Genes & Diseases (2014) 1, 106–112

HOSTED BY

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

REVIEW ARTICLE

The molecular pathogenic role of inflammatory stress in dysregulation of lipid homeostasis and hepatic steatosis

Yaxi Chen [a], Zac Varghese [b], Xiong Z. Ruan [a,b,*]

[a] *Centre for Lipid Research, Key Laboratory of Molecular Biology on Infectious Diseases, Ministry of Education, Chongqing Medical University, Chongqing, China*
[b] *John Moorhead Research Laboratory, Centre for Nephrology, University College London Medical School, Royal Free Campus, London, UK*

Received 10 July 2014; accepted 20 July 2014
Available online 27 July 2014

KEYWORDS
Ectopic lipid deposition;
Inflammation;
Lipid homeostasis;
Lipid redistribution;
Non-alcoholic fatty liver disease

Abstract Non-alcoholic Fatty Liver Disease (NAFLD) is becoming the leading cause of chronic liver injury in developed countries and China. Chronic systemic inflammation plays a decisive role and is fundamental in the progression of NAFLD from simple steatosis (SS) toward higher risk nonalcoholic steatohepatitis (NASH) states. However, the exact mechanisms by which inflammation leading to NASH are incompletely understood. In this review, we focus the role of the cross talk between inflammation and lipid homeostasis on the progression of NAFLD.

Introduction

Clinical studies revealed dramatically high prevalence of Non-alcoholic Fatty Liver Disease (NAFLD) worldwide and NAFLD has become the leading cause of chronic liver injury in developed countries. The prevalence of NAFLD among adults in the general population is approximately 15% (6.3–27.0%) in China.[1] NAFLD is strongly associated with obesity, insulin resistance, hypertension, and dyslipidemia, suggesting that NAFLD might be considered as the liver manifestation of the metabolic syndrome.

NAFLD, characterized by increased lipids accumulation (mainly triglycerides and cholesterol) in the liver represents a spectrum of disease ranging from "simple steatosis" (SS), which is considered relatively benign, to nonalcoholic steatohepatitis (NASH) and to NAFLD-associated cirrhosis and end-stage liver disease. SS is believed to be the basis for the development of NASH. However, only 10–20% of patients with SS develop to NASH, which can progress to

* Corresponding author. Chongqing Medical University, Chongqing, China and John Moorhead Research Laboratory, Centre for Nephrology, University College London Medical School, Royal Free Campus, London, UK. Tel.: +44 (0)20 7830 2190; fax: +44 (0)20 7830 2125.
E-mail addresses: x.ruan@ucl.ac.uk, xiongzruan@gmail.com (X.Z.Ruan).
Peer review under responsibility of Chongqing Medical University.

http://dx.doi.org/10.1016/j.gendis.2014.07.007

cirrhosis, end-stage liver disease, and hepatocellular carcinoma.[2] Why not all NAFLD patients progressed to NASH is largely unknown.

The "two-hit hypothesis" has become an important theoretical framework for understanding NAFLD pathogenesis.[3] The first hit mainly consists of lipid accumulation in the liver in the form of triglycerides and free fatty acids accompanied by small amounts of cholesterol, cholesterol ester, and phospholipid, a process that is closely linked with SS and insulin resistance.[4] The second hit involves an inflammatory insult to the liver. The cytokines or chemokines released by hepatocytes or activated neutrophils further exacerbate hepatic tissue injuries induced by the first hit and cause NASH.[5] However, this theory has been put into question based on insights on the interaction between lipid homeostasis, insulin resistance, and inflammatory stress. It is now appreciated that a more complex process including lipotoxicity, insulin resistance, oxidative stress and inflammatory cascade is probably responsible for liver injury and promote NAFLD progression. The "multiple-parallel-hit" hypothesis has arisen.[6]

Chronic systemic inflammation plays a decisive role and is fundamental in the progression of NAFLD from SS toward higher risk cirrhotic states.[7] However, the exact mechanisms by which inflammation leading to NASH are incompletely understood. In this review, we focus the role of the cross talk between inflammation and lipid homeostasis on the progression of NAFLD.

