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α_1 -Fetoprotein Transcription Factor (FTF)/Liver Receptor Homolog-1 (LRH-1) Is an Essential Lipogenic Regulator

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Abstract

α_1 -Fetoprotein transcription factor (FTF), also known as liver receptor homolog 1 (LRH-1) is highly expressed in liver and intestine, where it is implicated in the regulation of cholesterol, bile acid and steroid hormone homeostasis. FTF is an important regulator of bile acid metabolism. We show here that FTF plays a key regulatory role in lipid homeostasis including triglyceride and cholesterol homeostasis. FTF deficient mice developed lower levels of serum triglyceride and cholesterol as a result of lower expression of several hepatic FTF target genes. Chenodeoxycholic acid repressed FTF expression resulting in a decrease in serum triglyceride in wild-type mice. The absence of chenodeoxycholic acid-mediated repression in FTF^{+/-} mice demonstrated the essential role of FTF in triglyceride metabolism. Taken together, our results identify the nuclear receptor FTF as a central regulator of lipid metabolism.

Keywords

triglycerides; cholesterol; bile acids; nuclear receptor; knockout mice

1. Introduction

α_1 -Fetoprotein transcription factor (FTF), also known as liver receptor homolog-1 (LRH-1; NR5A2), CYP7A promoter-binding factor (CPF), human B1-binding factor (HB1F) and pancreas homolog receptor-1, is the mammalian homolog of the *Drosophila fushi tarazu F1* (FTZ-F1; NR5A3). FTF orthologs have been identified in several species, including rat, chicken, horse, zebrafish, frog, and human (1). FTF plays an important role in embryogenesis and hepatic metabolism. A targeted mutation of the gene coding for FTF in mouse results in embryonic lethality around the gastrulation period, indicating a requirement for this nuclear receptor early in development (2). FTF was originally considered an “orphan” nuclear receptor, because its natural ligand was unknown; however, several groups have recently shown by x-

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ray crystallography that the human FTF ligand binding pocket can bind various phospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and perhaps even phosphatidylinositol-phosphates (PIPs), linking phospholipids metabolism to gene transcription (3).

FTF is predominantly expressed in endoderm-derived organs such as liver, intestine and exocrine pancreas in adult mammals. Consistent with its expression profile in the enterohepatic system, FTF has been shown to control cholesterol and bile acid pathways that govern hepatic and intestinal sterol homeostasis (4,5). FTF is a very important regulator of two important genes for bile acid synthesis, cholesterol 7 α -hydroxylase (CYP7A1) and sterol 12 α -hydroxylase (CYP8B1) (4,6), as well as bile acid transport genes, such as the multidrug resistance protein 3 (mpr3) and the apical sodium-dependent bile acid transporter ABST (7-9). Several genes involved in HDL metabolism and reverse cholesterol transport have also been identified as targets for FTF, including CETP (10), ApoA-I (11), scavenger receptor class B type I (SRB1) (12), the ABCG5/ABCG8 transporters (13), and apoM (14). Recently, it has been suggested that FTF might play a role in hepatic lipogenesis based on tissue culture studies (15). Moreover, FTF has been reported to regulate microsomal triglyceride transfer protein (MTP) (16). Recently, the role of FTF as a modulator of intestinal crypt cell renewal and proliferation has emerged (17,18). Therefore, FTF plays a crucial role in many aspects of the enterohepatic system.

In addition to its role in cholesterol metabolism in the liver and the intestine, FTF is also expressed in other tissues, such as pre-adipocytes (19), the ovary and other tissues (2). FTF has been shown to control steroid hormone biosynthesis by regulating the expression of genes involved in steroidogenesis, including steroidogenic acute regulatory protein (STAR) (20), cholesterol side-chain cleavage (CYP11A1) (21), 17 α -hydroxylase (22), C17, 20 lyase (CYP17) (22), 3 β -hydroxysteroid dehydrogenase (3 β -HSD) (23), 11 β -hydroxylase (CYP11B1) (23) and P450 aromatase (CYP19) (19). FTF also plays a key role in progesterone production and reproductive function (24).

