Digestive Diseases

Dig Dis 2011;29:377–383
DOI: 10.1159/000329800

Obesity and Fatty Liver Are 'Grease' for the Machinery of Hepatic Fibrosis

Anja Bosserhoff[a] Claus Hellerbrand[b]

[a]Institute of Pathology and [b]Department of Internal Medicine I, University Hospital Regensburg, Regensburg, Germany

Key Words
Hepatic fibrosis · Hepatic steatosis · Nonalcoholic fatty liver disease · Metabolic syndrome

Abstract
Nonalcoholic fatty liver disease (NAFLD) starts with hepatic steatosis, which can progress with inflammation to nonalcoholic steatohepatitis, and a subset of patients develop progressive fibrosis and ultimately cirrhosis. In the majority of cases, NAFLD is associated with (components of) the metabolic syndrome. Obesity, diabetes and hepatic steatosis are also independent risk factors for hepatic fibrosis in different chronic liver diseases. However, the question is whether it is actually nonalcoholic steatohepatitis and not 'simple' steatosis that promotes fibrosis progression based on hepatocellular injury. In this review, the concept will be put forward that (1) hepatic steatosis per se is profibrogenic, and (2) that in NAFLD development and progression of hepatic fibrosis is not simply determined by (the degree of) hepatic inflammation. In addition to the liver, this view is expanded to other organs affected by the metabolic syndrome, which affects hepatic injury and fibrosis also via extrahepatic pathophysiological alterations. In conclusion, fatty liver and the metabolic syndrome, respectively, have to be recognized as significant lubricants of hepatic fibrosis, and simple hepatic steatosis cannot be considered as benign.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological condition of emerging importance, and is now recognized as the most common cause of abnormal liver tests. NAFLD is associated with obesity, type 2 diabetes, dyslipidemia and hypertension. These conditions have insulin resistance as the common factor and cluster to form the metabolic syndrome. Most patients with NAFLD have increased liver fat content alone (simple steatosis), but others develop increasing hepatic inflammation known as nonalcoholic steatohepatitis (NASH). A significant number of NASH patients – but not all – develop progressive fibrosis, ultimately leading to cirrhosis, hepatocellular carcinoma and end-stage liver disease [1].

Current Model of Disease Progression in NAFLD

A wealth of studies in animal models and humans with this disorder have increased our understanding of the pathogenesis and progression of NAFLD; however, the underlying mechanisms that influence the progression of steatosis to steatohepatitis are still largely unknown. We do know even less about the factors that cause development and progression of fibrosis in NAFLD.

The 'two-hit model' has been suggested to describe the development and progression of 'simple' fatty liver and NASH, respectively [2]. The first hit causes hepatic steatosis, i.e. an imbalance between hepatocellular lipid up-

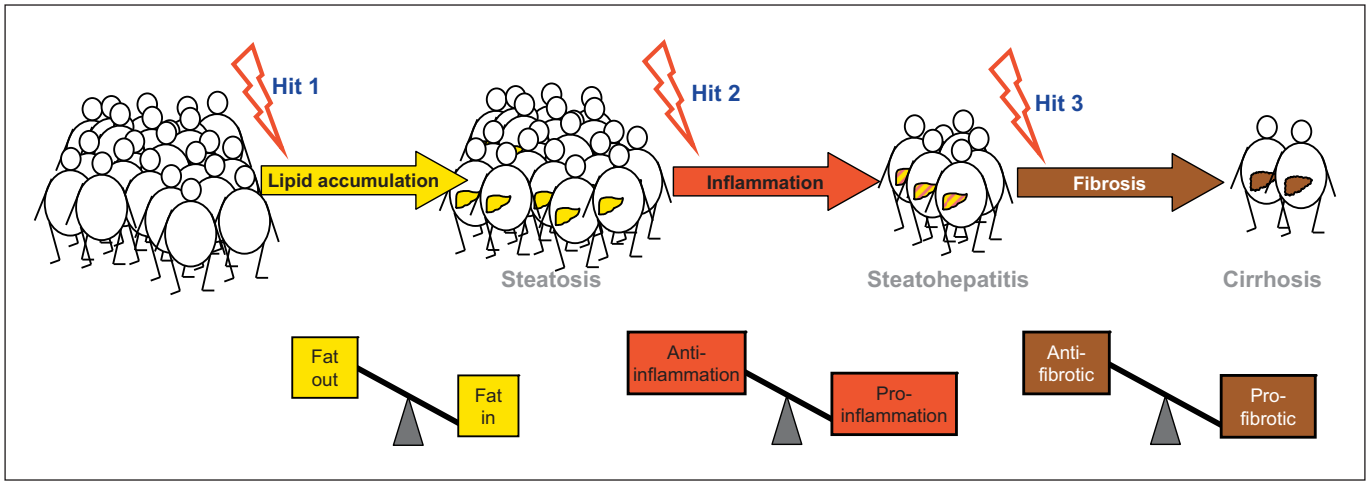
KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0257–2753/11/0294–0377$38.00/0