The role of inflammation in NAFLD

Hepatic steatosis is the common feature of NAFLD from early stage to advanced NASH-cirrhosis. The pathogenesis of NASH was originally conceptualised as a disease of consecutive hits: the accumulation of fat in the liver cells (steatosis), cascade of inflammation, fibrosis and cirrhosis. The lipotoxicity of hepatic fat induces inflammatory stress and oxidative stress.[8] Many studies have found that inflammatory stress is involved in the occurrence and development of NAFLD process, not only at the process of the second hit, suggesting that both inflammation and lipid are important risk factors for hepatic steatosis. NAFLD has been regarded as an inflammatory disorder, which is characterized by low-grade chronic systemic inflammation,[9] underscoring the similarities between NAFLD and the metabolic syndrome.[10,11] Circulating concentrations of C-reactive protein (CRP) and pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are considered to be the most important factors in causing and maintaining insulin resistance[12] and closely related to the development of NAFLD and other metabolic disorders.[13] TNF-α and IL-6 expression in liver is strongly induced in response to high fat diet and inhibition of TNF and IL-6 signaling prevents hepatic steatosis without a considerable effect on weight gain. Elevated serum TNF-α level is associated with the development of NAFLD and an independent factor for NASH in a clinical human study.[14] Recently, mice treated with the anti-TNF-α drug thalidomide show some improvements in the hepatic alterations mediated by a high-fat diet.[15] In a rat experimental model of NASH, anti-TNF-α antibodies decrease inflammation, necrosis, and fibrosis.[16] Moreover, several studies have shown that inflammatory cytokines increase serum free fatty acids (FFA) levels, causing dyslipidemia and NAFLD, which can be prevented by using anti-cytokine antibodies in mouse models.[17,18]

IL-6 is a key element in the acute phase response, mediating the synthesis of several acute phase proteins (such as CRP and serum amyloid A).[19] It also plays an indirect deleterious role in NAFLD pathogenesis. Serum IL-6 levels are higher in animal models and patients with NAFLD.[9,20,21] Diet-induced NASH is reduced in IL-6 knockout mice.[22] In humans with NASH, a positive correlation between IL-6 expression in hepatocytes and the severity of NAFLD was observed.[23]

Although many cytokines are shown to modulate the progression of NAFLD, the central mechanism that mediates the effects of these cytokines on the progression of NAFLD, are not fully clear. Nonetheless, several specific intracellular signaling pathways, including nuclear factor NF-κB, Jun kinases (JNK), activating protein-1 (AP-1), STAT3, AMPK-TORC1 pathway have emerged as potential targets for many of these cytokines and chemokines.[24]

Among the inducible transcription factors that control inflammatory gene expression, NF-κB plays a central and evolutionarily conserved role in coordinating the expression of various soluble pro-inflammatory mediators and leukocyte adhesion molecules. NF-κB is a collection of protein dimers that control the transcription of a host of target genes. NF-κB dimers are mainly kept inactive through binding to inhibitory proteins IκB. The IκB kinase (IKK) complex, which is responsive to many inflammatory stimuli, phosphorylates the IκBs, thereby triggering their degradation, and causing NF-κB activation. Recent data lend credence to the fact that hepatic steatosis activates IKK and NF-κB.[20] The degradation of IκB thus allows NF-κB to translocate into the nucleus where it can act as a transcription factor that upregulates IL-6 production and secretion. It has been demonstrated that high fat diet increases NF-κB activation, resulting in a sustained elevation of the IKK-related kinase IKKε in liver. IKKε ablation reduces expression of inflammatory cytokines and protects mice from high-fat diet-induced obesity, chronic inflammation in liver and adipose tissue, and hepatic steatosis.[25] In addition, JNK activation increases production of inflammatory cytokines capable.[26] The two main isoforms of JNK (JNK1 and JNK2) appear to have distinct specific effects on murine NASH. JNK1 promotes steatosis and hepatitis while JNK2 inhibits hepatocyte cell death.[27] These results suggest that chronic low-grade systemic inflammation play a key role on the progression from hepatic steatosis to NASH, fibrosis, cirrhosis.[24]

Effects of inflammation on FFA uptake, synthase and oxidation in the liver

NAFLD is a consequence of excessive lipids accumulation in liver. Hepatic triglycerides (TG) and FFA accumulation results from an imbalance between lipid availability from circulating lipid uptake or de novo lipogenesis (DNL) and lipid disposal via FFA β-oxidation or TG-rich lipoprotein secretion. Excessive intrahepatic TG or steatosis generally occurs when there is more fatty acid synthesis and less fatty acid oxidation. This

eventually triggers lipoperoxidative stress and hepatic injury. Studies on obese adults with hepatic steatosis have described an increased hepatic de novo lipogenesis accompanied with a decreased fatty acid oxidation.[28–30] In normal subjects, de novo lipogenesis contributes to less than 5% of fatty acid incorporated into secreted VLDL-TG. However, the rate of de novo fatty acid synthesis is greatly increased and accounts for approximately 20% of the fatty acid in intrahepatic TG in subjects with NAFLD.[31,32] Moreover, inhibition or activation of intrahepatic fatty acid oxidation can influence intrahepatic TG content. Genetic or experimentally-induced deficiencies in mitochondrial oxidative enzymes lead to hepatic steatosis,[33–35] whereas increasing the expression or activity of hepatic enzymes involved in fatty acid oxidation, reduces intrahepatic TG accumulation in rodent animal models.[36,37] This suggests that the balance between fatty acid production and consumption plays an important role in maintaining normal intrahepatic TG levels.

