

CYTOKINES AND THE PATHOGENESIS OF NON-ALCOHOLIC STEATOHEPATITIS

RECENT ADVANCES IN CLINICAL PRACTICE

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“PRIMARY” NASH AND THE DYSMETABOLIC SYNDROME

Histopathological characteristics distinguish steatohepatitis from other causes of chronic liver injury. For years, the main cause of steatohepatitis was thought to be excessive consumption of alcohol. Increasingly, steatohepatitis is being diagnosed in individuals who deny alcohol abuse. Arbitrarily, “non-alcoholic” steatohepatitis (NASH) is subcategorised into “primary” and “secondary” NASH.¹

Primary NASH refers to steatohepatitis that is associated with the dysmetabolic syndrome (that is, obesity, type 2 diabetes, dyslipidaemia).² Primary NASH is thought to be the predominant form of NASH, afflicting at least as many individuals in the USA as chronic hepatitis C. Secondary NASH refers to steatohepatitis that accompanies other syndromes (for example, lipodystrophy) or that is caused by certain drugs (for example, amiodarone).³ Accumulating evidence suggests that common mechanisms may mediate the pathogenesis of alcohol induced steatohepatitis and primary NASH.⁴ Although unproven, it is likely that primary and secondary NASH also share common pathogenic mechanisms.

PATHOGENIC RELEVANCE OF NASH ASSOCIATED DISORDERS

Obesity

Obesity, especially visceral adiposity, is a major risk factor for NASH in humans.⁵ Even if they do not have an elevated body mass index, patients with NASH are very likely to be insulin resistant.² Basic research on fat cell (adipocyte) biology provides some clues about why obesity, insulin resistance, and NASH may be interrelated.

Obesity increases adipose tissue mass. It has long been known that adipose tissue is a source of free fatty acids that are delivered to the liver and a depot for triglycerides that are synthesised by hepatocytes and released into the blood. Only recently however has the neuroendocrine role of adipose tissue been appreciated. Fat produces hormones, such as leptin, resistin, and adiponectin, that regulate metabolism in other tissues, as well as fat itself. Fat is also a source of neurotransmitters, such as noradrenaline and angiotensin II. In addition, because adipocytes produce immunomodulatory cytokines, such as tumour necrosis factor (TNF)- α and interleukin (IL)-6, fat is a component of the immune system.^{6–8}

Given the neuroendocrine and immunomodulatory functions of fat, it is not surprising that absolute increases in adipose tissue mass influence systemic energy homeostasis, intermediary metabolism, and immune function. The situation is further complicated by the likelihood that there are both qualitative and quantitative differences in the factors (aka, “adipokines”) that are produced by different fat depots. For example, visceral fat, which appears to be less “mature” than subcutaneous fat, produces more TNF- α and free fatty acids but less adiponectin than subcutaneous fat, which produces large amounts of leptin.⁷ Thus the ultimate consequences of obesity may differ depending on which fat depots predominate.

Visceral fat depots, TNF- α , hepatic insulin resistance, and fatty liver

Visceral adiposity in particular has been strongly associated with the dysmetabolic syndrome and NASH.^{9–12} Visceral fat promotes fatty liver disease by several mechanisms. Firstly, intra-abdominal fat provides an immediate source of free fatty acids that are delivered directly into the portal vein.¹¹ In mice, situations that increase the delivery of free fatty acids to tissues, including the liver, induce localised insulin resistance.¹³ In addition, gene disruption studies in mice have proven that interference with insulin signalling in hepatocytes (that is, hepatocyte insulin resistance) activates fat synthesising enzymes in these cells and results in hepatic steatosis.¹⁴ Secondly, individuals with truncal obesity have extremely low levels of adiponectin and relatively high levels of TNF- α .¹⁵ TNF- α is known to activate intracellular signalling molecules, including stress related kinases such as Jun N-terminal kinase and inhibitor kappa beta kinase beta, that

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make cells resistant to the actions of insulin.^{13 16 17} Adiponectin antagonises both the production and activity of TNF- α .¹⁸⁻²¹ Therefore, TNF- α actions are potentiated when adiponectin is scarce.²² In addition, TNF- α itself inhibits adiponectin.^{23 24} Adiponectin acts directly on hepatocytes to inhibit fatty acid synthesis and uptake while stimulating fatty acid oxidation.²⁵⁻²⁷ Therefore, adiponectin itself also enhances hepatocyte sensitivity to insulin.^{28 29} The combination of low adiponectin and high TNF- α levels in the context of increased hepatic exposure to free fatty acids results in hepatic steatosis and severe hepatic insulin resistance.³⁰⁻³² The importance of visceral fat in the pathogenesis of hepatic insulin resistance and steatosis has been demonstrated in Zucker fatty fatty (*fa/fa*) rats. In these animals with inherited leptin resistance, surgical resection of intra-abdominal fat depots reverses both conditions.³³