Accessible online at:
www.karger.com/ddi

Claus Hellerbrand, MD
University Hospital Regensburg
Department of Internal Medicine I
DE–93042 Regensburg (Germany)
Tel. +49 941 944 7155, E-Mail claus.hellerbrand@klinik.uni-regensburg.de



Color version available online

Fig. 1. The 'three-hit model' for the progression of NAFLD. Obesity and the metabolic syndrome reached a pandemic dimension, and most of these individuals have fatty liver also in the absence of significant chronic alcohol consumption (nonalcoholic fatty liver). In a significant number of cases, steatosis progresses to (nonalcoholic) steatohepatitis, and a subset of patients further develop fibrosis, which may lead to cirrhosis. The 'hit model' is based on the hypothesis that there is an imbalance between hepatocellular lipid uptake on the one hand and lipid combustion and secretion on the other, which constitutes the 'first hit'. The 'second hit' is caused by an imbalance of pro- and anti-inflammatory factors, which leads to hepatic inflammation. Finally, a 'third hit', i.e. an imbalance of pro- and antifibrotic factors, leads to progressive hepatic fibrosis, which may ultimately lead to cirrhosis.

take on the one hand and lipid combustion and secretion on the other. Subsequently, a second hit is required to induce NASH, i.e. a situation where proinflammatory mechanisms overcome anti-inflammatory mechanisms (fig. 1). Several excellent reviews have summarized our current knowledge of mechanisms, which tip the balance towards steatosis and inflammation, respectively [1, 3]. For example, lipid peroxidation has been identified to induce increased DNA oxidative damage and cell death, and herewith, hepatic inflammation. However, hepatic (necro)inflammation does not appear to be sufficient for progression to severe fibrosis since progression from NASH to cirrhosis develops only in a fraction of patients. Actually, several lines of evidence indicate that hepatic fibrosis is not simply the obligatory consequence of hepatic inflammation, but a 'third hit' (or 'third hits') is required to initiate and perpetuate fibrogenesis.

Direct Effect of Hepatic Steatosis on Hepatic Fibrosis

Hepatic fibrosis is characterized by an increased deposition of extracellular matrix proteins as a result of increased fibrogenesis and decreased fibrolysis. Activated hepatic stellate cells (HSCs) are the effector cells of hepatic fibrosis. After hepatic injury, HSCs undergo an activation process and transform to an activated myofibroblast-like phenotype. They are responsible for the excessive hepatic extracellular matrix deposition and their activity is recognized as a central event in the development of hepatic fibrosis [4, 5]. Also in NAFLD, HSC activation is the central pathophysiological mechanism underlying hepatic fibrosis, which has to be kept in mind when searching for factors causing or enhancing the 'third hit'.

To gain insight into the molecular mechanisms linking hepatic steatosis to fibrogenesis, we established an in vitro model to study the effect of hepatic steatosis on HSCs [6]. In this model, a dose-dependent lipid accumulation in primary human hepatocytes was induced by incubation with free fatty acids. Subsequently, HSCs were stimulated with conditioned medium collected from lipid-loaded and control hepatocytes, respectively. Applying this system, we found that steatotic hepatocytes release soluble factors, which enhance proinflammatory and profibrogenic gene expression in HSCs, and furthermore induce their activation, proliferation and resistance to apoptosis [6]. These in vitro data strongly indicate that pure fatty liver has the potential to promote liver fibrosis independent of inflammatory cells or hepatic inflammation. There is also increasing in vivo evidence that simple steatosis can 'bypass' the second hit, i.e. directly affecting

hepatic fibrosis. Thus, it has been shown by Deems at al. [7] that a high-fat diet exacerbates hepatic fibrosis in the bile duct-ligation model in mice.