Inflammation may modify hepatic lipid homeostasis, thereby enhancing lipid accumulation in the liver. There are several key transcription factors and enzymes regulating intrahepatic fatty acids synthesis and oxidation. Sterol regulatory element-binding protein-1,2 (SREBP-1,2) are nuclear transcription factors that transcriptionally activate genes involved in de novo lipogenesis.[38,39] The acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) are important downstream target genes of SREBP-1, which are the rate-limiting enzymes of palmitate molecule formation. Moreover, carnitine palmitoyltransferase 1A (CPT1A), hydroxyacyl-CoA dehydrogenase beta subunit (HADHB) and carnitine O-octanoyltransferase (CROT) are key enzymes of intrahepatocellular fatty acid oxidation, which mediate the transport of long-chain fatty acids inside the mitochondrial matrix, catalyze mitochondrial β-oxidation to liberate carbon units and catabolize very long-chain fatty acids (>20 carbons), respectively.[36,40,41]

Subcutaneous casein injection is an established method for induction of chronic systemic inflammation in mouse models. This has been used in many studies of atherosclerosis and hepatic steatosis.[42–44] In comparison with other sole cytokine or lipopolysaccharide (LPS) induced mouse models, casein-injection induces a lower degree inflammatory stress characterized by increased multiple cytokines (IL-1β, TNF-α and IL-6 etc.) and amyloid A (SAA, like CRP in human) levels in serum, which is more likely to mimic chronic systemic inflammatory state observed in patients with inflammatory stress.[45] We demonstrate that inflammation induced by casein injection promotes hepatic fatty acids synthesis mediated by SREBP1-ACCα/FAS and disturbs fatty acids β-oxidation mediated by CPT1A, CROT and HADHB in hepatic cells and livers of C57BL/6J mice. This imbalance of fatty acids metabolism under inflammatory stress exacerbates hepatic lipid accumulation and induces endoplasmic reticulum (ER) stress and oxidative stress, promoting progression of NAFLD.

Effects of inflammation on cholesterol accumulation in the liver

Dysregulation of both TG and FFA in NAFLD has been elucidated in many studies, while the role of cholesterol in NAFLD used to be neglected. Recent studies have indicated that liver cholesterol accumulation is more toxic than TG and may be a trigger for progression of NAFLD. Studies have demonstrated that free cholesterol levels are increased in the livers of NASH patients.[46] It has also been shown in experimental models that free cholesterol loading, but not TG or FFA loading, precipitates steatohepatitis.[47] We have demonstrated that chronic systemic inflammation induced by casein in mice enhances cholesterol accumulation by increasing cholesterol uptake via LDL receptor and cholesterol de novo synthesis via HMGCoA-reductase in livers.[42,48] In addition, inflammatory stress disrupts PPAR-LXR-CYP7A1/ABCA1-mediated bile acid synthesis and cholesterol efflux resulting in exacerbated cholesterol accumulation in livers.[49] Excess cholesterol accumulation in cells can trigger ER stress, oxidative stress and apoptosis, which aggravate NAFLD.[50–52]

Inflammation and ectopic lipid accumulation in the liver

NAFLD is associated with the increase in both obesity and type 2 diabetes (T2DM). It has been demonstrated that weight gain is an independent predictor of the development of NAFLD, however there are still relatively high proportion (~40%) of Chinese NAFLD patients with normal BMI under the ethnic-specific overweight and obesity criteria.[53,54]

