Lipase family member N is a novel target gene for CCAAT/enhancer-binding protein α in type 2 diabetic model mouse liver

Daisuke Aibara, Kohei Matsuo and Kimihiko Matsusue

Faculty of Pharmaceutical Science, Fukuoka University, Fukuoka 814-0180, Japan

Abstract. CCAAT/enhancer-binding protein α (C/EBPα) is a transcription factor abundantly expressed in the liver and white adipose tissue (WAT). In this study, we investigated the mechanism by which C/EBPα regulates the lipase family member N (Lipn) gene in the mouse liver. Mouse Lipn consists of non-coding exon 1 and the translation start site located in exon 2. Lipn expression in the fatty liver of ob/ob mice was significantly higher than that in OB/OB mice and was significantly repressed by liver-specific C/EBPα deficiency. Lipn expression in the liver, eWAT, and sWAT of wild-type mice was undetectable, although C/EBPα was highly expressed in these tissues. The database analysis revealed four putative C/EBP-responsive elements (CEBPREs), highly homologous with the typical CEBPRE consensus sequence at positions –2,686/–2,678, –1,364/–1,356, –106/–98, and –45/–37 from the transcription start site (+1) of Lipn. Reporter assays using reporter constructs with serial or internal deletions of the 5'-flanking regions of Lipn showed that two functional CEBPREs (–106/–98 and –45/–37) in the Lipn promoter region are essential for enhancing Lipn transcriptional activity by C/EBPα. Electrophoretic mobility shift assay showed that C/EBPα/β binds to CEBPRE (–106/–98). These results suggest that C/EBPα and type 2 diabetic environment may be required for hepatic Lipn expression.

Key words: CCAAT/enhancer-binding protein, C/EBP-responsive element, Liver, Lipase family member N, Lipn
decrease in plasma HDL as well as LDL and TC levels. Furthermore, a deficiency of hepatic C/EBPα expression exacerbates hyperglycemia because of decreased insulin secretion [5]. These results indicate that hepatic C/EBPα is essential for ammonia detoxification and glucose and lipid homeostasis.

Lipase family member N (LIPN), a member of the alpha/beta hydrolase superfamily, contains eight beta strands connected by six alpha-helices and is an acidic lipase of epidermal-localized members [6]. The amino acid sequences of human and mouse LIPN show high homology (81%) [6]. LIPN is exclusively expressed in the epidermis [7] and is strongly induced during keratinocyte differentiation [8]. Furthermore, a mutation in LIPN causes a late-onset form of autosomal-recessive congenital ichthyosis, characterized by a 2-bp deletion in exon 3 of LIPN, leading to the generation of a stop codon in the LIPN amino acid sequence [8]. These results suggest that LIPN plays a vital role in epidermal differentiation. However, the role of tissues other than the epidermis and mechanism of transcriptional regulation remain unclear.

Several studies on LIPN have focused on the physiological functions of the epidermis. Recently, our preliminary studies revealed that Lipn is highly expressed in the fatty liver of ob/ob mice and is considerably decreased by liver-specific deficiency of C/EBPα. Therefore, in this study, we investigated the role of C/EBPα in the transcriptional regulation of hepatic lipids.

**Materials and Methods**

**Animals and treatment**

This study was approved by the Center for Experimental Animals at Fukuoka University (Permission number: 2014113) and carried out in accordance with the guidelines of the center. Liver-specific C/EBPα knockout mice (C/EBPαLKO) were generated by breeding Cebpα-floxed mice with mice expressing Cre recombinase under the control of the albumin promoter [4, 5]. Additionally, Cebpα-floxed mice were generated in the ob/ob C57BL/6J (ob/ob-C/EBPαLKO) and C57BL/6J (OB-OB-C/EBPαLKO) backgrounds as a wild-type for leptin gene [5]. Eight-week-old male C/EBPαWT or LKO mice (n = 3) with or without ob/ob were fed ad libitum (MF, Oriental Yeast, Fukuoka, Japan). A high-fat (HF) diet experiment was performed with the mouse livers used in our previous study [9].

