Krüppel-like factor 2 promotes liver steatosis through upregulation of CD36

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Abstract The Krüppel-like factor (KLF) family of transcription factors regulates diverse biological processes that include proliferation, differentiation, apoptosis, development, and responses to external stress. In the present study, we aim to investigate the roles of KLF2 in hepatic steatosis. Our results showed that mRNA and protein levels of KLF2 were significantly elevated in livers from obese mice. Adenovirus-mediated overexpression of KLF2 induced accumulation of triglycerides in C57BL/6 mice, whereas KLF2 silencing ameliorates hepatosteatosis in ob/ob mice. At the molecular level, our data established CD36 as a novel transcriptional target of KLF2. KLF2 upregulated CD36 expression through a consensus binding site on its proximal promoter region. Additionally, the steatotic effect of KLF2 was dramatically inhibited in CD36-null mice.

Therefore, our study reveals a novel link between KLF2-induced hepatic triglyceride accumulation and the expression of CD36. —Chen, J.-L., X.-J. Lu, K.-L. Zou, and K. Ye. Krüppel-like factor 2 promotes liver steatosis through upregulation of CD36. J. Lipid Res. 2014. 55: 32–40.

Supplementary key words Krüppel-like factor 2 • nonalcoholic fatty liver disease • fatty acid uptake

Nonalcoholic fatty liver disease (NAFLD) is characterized by an increase in intrahepatic triglyceride content with or without inflammation and fibrosis (1, 2). NAFLD has become an important public health problem because of its high prevalence, potential progression to severe liver disease, and association with serious cardiometabolic abnormalities, including type 2 diabetes mellitus, the metabolic syndrome, and coronary heart disease (3–5).

Liver plays a central role in FFAs and triglyceride metabolism. Steatosis develops when the rate of FA input (uptake and de novo lipogenesis) is greater than the rate of FA output (β-oxidation and secretion) (6). FFAs released from white adipose tissues enter the systemic circulation and are then transported to the liver by the hepatic artery and portal vein. Upon uptake by hepatocytes, FFAs could be reesterized into triglycerides, especially when intrahepatic FFAs are in excess (7). Indeed, plasma FFA concentrations are usually increased in animals or patients with hepatic steatosis (8). Hepatic uptake of FFAs is mediated by cell surface receptors, including the FA translocase CD36/FAT, which belongs to the class B scavenger receptor family (9, 10). CD36 has been documented to play an important role in hepatic steatosis, and mouse models of high-fat-diet-induced steatohepatitis and genetic models of obesity have demonstrated increased CD36 expression in liver (11). Additionally, an increased level of hepatic CD36 was also found in NAFLD patients (9), indicating that CD36-mediated FFA uptake plays an important role in the initiation or progression of hepatosteatosis.

Krüppel-like factors (KLFs) are a subfamily of the zinc finger class of DNA binding transcriptional regulators (12). Previous studies have reported that KLF2 is required for mouse cardiac development and endothelial functions (13, 14). A recent study also suggests its roles in hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins (15). However, the exact cellular functions of KLF2 in hepatic lipid metabolism remain poorly understood.

In this study, we show that overexpression of KLF2 induces marked hepatic triglyceride accumulation, even when mice were maintained on a standard chow diet. We also showed that CD36 is a novel KLF2 target gene and plays an important role in the steatotic effect of KLF2.

MATERIALS AND METHODS

Animal studies

CD36 knockout mice were obtained from Jackson Laboratories (Bar Harbor, ME). Male C57BL/6 and ob/ob mice aged 8–10 weeks were used. The animal studies were approved by the Institutional Animal Care and Use Committee of Tongji University School of Medicine. Adult male C57BL/6 mice and ob/ob mice were used in all other experiments.

Abbreviations: Ad, adenovirus; HFD, high-fat diet; KLF, Krüppel-like factor; Luc, luciferase; NAFLD, nonalcoholic fatty liver disease; TF, transcription factor.