Ectopic lipid deposition is defined as excess lipids deposition in nonadipose tissues. In physiological conditions, FFA delivered to adipose tissue is converted to TG for storage during states of nutrient abundance. Therefore white adipose tissue has a unique capacity to store large amounts of excess lipid, while nonadipose tissues in which FFA is utilized have a limited capacity for storage of lipids.[55,56] Adipose lipids also can be released into circulation to form albumin/FFA complexes, which allow FFA transport into nonadipose tissues, such as muscle, heart, kidney and liver for beta-oxidation on demand. Insulin regulates this process to maintain FFA homeostasis in balance. In pathophysiological state, ectopic lipid accumulation in liver is the hepatic manifestation of the metabolic syndrome, which is known to be associated with insulin resistance and hyperlipidemia.[57] Increasing lipogenesis in nonadipose tissues plays a crucial role in ectopic lipid deposition.[18] In mammalian species, lipogenesis of cells is under control of SREBP-1,2. SREBP-1 is selectively involved in activation of genes in FFA metabolism by regulating the expression of genes encoding rate-limiting enzymes responsible for de novo lipogenesis, of which FAS and ACC seem to be particularly important since the expression of these genes is associated with the changes of FFA synthesis rates. We have demonstrated that chronic systemic inflammation up-regulates mRNA and protein expression of SREBP1, ACCα and FAS in liver and muscle, while decreasing the expression of these genes in adipose tissue.

FFA homeostasis of adipocytes is not only dependent on lipogenesis but also lipolysis in adipose tissues. Numerous studies have demonstrated that cytokines increase adipose lipolysis, but the underlying mechanism is unclear. Both adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) can initiate triglyceride (TG) degradation by cleaving the first ester bond. Importantly, ATGL represents

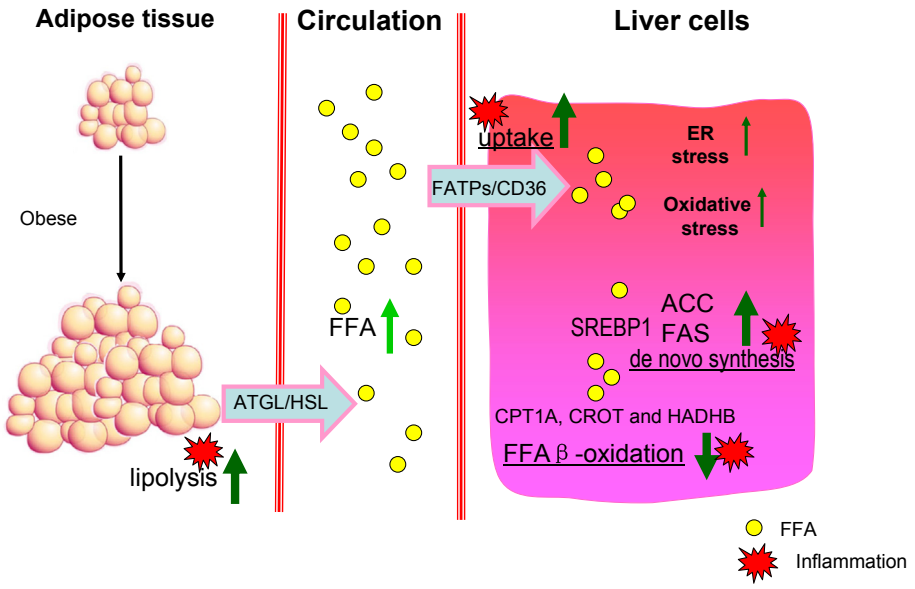

Figure 1 The role of inflammatory stress on hepatic lipid homeostasis. Inflammatory stress increases ATGL/HSL mediated lipolysis in white adipose tissue. FFAs are released into the circulation to form albumin/FA complexes, which are transported into hepatocytes by either passive transport or fatty acid transporting proteins (FATPs) including CD36. Inflammation up-regulates de novo FFA synthesis expression and reduces FFA β-oxidation in liver.

the rate limiting lipolytic enzyme in mammals, and HSL, increased by insulin resistance is unique in its capacity to break down the second ester bond, converting diglycerides (DG) to monoglycerides in adipose tissue. We have shown that inflammation increases both mRNA and protein expression of ATGL and HSL in white adipose tissue, suggesting that inflammation disrupts lipid metabolism by increasing lipolysis in addition to an impaired lipogenesis in adipose tissue. In addition, several studies have suggested that cytokines may affect the stability of lipid droplets in adipocytes by influencing the proteins that stabilize the lipid droplet. TNF-α decreases lipid droplet protein perilipin expression, presumably enhancing the ability of lipases to access TG within the lipid droplets.[59,60] TNF-α also decreases expression of a newly described lipid droplet protein fat-specific protein 27 (FSP27).[61] These data suggest that inflammation tissue-specifically 'switches on' lipogenesis in nonadipose tissues, especially in liver while 'switches off' lipogenesis and enhances lipolysis in adipose tissue,[58] promoting ectopic lipid accumulation in liver.

Inflammation and lipid redistribution in liver

Donnelly recently demonstrated that about 59% of liver fatty acids in NAFLD patients is derived from the circulation, 26% from de novo liver fatty acids synthesis and only 15% from the diet,[31] suggesting that liver fatty acid comes mainly from peripheral adipose tissue and abnormal fatty acid transport to liver may be play an important role in the development of NAFLD.