**RNA extraction and quantitative real-time polymerase chain reaction**

RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA), and quantitative polymerase chain reaction (qPCR) was performed using cDNA generated from 1 μg of total RNA using an Affinity Script qPCR cDNA Synthesis kit (Agilent Technologies, Santa Clara, CA, USA). Cebpa consists of one exon and no intron, indicating that genomic DNA in the generated cDNA prevents accurate measurement of Cebpa mRNA level. Thus, the cDNA for Cebpa analysis was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). This kit, involving an RNase-free DNase I treatment step, was used for Cebpa mRNA analysis to remove genomic DNA completely. The following primers were used for specific genes: Lipn: forward, 5'-CGCAGATGGTGACA TTCTCG-3′ and reverse, 5'-CAAGCCAGTAGGCATTG TCTG-3'; Cebpa: forward, 5'-CAAGAACAGCAACGA GTACCG-3′ and reverse, 5'-GTCACTGTGCTCACTCCA GCAC-3'; acidic ribosomal phosphoprotein P0 (36b4): forward, 5'-AACAAGTGTGCTCACTACGGGCGTCC 3′ and reverse, 5'-TGCTGCTTCTGAGATTTTGG-3′. The expression of sample mRNAs was normalized to that of 36b4 mRNA.

**Cell culture**

HEK293FT cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with a high level of glucose and pyruvate (Thermo Fisher Scientific), 10% fetal bovine serum (Biowest, Nuaillé, France), and 1% antibiotic–antimycotic agents (Thermo Fisher Scientific) at 37°C with 5% CO₂.

**Construction of reporter and expression plasmids**

The transcriptional start site of mouse Lipn was obtained from the Database of Transcriptional Start Sites (http://dbtss-old.hgc.jp/hg19_mm9/). The –2967 (D1), –929 (D2), and –466 (D3) bp fragments from the transcriptional start site (+1) of the mouse Lipn 5′-upstream region, containing the CACC sites at the 5′-end of the primers, were amplified by PCR and cloned into the Gateway entry vector pENTR/DTOPO (Thermo Fisher Scientific), and then recombined with the pGL4.17 vector (Promega, Madison, WI, USA), which was prepared using the Gateway Vector Conversion System according to the manufacturer’s instructions (Thermo Fisher Scientific). The following forward primer sequences were used: D1, 5′-CACCAGACTAAAAGAC TGTGGATG-3′; D2, 5′-CACCCTCTTCTCACCA TACAGTGAATC-3′; and D3, 5′-CACCAGTAAATTTT GCCTCTCACCC-3′. The reverse primer sequence, which was common for all constructs, was as follows: 5′-GCTGTGCAGTACAGTCTGACG3′. Internal deletion constructs for the Lipn 5′-upstream region were prepared by inverse PCR as previously described [10, 11]. The Lipn D3 construct was used as a template. The
primer sequences were as follows: D3-1 forward: 5'-CAAGTGTATATCATGCATTGGTCGC-3' and reverse, 5'-TAGTGGTGCACTGATGCGAAGTAA-3'; D3-2 forward: 5'-TCTCTTATTTCCACACCTTCCTGGT-3' and reverse, 5'-TACACGGCAGAGGGGTCCTTAG-3'; D3-3 forward: 5'-TCTCTTATTTCCACACCTTCCTGGT-3' and reverse, 5'-TAGTGGTGCACTGATGCGAAGTAA-3'. The C/EBPα/β expression vectors were provided by Dr. Frank J. Gonzalez (National Cancer Institute, Bethesda, MD, USA).

**Transient transfection and reporter assay**

HEK293FT cells were seeded at a density of 2.0 × 10⁵ cells/well in 24-well plates 24 h before transfection. The cells were transfected with plasmids using the PEI Max transfection reagent (Polysciences, Inc., Warrington, PA, USA) following the manufacturer’s instructions. Each well contained 1 μL of 1 μg/μL PEI Max transfection reagent, 0.15 μg of C/EBPα/β expression plasmids, 0.05 μg of luciferase reporter pGL4.17 containing the 5'-region of mouse Lipn, and 0.005 μg of pHLR/TK (Promega, Madison, WI, USA) as an internal control for transfection efficiency. The cells were harvested at 24 h after transfection for the reporter assay. The reporter assay was performed using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activity was measured using a Lumat 3 luminometer (Berthold Technologies, Bad Wildbad, Germany).