In spite of the remarkable therapeutic advances of the past 30 years, atherosclerotic cardiovascular diseases remain the major cause of mortality and morbidity in the world. Elevated triglycerides and low-density lipoprotein (LDL), and reduced high-density lipoprotein (HDL) are independent risk factors for atherosclerotic cardiovascular disease (25). During the past 20 years, strong evidence has shown that members of the nuclear receptor family of genes, especially the liver X receptor (LXR), the peroxisome proliferator-activated receptor (PPAR), and the Farnesoid X Receptor (FXR) are dominant regulators of sterol and fatty acid metabolism (26,27). New therapies targeted toward these nuclear receptors are attractive. Two PPAR-modulating classes of drugs are in widespread use today, i.e., fibrate drugs are PPAR α ligands, and thiazolidinediones (TDZs) are PPAR γ ligands used in diabetes treatment (28).

Some evidence supports that the nuclear receptor FTF plays an important role in lipid metabolism (19). However, evidence on the effect of FTF on overall lipid metabolism *in vivo* is lacking. To evaluate the effect of FTF on blood lipid *in vivo*, we used FTF heterozygous mice to show that FTF insufficiency specifically leads to a reduction of triglycerides, total cholesterol and high-density lipoprotein cholesterol, demonstrating for the first time, a role of FTF in systemic lipid metabolism.

2. Materials and Methods

2.1. Animals

FTF^{+/-} mice were a generous gift from Dr. L. Belanger (Laval University, Quebec) and have been described previously (2,4). FTF^{+/-} mice were mated with MF1 or 129SV, obtained from the Jackson Laboratory, for 10 generations before experiments. Heterozygous mice were detected by PCR analyses as described previously (4). All mice were maintained in a temperature-controlled (23°C) facility with a 12 hours light/dark cycle and were given free access to water and standard laboratory rodent food (Harlan 7012). The study protocols were approved by the Virginia Commonwealth University Animal Care and Use Committee. Male mice were used in all experiments shown. For treatment with bile acids, mice were fed a standard diet supplemented with 0.25 % (w/w) chenodeoxycholic acid. The mouse blood and tissue were harvested at the middle of the dark cycle. Abdominal aorta blood was collected and transferred into BD Vacutainer Plus serum tubes. All mice appeared to be in good health. FTF^{+/-} mice gained weight at a similar pace as wild-type mice.

2.2. Antibodies and western blot experiments

Anti-FTF antibodies were prepared against amino acids 179-197 of the mouse FTF sequence and were affinity-purified before use as described previously (4). Anti-histone deacetylase was purchased from Santa Cruz Biotechnology, Inc. Liver nuclear extracts were prepared using a kit from Pierce as described previously (29). 10 µg of nuclear protein was fractionated on an SDS-polyacrylamide gel, transferred to nitrocellulose, incubated with the corresponding antibodies and processed with a Western Lightning Chemiluminescence Reagent Plus kit (PerkinElmer Life Sciences) according to the manufacturer's recommendations.

2.3. RNA Isolation and real-time quantitative PCR

RNA preparation and quantitative RT-PCR analysis was performed as described using either a fluorescence probe or SYBR Green (29). Primers and probes sequences are shown in Table I.

2.4. Adenovirus Preparation and Propagation

Preparation, propagation and purification of the FTF and the control adenoviruses were carried out as described (4). C57BL/6J mice were administered a dose of 1×10^9 plaque-forming units per mouse through the tail vein. After 4 days, mice were killed and blood and tissues harvested for further analysis.

2.5. Serum lipid measurements and liver function test

Serum aspartate and alanine aminotransferase (AST and ALT), alkaline phosphatase, total serum cholesterol, HDL-C, and TG were measured on a Beckman Coulter DXC 800 automated analyzer.