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weeks were purchased from the Shanghai Laboratory Animal Co. (SLAC; Shanghai, China). High-fat-diet (HFD)-induced obese mice were maintained with free access to a high-fat chow (Research Diets, New Jersey) and water for 10 weeks. All mice were housed at 21 ± 1°C with a humidity of 50 ± 10% and a 12 h light–dark cycle. The animal protocol was approved by the Animal Care Committee of Shanghai East Hospital, Tongji University School of Medicine.

Adenovirus administration

Adenovirus-expressing murine KLF2 or luciferase (Ad-KLF2, Ad-Luc) was constructed by Invitrogen (Shanghai, China) with a full-length KLF2 or luciferase cDNA coding sequence. Overexpression of hepatic KLF2 or luciferase was achieved by means of a single-dose tail vein injection of Ad-KLF2 or luciferase (5 × 10⁸ plaque-forming units) in normal C57BL/6 mice. To knock down KLF2 expression in ob/ob mice, adenoviruses expressing KLF2 or LacZ shRNA (sh-KLF2, sh-LacZ) were generated using pAD BLOCK_IT_DEST vectors (Invitrogen). All viruses were purified by the cesium chloride method and dialyzed in PBS-containing 10% glycerol prior to animal injection. After a 4 h fast, C57BL/6 and ob/ob mice aged 8–10 weeks were injected with adenoviruses through tail vein injection.

Hepatic triglyceride measurement

Crushed liver tissues from mice following a 12 h fast were harvested and homogenized in chloroform-methanol (2:1 v/v) using a polytron tissue grinder. The extracts were dried under N₂ flow and resuspended in isopropanol. Triglyceride concentrations were assayed using commercial kits (Biovision) according to the manufacturer’s instructions.

Western blotting

Equal amounts of protein from hepatic tissues and cells were lysed in radioimmunoprecipitation buffer containing 50 mM Tris-HCl, 150 mM NaCl, 5 mM MgCl₂, 2 mM EDTA, 1 mM NaF, 1% NP40, and 0.1% sodium dodecyl sulfate. After centrifugation at 15,000 g for 20 min, the supernatant was transferred to a new tube and the protein concentration was determined. Equivalent samples were subjected to SDS-PAGE on 12% gels. The gels were then transferred to nitrocellulose membranes and probed with the indicated primary antibodies. The immunoreactive proteins were visualized by incubating in HRP-conjugated secondary antibodies. The signals were detected by the Millipore SuperSignal HRP Substrate Kit according to the manufacturer’s instructions. Antibodies against KFL2 and CD36 were purchased from Abcam, and β-actin antibody was from Santa Cruz.

Fig. 1. Hepatic expression of KLF2 is increased in fatty livers. A,B: Quantitative real-time PCR and Western blot analysis of KLF2 mRNA (A) and protein (B) levels in livers, epididymal white adipose tissue (WAT), and gastrocnemius muscle (SM) of ob/ob mice and wild-type littermates (WT). N = 8 per group. C,D: Quantitative real-time PCR and Western blot analysis of KLF2 mRNA (A) and protein (B) levels in livers, abdominal white adipose tissues (WAT), and gastrocnemius muscle (SM) of mice fed with normal regular diet (RD) or high-fat-diet (HFD). N = 6 per group.
siRNA and quantitative real-time PCR
KLF2 oligo-siRNA was obtained from Dharmacon (Thermo Scientific; Brookfield, WI). Total RNA was isolated from hepatic tissues or cell lysates using the standard TRizol method (Invitrogen) according to the manufacturer’s instructions. Quantitative real-time PCR was performed using Superscript III real-time PCR kits (Invitrogen) on 7000HT Fast System (Applied Biosystems). The expression of the β-actin gene was determined as an internal control. The primers used are listed in the supplementary Table.