Further, we demonstrated significantly elevated hepatic expression of RANTES and MCP-1 to be an early event in NAFLD progression, which occurs in hepatic steatosis without hepatic inflammation [8]. Both chemokines have been shown to play a critical role in hepatic fibrosis, and part of the profibrogenic effect is also mediated via direct effects on HSCs and herewith independent of hepatic inflammation [10]. Moreover, Tarantino et al. [11] found enhanced serum concentrations of transforming growth factor (TGF)-β1 in NAFLD patients, which were similar in patients with steatosis and NASH. TGF-β1 is one of the most potent profibrogenic cytokines; therefore, this study further suggests that hepatic steatosis cannot be considered as benign, but as harboring direct profibrogenic potential. Furthermore, histological examination of NAFLD patients revealed that severe steatosis and panacinar steatosis were more often associated with advanced fibrosis and herewith indicates that the location and degree of lipid accumulation affect the pathophysiological potential of hepatic steatosis [12].

Insulin Resistance and Fibrosis

In the majority of cases, NAFLD is associated with (components of) the metabolic syndrome. In fact, NAFLD itself is considered as a component of the metabolic syndrome. Therefore, it has to be considered that fatty liver is flanked by diabetes and/or high insulin levels in most cases, and that this is critical for hepatic fibrosis. Thus, it has been shown that high glucose concentrations induce proliferation and collagen production by HSCs [13]. Further, high glucose and hyperinsulinemia stimulate expression of further fibrogenic factors in HSC in vitro and in vivo in experimental models of obesity and type II diabetes [14]. Moreover, a recent study by Ota et al. [15] compared the effect of a high-fat diet on two strains of rats. OLETF rats are prone to obesity, insulin resistance, hyperinsulinemia and diabetes, while LETO rats are lean and insulin-sensitive. Interestingly, in OLETF rats, a high-fat diet led to increased hepatic triglyceride accumulation and inflammation as well as HSC activation and fibrosis, while the hepatic response was significantly milder in LETO rats. In line with this study, Lo et al. [16] described that diabetes is a progression factor for hepatic fibrosis in a high-fat diet model of NASH, and Nan et al. [17] showed that treatment with an antidiabetic drug ameliorates fibrosis in an experimental murine NASH model. Accordingly, a long-term follow-up study of NAFLD patients revealed that progression of liver fibrosis is associated with more pronounced insulin resistance and significant weight gain [18].

Together, these findings indicate that insulin resistance and/or diabetes may accelerate the entire pathologic spectrum of NAFLD. Intriguingly, fibrosis is affected directly and (at least in part) independently of the effect on hepatic inflammation. Of note, the profibrotic action does not even necessarily require hepatic lipid accumulation. Thus, the metabolic syndrome is independently associated with more severe fibrosis, but not with the severity of steatosis, both in chronic viral hepatitis and NASH [19]. Consequently, a recent study of livers of diabetics found activation of HSCs and sinusoidal fibrosis without histologically detectable NASH and irrespective of the degree of steatosis [20]. Similarly, Abrams et al. [21] showed that a significant subset of morbidly obese individuals had portal fibrosis in the absence of NASH, which is associated with glycemic dysregulation.

Although the pathogenic mechanisms implicated in the association of diabetes and fibrosis are presently unknown, high extracellular and intracellular glucose environment may activate several pathways related to the production of cytokines, growth factors and reactive oxidative species, which can mediate tissue damage and fibrosis in diabetes. In 2001, Paradis et al. [14] suggested that hyperglycemia and insulin are key factors in the progression of fibrosis in patients with NASH through the upregulation of connective tissue growth factor, a key factor of hepatic fibrosis [22]. Of note, there are intriguing similarities between the association of diabetes and fibrosis in the liver and in other organs. Thus, experimental models have indicated that hyperglycemia influences the severity of pulmonary fibrosis [23]. Additionally, several clinical studies have revealed an association between diabetes and pulmonary fibrosis [24, 25]. Moreover, experimental diabetes mellitus leads to upregulation of profibrotic genes and fibrosis in the myocardium [26, 27]. In the heart, connective tissue growth factor also appears as a mediator of adverse effects of high glucose and fatty acids in cardiomyocytes [28]. Interestingly, connective tissue growth factor has also been implicated in the epithelial-to-mesenchymal transition of renal tubular epithelial cells, which contributes to the renal fibrosis associated with diabetic nephropathy [29].