Cytokines, adipokines, and steatohepatitis

As mentioned previously, similarities in the histopathology of alcohol induced steatohepatitis and obesity related NASH suggest that common mechanisms may mediate both diseases. In non-obese experimental animals in which chronic ingestion of alcohol containing diets was used to induce steatohepatitis, various treatments that inhibit TNF- α activity prevent alcohol induced steatohepatitis (reviewed by Tsukamoto and Lu³⁴). Work by Sasaki and Wands³⁵ and Mohr and colleagues³⁶ has also suggested that chronic consumption of ethanol causes insulin resistance in rats and mice. Given evidence that the cellular mechanisms for insulin resistance also activate TNF- α production,¹⁶ it is not so surprising that insulin sensitising drugs that inhibit TNF- α ^{37 38} protect mice from alcohol induced steatohepatitis.³⁹ Recently, in a mouse model of NASH (for example, leptin deficient *ob/ob* mice), we showed that anti-TNF- α antibodies improve hepatic insulin sensitivity and NASH.⁴⁰ Xu *et al* reported that non-obese ethanol fed mice and obese *ob/ob* mice both exhibit increased TNF- α and reduced adiponectin levels. Injections of recombinant adiponectin inhibited TNF- α , improved insulin sensitivity, and reversed steatohepatitis in lean (leptin sufficient) ethanol fed mice, as well as in obese mice with genetic leptin deficiency.⁴¹

Obese humans almost always have high serum levels of leptin.¹⁰ Therefore, it has been argued that results from studies of leptin deficient *ob/ob* mice may have little relevance to the pathogenesis of insulin resistance and NASH in obese humans.^{3 42} While there is no question that leptin levels in *ob/ob* mice and obese humans differ dramatically, both *ob/ob* mice and obese humans with the dysmetabolic syndrome have low levels of adiponectin and relatively high levels of TNF- α .^{10 41} In humans, various treatments that improve the dysmetabolic syndrome (for example, diet and exercise, thiazolidinediones) have been shown to increase adiponectin levels and reduce TNF- α .^{38 43} Therefore, it is becoming apparent that similar factors (that is, TNF- α and adiponectin) mediate NASH pathogenesis when leptin levels are increased and when leptin levels are reduced. Given this insight, it will be interesting to determine if supplementing adiponectin directly improves human insulin resistance and NASH.

NASH, FIBROGENIC FACTORS, AND CIRRHOSIS

In most liver diseases, including NASH, liver related morbidity and mortality are greatest in the subpopulation that progresses to cirrhosis.^{44 45} Because the epidemiology of

fatty liver diseases in humans demonstrates that cirrhosis is more likely to occur in those with NASH than in those with simple steatosis,⁴⁶ it has been suggested that the severity of chronic hepatic necroinflammation dictates the ultimate outcome of NASH in humans.³ Interestingly, this does not appear to be the case in rodents. Many genetically altered mice with severe steatohepatitis (for example, MAT-1 α null mice, PPAR α null mice) do not develop cirrhosis^{47 48} while cirrhosis is common in other strains (for example, microsomal triglyceride transferase deficient mice) that have significant steatosis but relatively trivial steatohepatitis (reviewed by Koteish and Diehl⁴⁹). Studies of *ob/ob* mice have identified two obesity related factors that regulate hepatic fibrosis.

Leptin

Direct comparison of *ob/ob* mice with steatohepatitis and wild-type mice with steatohepatitis induced by feeding antioxidant depleted methionine choline deficient (MCD) diets, demonstrates that hepatic oxidant stress and necroinflammation are comparable in mice with and without genetic leptin deficiency.⁵⁰ However, leptin deficient mice develop significantly less hepatic fibrosis than wild-type mice, even when challenged by hepatotoxins, such as thioacetamide, or infections, such as schistosomiasis.⁵¹⁻⁵⁵ Leptin replacement therapy normalises the hepatic fibrogenic response of *ob/ob* mice, demonstrating unequivocally that leptin is a critical regulator of liver fibrosis.⁵⁵ However, the mechanisms by which leptin promotes hepatic fibrosis are poorly understood. Direct effects of leptin on hepatic stellate cells have been demonstrated by several groups.^{56 57} Although the particular type of leptin receptor that is involved remains controversial, there appears to be consensus that leptin induces PI3 kinase, promotes the growth of cultured stellate cells, and upregulates their collagen gene expression.^{58 59}