Promoter analysis and luciferase assays
The CD36 promoter was amplified from the mouse genomic DNA and inserted into the pGL3 vector (Promega). For the luciferase reporter assays, Hep1-6 cells were seeded in 12-well plates and transfected with KLF2 expression plasmids and reporter vectors, using Lipofectamine 2000 (Invitrogen). Cell lysates were harvested 30 h after transfection. Luciferase activity was measured using the Dual Luciferase Reporter Assay System (Promega).

Chromatin immunoprecipitation assays
Chromatin immunoprecipitation assays were done following the manufacturer’s instructions using the EZ ChIP/MagnaChIP kit from Upstate Biotechnology (Millipore). Briefly, Hep1-6 cells in 10 cm dishes were fixed with 1% formaldehyde. After lysis, genomic DNA was sheared to fragments of 200–1,000 bp using several sonications. Chromatin was incubated and precipitated with antibodies against 3 µg KLF2 antibody (Abcam) or IgG (Abcam) overnight at 4°C.

FA uptake experiments
After 6 h of fasting, mice aged 8–10 weeks were administered via the tail vein 1 mCi of the radio-labeled FA analog BMIPP (15-p-[123I] iodophenyl-3-(R, S)-methylpentadecanoic acid; Molecular Insight Pharmaceuticals). After 2 h, mice were euthanized, tissues were removed, and the level of incorporated 123I was determined directly using a γ counter.

Statistical analysis
Values were expressed as mean ± SEM. Comparisons between groups were performed using the Student’s two-tailed t-test or ANOVA with a Bonferroni post hoc test of pairwise comparisons where appropriate. Statistical significance is shown as *P < 0.05, **P < 0.01, or ***P < 0.001.

RESULTS
Upregulation of hepatic KLF2 in mice with hepatosteatosis
Leptin-deficient ob/ob mice are a well-characterized mouse model of obesity, insulin resistance, and liver HOMEGRAPH

![Graphs showing results](image-url)

Fig. 2. KLF2 overexpression aggravates liver triglyceride accumulation. A: Western blot of liver extracts from representative mice injected with luciferase or KLF2 adenoviruses (Ad-Luc, Ad-KLF2). N = 8 per group. B–J: Serum alanineaminotransferase levels (B), blood glucose (C), body weight (D), total body fat content (E), liver weight (F), triglyceride contents (G), serum triglyceride content (H), liver and serum cholesterol content (I, J), from two groups of mice were determined.
KLF2 overexpression promotes hepatosteatosis in C57BL/6 mice

To address the liver-specific function of KLF2, we overexpressed it in livers of lean wild-type mice by delivering an adenovirus expressing KLF2 or luciferase as negative control via tail vein injection. KLF2 adenovirus dramatically increased its protein abundance in the livers, whereas its expression in white adipose tissue and skeletal muscle remained unaffected (Fig. 2A). In addition, serum alanine aminotransferase levels were not changed in the adenovirus-infected groups, compared with nonadenoviral-infected mice, suggesting that adenovirus delivery caused no obvious signs of liver damage (Fig. 2B). At day 9 after injection, acute overexpression of KLF2 caused no major differences in blood glucose levels, body weight, and total body fat content compared with controls (Fig. 2C–E). In contrast, liver weights and triglyceride contents were significantly elevated (Fig. 2F, G). In parallel, overexpression of hepatic KLF2 resulted in an increase in serum triglyceride levels (Fig. 2H), whereas hepatic and serum cholesterol levels remained unchanged between the two groups (Fig. 2I, J).

KLF2 positively regulates CD36 expression

Liver triglyceride stores are determined by the relative balance of lipid uptake and release, de novo lipogenesis, and FA oxidation (5, 6). To determine the mechanism by which KLF2 promotes steatosis, we profiled the expression of genes in the liver by quantitative real-time PCR. Our results suggested that the expression of CD36 was induced in the liver of mice with KLF2 overexpression (Fig. 3A). The induction of CD36 was also confirmed by Western blotting analyses (Fig. 3B). Consistent with the elevation of CD36 content, hepatic FA uptake was significantly increased in Ad-KLF2-expressing mice compared with Ad-luciferase-expressing mice (Fig. 3C). However, there was no difference in FA uptake in other tissues, including skeletal muscle, white adipose tissue, and heart (Fig. 3C). In addition, genes in triglyceride synthesis

