Fig. 2). In addition, the livers of mice on a chow diet with LXR_T showed substantial lipid accumulation in the liver compared to mice gavaged vehicle alone both in term of droplet number and size. There was little added effect of SAT diet together with LXR_T on hepatic lipid accumulation (Fig. 2). In contrast, the n-3 diet resulted in substantial decreases in the lipid accumulation in the liver of LXR_T mice compared to the chow diet (Fig. 2).

We examined whether an n-3 diet can protect the liver from LXR_T-induced liver injury. Others have previously demonstrated that T0901317 treatment resulted in an increase of plasma ALT and AST levels, indicating liver damage following treatment with LXR agonists [28]. In our experiments, plasma ALT and AST in the T0901317 LXR_T group were

3- and 2-fold higher than control mice, respectively, in chow fed mice (Fig. 3 A&B). Also, in both control and LXR_T mice, the SAT diet increased plasma ALT and AST levels compared to the chow diet. In contrast, LXR_T mice fed the n-3 diet had 31% decreases in plasma ALT levels compared to chow-fed mice and the n-3 diet normalized plasma AST to levels similar to those of control mice. These results indicate that LXR_T-associated liver dysfunction was ameliorated by n-3 FA, as evidenced by both decreased steatosis and normalization of transaminases in mice on n-3 FA-rich diets.

Effects of LXR agonist and dietary fats on hepatic SREBP-1c and FAS mRNA expression

In contrast to SAT, n-3 diets lowered hepatic lipid content, and improved adverse histological changes in liver. We next examined expression of genes that can regulate hepatic triglyceride accumulation. mRNA levels of SREBP1-c and FAS, known LXR target genes, were determined in livers of mice on the different diets by real-time PCR. Consistent with results of previous study by others [27], expression of SREBP1-c and FAS mRNA was significantly enhanced by LXR_T treatment in chow-fed mice by ~ 1.6- and 7-fold, respectively, compared to the control group (Fig. 4 A&B). Hepatic SREBP-1c mRNA levels were increased by SAT diet by ~ 1.6-fold in control mice compared to chow diet, SAT diet in LXR_T mice had little or no further effect on hepatic SREBP1-c and FAS mRNA expression (Fig. 4 A&B). In contrast, mice fed the n-3 diet had marked reductions on increased hepatic SREBP1-c and FAS mRNA levels induced by LXR_T (43% and 53%, respectively) compared to SAT diet (Fig. 4 A&B).

4. Discussion

In this study, we determined whether hepatic steatosis and injury are influenced by LXR activation and how different dietary fats might influence hepatic steatosis and dysfunction in C57BL/6 mice given a LXR_T. All mice on LXR_T showed hepatomegaly, a significant increase of hepatic triglyceride levels, and impaired liver function tests as indicated by elevations in plasma ALT and AST. We demonstrated that a SAT-rich diet further increased these parameters. In contrast, LXR_T-induced hepatic steatosis and dysfunction (ALT & AST) was ameliorated by an n-3 diet and this was accompanied by blunted upregulation of SREBP1-c and FAS expression caused by LXR activation.

LXR induces a variety of molecules involved in mediating macrophage cholesterol efflux and reverse cholesterol transport [4,5,29,30], has anti-inflammatory effects [31], inhibits apoptosis [32], and promotes efferocytosis [33]. LXR also stimulates cholesterol catabolism by upregulating expression of cholesterol 7 α -hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid synthesis. Treatment with LXR agonist T0901317 decreased the cholesterol content in the liver of mice and this was associated with the increase in biliary cholesterol secretion [34]. These findings led to identification of LXR agonists as potentially ideal anti-atherogenic drugs. We also found that hepatic cholesterol content was markedly decreased by LXR_T treatment in all diet groups. In contrast, plasma total cholesterol was significantly increased after LXR_T treatment likely because of increases in HDL cholesterol. T0901317 elevates HDL cholesterol and generates enlarged HDL particles in C57BL/6 mice [34]. In the current study, SAT diet led to a marked increase in plasma total cholesterol levels compared to chow and n-3 diet in both control and LXR_T mice diets, presumably because of the higher plasma LDL fractions in SAT diet compared to chow and n-3 diet [35].

Despite beneficial effects of LXR agonist on cholesterol homeostasis and atherosclerosis, the induction of fatty liver and hepatic dysfunction induced by LXR activators has so far limited their clinical development [3,10]. The present study suggests one potential way around this problem would be the combination of LXR activator treatment with diets

enriched in n-3 FA. Our study indicates that this minimizes fatty liver formation and transaminase elevation in mice treated with an LXR_T. Of interest, highly purified EPA, an n-3 FA, improves hepatic steatosis and fibrosis through its anti-inflammatory and antioxidative properties in patients with hepatic steatohepatitis [36].