Together, these findings clearly indicate that diabetes and insulin resistance are significant promoters of fibro-

sis in general, and with regard to NAFLD, they further show that the metabolic syndrome can bypass the first and second hit, i.e. can directly affect hepatic fibrosis.

Extrahepatic Manifestations of the Metabolic Syndrome Promote Hepatic Fibrosis

Diabetes and insulin resistance highlight how important it is to consider that NAFLD occurs in the context of a systemic disease, and thus differs from most other liver diseases. Moreover, in NAFLD several other organs and biological functions are affected in addition to the liver, and these alterations directly or indirectly promote inflammation and fibrosis in fatty liver.

One example is visceral adipose tissue, in which expression and secretion of adipocytokines is quantitatively and qualitatively altered in obese patients [30]. On the one hand, expression of adiponectin, which is known to be hepatoprotective and antifibrotic, is significantly reduced. On the other hand, obese patients frequently have increased levels of leptin, another adipokine mainly released by adipocytes, and associated leptin resistance [31]. Leptin is profibrogenic and liver fibrosis is decreased in leptin- or leptin receptor-deficient mice [31, 32]. A recent study found that leptin directly promotes the myofibroblastic phenotype in HSCs by activating the hedgehog pathway [33].

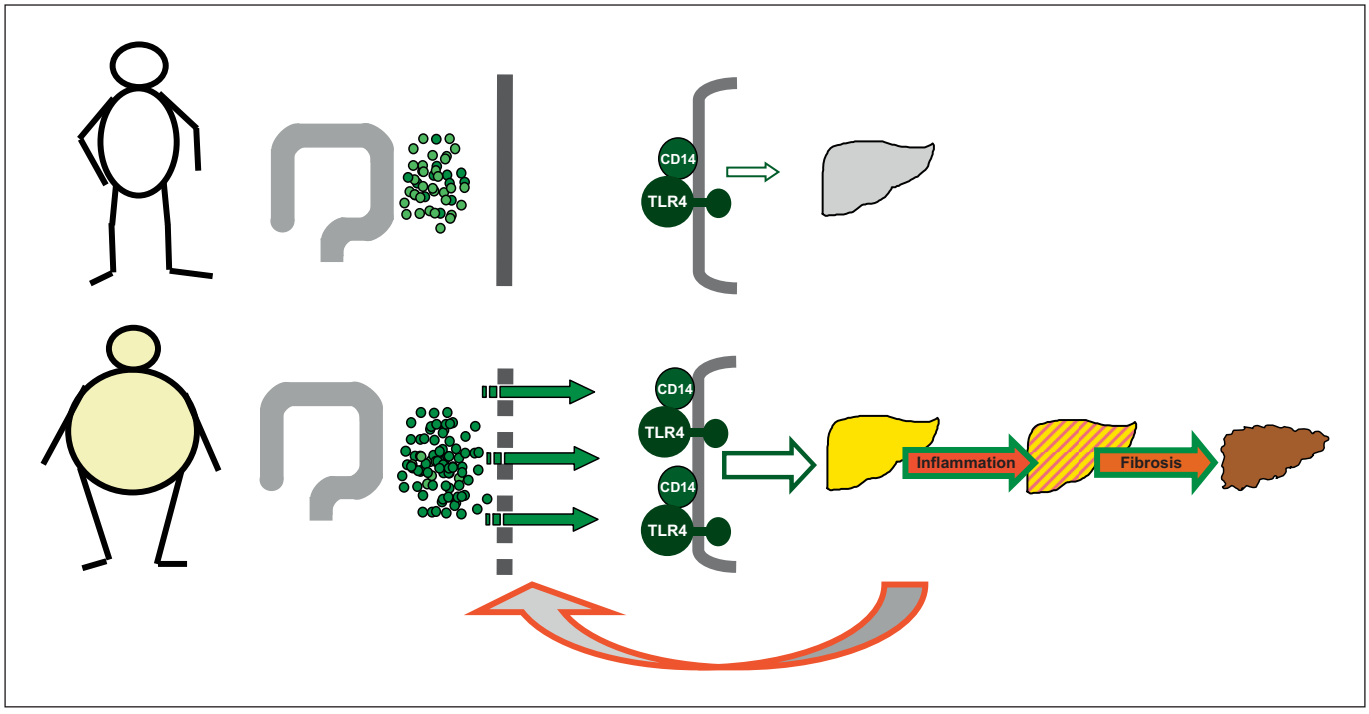
Another example how the complexity of the metabolic syndrome or pathophysiological alterations associated with the metabolic syndrome may affect the progression of NAFLD is obstructive sleep apnea that causes chronic intermittent hypoxia during sleep. Obstructive sleep apnea is frequent in obese individuals, and it has been shown that chronic intermittent hypoxia induces oxidative stress in hepatocytes and predisposes to liver injury [34]. Similarly, chronic intermittent hypoxia promotes the progression of NASH in animal models [35, 36]. After exposure to chronic intermittent hypoxia, mice with diet-induced hepatic steatosis developed lobular inflammation and fibrosis in the liver, which were not evident in control mice [35]. Moreover, severe obstructive sleep apnea has been identified as a risk factor for elevated liver enzymes and steatohepatitis independent of body weight in patients [37]. Hypoxia leads to the activation of hypoxia-inducible factor 1 and 2. These oxygen-sensitive transcription factors are important regulators of hepatic lipid metabolism and are known to play a critical role in hepatic inflammation and fibrosis [38]. Interestingly, a recent study revealed that hypoxia also underlies the development of the inflammatory response in adipocytes [39], which significantly contributes to systemic as well as hepatic inflammation in the metabolic syndrome [40]. This further indicates the complexity of the interaction of hepatic and extrahepatic pathophysiological mechanisms, which promote the progression in NAFLD.