Fig. 3. Control of CD36 expression by KLF2. A: Quantitative real-time PCR and Western blot analysis of CD36 mRNA (A) and protein (B) levels in livers from mice transfected with viruses. N = 8 per group. C: FA uptake in liver, skeletal muscle (SM), white adipose tissue (WAT), and heart was determined in two groups of mice. Mice that were euthanized 2 h postiodofofitic acid injection were quantified for the level of incorporation using a γ counter and shown as μCi/g body weight. N = 6 per group. D: Real-time PCR analysis of genes in triglyceride synthesis (GPAT1, AGPAT1, lipin1, DGAT1) and lipid droplet storage (perlipin 1, perlipin 2, and Cidea) in livers. N = 8 per group. E: Quantitative real-time PCR analysis of genes related to lipid metabolism in livers from two groups of mice. N = 8 per group.
(GPAT1, AGPAT1, Lipin1, DGAT1) and lipid droplet storage (perilipin 1, perilipin 2, and Cidea) were upregulated in liver overexpressing KLF2 (Fig. 3D).

Additionally, the expression of Srebp-1c and its target lipogenic enzymes, Fasn, and Acc1, was unchanged (Fig. 3E). PPARγ, a master regulator of β-oxidation, and its target genes, such as Acox1 and Lcad, were also unaffected (Fig. 3E). Expression of PPARγ was also unchanged by KLF2 overexpression (Fig. 3E). Moreover, we examined other FA transfer proteins and cell surface lipoprotein receptors and found that the expression of Fatp1 and Fatp2 was slightly increased in mice (Fig. 3E), whereas the expression of LDLR, VLDLR, and SR-B was not affected (data not shown).

The mouse CD36 gene promoter is a transcriptional target of KLF2

To further understand this regulation in an independent setting, Hep1-6 cells were transfected with equal amounts of adenoviruses containing KLF2 or luciferase. As shown in Fig. 4A, B, KLF2 overexpression resulted in a significant increase of CD36. A similar induction was also consistently observed in mouse primary hepatocytes (Fig. 4C, D). In contrast, knockdown of KLF2 by two independent siRNA sequences led to a downregulation of CD36 mRNA and protein levels (Fig. 4E, F). Therefore, our data indicate that CD36 could be a molecular target of KLF2.

Next, we examined the activities of reporters driven by a promoter region in the mouse CD36 gene in Hep1-6 cells (Fig. 4G). In accordance with the regulation of the CD36 expression in the liver or cultured hepatocytes, KLF2 activated mouse CD36 promoter activity in a dose-dependent manner (Fig. 4G). Through serial deletion of this promoter, we defined a minimal KLF2-responsive region (95 to 91 bp upstream of the transcriptional start site) that contained a consensus binding site for KLF2 (5′-CACCC-3′) (Fig. 4H). Site-directed mutagenesis experiments further confirmed that this site was required for the induction of promoter activities by KLF2 (Fig. 4I). Indeed, mutation of this site completely abolished the effect of KLF2 on CD36 transcriptional activity (Fig. 4I). In addition, chromatin immunoprecipitation assays also showed that KLF2 is bound to the promoter region of the CD36 gene in Hep1-6 cells (Fig. 4J).

Liver-specific KLF2 deficiency improves hepatosteatosis and hypertriglyceridemia

Next, to address the liver-specific function of KLF2, we disrupted the activity of KLF2 by adenoiral shRNA against a KLF2 coding region (Ad-shKLF2) or nonspecific control shRNA (Ad-shLacZ) in ob/ob mice. KLF2 shRNA treatment significantly reduced hepatic KLF2 mRNA and protein levels as compared with LacZ shRNA-injected littersmates (Fig. 5A, B). As a result of KLF2 deficiency, hepatic triglyceride contents were reduced (Fig. 5C, D). The liver weight was also reduced in ob/ob mice treated with Ad-shKLF2 (Fig. 5E). Consistently, abrogation of hepatic KLF2 resulted in a reduction in serum triglyceride levels (Fig. 5F). Interestingly, the body weight, total body fat content, blood glucose, and cholesterol levels were comparable in the two groups (data not shown). Consistently, knockdown of hepatic KLF2 in ob/ob mice resulted in a decreased expression of CD36 (Fig. 5G, H), but not genes related to lipogenesis or FAA oxidation (data not shown).