Th-2 cytokines

Our group has suggested that leptin may also regulate hepatic fibrosis indirectly by modulating the production of cytokines and neurotransmitters. As mentioned previously, leptin deficient mice are relatively resistant to schistosoma induced hepatic fibrosis.⁵⁴ In wild-type mice, the balance of Th-1 (proinflammatory) and Th-2 (anti-inflammatory, profibrogenic) cytokines regulates this process. Schistosoma infected mice with targeted disruption of the genes for both IL-4 and IL-10 do not develop liver fibrosis despite having a severe hepatic necroinflammatory response to infection. In contrast, schistosoma infected mice with targeted disruption of both IL-4 and IL-12 develop severe hepatic fibrosis despite having only trivial hepatitis.⁶⁰ Leptin deficient *ob/ob* mice have reduced hepatic production of both IL-4 and IL-10, similar to leptin sufficient mice that are protected from schistosoma induced hepatic fibrosis. We showed that treating *ob/ob* mice with leptin (which normalises their fibrogenic response to liver injury) also increases their production of Th-2 profibrogenic cytokines, including IL-10.⁶¹

Natural killer T (NKT) cells

Components of the innate immune system, particularly NKT cells, play a key role in balancing the types of cytokines that are produced in the liver.⁶² We found that with age, the livers of leptin deficient *ob/ob* mice become selectively depleted of NKT cells due to increased rates of hepatic NKT cell apoptosis.⁶³ Treatment with leptin increases hepatic NKT cell

populations in ob/ob mice, suggesting that leptin directly or indirectly regulates NKT cell viability.⁶¹ Consistent with this concept, we showed that leptin increases IL-15, a cytokine that is known to expand NK T cell populations in the liver. Treating ob/ob mice with IL-15 dramatically increases their numbers of hepatic NKT cells despite persistent leptin deficiency. However, the effects of IL-15 are not restricted to NKT cells (IL-15 also dramatically expands NK cell populations). Moreover, IL-15 only slightly reduces NKT cell apoptosis, suggesting that other factors may play a more important role than IL-15 deficiency in mediating the reductions in hepatic NKT cell populations that occur during leptin deficiency.

Neurotransmitters

Recent studies in our laboratory suggest that the neurotransmitter noradrenaline is likely to be a key regulator of hepatic NKT cell viability (unpublished data, see below). Leptin induces the production of noradrenaline. Thus leptin deficient ob/ob mice have reduced levels of noradrenaline.⁶⁴ Recently, noradrenaline deficiency has been implicated in several of the peripheral consequences of leptin deficiency.⁶⁵ In this regard, it is interesting that NKT cells express adrenoceptors⁶⁶ because changes in noradrenaline might influence these cells. We have explored this possibility by studying both leptin sufficient and leptin deficient mice.

Our preliminary studies suggest that wild type mice with targeted disruption of the gene that encodes dopamine beta hydroxylase (*Dbh*, the enzyme catalyses the synthesis of noradrenaline from dopamine) have reduced hepatic NKT cell populations. Noradrenaline deficiency underlies depletion of hepatic NKT cell populations in *Dbh* null mice because treatment with adrenergic agonists restores their hepatic NKT cell numbers. This finding suggested to us that noradrenaline deficiency may also contribute to the decreased hepatic NKT cell populations in ob/ob mice. Therefore, we treated ob/ob mice with noradrenaline. This significantly reduced apoptosis of hepatic NKT cells and restored liver NKT cell populations to normal levels. Moreover, noradrenaline induced reconstitution of liver NKT cell populations terminated the overproduction of Th-1 cytokines (for example, TNF- α and interferon γ) by other types of cytokine producing cells, re-establishing balanced hepatic production of Th-1 and Th-2 cytokines despite persistent leptin deficiency (unpublished data). Therefore, both leptin (which increases noradrenaline) and noradrenaline itself enhance the activity of Th-2 cytokines. Th-2 cytokines are necessary to antagonise the activity of pro-inflammatory cytokines, such as TNF- α . However, as demonstrated by studies of schistosoma infected mice with targeted disruption of Th-2 cytokine genes, Th-2 cytokine activity is also necessary for hepatic fibrosis to develop during liver injury.