In insulin resistance, insulin does not suppress TG hydrolysis in adipose tissue; FFA are released into the circulation to form albumin/FA complexes, which are transported into hepatocytes by either passive transport or fatty acid transporting proteins (FATPs) including CD36 (also called 'fatty acid translocase), plasma membrane fatty acid binding protein and caveolin. Genetic rodent models that alter expression of lipid transport proteins provide evidence for the role of ectopic lipid accumulation in the pathogenesis of insulin resistance.[62] Lipoprotein lipase (LpL) is a key enzyme that hydrolyzes circulating triglyceride, permitting tissue FFA uptake through FATPs. Hepatic specific overexpression of LpL leads specifically to hepatic steatosis and hepatic insulin resistance.[63,64] Loss of hepatic fatty acid transport, specifically through deletion of FATP2[65] or FATP5,[66] protects against the development of hepatic steatosis.

CD36, a type B scavenger receptor plays an important role in hepatic FFA transport. CD36 is a transmembrane glycoprotein, which is expressed in various cells associated with energy metabolism, including adipocytes, pancreatic beta cells, skeletal myocytes and hepatocytes. This multifunctional receptor has been studied extensively with regard to its role in facilitating the uptake of FFA and oxidised low-density lipoproteins, which are involved in the aetiology of such metabolic disorders as diabetes,[67] atherosclerosis[68] and NAFLD.[69] Overexpression of CD36 aggravates FFA and TG storage in human hepatoma cells and the livers of C57BL/6J mice.[70,71] In patients with NAFLD, CD36 up-regulation is significantly associated with liver fat accumulation.[72] These findings suggest that hepatic CD36 expression is closely related to hepatic steatosis. Both in vitro and in vivo, we have demonstrated that inflammatory stress enhances hepatic CD36 expression and increases CD36 mediated- uptake of FFA, suggesting that increased FFA uptake via CD36 is the one of mechanisms for lipid redistribution to liver in addition to the enhanced hepatic lipogenesis under inflammatory stress.

Conclusions

NAFLD is becoming a serious public health problem in the world due to its effects on many metabolic disorders. It

seems that inflammation and lipid disorder are two leading causes for the progression of NAFLD. Inflammatory stress initiates and promotes hepatic steatosis by modifying FFA homeostasis including increasing hepatic FFA uptake, synthase and reducing oxidation. Inflammation increases ectopic lipid deposition and lipid redistribution from adipose tissue to liver (Fig. 1). Excess hepatic cholesterol accumulation under inflammatory stress can trigger ER stress, oxidative stress and apoptosis, which may lead to NASH-associated cirrhosis and end-stage liver disease. However, there are many remaining questions, including the deep mechanisms that control the progression from SS to NASH that need to be answered. Since most of hepatic lipids in NAFLD patients are derived from peripheral adipose tissue and abnormal redistribution, more studies on lipid homeostasis which links to organ crosstalk should be investigated. Blocking abnormal lipid redistribution could be a new therapeutic target for NAFLD prevention and treatment.

Conflict of interest statement

All the authors declared no competing interests.

Acknowledgement

This study was supported by Major State Basic Research Development Program of China (973 Program, NO. 2012CB517700 & 2012CB517500), the National Natural Science Foundation of China (81270493, 81270789, 81200567 and Key Program, No. 81030008, 81390354), the Moorhead Trust, the Royal Free Hospital Special Trustees Grant-115 through Dr. Zac Varghese, and Kidney Research UK (RP37/2008).