CD36 is required for KLF2-mediated hepatosteatosis

Having established CD36 as a KLF2 target gene, we went on to determine whether the expression of CD36 was required for the steatotic effect of KLF2. Therefore, both wild-type and CD36-null mice were equally infected with KLF2 adenoviruses (17, 18) (Fig. 6A). Our data showed that KLF2 overexpression increased triglyceride content in wild-type but not in CD36-null mice (Fig. 6B). In addition, FAA uptake in wild-type mice was significantly increased by KLF2, but was abolished in CD36-null mice (Fig. 6C). Therefore, these results demonstrate that the role of KLF2 in the initiation or progression of liver steatosis is, at least in part, CD36 dependent.

DISCUSSION

In this study, for the first time, we provide insights into tissue-specific functions of KLF2, with further implications for the pathogenesis of obesity-related NAFLD. KLF2 expression was found to be upregulated in both genetic and dietary obese mice. Overexpression of KLF2 in liver cells induced CD36 gene expression and increased FAA uptake, whereas KLF2 inhibition is sufficient to improve liver triglyceride accumulation in ob/ob mice. Moreover, our
Elevated levels of serum FA is tightly associated with hepatic insulin resistance, increased hepatic glucose production, hepatic steatosis, and increased hepatic triglyceride secretion in VLDL. Although exposure of the liver to elevated levels of serum FA may have a number of deleterious effects on liver function, the increased hepatic CD36 protein expression is a regulatory mechanism that exists in the mouse liver to control FA uptake. Indeed, the implication of CD36 in steatosis has been recognized by several

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**Fig. 5.** Liver-specific ablation of KLF2 improves triglyceride accumulation in ob/ob mice. A,B: Quantitative real-time PCR and Western blot analysis of KLF2 mRNA (A) and protein (B) levels in livers from ob/ob mice transfected with shRNA adenoviruses targeting LacZ or KLF2 (shLacZ, shKLF2). N = 7 per group. C,E: Liver triglyceride contents (C), hematoxylin and eosin staining (D), liver weights (E), and serum triglyceride contents (F) from two groups of mice were determined. Scale bar = 100 μM. G,H: Quantitative real-time PCR and Western blot analysis of CD36 mRNA (G) and protein (H) levels in livers from ob/ob mice.

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results indicate that CD36 is a transcriptional target of KLF2 and required for the FA uptake and steatotic effect of KLF2.
An increased expression of CD36 was observed in the livers of high-fat-diet-fed mice and ob/ob mice (11, 19). A forced expression of CD36 in the mouse liver increased FA uptake and triglyceride storage (20). Together, these results suggest a causative role for CD36 in the pathogenesis of hepatic steatosis. Activation of CD36 gene expression has also been proposed to play a role in the steatotic effect of nuclear receptors PPARγ, preg- nane X receptor, liver X receptor, and the aryl hydrocarbon receptor (21–23). Promoter analysis has established CD36 as a transcription target of these transcription factors (TFs). Therefore, it would be interesting to investigate whether KLF2 has a cooperative regulatory effect with these TFs. Moreover, previous reports showed that other members of the KLF family, including KLF3, KLF4, and KLF5, could regulate triglyceride accumulation in white adipocyte tissue (24–26), whereas KLF15 promotes glucose production and metformin action in livers (27). Therefore, whether these KLF proteins could also regulate liver steatosis remains to be explored in the future.