Intestinal Effects of the Metabolic Syndrome Promote Hepatic Fibrosis

Obesity leads to quantitative and qualitative changes of the intestinal flora and an impaired intestinal barrier, which together lead to increased translocation of bacteria and bacterial compounds [41, 42] known to be proinflammatory and profibrogenic (fig. 2). Thus, in dietary NASH as well as other models of chronic liver injury, reduction of the number of intestinal bacteria by antibiotics ameliorates hepatic fibrosis [43, 44]. In addition to the quantity, obesity and high-fat diets lead to changes in the composition of the intestinal flora in a deleterious way [45–49]. Thus, high-fat diets promote the growth of endotoxin-producing bacteria and bacteria with an increased capacity for uptake of carbohydrates typical of a 'Western diet' [45–47].

In NAFLD, intestinal permeability is impaired by a loss of the integrity of epithelial tight junctions [42]. Recently, we have shown that experimental colitis leads to an increased expression of intestinal defensins which apparently fosters the intestinal barrier [50]. It is noteworthy, however, that this protective defensin expression was almost completely abrogated in mice fed a high-fat diet, and also portal endotoxin levels were significantly higher in these mice compared to animals with colitis but fed with normal chow [50]. Further studies have to show whether this inhibitory effect of a high-fat diet on the intestinal barrier is also operative under less pronounced subchronic intestinal inflammation as observed in NAFLD patients.

Furthermore and surprisingly, we recently revealed in experimental murine models that application of a high-fat diet leads to a markedly increased hepatic expression of the endotoxin receptor Toll-like receptor 4 (TLR4), which is known to play a crucial role in hepatic fibrosis [50, 51]. Under normal conditions, TLR-signaling pathways are inactivated although the liver is constantly exposed to gut-derived bacterial products. Under pathological conditions, however, signaling via TLRs promotes antimicrobial responses and hepatic injury [52], and the crucial role of TLR4-signaling in hepatic fibrosis has re-



Color version available online

Fig. 2. The metabolic syndrome affects the gut-liver interaction. Schematic depiction of the pathological alterations in intestine and endotoxin-induced signal transduction, respectively, by which the metabolic syndrome promotes hepatic inflammation and fibrosis. Obesity leads to quantitative and qualitative changes of the intestinal flora, i.e. higher number as well as a higher percentage of endotoxin-producing bacteria. Moreover, the intestinal barrier is paired, which leads to increased translocation of bacteria and bacterial compounds. Hepatic steatosis and inflammation induce expression of the endotoxin receptor Toll-like receptor 4 (TLR4), which (together with CD14) plays a crucial role in the development and progression of hepatic inflammation and fibrosis. With the progression of hepatocellular injury the intestinal barrier is further impaired, leading to a vicious circle.

cently been highlighted [53]. Moreover, it has been shown that free fatty acids can activate TLR4 as pattern recognition receptors by mimicking pathogens [54]. It is noteworthy that free fatty acids are frequently elevated in obese patients and patients with NAFLD.