2.6. Hepatic lipids content

Hepatic lipids were extracted by using the Folch method (30), and were quantified by enzymatic assays. Briefly, 100 mg aliquots of liver were homogenized in 1 ml of phosphate-buffered saline solution as extracted with 20 ml of chloroform:methanol (2:1). After phase separation the chloroform phase was dried and dissolved in 1 ml of saline solution containing 1% tritonX-100 (31). Triglycerides, fatty acids and cholesterol were quantified using a kit from Wako following the manufacturer's protocols.

2.7. Statistic Analysis

Values are expressed as mean $\pm$ SD. The differences between different genotypes were calculated by the 2-tailed Student's *t* test.

3. Results

3.1. Decreased serum triglycerides and cholesterol in FTF^{+/-} mice

To study the role of FTF on overall lipid homeostasis *in vivo*, we measured serum triglyceride and cholesterol in wild-type and in FTF^{+/-} mice (4,5). As we and others reported earlier, FTF^{+/-} mice exhibited less than half of the normal amount of FTF mRNA and half of FTF protein compared with wild-type mice (Fig. 1), as shown by Q-RT-PCR analysis and western blotting. The FTF^{+/-} litter sizes were smaller, although there was no difference in weight between wild-type and FTF^{+/-} mice. Liver function tests (AST, ALT and alkaline phosphatase) were normal in FTF^{+/-} mice (data not shown).

We then investigated lipid homeostasis in FTF^{+/-} mice. Serum triglycerides of *ad libitum*-fed FTF^{+/-} mice were approximately 66 % lower than those of wild-type mice, both in a MF-1 and 129SV background (Table 2 and 3). Fasted (4 hours) FTF^{+/-} mice also had lower serum triglycerides levels (71 % compared to wild-type) (Table 4). Serum total cholesterol and HDL-cholesterol was lower both in fed and 4-hour fasted FTF^{+/-} mice than in wild-type mice (Table 2, 3 and 4).

3.2. FTF overexpression increases serum triglycerides

We then turned to an FTF-overexpressing mouse model. Male C57BL/6 mice were injected with an adenovirus containing the human FTF cDNA driven by the CMV promoter. As control we used mice injected with saline solution or injected with a control virus encoding the bacterial β -galactosidase gene instead of the FTF cDNA. FTF mRNA was elevated more than 20-fold in mice injected with the FTF virus compared with control virus (4). Figure 2 (top panel) shows that mice injected with the FTF virus had more than 3-fold higher serum TG levels compared with control mice, in agreement with the lower serum TG levels found in the FTF^{+/-} mice (Tables 2 and 3). Serum cholesterol levels were unchanged. FTF overexpression in 129SV mice resulted in a similar increase in serum TG levels (data not shown).

Since the transcriptional factor SREBP-1c has been shown to be a crucial factor on fatty acid and TG synthesis (32), we investigated whether FTF overexpression had an effect on the nuclear levels of the mature SREBP-1c protein. Figure 2 (bottom panel) shows that injection of the FTF-containing adenovirus in mice increased FTF expression (about 20-fold) in the liver compared with non-injected mice or mice injected with a control virus. Most importantly, that increase in FTF expression was accompanied by a similar increase in the nuclear (mature) form of SREBP-1 (Fig 2 bottom panel). Injection of the control virus slightly elevated SREBP-1c levels compared with non-injected mice.

3.3. Chenodeoxycholic acid lowers plasma TG levels in wild-type mice but not in FTF^{+/-} mice

It is well known that bile acids affect TG homeostasis (33). Thus, human bile acid-binding resins induce the production of very low-density lipoproteins (VLDL) TG. It has been shown that in cholesterol gallstone patients treated with chenodeoxycholic acid (CDCA) serum TG levels are lower than in untreated individuals (34). The same phenomenon has been observed in mice, *via* a pathway involving FXR, SHP and SREBP-1c (35). In an attempt to further define the role of FTF in lipid homeostasis, wild-type and FTF^{+/-} mice were fed with a diet containing 0.25 % CDCA for 2 days. The CDCA-containing diet was well tolerated by the mice, based on the fact that liver trans-aminases in serum did not change (data not shown). The intake of CDCA-containing food was normal. The CDCA-containing diet strongly lowered circulating