In summary, our study provides an unexpected link between KLF2-induced hepatic steatosis and the expression of CD36. We propose that KLF2 and its target, CD36, may represent novel therapeutic targets to manage NAFLD and related metabolic diseases or liver diseases.

### REFERENCES

1. Marchesini, G., E. Bugianesi, G. Forlani, F. Cerrelli, M. Lenzi, R. Manini, S. Natale, E. Vanni, N. Villanova, N. Melchionda, et al. 2003. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*. **37**: 917–923.

2. Adams, L. A., J. F. Lymp, J. St Sauver, S. O. Sanderson, K. D. Lindor, A. Feldstein, and P. Angulo. 2005. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology*. **129**: 113–121.

3. Petersen, K. F., S. Dufour, J. F. Ying, D. Befroy, J. Dziura, C. Dalla Man, C. Cobelli, and I. Shulman. 2006. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc. Natl. Acad. Sci. USA*. **103**: 18273–18277.

4. Korenblat, K. M., E. Fabbrini, B. S. Mohammed, and S. Klein. 2008. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. *Gastroenterology*. **134**: 1369–1375.

5. Cohen, J. C., J. D. Horton, and H. H. Hobbs. 2011. Human fatty liver disease: old questions and new insights. *Science*. **332**: 1519–1523.

6. Fabbrini, E., S. Sullivan, and S. Klein. 2010. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology*. **51**: 679–689.

7. Nielsen, S. Z., Guo, C. M., Johnson, D. D., Hensrud, and M. D. Jensen. 2004. Splanchnic lipolysis in human obesity. *J. Clin. Invest*. **113**: 1582–1588.

8. Mittendorfer, B., F. Magkos, E. Fabbrini, B. S. Mohammed, and S. Klein. 2009. Relationship between body fat mass and free fatty acids kinetics in men and women. *Obesity (Silver Spring)*. **17**: 1872–1877.

9. Greco, D., A. Kotronen, J. Westerbacka, O. Puig, P. Arkkila, T. Kivistö, S. Laitinen, M. Kolak, R. M. Fisher, A. Hamsten, et al. 2008. Gene expression in human NAFLD. *Am. J. Physiol. Gastrointest. Liver Physiol.* **294**: G1281–G1287.

10. Fabbrini, E., F. Magkos, B. S. Mohammed, T. Pietka, N. A. Abumrad, B. W. Patterson, A. Okunade, and S. Klein. 2009. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc. Natl. Acad. Sci. USA*. **106**: 15430–15435.

11. Inoue, M., T. Ohtake, W. Motomura, N. Takahashi, Y. Kosaki, S. Miyoshi, Y. Suzuki, H. Saito, Y. Kohgo, and T. Okumura. 2005. Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. *Biochem. Biophys. Res. Commun.* **336**: 215–222.

12. McConnell, B. B., and V. W. Yang. 2010. Mammalian Kruppel-like factors in health and diseases. *Physiol. Rev.* **90**: 1337–1381.

13. Chipplunkar, A. R., T. K. Lung, Y. Alhashem, B. A. Koppenhaver, F. N. Saltouni, R. C. Kukreja, J. L. Haar, and J. A. Lloyd. 2013.
Kruppel-like factor 2 is required for normal mouse cardiac development. *PLoS ONE* 8: e54891.

14. Lin, Z., V. Natesan, H. Shi, F. Dong, D. Kawanami, G. H. Mahabaleshwar, G. B. Atkins, L. Nayak, Y. Cui, J. H. Finigan, et al. 2010. Kruppel-like factor 2 regulates endothelial barrier function. *Atheroscler. Thromb. Vasc. Biol.* 30: 1952–1959.

15. Marrone, G., L. Russo, E. Rosado, D. Hide, G. Garcia-Cardenas, J. C. Garcia-Pagan, J. Bosch, and J. Gracia-Sancho. 2013. The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellar cell deactivation induced by statins. *J. Hepatol.* 58: 98–103.