Conclusion

The 'hit model' is a valuable framework for understanding the molecular mechanisms of disease progression in NAFLD. However, like every model it simplifies and depicts only part of the complexity of the pathophysiological mechanisms promoting progression of hepatic steatosis to inflammation and fibrosis. Thus, one has to consider that in addition to the liver, the metabolic syndrome pathophysiologically affects several other organs. Furthermore and importantly, there is clear evidence that steatosis is not only the basis for hepatic inflammation, but development and progression of hepatic fibrosis are subsequently determined by the degree of steatohepatitis. In contrast, fatty liver directly affects hepatic fibrosis in NAFLD as well as in chronic liver disease in general. Considering their epidemiological dimensions, it is important to further unravel the underlying mechanisms of how the metabolic syndrome and fatty liver, respectively, lubricate hepatic fibrogenesis. This knowledge could be the basis for novel prognostic markers and therapeutic targets of liver fibrosis in NAFLD and chronic liver disease in general.

Disclosure Statement

Both authors have no conflicts of interest to declare.

References

1 Straub BK, Schirmacher P: Pathology and biopsy assessment of non-alcoholic fatty liver disease. Dig Dis 2010;28:197–202.
2 Day CP, James OF: Steatohepatitis: a tale of two 'hits'? Gastroenterology 1998;114:842–845.
3 Tilg H, Moschen AR: Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology 2010;52:1836–1846.
4 Bataller R, Brenner DA: Liver fibrosis. J Clin Invest 2005;115:209–218.
5 Friedman SL: Mechanisms of disease: mechanisms of hepatic fibrosis and therapeutic implications. Nat Clin Pract Gastroenterol Hepatol 2004;1:98–105.
6 Wobser H, Dorn C, Weiss TS, Amann T, Bollheimer C, Buttner R, Scholmerich J, Hellerbrand C: Lipid accumulation in hepatocytes induces fibrogenic activation of hepatic stellate cells. Cell Res 2009;19:996–1005.
7 Deems RO, Skypala PL, Martinez-Hernandez A, Friedman LS, Friedman MI: Dietary fat exacerbates liver disease in bile duct-ligated rats. J Nutr 1993;123:1414–1420.
8 Kirovski G, Gabele E, Dorn C, Moleda L, Niessen C, Weiss TS, Wobser H, Schacherer D, Buechler C, Wasmuth HE, Hellerbrand C: Hepatic steatosis causes induction of the chemokine RANTES in the absence of significant hepatic inflammation. Int J Clin Exp Pathol 2010;3:675–680.
9 Kirovski G, Dorn C, Huber H, Beer C, Wobsser H, Schacherer D, Buechler C, Wiest R, Hellerbrand C: Elevated systemic monocyte chemoattractrant protein-1 levels in hepatic steatosis without significant hepatic inflammation. submitted.
10 Marra F, Romanelli RG, Giannini C, Failli P, Pastacaldi S, Arrighi MC, Pinzani M, Laffi G, Montalto P, Gentilini P: Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells. Hepatology 1999;29:140–148.
11 Tarantino G, Conca P, Riccio A, Tarantino M, Di Minno MN, Chianese D, Pasanisi F, Contaldo F, Scopacasa F, Capone D: Enhanced serum concentrations of transforming growth factor-beta1 in simple fatty liver: is it really benign? J Transl Med 2008;6:72.
12 Chalasani N, Wilson L, Kleiner DE, Cummings OW, Brunt EM, Unalp A: Relationship of steatosis grade and zonal location to histological features of steatohepatitis in adult patients with non-alcoholic fatty liver disease. J Hepatol 2008;48:829–834.
13 Sugimoto R, Enjoji M, Kohjima M, Tsuruta S, Fukushima M, Iwao M, Sonta T, Kotoh K, Inoguchi T, Nakamuta M: High glucose stimulates hepatic stellate cells to proliferate and to produce collagen through free radical production and activation of mitogen-activated protein kinase. Liver Int 2005;25:1018–1026.
14 Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, Conti M, Huet S, Ba N, Buffet C, Bedossa P: High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology 2001;34:738–744.
15 Ota T, Takamura T, Kurita S, Matsuzawa N, Kita Y, Uno M, Akahori H, Misu H, Sakurai M, Zen Y, Nakanuma Y, Kaneko S: Insulin resistance accelerates a dietary rat model of nonalcoholic steatohepatitis. Gastroenterology 2007;132:282–293.