plasma TG, and total plasma cholesterol in wild-type mice (Fig. 3, top panel). However, a decrease in serum TG and total cholesterol was not observed in FTF^{+/-} mice. CDCA suppressed FTF mRNA slightly, but significantly, in wild-type mice, but not in FTF^{+/-} mice (Fig. 3, bottom panel). As a positive control we quantified small heterodimer partner (SHP) mRNA (Fig. 3, bottom panel), which is well established to be induced by bile acids treatment with CDCA induced in both wild-type and FTF^{+/-} mice (Fig. 3, bottom panel). Nuclear FTF protein levels correlated with the mRNA levels (bottom panel insert). These experiments strongly suggest that FTF is essential for bile acid lowering of serum TG *in vivo*.

3.3. Decreased hepatic lipid content in FTF^{+/-} mice

To study whether the pivotal role that FTF plays in serum lipid content acts through the liver, we quantified both TG and FA in the liver of wild-type and FTF^{+/-} mice. Figure 4 shows that both TG and FA are significantly decreased in the liver of FTF^{+/-} mice. Liver cholesterol content did not change.

3.4. Decreased expression liver target genes related to lipid homeostasis in FTF^{+/-} mice

To better understand the molecular mechanisms underlying the role of FTF in lowering serum TG levels, we used quantitative RT-PCR to measure hepatic mRNA levels of several important genes involved in lipid homeostasis in wild-type and FTF^{+/-} mice (Table 4). No major changes were observed in the expression of HMG-CoA reductase, LDL receptor and cholesterol transport protein ABCA1. ApoA1 was slightly decreased although these differences did not reach statistical significance. ApoM, which plays a role in pre-β-HDL formation, was decreased in FTF^{+/-} mice, suggesting that FTF play an important role in HDL formation. FTF^{+/-} mice exhibited about 50 % scavenger receptor B1 (SR-B1) mRNA levels, which mediates selective uptake of HDL-CE by liver, compared to wild-type mice. Genes encoding transporters involved in biliary cholesterol secretion, ABCG5/ABCG8, were significantly decreased in FTF^{+/-} mice. Expression of the SREBP-1c and its target genes encoding enzymes involved in fatty acid and TG biosynthesis, such as acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS), was significantly reduced in FTF^{+/-} mice. These changes were similar in a 129SV background (Table 4) and in a MF-1 background (data not shown).

4. Discussion

Regulation of hepatic lipid metabolism plays a key role in whole body energy balance, since the liver is the major site of triglyceride synthesis (lipogenesis) and carbohydrate metabolism. The liver is responsible for the conversion of excess dietary carbohydrate into triglyceride through *de novo* lipogenesis. The balance between TG production and clearance determines the circulating TG levels. Liver TG production is mainly determined by the fatty acid synthesis rate. The present study provides, for the first time, *in vivo* evidence that the nuclear receptor FTF is a key regulator of lipid homeostasis. There are several pieces of evidences that support it. First, FTF^{+/-} mice show markedly decreased serum triglyceride and cholesterol levels compared to wild-type mice. This is associated with a decrease in the mRNA of hepatic genes known to control lipid metabolism, suggesting that FTF is an essential regulatory factor in hepatic lipid production and cholesterol transport. Second, FTF suppression by CDCA led to a decrease in serum triglycerides and cholesterol in wild-type mice but not in FTF^{+/-} mice. These results further indicate that FTF is a positive regulator of lipid metabolism. Third, FTF overexpression led to hypertriglyceridemia in C57BL/6 mice. Taken together, the present study suggests that FTF is an important factor in lipid metabolism.