Noradrenaline, hepatic fibrosis, and leptin deficiency

Given this, we wondered if enhancing adrenergic activity in ob/ob mice might permit these animals to develop hepatic fibrosis despite ongoing leptin deficiency. To test this possibility, we gave ob/ob mice (which have mild chronic steatohepatitis) continuous infusions of noradrenaline for 2–4 weeks and evaluated the effects on hepatic stellate cells, transforming growth factor β (TGF- β), a key profibrogenic cytokine, collagen gene expression, and hepatic fibrosis.⁶⁷ Noradrenaline treatment increased the numbers of activated

stellate cells, induced TGF- β mRNA, upregulated collagen gene expression, and led to the pericellular and perisinusoidal accumulation of fibrous tissue in ob/ob mice. Enhancement in hepatic fibrogenesis occurred despite noradrenaline associated reductions in proinflammatory cytokine production by liver mononuclear cells and accompanying decreases in serum alanine aminotransferase levels. The mechanisms by which noradrenaline promotes hepatic fibrosis are likely to be complex. In addition to its effects on hepatic cytokine production, adrenergic activity is necessary for leptin to activate cultured stellate cells because prazosin, an alpha adrenergic antagonist, partially blocks leptin mediated increases in the growth of cultured stellate cells.⁶⁷

SUMMARY

The pathogenesis of NASH is being unravelled by studies of animal models and humans with this disorder. The necro-inflammatory component of NASH appears to be modulated by interactions among various factors (for example, cytokines, hormones, neurotransmitters) that regulate the biological activity of TNF- α and other proinflammatory (Th-1) cytokines. Hepatic necroinflammation is necessary, but not sufficient, for progression to cirrhosis. Factors such as leptin and certain leptin inducible factors (for example, noradrenaline), that regulate the activity of profibrogenic cytokines, such as IL-10 and TGF- β , dictate the extent of fibrosis that occurs during liver injury. A better understanding of how these and other soluble and cell associated factors regulate the phenotypes of different types of liver cells should help us to develop rationale treatments for NASH and other disorders in the dysmetabolic syndrome.

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REFERENCES

- 1 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003;**37**:1202–19.
- 2 **Marchesini G**, Bugianesi E, Forlani G, *et al*. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003;**37**:917–23.
- 3 **Chituri S**, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 2001;**21**:27–41.
- 4 **Tilg H**, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. *N Engl J Med* 2000;**343**:1467–76.
- 5 **Clark JM**, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003;**98**:960–7.
- 6 **Rajala MW**, Scherer PE. Minireview: The adipocyte—at the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology* 2003;**144**:3765–73.
- 7 **Lyon CJ**, Law RE, Hsueh WA. Minireview: adiposity, inflammation, and atherogenesis. *Endocrinology* 2003;**144**:2195–200.
- 8 **Chaladakov GN**, Stankulov IS, Hristova M, *et al*. Adipobiology of disease: adipokines and adipokine-targeted pharmacology. *Curr Pharm Des* 2003;**9**:1023–31.
- 9 **Katsuki A**, Sumida Y, Urakawa H, *et al*. Increased visceral fat and serum levels of triglyceride are associated with insulin resistance in Japanese metabolically obese, normal weight subjects with normal glucose tolerance. *Diabetes Care* 2003;**26**:2341–4.
- 10 **Lebovitz HE**. The relationship of obesity to the metabolic syndrome. *Int J Clin Pract Suppl* 2003;**134**:18–27.
- 11 **Kelley DE**, McKolanis TM, Hegazi RA, *et al*. Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. *Am J Physiol Endocrinol Metab* 2003;**285**:E906–16.