Our experiments demonstrated that all mice on LXR_T showed hepatomegaly, a significant increase of hepatic triglyceride levels, and impaired liver function tests as indicated by elevations in plasma ALT and AST, in accordance with previous studies [27,28,37] and that a SAT-rich diet further increased these parameters. In contrast, LXR_T-induced hepatic steatosis and dysfunction (ALT & AST) was ameliorated by an n-3 diet, suggesting that n-3 FA inhibits hepatocyte injury associated with excess triglyceride deposition. Similar findings of improved hepatic steatosis by the n-3 diet were observed in insulin-resistant mice; where dietary SAT feeding further exacerbated hepatic lipid accumulation (supplemental Figure); Our previous data on insulin-resistant mice [35] showed that n-3 diet maintained plasma glucose and insulin levels similar to chow-fed mice, but the SAT diet led to hyperglycemia with lower plasma insulin levels, likely associated with decreasing pancreatic beta cell function. These findings are in accordance with previous experimental data showing that increased *de novo* lipogenesis, accumulation of triglyceride and depletion of n-3 FA have been demonstrated in hepatic tissue of patients with NAFLD as compared to control subjects [38]. Sekiya et al. [39] showed that n-3 FA attenuate hepatic steatosis in insulin resistance *ob/ob* mice, regardless of hepatic triglyceride storage, FA composition and lipogenesis. Furthermore, n-3 FA treatment was associated with a reduction in plasma ALT in humans with NAFLD [40], whereas a SAT-rich diet positively correlates with ALT levels in patients with hepatic steatohepatitis [41].

Our results showed that treatment with LXR_T for 4 weeks did not affect plasma FFA and triglyceride levels in any diet group. Several studies have been shown that LXR agonists induce hypertriglyceridemia in animal models [9,42,43]. However, the increase of plasma triglycerides by T0901317 was transient and reversible [42]. Chisholm et al. [43] showed that the elevation in plasma triglycerides with T0901317 normalized after one week of treatment in C57BL/6 mice, but hepatic triglyceride accumulation persisted, suggesting hepatic lipid accumulation may be a more reliable marker of increased lipogenesis.

Hepatic lipid metabolism is controlled in part by SREBP-1c, a transcription factor with preferential specificity for FA and triglyceride metabolism. Activation of hepatic SREBP-1c accelerates triglyceride accumulation in the liver through induction of lipogenic genes such as FAS [3]. Activation of the SREBP-1c and FAS after LXR agonist treatment leads to marked increase in hepatic steatosis [6,28], suggesting increased SREBP-1c is postulated as a mediator of the lipogenic effect of LXR agonists in the liver. Hepatic SREBP-1c expression is also induced by dietary saturated FA [11,12], whereas n-3 FA have been reported to inhibit hepatic FA synthesis by suppressing SREBP-1c through multiple mechanisms [44]. In a separate study to that reported herein, we found the same n-3 diet used for this study markedly depressed the active or nuclear n-terminal SREBP-1c in liver as well as adipose tissue (unpublished data). In the current study, similar to others, administration of LXR_T to C57BL/6 mice resulted in induction of SREBP-1c as well as FAS mRNA levels in the liver. In parallel with hepatic triglyceride reduction, an n-3 rich diet inhibited LXR_T-induced increases in mRNA expression of SREBP-1c and its target gene, FAS. Ou et al. [16] demonstrated that unsaturated FA lower SREBP-1c mRNA levels in part by antagonizing the actions of LXR. LXR activation by T090131 increased precursor and mature forms of SREBP-1 and endogenous fatty acid synthesis, whereas polyunsaturated FA decreased protein expression of precursor and mature SREBP-1 and its mRNA as well as fatty acid synthesis through interference with LXR activity [45]. Furthermore, several studies indicated that n-3 FA inhibit genes or activities of lipogenic enzymes including

acetyl-CoA carboxylase and stearoyl-CoA desaturase-1 as well as de novo hepatic lipogenesis [46–48]. However, Pawar et al. [49] demonstrated that LXR agonist (TO901317) induced mRNA expression of ABCG5 and ABCG8 but n-3 FA, EPA, had no effect on the level of these transcripts in hepatocytes (FTO-2B cells). They also showed that feeding rats a diet supplemented with 10% n-3 rich fish oil for 5 days did not change the LXR-regulated transcripts such as CYP7A1, ABCG5 or ABCG8, suggesting that the n-3 FA suppression of SREBP-1c and its targeted lipogenic genes was independent of LXR α . Deng et al. [50] also reported similar results: fish oil feeding effectively decreased hepatic expression of lipogenic genes whereas the expression of more traditional LXR α target genes such as ABCG5, ABCG8 or CYP7A1 are unchanged. In the present study, we also found that an n-3 rich diet did not affect hepatic ABCA1 mRNA expression (data not shown).