The studies presented here strongly suggest that FTF is an important lipogenic regulator that controls the transcription factor SREBP-1c and some of its target genes. SREBP-1c has emerged as a major mediator of lipogenic gene expression (36). SREBP-1c controls the fatty

acid biosynthetic pathway, such as the ATP-dependent citrate lysate (37), acetyl-CoA carboxylase (ACC) (38) and fatty acid synthase (FAS) (39,40). Consistent with these studies, SREBP-1c expression, as well as the expression of two lipogenesis regulatory hepatic SREBP-1c target genes, ACC and FAS, are significantly decreased in FTF^{+/-} mice compared to wild-type mice (Table 4).

A key factor in the control of SREBP-1c expression is the nuclear receptor LXR (41). LXR is also a key factor for the transcription of the FAS gene (42). It has recently been described that FTF binds a distinct nuclear receptor half-site 21 bases downstream of the DR-4 element of the FAS promoter. This binding is required for full LXR responsiveness of the endogenous FAS gene as well as for promoter reporter constructs in tissue culture cells (15), and provides a mechanistic explanation for our findings. It has also been established that LXRs are potential regulators of lipogenesis by controlling basal transcription of SREBP-1c (43,44). Additional studies will be required to determine if the effect of FTF on lipogenic target genes is independent of LXR or acting as a competence factor for LXR *in vivo*.

Heterodimer ABCG5/ABCG8 is responsible for secretion of biliary cholesterol from liver into bile (45). Expression of both ABCG5 and ABCG8 is significantly decreased in FTF^{+/-} mice, which is consistent with other FTF knockout mouse models (31,46) and confirms tissue culture studies that showed that FTF activates the ABCG5/ABCG8 intergenic promoter (13). Interestingly, expression of another ABC transporter, ABCB11, also known as bile salt export pump or BSEP, which is responsible for most of the bile salt transport from hepatocytes into the bile canicular lumen, is unchanged in FTF^{+/-} mice. Similarly, expression of the cholesterol transporter ABCA1 is virtually identical in these two groups.

We already reported that plasma cholesterol levels are lower in the FTF^{+/-} mouse than in wild-type mice (4). The data presented in this study support those preliminary observations and add a mechanistic explanation. Because cholesteryl-ester-transfer protein (CETP) is absent from mice, mice transport the majority of plasma cholesterol in HDL particles. Accordingly, HDL-C is between 80 and 88 % of total cholesterol both in wild-type and in FTF^{+/-} mice (4-hour fasted). Both total and HDL-C are significantly reduced in FTF^{+/-} mice compared to wild-type mice (Table 2 and 3). ApoM, which is a 26-kDa apolipoprotein associated mainly with HDL required for pre- β -HDL formation and cholesterol efflux to HDL (47), is also decreased in FTF^{+/-} mice. This supports the tissue culture studies that identified FTF as an activator of human and mouse apoM transcription that binds to an FTF response element located in the proximal apoM promoter region (14). The correlation that we observed between lower circulating HDL-C and total cholesterol and decreased apoM expression strongly suggests that lower HDL synthesis in the liver of FTF^{+/-} mice is one of the molecular mechanisms underlying decreased plasma cholesterol. Finally, the observation that liver cholesterol content does not change in the FTF^{+/-} mouse compared to wild-type (Fig. 4) further suggest that the reduction in plasma cholesterol in the FTF^{+/-} mice is due to an accelerated cholesterol degradation consequence of an increase in bile acid synthesis in the FTF^{+/-} mouse, as we previously showed (4).

Expression of apoA1, which is the major protein component of HDL, is not differently expressed in FTF^{+/-} mice, supporting recent studies that suggest that FTF regulates ApoA1 gene transcription in a species-specific manner; it activates human ApoA1 but not mouse ApoA1 expression (11). Interestingly, expression of SR-BI is also decreased in FTF^{+/-} mice, consistent with data showing that FTF induces both murine and human SR-BI promoter activity in tissue culture cells (12). SR-BI mediates the selective hepatic uptake of cholesteryl esters from HDL and plays a key role in reverse cholesterol transport. As such, SR-BI overexpression in the liver reduces plasma HDL levels and increases cholesterol excretion (48). Furthermore, plasma HDL levels are elevated in SR-BI^{-/-} mice (49,50). This suggests that the lower plasma

cholesterol levels that we have observed in the FTF^{+/-} mice compared with wild-type is not due to an increased uptake of lipoproteins from the blood but to an augmented hepatic cholesterol metabolism as a result of higher cholesterol 7 α -hydroxylase expression in the FTF^{+/-} mice (4), in addition to the lower HDL synthesis in the FTF^{+/-} mice discussed above.