References

1. Fan JG. Epidemiology of alcoholic and nonalcoholic fatty liver disease in China. Journal of gastroenterology and hepatology. Aug 2013;28(suppl 1):11—17.
2. Schattenberg JM, Schuppan D. Nonalcoholic steatohepatitis: the therapeutic challenge of a global epidemic. *Curr Opin Lipidol*. Dec 2011;22(6):479—488.
3. Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology*. Apr 1998;114(4):842—845.
4. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology*. Apr 2001;120(5):1183—1192.
5. Lalor PF, Faint J, Aarbodem Y, Hubscher SG, Adams DH. The role of cytokines and chemokines in the development of steatohepatitis. *Sem Liver Dis*. May 2007;27(2):173—193.
6. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology (Baltimore, Md.)*. Nov 2010;52(5):1836—1846.
7. Peverill W, Powell LW, Skoien R. Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. *Int J Mol Sci*. 2014;15(5):8591—8638.
8. Takaki A, Kawai D, Yamamoto K. Molecular mechanisms and new treatment strategies for non-alcoholic steatohepatitis (NASH). *Int J Mol Sci*. 2014;15(5):7352—7379.
9. Haukeland JW, Damas JK, Konopski Z, et al. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. *J Hepatol*. Jun 2006;44(6):1167—1174.
10. Ford ES. The metabolic syndrome and C-reactive protein, fibrinogen, and leukocyte count: findings from the Third National Health and Nutrition Examination Survey. *Atherosclerosis*. Jun 2003;168(2):351—358.
11. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology (Baltimore, Md.)*. Apr 2003;37(4):917—923.
12. Tarantino G, Savastano S, Colao A. Hepatic steatosis, low-grade chronic inflammation and hormone/growth factor/-adipokine imbalance. *World J Gastroenterol*. Oct 14 2010;16(38):4773—4783.
13. Greenfield JR, Campbell LV. Relationship between inflammation, insulin resistance and type 2 diabetes: 'cause or effect'? *Curr Diabetes Rev*. May 2006;2(2):195—211.
14. Wong VW, Hui AY, Tsang SW, et al. Metabolic and adipokine profile of Chinese patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. Sep 2006;4(9):1154—1161.
15. Pinto Lde F, Compri CM, Fornari JV, et al. The immunosuppressant drug, thalidomide, improves hepatic alterations induced by a high-fat diet in mice. *Liver Int*. Apr 2010;30(4):603—610.
16. Koca SS, Bahcecioglu IH, Poyrazoglu OK, Ozercan IH, Sahin K, Ustundag B. The treatment with antibody of TNF-alpha reduces the inflammation, necrosis and fibrosis in the non-alcoholic steatohepatitis induced by methionine- and choline-deficient diet. *Inflammation*. Apr 2008;31(2):91—98.
17. Li Z, Yang S, Lin H, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology (Baltimore, Md.)*. Feb 2003;37(2):343—350.
18. Marra F, Bertolani C. Adipokines in liver diseases. *Hepatology (Baltimore, Md.)*. Sep 2009;50(3):957—969.
19. El-Assal O, Hong F, Kim WH, Radaeva S, Gao B. IL-6-deficient mice are susceptible to ethanol-induced hepatic steatosis: IL-6 protects against ethanol-induced oxidative stress and mitochondrial permeability transition in the liver. *Cell Mol Immunol*. Jun 2004;1(3):205—211.
20. Cai D, Yuan M, Frantz DF, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med*. Feb 2005;11(2):183—190.
21. Kugelmas M, Hill DB, Vivian B, Marsano L, McClain CJ. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. *Hepatology (Baltimore, Md.)*. Aug 2003;38(2):413—419.
22. Mas E, Danjoux M, Garcia V, Carpentier S, Segui B, Levade T. IL-6 deficiency attenuates murine diet-induced non-alcoholic steatohepatitis. *PloS One*. 2009;4(11):e7929.
23. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol*. Jun 2008;103(6):1372—1379.
24. Sun B, Karin M. Obesity, inflammation, and liver cancer. *J Hepatol*. Mar 2012;56(3):704—713.
25. Chiang SH, Bazuine M, Lumeng CN, et al. The protein kinase IKKepsilon regulates energy balance in obese mice. *Cell*. Sep 4 2009;138(5):961—975.
26. Solinas G, Vilcu C, Neels JG, et al. JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell Metab*. Nov 2007;6(5):386—397.
27. Singh R, Wang Y, Xiang Y, Tanaka KE, Gaarde WA, Czaja MJ. Differential effects of JNK1 and JNK2 inhibition on murine steatohepatitis and insulin resistance. *Hepatology (Baltimore, Md.)*. Jan 2009;49(1):87—96.