**Electrophoretic mobility shift assay**

Electrophoretic mobility shift assay (EMSA) was performed as described previously [12]. For the supershift assay, samples were incubated with 0.5 μg of anti-C/EBPα IgG (Cell Signaling Technology, Danvers, MA, USA) and anti-C/EBPβ IgG (Abcam, Cambridge, UK) for 30 min after the binding reactions. The gels were exposed to an imaging plate (Fujifilm, Tokyo, Japan) and visualized using an FLA-7000 imaging analyzer (Fujifilm). Probe sequences for the EMSA assay were as follows: Vaspin CEBPRE as the control probe [11], 5'-AAGAAGAAAGATGCAAAAGTGTAG-3'; CEBPRE-3, 5'-CCACTATTCCCACACCTAACAGTTG-3'; CEBPRE-4, 5'-GTGTCATTTCAGGTTTCCTCA-3'.

**Statistical analysis**

Experimental values are expressed as mean ± standard error of the mean. Statistical analysis was performed using Student’s t-test for unpaired data, with p < 0.05 considered to indicate statistically significant differences.

---

**Results**

**Non-coding exon 1 in mouse Lipn gene**

Database analysis was performed to identify the transcription start sites of mouse Lipn. The search of DBTSS (https://dbtss.hgc.jp/) and NCBI gene (https://www.ncbi.nlm.nih.gov/gene/) indicated the existence of putative non-coding exon 1 in mouse Lipn, with the translation start site located in exon 2 (Fig. 1A). The expression of exon 1 in the mouse liver was examined by sequencing the PCR products from the mouse liver cDNA library.

**C/EBPα regulates the expression of Lipn gene in the mouse liver**

The RT-qPCR analysis was performed using liver-specific C/EBPα knockout mice in the C57BL/6J (OB/OB-C/EBPαLKO) or ob/ob C57BL/6J (ob/ob-C/EBPαLKO) backgrounds to determine the contribution of hepatic C/EBPα to Lipn expression in the mouse liver. A previous study demonstrated that the c/ebpa mRNA level in the OB/OB- or ob/ob-C/EBPαLKO mouse liver was very low at 12% or 5% compared with that in the OB/OB- or ob/ob-C/EBPαWT mouse liver, respectively [11]. The expression of Lipn mRNA was undetectable in the liver of OB/OB-C/EBPαWT mice but was considerably induced in the fatty liver of ob/ob-C/EBPαWT mice (Fig. 1Ba). Furthermore, the expression of Lipn mRNA in the liver of ob/ob-C/EBPα LKO mice was significantly lower than that in ob/ob-C/EBPαWT mice, at 23% (Fig. 1Ba). A high expression of Lipn was also observed in HF-inducible fatty liver as in other fatty liver model (Fig. 1Bb). These results indicate that hepatic C/EBPα is involved in transcriptional regulation of Lipn expression in the fatty liver of ob/ob mice.

**Expression of hepatic Lipn gene requires C/EBPα and a type 2 diabetic environment**

Although C/EBPα is highly expressed in the liver and fat tissue [2], the expression of Lipn in other mouse tissues remains unclear. Thus, the expression of Lipn in ob/ob mice was confirmed in tissues other than the fatty liver. Lipn mRNA was detected in the fatty liver, epididymal white adipose tissue (eWAT), brown adipose tissue (BAT), and skeletal muscle (muscle), but was undetectable in the heart, kidney, and brain (Fig. 2A). In the liver, Lipn expression was observed in the fatty liver of ob/ob mice but not in the liver of OB/OB mice, although C/EBPα was highly expressed in the OB/OB mouse liver. To examine the expression of Lipn mRNA in the eWAT and subcutaneous WAT (sWAT) of OB/OB mice, Lipn mRNA expression in the eWAT or sWAT was compared between ob/ob and OB/OB mice. The Lipn...
mRNA level in the eWAT, sWAT, and liver of ob/ob mice was considerably higher than that in the liver of OB/OB mice (Fig. 2B and D). In contrast, Cebpa mRNA expression in the eWAT, sWAT, and liver of ob/ob mice was significantly lower than that of OB/OB mice (Fig. 2C and E). These results suggest that Lipn expression is associated with C/EBPα in the liver and WAT as well as in the type 2 diabetic environment of ob/ob mice.