Chenodeoxycholic acid has long been known to reduce plasma triglycerides and VLDL (33, 51). The observation that CDCA reduces serum triglycerides and cholesterol in wild-type mice but not in FTF^{+/-} mice suggests that FTF is an important nuclear receptor mediating repression of triglycerides and cholesterol by CDCA, mimicking the lack of TG-lowering effect by bile acids in the LXR knockout mouse by lowering SREBP-1c and FAS expression (35). CDCA is a ligand of the FXR receptor that, when activated, induces SHP expression (52,53). It has been shown that SHP suppresses SREBP-1c expression by inhibiting the activity of LXR, a required factor for SREBP-1c transcription (35). Perhaps FTF levels are so low in the FTF^{+/-} mouse that the CDCA-mediated induction of SHP expression cannot further reduce LXR activity to result in an additional reduction of plasma TG levels.

Interestingly, plasma TG levels are unaffected in two conditional FTF null mouse models (31,46). Plasma cholesterol, on the other hand, is lower in one model (46), but not in the other (31). The most likely explanation for these contradictory results is the differences in genetic background of these mouse lines. Both of the conditional mouse lines had a mixed C57BL6/129SV genetic background, whereas the studies presented here were performed in mice with a genetic background more than 99 % MF-1 or 129SV. More studies will be required to further explain these apparent discrepancies.

In summary, the studies presented here show that FTF affects plasma triglyceride and cholesterol levels, through the regulation of a number of liver genes involved in lipogenesis and cholesterol transport. These findings reveal a key role for FTF in regulating whole-body lipid homeostasis, which suggests that FTF may become another therapeutic target for dyslipidemia.

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Fig. 1. Differential FTF protein and mRNA levels in wild-type and FTF^{+/-} mice fed *ad libitum* (129 SV background). FTF mRNA and protein were quantified by RT-PCR and western blotting respectively, as indicated in Materials and methods. An autoradiograph for the western blot is shown at the bottom, with HDAC as a loading control. Values represent the means $\pm$ S.D. from three mice/group. * $p < 0.05$ compare to wild-type mice.

Fig. 2. FTF overexpression increased serum triglyceride and mature nuclear SREBP-1c in C57BL/6 mice. Male mice (three to five/group, 40 days old, fed *ad libitum*) were injected with the indicated viruses. Four days later, blood was collected and triglycerides and cholesterol quantified (top panel). Values represent the means $\pm$ S.D. * $p < 0.05$ compared to control virus. Bottom panel shows a Western blot performed on nuclear extracts to quantify FTF and SREBP-1. Histone deacetylase (HDAC) was used as a control.

Fig. 3.

Chenodeoxycholic feeding decreased serum triglyceride wild-type mice but not in FTF^{+/-} mice. Wild-type and FTF^{+/-} mice (50 days old, 129SV background, three per group) were fed *ad libitum* either a control diet or the same diet supplemented with 0.25 % chenodeoxycholic acid for 2 days. Serum and liver were collected and used to quantify triglycerides and total cholesterol (top panel), FTF and SHP mRNA (bottom panel), and FTF and HDAC nuclear proteins (bottom panel, insert) as indicated in Materials and methods. * $p < 0.05$, ** $p = 0.07$ compare to wild-type mice fed the control diet.

Fig. 4. FTF^{+/-} mice have lower liver triglyceride content than wild-type mice. Triglycerides, fatty acids and cholesterol were quantified in the liver of wild-type and FTF^{+/-} male mice (50 days old, 129SV background, three to five/group) as indicated in Materials and methods. * $p < 0.05$ compare to wild-type mice; ** $p = 0.07$.