Another possible mechanism by which an n-3 FA diet improves hepatic steatosis may be due to inhibition of lipogenesis by down-regulating carbohydrate-responsive element-binding protein (ChREBP). ChREBP is also known to regulate lipogenic genes in liver [51] and upregulated by LXR activation [52]. Dentin et al. [53] suggested that ChREBP is an important transcription factor responsible for coordinated suppression of lipogenic genes by n-3 FA. Another possibility is that n-3 FA modulate gene transcription by interacting with peroxisome proliferators-activated receptor (PPAR) α [54]. PPAR α activation was shown to inhibit activation of LXR-regulated genes and reciprocally, LXR activation inhibited PPAR α -regulated gene expression [55, 56].

5. Conclusions

We conclude that an n-3 diet can block the adverse hepatic effects of LXR_T on hepatic steatosis. These are related to suppression of the transcription of hepatic FAS via inhibition of the expression of SREBP-1c. Combining n-3 FA with LXR_T could prove promising in decreasing atherosclerosis without hepatotoxicity.

Research Highlights

- ✓ LXR agonist and saturated fat ingestion lead to hepatic steatosis and dysfunction.
- ✓ n-3 fatty acids decrease LXR agonist-mediated hepatic steatosis.
- ✓ This is linked to decreasing hepatic SREBP-1c and fatty acid synthase mRNA.
- ✓ n-3 diet also normalize LXR agonist-mediated increases of hepatotoxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

NAFLD	nonalcoholic fatty liver diseases
LXR	liver X receptor
SREBP-1c	sterol regulatory binding protein-1c
KO	knockout
SAT	saturated fat

FA	fatty acids
ALT	alanine aminotransferase
AST	aspartate aminotransferase
FAS	fatty acid synthase
FFA	free fatty acid
CYP7A1	cholesterol 7 α -hydroxylase
ChREBP	carbohydrate-responsive element-binding protein

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Figure 1. Effects of LXR_T and diets on body weight (A), liver/body weight ratio (B) and hepatic triglyceride (C) and cholesterol (D) contents in C57BL/6 mice

C57BL/6 mice received daily gavages of LXR agonist T0901317 (LXR_T) or vehicle, and were fed a chow, SAT or n-3 diet for 4 weeks. Data are expressed as the mean $\pm$ SE (n=8–9 for each group). Two-way ANOVA was performed and significant effects of LXR_T and diet are indicated in italics ([#]p<0.05, ^{##}p<0.01, ^{###}p<0.001). *p<0.05, **p<0.01, chow vs SAT or n-3; §§p<0.01, SAT vs n-3. □ White bar is control, ■ Black bar is LXR_T

Figure 2. Histologic analysis of liver from vehicle (control) or LXR_T mice fed chow, SAT or n-3 diet

Oil Red O staining shows hepatic neutral lipid accumulation in red. Representative photomicrographs of liver sections are shown at 10× magnification (n=4).

Figure 3. Plasma transaminase levels in vehicle (control) or LXR_T mice fed chow, SAT or n-3 diets

Plasma ALT (A) and AST (B) levels were measured. Data are expressed as the mean $\pm$ SE (n=8–9 for each group). Two-way ANOVA was performed and significant effects of LXR_T and diet are indicated in italics ([#]p<0.05, ^{##}p<0.01). [§]p<0.05, SAT vs n-3. ALT: alanine aminotransferase, AST: aspartate aminotransferase. □ White bar is control, ■ Black bar is LXR_T

Figure 4. Effects of LXR_T and diets on hepatic mRNA expression of SREBP-1c and FAS
Total RNA was prepared for assessment of SREBP-1c (A) and FAS (B) by real-time PCR and were normalized to the actin mRNA. Data are expressed as the mean $\pm$ SE (n=8–9 for each group). Two-way ANOVA was performed and significant effects of LXR_T and diet are indicated in italics ([#]p<0.05, ^{##}p<0.01, ^{###}p<0.001). *p<0.05, chow vs n-3; §p<0.05, SAT vs n-3. □ White bar is control, ■ Black bar is LXR_T

Table 1

Plasma FFA, triglyceride and total cholesterol levels of vehicle (control) or LXR_T-treated mice fed a chow, SAT or n-3 diet.

		Triglyceride (mg/dL)	FFA (μM)	Cholesterol (mg/dL)
Control	CHOW	17.43±2.55	0.51±0.08	61.13±8.94
	SAT	33.68±2.75**	0.70±0.04	136.15±15.64***
	n-3	18.30±1.7§§	0.47±0.03§	73.36±4.75§§
LXR _T	CHOW	20.92±5.17	0.54±0.02	124.49±4.31###
	SAT	26.76±5.56	0.63±0.05	220.30±10.44***
	n-3	21.36±3.96	0.56±0.06	114.53±13.90§§§

Values are mean ± SE (n=8–9 for each group).

p<0.001, control vs LXR_T;

**
p<0.01,

p<0.001, chow vs SAT;

§§
p<0.01,

§§§
p<0.001, SAT vs n-3.

FFA: free fatty acid.