16 Lo L, McLennan SV, Williams PF, Bonner J, Chowdhury S, McCaughan GW, Gorrell MD, Yue DK, Twigg SM: Diabetes is a progression factor for hepatic fibrosis in a high fat fed mouse obesity model of non-alcoholic steatohepatitis. J Hepatol 2010, E-pub ahead of print.
17 Nan YM, Fu N, Wu WJ, Liang BL, Wang RQ, Zhao SX, Zhao JM, Yu J: Rosiglitazone prevents nutritional fibrosis and steatohepatitis in mice. Scand J Gastroenterol 2009;44:358–365.
18 Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S: Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865–873.
19 Tsochatzis E, Papatheodoridis GV, Manesis EK, Kafiri G, Tiniakos DG, Archimandritis AJ: Metabolic syndrome is associated with severe fibrosis in chronic viral hepatitis and non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2008;27:80–89.
20 Jaskiewicz K, Rzepko R, Sledzinski Z: Fibrogenesis in fatty liver associated with obesity and diabetes mellitus type 2. Dig Dis Sci 2008;53:785–788.
21 Abrams GA, Kunde SS, Lazenby AJ, Clements RH: Portal fibrosis and hepatic steatosis in morbidly obese subjects: a spectrum of nonalcoholic fatty liver disease. Hepatology 2004;40:475–483.
22 Gressner OA, Gressner AM: Connective tissue growth factor: a fibrogenic master switch in fibrotic liver diseases. Liver Int 2008;28:1065–1079.
23 Usuki J, Enomoto T, Azuma A, Matsuda K, Aoyama A, Kudoh S: Influence of hyperglycemia to the severity of pulmonary fibrosis. Chest 2001;120:71S.
24 Gribbin J, Hubbard R, Smith C: Role of diabetes mellitus and gastro-oesophageal reflux in the aetiology of idiopathic pulmonary fibrosis. Respir Med 2009;103:927–931.
25 Garcia-Sancho Figueroa MC, Carrillo G, Perez-Padilla R, Fernandez-Plata MR, Buendia-Roldan I, Vargas MH, Selman M: Risk factors for idiopathic pulmonary fibrosis in a Mexican population. A case-control study. Respir Med 2010;104:305–309.
26 Falcao-Pires I, Goncalves N, Moura C, Lamego I, Eloy C, Lopes JM, Begieneman MP, Niessen HW, Areias JC, Leite-Moreira AF: Effects of diabetes mellitus, pressure-overload and their association on myocardial structure and function. Am J Hypertens 2009;22:1190–1198.
27 Black MJ, D'Amore A, Auden A, Stamp L, Osicka T, Panagiotopoulos S, Jerums G: Chronic type 1 diabetes in spontaneously hypertensive rats leads to exacerbated cardiac fibrosis. Cardiovasc Pathol 2010;19:361–370.
28 Wang X, McLennan SV, Allen TJ, Tsoutsman T, Semsarian C, Twigg SM: Adverse effects of high glucose and free fatty acid on cardiomyocytes are mediated by connective tissue growth factor. Am J Physiol Cell Physiol 2009;297:C1490–C1500.
29 Burns WC, Twigg SM, Forbes JM, Pete J, Tikellis C, Thallas-Bonke V, Thomas MC, Cooper ME, Kantharidis P: Connective tissue growth factor plays an important role in advanced glycation end product-induced tubular epithelial-to-mesenchymal transition: implications for diabetic renal disease. J Am Soc Nephrol 2006;17:2484–2494.
30 Marra F, Bertolani C: Adipokines in liver diseases. Hepatology 2009;50:957–969.
31 Tsochatzis E, Papatheodoridis GV, Archimandritis AJ: The evolving role of leptin and adiponectin in chronic liver diseases. Am J Gastroenterol 2006;101:2629–2640.
32 Marra F, Aleffi S, Bertolani C, Petrai I, Vizzutti F: Adipokines and liver fibrosis. Eur Rev Med Pharmacol Sci 2005;9:279–284.
33 Choi SS, Syn WK, Karaca GF, Omenetti A, Moylan CA, Witek RP, Agboola KM, Jung Y, Michelotti GA, Diehl AM: Leptin promotes the myofibroblastic phenotype in hepatic stellate cells by activating the hedgehog pathway. J Biol Chem 2010;285:36551–36560.
34 Savransky V, Nanayakkara A, Vivero A, Li J, Bevans S, Smith PL, Torbenson MS, Polotsky VY: Chronic intermittent hypoxia predisposes to liver injury. Hepatology 2007;45:1007–1013.
35 Savransky V, Bevans S, Nanayakkara A, Li J, Smith PL, Torbenson MS, Polotsky VY: Chronic intermittent hypoxia causes hepatitis in a mouse model of diet-induced fatty liver. Am J Physiol Gastrointest Liver Physiol 2007;293:G871–G877.
36 Zamora-Valdes D, Mendez-Sanchez N: Experimental evidence of obstructive sleep apnea syndrome as a second hit accomplice in nonalcoholic steatohepatitis pathogenesis. Ann Hepatol 2007;6:281–283.
37 Tanne F, Gagnadoux F, Chazouilleres O, Fleury B, Wendum D, Lasnier E, Lebeau B, Poupon R, Serfaty L: Chronic liver injury during obstructive sleep apnea. Hepatology 2005;41:1290–1296.