Four putative CEBPREs in the 5'-upstream region of Lipn

A database analysis was performed to identify functional CEBPRE in the 5'-upstream region of Lipn. A search of the JASPAR database (http://jaspar.genereg.net/) revealed four putative CEBPREs (Fig. 3A). CEBPRE-1, CEBPRE-2, CEBPRE-3, and CEBPRE-4 showed 100%, 89%, 78%, and 89% homology with the typical CEBPRE consensus sequence [11], respectively. Lipn luciferase reporter plasmids (−2,967/+27) containing CEBPRE1-4 were transfected into HEK293FT cells with or without C/EBPα or C/EBPβ expression plasmids (Fig. 3B). The Lipn promoter activities with C/EBPα and C/EBPβ were induced by approximately 5.4- and 3.7-fold, respectively, compared with those in cells without C/EBPα/β, indicating the presence of functional CEBPRE in the 5'-upstream region of Lipn.

Identification of functional CEBPRE in Lipn 5'-upstream region

Reporter constructs with serial deletions of the 5'-flanking region of Lipn were prepared to identify the cis-element responsible for the binding of C/EBPα/β. C/EBPα (Fig. 4Aa) or C/EBPβ (Fig. 4Ab) induced the luciferase activity of the Lipn D1 construct by approximately 4.1- or 3.4-fold, respectively. The D2 and D3 constructs lacking CEBPRE-1 and CEBPRE-2 remained unchanged with C/EBPα or C/EBPβ expression compared with the D1 construct, indicating that CEBPRE-1 and CEBPRE-2 are not functional in inducing promoter activities by C/EBPα/β (Fig. 4Aa and b). Each reporter construct
lacking CEBPRE-3 or -4 was prepared by internally deleting these CEBPREs (Fig. 4Ba and b). The luciferase activity of the \( \text{Lipn} \) construct was induced by approximately 4.9- or 3.3-fold with C/EBPα or C/EBPβ, respectively. C/EBPα or C/EBPβ slightly induced the luciferase activities of the \( \text{Lipn} \) construct lacking CEBPRE-3 by approximately 1.6- or 1.2-fold, respectively. C/EBPα or C/EBPβ induced the activities of the \( \text{Lipn} \) construct lacking CEBPRE-4 by approximately 3.0- or 2.2-fold, respectively. C/EBPα or C/EBPβ could not induce luciferase activity of the \( \text{Lipn} \) construct lacking both CEBPREs. These results suggest that CEBPRE-3 and -4 on the \( \text{Lipn} \) promoter function in transcriptional regulation with C/EBPα/β.

C/EBPα/β directly binds to CEBPRE-3 in the \( \text{Lipn} \) promoter region

To examine whether C/EBPα/β directly binds to CEBPRE-3 and CEBPRE-4, EMSA using CEBPRE-3/-4 probes was performed. C/EBPα directly binds to

---

Fig. 2  Expression of \( \text{Lipn} \) is induced in \( \text{ob/ob} \) tissues which express C/EBPa. qPCR analyses of \( \text{Lipn} \) (A, B, and D) and \( \text{Cebpa} \) (C and E) mRNAs were performed using the liver, eWAT, sWAT, BAT, muscle, heart, kidney, or brain of \( \text{OB/OB} \) or \( \text{ob/ob} \) mice. Expression was normalized to \( 36b4 \) mRNA expression, and each bar represents average ± standard error of the mean of three individual experiments. eWAT, epididymal white adipose tissue; sWAT, subcutaneous white adipose tissue; BAT, brown adipose tissue; Muscle, skeletal muscle; N.D., not detected. Significant differences from \( \text{ob/ob} \) liver, eWAT, or sWAT: * \( p < 0.001 \).
CEBPRE of the vaspin gene as a positive control (control-CEBPRE) [11] and CEBPRE-3. The binding of C/EBPα to CEBPRE-3 was supershifted by anti-C/EBPα IgG. However, C/EBPα could not directly bind to CEBPRE-4 (Fig. 5A). The binding of C/EBPβ to CEBPRE-3/-4 also showed a similar tendency toward C/EBPα (Fig. 5B). These results suggest that C/EBPα and C/EBPβ can directly bind to CEBPRE-3.