- 12 **Chalasan N**, Deeg MA, Persohn S, *et al*. Metabolic and anthropometric evaluation of insulin resistance in nondiabetic patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2003;**98**:1849–55.
- 13 **Kim JK**, Fillmore JJ, Chen Y, *et al*. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci U S A* 2001;**98**:7522–7.
- 14 **Tobe K**, Suzuki R, Aoyama M, *et al*. Increased expression of the sterol regulatory element-binding protein-1 gene in insulin receptor substrate-2(–/–) mouse liver. *J Biol Chem* 2001;**276**:38337–40.
- 15 **Misra A**, Garg A. Clinical features and metabolic derangements in acquired generalized lipodystrophy: case reports and review of the literature. *Medicine* 2003;**82**:129–46.
- 16 **Kim JK**, Kim YJ, Fillmore JJ, *et al*. Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest* 2001;**108**:437–46.
- 17 **Hirosumi J**, Tuncman G, Chang L, *et al*. A central role for JNK in obesity and insulin resistance. *Nature* 2002;**420**:333–6.
- 18 **Ruan H**, Lodish HF. Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor- α . *Cytokine Growth Factor Rev* 2003;**14**:447–55.
- 19 **Fernandez-Real JM**, Lopez-Bermejo A, Casamitjana R, *et al*. Novel interactions of adiponectin with the endocrine system and inflammatory parameters. *J Clin Endocrinol Metab* 2003;**88**:2714–18.
- 20 **Ouchi N**, Kihara S, Arita Y, *et al*. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF- κ B signaling through a cAMP-dependent pathway. *Circulation* 2000;**102**:1296–301.
- 21 **Yokota T**, Oritani K, Takahashi I, *et al*. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 2000;**96**:1723–32.
- 22 **Kern PA**, Di Gregorio GB, Lu T, *et al*. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor- α expression. *Diabetes* 2003;**52**:1779–85.
- 23 **Bruun JM**, Lihn AS, Verdich C, *et al*. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. *Am J Physiol Endocrinol Metab* 2003;**285**:E527–33.
- 24 **Arner P**. The adipocyte in insulin resistance: key molecules and the impact of the thiazolidinediones. *Trends Endocrinol Metab* 2003;**14**:137–45.
- 25 **Yamauchi T**, Kamon J, Minokoshi Y, *et al*. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002;**8**:1288–95.
- 26 **Pajvani UB**, Du X, Combs TP, *et al*. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. *J Biol Chem* 2003;**278**:9073–85.
- 27 **Yamauchi T**, Kamon J, Ito Y, *et al*. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003;**423**:762–9.
- 28 **Stefan N**, Stumvoll M. Adiponectin—its role in metabolism and beyond. *Horm Metab Res* 2002;**34**:469–74.
- 29 **Wang Y**, Xu A, Knight C, *et al*. Hydroxylation and glycosylation of the four conserved lysine residues in the collagenous domain of adiponectin. Potential role in the modulation of its insulin-sensitizing activity. *J Biol Chem* 2002;**277**:19521–9.
- 30 **Yamauchi T**, Kamon J, Waki H, *et al*. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipodystrophy and obesity. *Nat Med* 2001;**7**:941–6.
- 31 **Harada K**, Shen WJ, Patel S, *et al*. Resistance to high fat diet-induced obesity and altered expression of adipose-specific genes in HSL-deficient mice. *Am J Physiol Endocrinol Metab* 2003;**285**:E1182–95.
- 32 **Diez JJ**, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur J Endocrinol* 2003;**148**:293–300.
- 33 **Gabrieli I**, Barzilai N. Surgical removal of visceral adipose tissue: effects on insulin action. *Curr Diab Rep* 2003;**3**:201–6.
- 34 **Tsukamoto H**, Lu SC. Current concepts in the pathogenesis of alcoholic liver injury. *FASEB J* 2001;**15**:1335–49.
- 35 **Sasaki Y**, Wands JR. Ethanol impairs insulin receptor substrate-1 mediated signal transduction during rat liver regeneration. *Biochem Biophys Res Commun* 1994;**199**:403–9.
- 36 **Mohr L**, Tanaka S, Wands JR. Ethanol inhibits hepatocyte proliferation in insulin receptor substrate 1 transgenic mice. *Gastroenterology* 1998;**115**:1158–65.
- 37 **Solomon SS**, Mishra SK, Cwik C, *et al*. Pioglitazone and metformin reverse insulin resistance induced by tumor necrosis factor- α in liver cells. *Horm Metab Res* 1997;**29**:379–82.
- 38 **Yu JG**, Javorschi S, Hevener AL, *et al*. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes* 2002;**51**:2968–74.