Table 1
Oligonucleotides used in the Q-RT-PCR quantification of mRNAs

Oligo	Gene	Sequence	GenBank™ no.
ABC transporters	ABCB11-F	CCA GAT CAC CAA CGA AGC CC	NM_021022
	ABCB11-R	CCA GCT CAA CCT CAA ATG CTT T	
	ABCB11-P	AGC AAT ATC CGC ACC GTG GCT GGA	
	ABCA1-F	ATT GCC AGA CGG AGC CG	NM_013454
	ABCA1-R	TGC CAA AGG GTG GCA CA	
	ABCG5-F	TGG ATC CAA CAC CTC TAT GCT AAA	AF312713
	ABCG5-R	GGC AGG TTT TCT CGA TGA ACT G	
	ABCG8-F	TGC CCA CCT TCC ACA TGT C	AF312713
HDL and receptors	ABCG8-R	ATG AAG CCG GCA GTA AGG TAG A	
	ApoA1-F	CCA GAG TGT GAT CGA CAA GGC	NM_009692
	ApoA1-R	ATT GTA AGA AAG CCA ATG CGG	
	ApoA1-P	TCT GAC TGC CCA GTG AGG TGC CCG	
	SR-B1-F	GCT GCG CTC GGC GTT GTC AT	NM_016741
	SR-B1-R	GGG ACG GGG ATC TCC TTC CA	
	ApoM-F	GGA ATG GAC GAC ACA GAG AC	NM_018816
	ApoM-R	CGG ATG GTA GCA CGA AGC	
Fatty Acid and TG Synthesis	SREBP1-F	AGC AGC CCC TAG AAC AAA CAC	NM_011480
	SREBP1-R	CAG CAG TGA GTC TGC CTT GAT	
	FAS-F	GGC ATC ATT GGG CAC TCC TT	AF127033
	FAS-R	GCT GCA AGC ACA GCC TCT CT	
	ACC-F	AGG ATT TGC TGT TTC TCA GAG CTT	NM_133360
	ACC-R	CAG GAT CTA CCC AGG CCA CAT	
Nuclear receptors	SHP-F	CTG AAG GGC ACG ATC CTC TTC	NM_011850

Oligo	Gene	Sequence	GenBank™ no.
Others	SHP-R	ACC AGG GCT CCA AGA CTT CAC	NM_013839
	LXR-F	ATG CCT GAT GTT TCT CCT GAT TCT	
	LXR-R	CAT CCT GGC TTC CTC CCT GA	
	LXR-P	AGT GTT GCC TCC CTG GTC TCC TGC	
	H. Lipase-F	CAT ACC AGC ACT ACA CCA TTG	NM_011388
	H. Lipase-R	ACT TCG CAG ATT CCT CCA G	
	ABST-F	TGG GTT TCT TCC TGG CTA GAC T	NM_010700
	ABST-R	TGT TCT GCA TTC CAG TTT CCA A	
	LDL-R-F	AGG TGT GAA GAT ATT GAC GAG TG	
	LDL-R-R	GGT TGG TGA AGA GCA GAT AGC	

An F in the oligonucleotide name means forward; and R, reversed; and a P, TaqMan probe

Table 2
FTF^{+/-} mice (MF-1 background) display a hypo-lipid profile under normal feeding conditions

Wild-type and FTF^{+/-} mice of approximately 35 days of age in a MF-1 genetic background, generated by the crossing of FTF^{+/-} mice with MF1, as explained in Material and methods, were fed *ad libitum* and the indicated blood parameter quantified. Both wild-type and FTF^{+/-} mice were from the same litter. Values represent the average $\pm$ SD (n = 3). TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol.