38 Moon JO, Welch TP, Gonzalez FJ, Copple BL: Reduced liver fibrosis in hypoxia-inducible factor-1alpha-deficient mice. Am J Physiol Gastrointest Liver Physiol 2009;296:G582–G592.
39 Wang B, Wood IS, Trayhurn P: Dysregulation of the expression and secretion of inflammation-related adipokines by hypoxia in human adipocytes. Pflugers Arch 2007; 455:479–492.
40 Taubes G: Insulin resistance. Prosperity's plague. Science 2009;325:256–260.
41 Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG: The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. Gut 2001;48:206–211.
42 Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Masciana R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A: Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009;49: 1877–1887.
43 Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG: Antibiotics prevent liver injury in rats following long-term exposure to ethanol. Gastroenterology 1995;108:218–224.
44 Solga SF, Diehl AM: Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. J Hepatol 2003;38: 681–687.
45 Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI: An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006; 444:1027–1031.
46 Ley RE, Turnbaugh PJ, Klein S, Gordon JI: Microbial ecology: human gut microbes associated with obesity. Nature 2006;444: 1022–1023.
47 Turnbaugh PJ, Backhed F, Fulton L, Gordon JI: Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 2008;3: 213–223.
48 Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmee E, Cousin B, Sulpice T, Chamontin B, Ferrieres J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R: Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–1772.
49 Cani PD, Delzenne NM: Gut microflora as a target for energy and metabolic homeostasis. Curr Opin Clin Nutr Metab Care 2007;10: 729–734.
50 Gaebele E, Dostert K, Hofmann C, Wiest R, Scholmerich J, Hellerbrand C, Obermeier F: DSS induced colitis increases portal LPS levels and enhances hepatic inflammation and fibrogenesis in experimental NASH. J Hepatol 2011, E-pub ahead of print.
51 Gabele E, Dostert K, Dorn C, Patsenker E, Stickel F, Hellerbrand C: A new model of interactive effects of alcohol and high-fat diet on hepatic fibrosis. Alcohol Clin Exp Res 2011;35:1361–1367.
52 Schwabe RF, Seki E, Brenner DA: Toll-like receptor signaling in the liver. Gastroenterology 2006;130:1886–1900.
53 Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF: TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 2007;13:1324–1332.
54 Valenti L, Fracanzani AL, Fargion S: The immunopathogenesis of alcoholic and nonalcoholic steatohepatitis: two triggers for one disease? Semin Immunopathol 2009;31:359–369.