16. Tsuji, H., Y. Ikeda, Y. Ebata, C. Kojima, R. Katsuma, T. Tsuneyama, T. Sakabe, K. Shomori, N. Komeda, S. Oshiro, et al. 2012. Retinoids ameliorate insulin resistance in a leptin-dependent manner in mice. *Hepatol.* 56: 1319–1330.

17. Beigle, V., K. Wouters, P. J. van Gorp, M. JGijbs, M. P. de Winther, C. J. Binder, D. Lutjohann, M. Febbraio, K. J. Moore, M. van Bilsen, et al. 2010. Role of scavenger receptor A and CD36 in diet-induced nonalcoholic steatohepatitis in hyperlipidemic mice. *Gastroenterology.* 138: 2477–2486.

18. Beigle, V., P. J. van Gorp, S. M. Walenbergh, M. J. Gijbs, F. Verheyen, W. A. Buurman, D. E. Briles, M. H. Hofker, C. J. Binder, and R. Shiri-Sverdlov. 2012. Specific immunization strategies against oxidized low-density lipoprotein: a novel way to reduce nonalcoholic steatohepatitis in mice. *Hepatol.* 56: 894–903.

19. Degrace, P., B. Moindrot, I. Mohamed, J. Gresti, Z. Y. Du, J. M. Chardigny, J. L. Sebedio, and P. Clouet. 2006. Upregulation of liver VLDL receptor and FAT/CD36 expression in LDLR−/−apoB100/100 mice fed trans-10,cis-12 conjugated linoleic acid. *J. Lipid Res.* 47: 2647–2655.

20. Koonen, D. P., R. L. Jacobs, M. Febbraio, M. E. Young, C. L. Soltys, H. Ong, D. E. Vance, and J. R. Dyck. 2007. Increased hepatic CD36 expression contributes to dyslipidemia associated with diet-induced obesity. *Diabetes.* 56: 2863–2871.

21. Zhou, J., Y. Zhai, Y. Mu, H. Gong, H. Uppal, D. Toma, S. Ren, R. M. Evans, and W. Xie. 2006. A novel pregnane X receptor-mediated and sterol regulatory element-binding protein-independent lipogenic pathway. *J. Biol. Chem.* 281: 15013–15020.

22. Zhou, J., M. Febbraio, T. Wada, Y. Zhai, R. Kuruba, J. He, J. H. Lee, S. Khadem, S. Ren, S. Li, et al. 2008. Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPARgamma in promoting steatosis. *Gastroenterology.* 134: 556–567.

23. Lee, J. H., T. Wada, M. Febbraio, J. He, T. Matsubara, M. J. Lee, F. J. Gonzalez, and W. Xie. 2010. A novel role for the dioxin receptor in fatty acid metabolism and hepatic steatosis. *Gastroenterology.* 139: 653–663.

24. Sue, N., B. H. Jack, S. A. Eaton, R. C. Pearson, A. P. Funnell, J. Turner, R. Czolij, G. Denyer, S. Bao, J. C. Molero-Navajas, et al. 2008. Targeted disruption of the basic Kruppel-like factor gene (Klf3) reveals a role in adipogenesis. *Mol. Cell. Biol.* 28: 3967–3978.

25. Birsoy, K., Z. Chen, and J. Friedman. 2008. Transcriptional regulation of adipogenesis by KLF4. *Cell Metab.* 7: 339–347.

26. Oishi, Y., I. Manabe, K. Tobe, K. Tsushima, T. Shindo, K. Fujiu, G. Nishimura, K. Maemura, T. Yamauchi, N. Kubota, et al. 2005. Kruppel-like transcription factor KLF5 is a key regulator of adipocyte differentiation. *Cell Metab.* 1: 27–39.

27. Takashima, M., W. Ogawa, K. Hayashi, H. Inoue, S. Kinoshita, Y. Okamoto, H. Sakaue, S. Wataoka, A. Emi, Y. Senga, et al. 2010. Role of KLF15 in regulation of hepatic gluconeogenesis and metformin action. *Diabetes.* 59: 1608–1615.