Parameter	Wild-type	FTF ^{+/-}	p value
Weight (g)	22.8 $\pm$ 0.5	22.3 $\pm$ 1.2	0.4
TG (mg/dL)	202.7 $\pm$ 22.7	134.3 $\pm$ 19.2	0.03
TC (mg/dL)	110.3 $\pm$ 2.5	89.0 $\pm$ 1.4	<0.01
HDL-C (mg/dL)	82.2 $\pm$ 2.6	63.0 $\pm$ 4.8	0.01

Table 3
FTF^{+/-} mice (129SV background) display a hypo-lipid profile under normal feeding conditions

Wild-type and FTF^{+/-} mice of approximately 50 days of age in a 129SV genetic background, generated by the crossing of FTF^{+/-} mice with MF1, as explained in Material and methods, were fed *ad libitum* and the indicated blood parameter quantified. Values represent the average $\pm$ SD (n = 8). Same abbreviations are used as in Table 2

Parameter	Wild-type	FTF ^{+/-}	p value
Weight (g)	22.6 $\pm$ 1.75	22.5 $\pm$ 1.2	0.97
TG (mg/dL)	213.7 $\pm$ 29.1	143.4 $\pm$ 28.2	<0.01
TC (mg/dL)	133.1 $\pm$ 2.8	114.4 $\pm$ 12.8	<0.01
HDL-C (mg/dL)	116.5 $\pm$ 3.8	100.9.0 $\pm$ 9.6	<0.01

Table 4
FTF^{+/-} mice display hypo-lipid profile after 4 hours fasting

Wild-type and FTF^{+/-} mice generated as in Table 3 (129SV background) of 50 days of age, were fasted for 4 hours before blood and livers were harvested and the same blood parameter quantified. Values represent the average $\pm$ SD (n = 3). Same abbreviations are used as in Table 2

Parameter	Wild-type	FTF ^{+/-}	p
Weight (g)	30.5 $\pm$ 1.2	30.2 $\pm$ 2.7	0.8
TG	147.0 $\pm$ 20.2	104.7 $\pm$ 12.7	0.05
TC	113.2 $\pm$ 2.0	85.3 $\pm$ 1.0	<0.01
HDL-C	91.0 $\pm$ 1.9	74.9 $\pm$ 2.5	<0.01
Glucose	198.7 $\pm$ 14.6	197.0 $\pm$ 19.5	0.9

Table 5
Expression of lipogenic genes and other genes related to lipid metabolism

Values represent the average $\pm$ SD (n = 3). Mice were in 129SV background of 35 days of age. Abbreviations are the same as in Table 2.

Category	Gene	Wild-type	FTF ^{+/-}	p
ABC transporters	ABCG5	100 $\pm$ 6.0	54.3 $\pm$ 9.5	0.002
	ABCG8	100 $\pm$ 36.1	45.4 $\pm$ 5.5	0.02
	ABCB11	100 $\pm$ 15.9	98.8 $\pm$ 8.5	0.95
	ABCA1	100 $\pm$ 6.6	108.6 $\pm$ 19.3	0.53
HDL and receptors	ApoA1	100 $\pm$ 31.5	67.7 $\pm$ 6.1	0.16
	SR-B1	100 $\pm$ 22.1	53.4 $\pm$ 11.7	0.034
	ApoM	100 $\pm$ 36.7	49.1 $\pm$ 18.1	0.05
	SREBP1	100 $\pm$ 12.9	53.5 $\pm$ 10.4	0.007
Fatty Acid and TG Synthesis	FAS	100 $\pm$ 15.8	51.1 $\pm$ 8.5	0.008
	ACC	100 $\pm$ 11.4	61.6 $\pm$ 17.6	0.03
	HNF-4 α	100 $\pm$ 22.6	93.1 $\pm$ 13.5	0.95
Nuclear receptors	LXR	100 $\pm$ 18.0	124.3 $\pm$ 18.6	0.18
	Hepatic Lipase	100 $\pm$ 39	92 $\pm$ 13	0.57
Others	LDL-R	100 $\pm$ 44.2	92.2 $\pm$ 50.2	0.84
	HMG-CoA Reductase	100 $\pm$ 10.1	91.6 $\pm$ 25.9	0.59
	ABST	100 $\pm$ 14.3	108.0 $\pm$ 11.2	0.54