- 39 **Enomoto N**, Takei Y, Hirose M, *et al*. Prevention of ethanol-induced liver injury in rats by an agonist of peroxisome proliferator-activated receptor-(γ), Pioglitazone. *J Pharmacol Exp Ther* 2003;**306**:846–54.
- 40 **Li Z**, Yang S, Lin H, *et al*. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 2003;**37**:343–50.
- 41 **Xu A**, Wang Y, Keshaw H, *et al*. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003;**112**:91–100.
- 42 **Green RM**. Hepatic metabolism and not simply the metabolic syndrome. *Hepatology* 2003;**38**:14–17.
- 43 **Esposito K**, Pontillo A, Di Palo C, *et al*. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA* 2003;**289**:1799–804.
- 44 **Bugianesi E**, Leone N, Vann E, *et al*. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002;**123**:134–40.
- 45 **Hui JM**, Kench JG, Chitturi S, *et al*. Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. *Hepatology* 2003;**38**:420–7.
- 46 **Matteoni C**, Younossi ZM, McCullough A. Nonalcoholic fatty liver disease: A spectrum of clinical pathological severity. *Gastroenterology* 1999;**116**:1413–19.
- 47 **Martinez-Chantar ML**, Corrales FJ, Martinez-Cruz LA, *et al*. Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A. *FASEB J* 2002;**16**:1292–4.
- 48 **Ip E**, Farrell GC, Robertson G, *et al*. Central role of PPAR α -dependent hepatic lipid turnover in dietary steatohepatitis in mice. *Hepatology* 2003;**38**:123–32.
- 49 **Koteish A**, Diehl AM. Animal models of steatosis. *Semin Liver Dis* 2001;**21**:89–104.
- 50 **Roskams T**, Yang SQ, Koteish A, *et al*. Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease. *Am J Pathol* 2003;**163**:1301–11.
- 51 **Honda H**, Ikejima K, Hirose M, *et al*. Leptin is required for fibrogenic responses induced by thioacetamide in the murine liver. *Hepatology* 2002;**36**:12–21.
- 52 **Saxena NK**, Ikeda K, Rockey DC, *et al*. Leptin in hepatic fibrosis: evidence for increased collagen production in stellate cells and lean littermates of ob/ob mice. *Hepatology* 2002;**35**:762–71.
- 53 **Ikejima K**, Takei Y, Honda H, *et al*. Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat. *Gastroenterology* 2002;**122**:1399–410.
- 54 **Potter JJ**, Mezey E. Leptin deficiency reduces but does not eliminate the development of hepatic fibrosis in mice infected with *Schistosoma mansoni*. *Liver* 2002;**22**:173–7.
- 55 **Leclercq IA**, Farrell GC, Schriemer R, *et al*. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. *J Hepatol* 2002;**37**:206–13.
- 56 **Tang M**, Potter JJ, Mezey E. Leptin enhances the effect of transforming growth factor beta in increasing type I collagen formation. *Biochem Biophys Res Commun* 2002;**297**:906–11.
- 57 **Saxena NK**, Saliba G, Floyd JJ, *et al*. Leptin induces increased α 2(I) collagen gene expression in cultured rat hepatic stellate cells. *J Cell Biochem* 2003;**89**:311–20.
- 58 **Marra F**. Leptin and liver fibrosis: a matter of fat. *Gastroenterology* 2002;**122**:1529–32.
- 59 **Anania FA**. Leptin, liver, and obese mice—fibrosis in the fat lane. *Hepatology* 2002;**36**:246–8.
- 60 **Hoffmann KF**, Cheever AW, Wynn TA. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. *J Immunol* 2000;**164**:6406–14.
- 61 **Li Z**, Lin HZ, Yang SQ, *et al*. Murine leptin deficiency alters Kupffer cell production of cytokines that regulate the innate immune system. *Gastroenterology* 2002;**123**:1304–10.
- 62 **Godfrey DI**, Hammond JL, Poulton LD, *et al*. NKT cells: facts, functions and fallacies. *Immunol Today* 2000;**21**:573.
- 63 **Guebre-Xabier M**, Yang SQ, Lin HZ, *et al*. Altered hepatic lymphocyte subpopulations in obesity-related fatty livers. *Hepatology* 1999;**31**:633–40.
- 64 **Commins SP**, Marsh DJ, Thomas SA, *et al*. Norepinephrine is required for leptin effects on gene expression in brown and white adipose tissue. *Endocrinology* 1999;**140**:4772–8.
- 65 **Takeda S**, Elefteriou F, Levasseur R, *et al*. Leptin regulates bone formation via the sympathetic nervous system. *Cell* 2002;**111**:305–17.
- 66 **Crispe IN**. Do natural T cells promote liver regeneration? *Hepatology* 2000;**31**:1022–4.
- 67 **Oben JA**, Roskams T, Yang S, *et al*. Norepinephrine induces hepatic fibrogenesis in leptin deficient ob/ob mice. *Biochem Biophys Res Commun* 2003;**308**:284–92.