28. Nakamuta M, Kohjima M, Morizono S, et al. Evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med*. Oct 2005;16(4):631—635.
29. Kohjima M, Enjoji M, Higuchi N, et al. Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. *Int J Mol Med*. Sep 2007;20(3):351—358.
30. Moylan CA, Pang H, Dellinger A, et al. Hepatic gene expression profiles differentiate presymptomatic patients with mild versus severe nonalcoholic fatty liver disease. *Hepatology (Baltimore, Md.)*. Feb 2014;59(2):471—482.
31. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest*. May 2005;115(5):1343—1351.
32. Diraison F, Moulin P, Beylot M. Contribution of hepatic de novo lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metab*. Nov 2003;29(5):478—485.
33. Rector RS, Thyfault JP, Uptergrove GM, et al. Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model. *J Hepatol*. May 2010; 52(5):727—736.
34. Zhang D, Christianson J, Liu ZX, et al. Resistance to high-fat diet-induced obesity and insulin resistance in mice with very long-chain acyl-CoA dehydrogenase deficiency. *Cell Metab*. May 5 2010;11(5):402—411.
35. Zhang D, Liu ZX, Choi CS, et al. Mitochondrial dysfunction due to long-chain Acyl-CoA dehydrogenase deficiency causes hepatic steatosis and hepatic insulin resistance. *Proc Natl Acad Sci USA*. Oct 23 2007;104(43):17075—17080.
36. Stefanovic-Racic M, Perdomo G, Mantell BS, Sipula IJ, Brown NF, O'Doherty RM. A moderate increase in carnitine palmitoyltransferase 1a activity is sufficient to substantially reduce hepatic triglyceride levels. *Am J Physiol*. May 2008; 294(5):E969—E977.
37. Savage DB, Choi CS, Samuel VT, et al. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *J Clin Investig*. Mar 2006;116(3):817—824.
38. Frederico MJ, Vitto MF, Cesconetto PA, et al. Short-term inhibition of SREBP-1c expression reverses diet-induced nonalcoholic fatty liver disease in mice. *Scand J Gastroenterol*. Nov 2011;46(11):1381—1388.
39. Knebel B, Haas J, Hartwig S, et al. Liver-specific expression of transcriptionally active SREBP-1c is associated with fatty liver and increased visceral fat mass. *PloS One*. 2012;7(2):e31812.
40. Kao HJ, Cheng CF, Chen YH, et al. ENU mutagenesis identifies mice with cardiac fibrosis and hepatic steatosis caused by a mutation in the mitochondrial trifunctional protein beta-subunit. *Human Mole Genet*. Dec 15 2006;15(24):3569—3577.
41. Le Borgne F, Ben Mohamed A, Logerot M, Garnier E, Demarquoy J. Changes in carnitine octanoyltransferase activity induce alteration in fatty acid metabolism. *Biochem Biophysical Res Commun*. Jun 17 2011;409(4):699—704.
42. Ma KL, Ruan XZ, Powis SH, Chen Y, Moorhead JF, Varghese Z. Inflammatory stress exacerbates lipid accumulation in hepatic cells and fatty livers of apolipoprotein E knockout mice. *Diabetes Metab*. Sep 2008;48(3):770—781.
43. Wal JM. Cow's milk proteins/allergens. Annals of allergy, asthma & immunology : official publication of the American College of Allergy. *Asthma Immunol*. Dec 2002;89(6 suppl 1): 3—10.
44. Miura K, Takahashi Y, Shirasawa H. Immunohistochemical detection of serum amyloid A protein in the liver and the kidney after casein injection. *Lab Invest*. Oct 1985;53(4): 453—463.
45. Zhang H, Ching S, Chen Q, Li Q, An Y, Quan N. Localized inflammation in peripheral tissue signals the CNS for sickness response in the absence of interleukin-1 and cyclooxygenase-2 in the blood and brain. *Neuroscience*. Dec 10 2008;157(4): 895—907.
46. Caballero F, Fernandez A, De Lacy AM, Fernandez-Checa JC, Caballeria J, Garcia-Ruiz C. Enhanced free cholesterol, SREBP-2 and StAR expression in human NASH. *J Hepatol*. Apr 2009;50(4):789—796.
47. Mari M, Caballero F, Colell A, et al. Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. *Cell Metab*. Sep 2006;4(3):185—198.
48. Zhao L, Chen Y, Tang R, et al. Inflammatory stress exacerbates hepatic cholesterol accumulation via increasing cholesterol uptake and de novo synthesis. *J Gastroenterol Hepatol*. May 2011;26(5):875—883.
49. Chen Y, Chen Y, Zhao L, et al. Inflammatory stress exacerbates hepatic cholesterol accumulation via disrupting cellular cholesterol export. *J Gastroenterol Hepatol*. May 2012;27(5): 974—984.
50. Zhang W, Kudo H, Kawai K, et al. Tumor necrosis factor-alpha accelerates apoptosis of steatotic hepatocytes from a murine model of non-alcoholic fatty liver disease. *Biochem Biophys Res Commun*. Jan 22 2010;391(4):1731—1736.
51. Senokuchi T, Liang CP, Seimon TA, et al. Forkhead transcription factors (FoxOs) promote apoptosis of insulin-resistant macrophages during cholesterol-induced endoplasmic reticulum stress. *Diabetes*. Nov 2008;57(11):2967—2976.
52. Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology (Baltimore, Md.)*. Jul 2006;44(1):27—33.
53. Chen CH, Huang MH, Yang JC, et al. Prevalence and risk factors of nonalcoholic fatty liver disease in an adult population of Taiwan: metabolic significance of nonalcoholic fatty liver disease in nonobese adults. *J Clin Gastroenterol*. Sep 2006;40(8):745—752.
54. Wong RJ, Ahmed A. Obesity and non-alcoholic fatty liver disease: disparate associations among Asian populations. *World J Hepatol*. May 27 2014;6(5):263—273.
55. Manco M, Calvani M, Mingrone G. Effects of dietary fatty acids on insulin sensitivity and secretion. *Diabetes Obes Metab*. Nov 2004;6(6):402—413.
56. Yu YH, Ginsberg HN. Adipocyte signaling and lipid homeostasis: sequelae of insulin-resistant adipose tissue. *Circ Res*. May 27 2005;96(10):1042—1052.
57. Asrih M, Jornayvaz FR. Inflammation as a potential link between nonalcoholic fatty liver disease and insulin resistance. *J Endocrinol*. Sep 2013;218(3):R25—R36.
58. Mei M, Zhao L, Li Q, et al. Inflammatory stress exacerbates ectopic lipid deposition in C57BL/6J mice. *Lipids Health Dis*. 2011;10:110.
59. Bezaire V, Mairal A, Anesia R, Lefort C, Langin D. Chronic TNFalpha and cAMP pre-treatment of human adipocytes alter HSL, ATGL and perilipin to regulate basal and stimulated lipolysis. *FEBS Lett*. Sep 17 2009;583(18):3045—3049.
60. Laurencikiene J, van Harmelen V, Arvidsson Nordstrom E, et al. NF-kappaB is important for TNF-alpha-induced lipolysis in human adipocytes. *J Lipid Res*. May 2007;48(5):1069—1077.
61. Ranjit S, Boutet E, Gandhi P, et al. Regulation of fat specific protein 27 by isoproterenol and TNF-alpha to control lipolysis in murine adipocytes. *J Lipid Res*. Feb 2011;52(2):221—236.
62. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. *Cell*. Mar 2 2012;148(5): 852—871.
63. Kim JK, Fillmore JJ, Chen Y, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin

resistance. *Proc Natl Acad Sci USA*. Jun 19 2001;98(13):7522—7527.
64. Merkel M, Weinstock PH, Chajek-Shaul T, et al. Lipoprotein lipase expression exclusively in liver. A mouse model for metabolism in the neonatal period and during cachexia. *J Clin Invest*. Sep 1 1998;102(5):893—901.
65. Falcon A, Doege H, Fluitt A, et al. FATP2 is a hepatic fatty acid transporter and peroxisomal very long-chain acyl-CoA synthetase. *Am J Physiol Endocrinol Metab*. Sep 2010;299(3):E384—E393.
66. Doege H, Grimm D, Falcon A, et al. Silencing of hepatic fatty acid transporter protein 5 in vivo reverses diet-induced non-alcoholic fatty liver disease and improves hyperglycemia. *J Biol Chem*. Aug 8 2008;283(32):22186—22192.
67. Kennedy DJ, Kuchibhotla S, Westfall KM, Silverstein RL, Morton RE, Febbraio M. A CD36-dependent pathway enhances macrophage and adipose tissue inflammation and impairs insulin signalling. *Cardiovasc Res*. Feb 15 2011;89(3):604—613.
68. Lauzier B, Merlen C, Vaillant F, et al. Post-translational modifications, a key process in CD36 function: lessons from the spontaneously hypertensive rat heart. *J Mol Cell Cardiol*. Jul 2011;51(1):99—108.
69. Greco D, Kotronen A, Westerbacka J, et al. Gene expression in human NAFLD. *Am J Physiol Gastrointest Liver Physiol*. May 2008;294(5):G1281—G1287.
70. Krammer J, Digel M, Ehehalt F, Stremmel W, Fullekrug J, Ehehalt R. Overexpression of CD36 and acyl-CoA synthetases FATP2, FATP4 and ACSL1 increases fatty acid uptake in human hepatoma cells. *Int J Med Sci*. 2011;8(7):599—614.
71. Koonen DP, Jacobs RL, Febbraio M, et al. Increased hepatic CD36 expression contributes to dyslipidemia associated with diet-induced obesity. *Diabetes*. Dec 2007;56(12):2863—2871.
72. Miquilena-Colina ME, Lima-Cabello E, Sanchez-Campos S, et al. Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in non-alcoholic steatohepatitis and chronic hepatitis C. *Gut*. Oct 2011;60(10):1394—1402.