Discussion

The expression of Lipn in the type 2 diabetic model, ob/ob mice, was considerably induced in the liver and WAT compared with that in OB/OB mice although C/EBPα was highly expressed in the liver and WAT of both types of mice. Interestingly, the induction of Lipn was not observed in the kidney, brain, or heart of ob/ob mice, which showed undetectable levels of C/EBPα. These results suggest that the regulation of Lipn expression is associated with C/EBPα, and a type 2 diabetic environment, such as hyperglycemia, hyperinsulinemia, and insulin resistance in tissues. Although the promotion of C/EBPα-dependent transcription in a diabetic environment remains unclear, we previously showed that serine protease inhibitor clade A member 12 (vaspin) is a novel target gene of hepatic C/EBPα and that the expression of vaspin is not induced in the fatty liver of ob/ob mice compared with that in OB/OB mice [11]. Therefore, the promotion of C/EBPα in a diabetic environment is likely Lipn specific.

Tumor necrosis factor α (TNFα) and glucose levels are known to be elevated in type 2 diabetic environments such as in ob/ob mice [13, 14]. It has been demonstrated that TNFα promotes the nuclear translocation of C/EBPβ...
Induction of the promoter activity of *Lipn* by C/EBPα/β depends on CEBPRE-3 and -4. (A) Serially deleted *Lipn* D1–D3 were transfected into HEK293FT cells with or without (a) C/EBPα or (b) C/EBPβ expression plasmid. (B) Internally deleted *Lipn* D3-1, -2, and -3 reporter plasmids were transfected into HEK293FT cells with or without (a) C/EBPα or (b) C/EBPβ expression plasmid. The cells were harvested at 24 h after transfection, and luciferase activity was measured. Each bar represents average ± standard error of the mean of three individual experiments. Significant differences from cells without C/EBP α and C/EBP β expression plasmid: * p < 0.001.
and C/EBPδ in hepatocytes [15]. Another study reported that a high blood glucose level activates the transcriptional activity of C/EBP families through MAPK signals [16]. Therefore, these factors, TNFα and high glucose in type 2 diabetic environment, might also be involved in C/EBPα-dependent upregulation in Lipn expression in the fatty liver and WAT of ob/ob mice. The role of human LIPN has been analyzed mainly in the epidermis [7, 8, 17]. However, the role of protein and transcriptional regulation of this gene in tissues other than the epidermis remains largely unknown [7, 8, 17].

C/EBPβ is a major member of the C/EBP family expressed in the liver and WAT of both OB/OB and ob/ob mice [11, 18]. In the present study, the Lipn promoter activity was significantly promoted by C/EBPβ expression. These results suggest that C/EBPβ in ob/ob mouse livers is involved in Lipn regulation. However, Lipn expression in ob/ob mouse livers was significantly repressed by liver-specific C/EBPα deficiency by approximately 80% compared with that in wild-type for C/EBPα. Therefore, hepatic C/EBPβ appears to be insufficient to compensate for the regulation of Lipn expression by C/EBPα.

The results of the reporter assay demonstrated that hepatic C/EBPα positively regulates Lipn expression in the mouse liver through CEBPRE-3 and -4 in the promoter region of Lipn. However, the EMASA results showed that C/EBPα/β binds to CEBPRE-3 but not to CEBPRE-4. Although the reason for the discrepancy remains unknown, it might be explained by the weak binding between C/EBPα/β and CEBPRE-4. In fact, the luciferase activity of the Lipn D3 construct was repressed by approximately 85% or 91% in the absence of CEBPRE-3, but only by approximately 49% or 48% in the absence of CEBPRE-4. The binding between C/EBPα and CEBPRE-4 should be evaluated in future study.

LIPN is an epidermal-localized member of the acidic lipase superfamily [6, 7]. LIPN has been suggested to be a part of the human keratinocyte differentiation program and one of the causative genes of autosomal-recessive congenital ichthyoses [8]. Although Lipn is expressed in the fatty liver and WAT, its physiological functions in these tissues remain largely unknown. Mice with lysosomal acid lipase knockout, an acidic lipase superfamily member, showed depletion of WAT and BAT, severe hepatosplenomegaly, and a shortened life span [19]. Furthermore, hepatocyte-specific null mice of lysosomal acid lipase also showed hepatosplenomegaly, accumulation of hepatic cholesterol ester, and reduction in adipocyte size in the WAT [20]. These results suggest that lysosomal acid lipase is critical for cellular lipid metabolism, adipocyte differentiation, and fat mobilization. In the present study, Lipn was expressed in fat-accumulated cells, adipocytes in the WAT, and hepatocytes in the fatty liver. Thus, Lipn in these tissues is also likely associated with cellular lipid metabolism.

In summary, we demonstrated that Lipn is considerably induced in the fatty liver or WAT of ob/ob mice with

---

Fig. 5  C/EBPα/β directly binds to CEBPRE-3 in the Lipn 5'-upstream region. EMASA was performed using 32P-labeled oligonucleotide probes, which were incubated with extracts of HEK293FT cells transfected with C/EBPα (A) or C/EBPβ (B) expression plasmid. Supershift assays were performed using anti-C/EBPα IgG or anti-C/EBPβ IgG. The location of C/EBPα or C/EBPβ binding is indicated with the black arrowhead, whereas the supershifted complex is indicated with the open arrowhead.
type 2 diabetes, suggesting that Lipn is involved in the symptoms of type 2 diabetes via an unknown mechanism. Indeed, hepatocyte-specific null mice of lysosomal acid lipase showed improved insulin sensitivity [20]. Hepatic Lipn expression requires C/EBPα and a type 2 diabetic environment. Therefore, hepatic C/EBPα may be associated with the pathogenic mechanism of type 2 diabetes by inducing Lipn. The promotion mechanism of Lipn expression by C/EBPα in a type 2 diabetic environment and physiological function of Lipn induced in the fatty liver and WAT should be further evaluated.

Acknowledgments

This work was supported by a grant from KAKENHI (grant numbers 17K08799 and 21K15424). We would like to thank Editage (www.editage.jp) for English language editing.

Conflicts of Interest

None of the authors have any potential conflicts of interest associated with this research.

References

1. Ramji DP, Foka P (2002) CCAAT/enhancer-binding proteins: structure, function and regulation. Biochem J 365: 561–575.
2. Birkenmeier EH, Gwynn B, Howard S, Jerry J, Gordon JI, et al. (1989) Tissue-specific expression, developmental regulation, and genetic mapping of the gene encoding CCAAT/enhancer binding protein. Genes Dev 3: 1146–1156.
3. Wang ND, Finegold MJ, Bradley A, Ou CN, Abdelsayed SV, et al. (1995) Impaired energy homeostasis in C/EBP alpha knockout mice. Science 269: 1108–1112.
4. Inoue Y, Inoue J, Lambert G, Yim SH, Gonzalez FJ (1996) Disruption of hepatic C/EBPalpha results in impaired glucose tolerance and age-dependent hepatosteatosis. J Biol Chem 279: 44740–44748.
5. Matsusue K, Gavrilova O, Lambert G, Brewer HB Jr, Ward JM, et al. (2004) Hepatic CCAAT/enhancer binding protein alpha mediates induction of lipogenesis and regulation of glucose homeostasis in leptin-deficient mice. Mol Endocrinol 18: 2751–2764.
6. Holmes RS, Cox LA, VandeBerg JL (2010) Comparative studies of mammalian acid lipases: evidence for a new gene family in mouse and rat (Lipo). Comp Biochem Physiol Part D Genomics Proteomics 5: 217-226.
7. Toulza E, Mattiuzzo NR, Galliano MF, Jonca N, Dossat C, et al. (2007) Large-scale identification of human genes implicated in epidermal barrier function. Genome Biol 8: R107.
8. Israeli S, Khamaysi Z, Fuchs-Telem D, Nousbeck J, Bergman R, et al. (2011) A mutation in LIPN, encoding epidermal lipase N, causes a late-onset form of autosomal-recessive congenital ichthyosis. Am J Hum Genet 88: 482–487.
9. Aibara D, Matsusue K, Matsuou K, Takiguchi S, Gonzalez FJ, et al. (2013) Expression of hepatic fat-specific protein 27 depends on the specific etiology of fatty liver. Biol Pharm Bull 36: 1766–1772.
10. Aibara D, Matsuou K, Takiguchi S, Gonzalez FJ, Yamano S (2018) Fat-specific protein 27 is a novel target gene of liver X receptor α. Mol Cell Endocrinol 474: 48–56.