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• *BASIC RESEARCH* •

Leptin administration exacerbates thioacetamide-induced liver fibrosis in mice

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Abstract

AIM: To investigate the effects of leptin administration on liver fibrosis induced by thioacetamide (TAA).

METHODS: Twenty-four male C57Bl/6 mice were randomly allocated into four groups, which were intra-peritoneally given saline (2 mL/kg), leptin (1 mg/kg), TAA (200 mg/kg), TAA (200 mg/kg) plus leptin (1 mg/kg) respectively, thrice a week. All mice were killed after 4 wk. The changes in biochemical markers, such as the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum and superoxide dismutase (SOD), malondialdehyde (MDA) in liver were determined. For histological analysis, liver tissues were fixed with 10% buffered formalin, embedded with paraffin. Hematoxylin-eosin (HE) staining and picric acid-Sirius red dyeing were performed. The level of α1(I) procollagen mRNA in liver tissues was analyzed by RT-PCR.

RESULTS: Apparent liver fibrosis was found in TAA group and TAA plus leptin group. Compared to saline group, the levels of ALT and AST in serum and MDA in liver increased in TAA group (205.67±27.69 U/L *vs* 50.67±10.46 U/L, 177.50±23.65 U/L *vs* 76.33±12.27 U/L, 2.60±0.18 nmol/mg pro *vs* 1.91±0.14 nmol/mg pro, $P<0.01$) and in TAA plus leptin group (256.17±22.50 U/L *vs* 50.67±10.46 U/L, 234.17±27.37 U/L *vs* 76.33±12.27 U/L, 2.97±0.19 nmol/mg pro *vs* 1.91±0.14 nmol/mg pro, $P<0.01$). The level of SOD in livers decreased (51.80±8.36 U/mg pro *vs* 81.52±11.40 U/mg pro, 35.78±6.11 U/mg pro *vs* 81.52±11.40 U/mg pro, $P<0.01$) and the level of α1(I) procollagen mRNA in liver tissues also increased (0.28±0.04 *vs* 0.11±0.02, 0.54±0.07 *vs* 0.11±0.02, $P<0.01$). But no significant changes were found in leptin group and saline group. Compared to TAA group, ALT, AST, MDA, and α1(I) procollagen mRNA and grade of liver fibrosis in TAA plus leptin group increased (256.17±22.50 U/L *vs* 205.67±27.69 U/L, $P<0.05$; 234.17±27.37 U/L *vs* 177.50±23.65 U/L, $P<0.05$; 2.97±0.19 nmol/mg pro *vs* 2.60±0.18 nmol/mg pro, $P<0.05$; 0.54±0.07 *vs* 0.28±0.04, $P<0.01$; 3.17 *vs* 2.00, $P<0.05$), and the level of SOD in liver decreased (35.78±6.11 U/mg pro *vs* 51.80±8.36 U/mg pro, $P<0.05$). There were similar changes in the degree of type I collagen deposition confirmed by picric acid–Sirius red dyeing.

CONCLUSION: Leptin can exacerbate the degree of TAA-induced liver fibrosis in mice. Leptin may be an important factor in the development of liver fibrosis.

Key words: Liver fibrosis; Leptin; α1(I) procollagen

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INTRODUCTION

Leptin, an obese gene product, is a 16-ku peptide hormone expressed and secreted predominantly by adipose tissue[1], and plays an essential role in the regulation of body weight mainly by reducing food intake and increasing energy expenditure[2]. In addition to the effect of controlling body fat mass, leptin has a variety of other biological functions, such as wound healing, angiogenesis, immune response, etc.[3-6], thus exerting its effects on many tissues or organs, including the liver. Potter *et al*.[7], found that isolated hepatic stellate cells (HSCs) also produce leptin during the *in vitro* transactivation process in 1998. Since then researchers have paid more attention to the correlation between leptin and liver diseases. Recently, it was reported that the serum leptin levels are elevated in patients with chronic viral hepatitis, alcohol-induced cirrhosis, or non-alcoholic steatohepatitis (NASH)[8-11]. These observations suggest that leptin may be involved in the progression of liver fibrosis. Accordingly, in the present study we investigated the effect of leptin administration on liver fibrosis caused by thioacetamide (TAA).

MATERIALS AND METHODS

Animals and treatment

Twenty-four, 6-wk-old male C57Bl/6 mice, weighing 18.4-24.2 g, were obtained from Institute of Transplantation, Tongji Medical College, Huazhong University of Science and Technology. All mice were housed in a temperature-humidity-controlled environment in a 12 h light-dark cycle

with free access to food and water. The mice were randomly divided into four groups with six mice in each, which were given an intra-peritoneal injection of saline (2 mL/kg), recombinant murine leptin (1 mg/kg, R&D Systems Inc., USA), TAA (200 mg/kg), TAA (200 mg/kg) plus leptin (1 mg/kg) thrice a week. All mice were killed after 4 wk. Blood and livers were collected for further examination.

Histological examination

Liver tissues were fixed with 10% buffered formalin, embedded with paraffin, and then hematoxylin-eosin (HE) staining and picric acid-Sirius red dyeing were performed. Liver fibrosis was evaluated by a semi-quantitative method to assess the degree of histological injury using the following criteria: grade 0: normal liver; grade 1: few collagen fibrils extended from the central vein to the portal tract; grade 2: apparent collagen fibril extension without encompassing the whole lobule; grade 3: collagen fibrils extended into and encompassed the whole lobule; grade 4: diffuse extension of collagen fibrils and formation of pseudo-lobule.

Estimation of liver function

Blood was obtained at the time of killing. The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured by the Olympus AU-1000 biochemical autoanalyzer, as markers of hepatic damage.

Level of malondialdehyde and superoxide dismutase

Malondialdehyde (MDA) and superoxide dismutase (SOD) contents in liver tissue were assayed by assay kits (Jiancheng Biotech Ltd, Nanjing, China).

RNA extraction and RT-PCR assay

Expression of α1(I) procollagen mRNA was evaluated with RT-PCR. Total RNA was isolated from liver specimens with RNAex reagent (Watson Biotechnologies, Inc., Shanghai, China) according to the manufacturer's descriptions. Total RNA was quantified spectrometrically at 260 nm, and the quality of isolated RNA was analyzed on agarose gels under standard conditions. Two-step RT-PCR was performed as recommended by the suppliers. Primer sequences were α1 (I) procollagen: forward 5'-CCT GGA CGC CAT CAA GGT CTA C-3' and reverse 5'-CCA AGT TCC GGT GTG ACT CG-3', fragment length 419 bp; β-actin: forward 5'-ACC ACA GCT GAG AGG GAA ATC G-3' and reverse 5'-AGA GGT CTT TAC GGA TGT CAA CG-3', fragment length 277 bp. Amplification conditions were as follows: pre-denaturation at 95 ℃ for 2 min, then in a thermal controller for 35 cycles (denaturation at 95 ℃ for 45 s, annealing at 56 ℃ for 45 s and extension at 72 ℃ for 1 min), and a final extension at 72 ℃ for 7 min after the last cycle. Ten milliliters of the PCR products was analyzed on 2% agarose gel containing ethidium bromide with TAE buffer at 80 V for 30 min and photographed under UV illumination. The band intensities were quantified by densitometry. α1 (I) procollagen/β-actin quotient indicated the relative expression of α1(I) procollagen.

Statistical analysis

The results were expressed as mean±SD. One-way analysis of variance (ANOVA) with LSD post hoc comparison was used to test for differences in means of variables between groups. $P<0.05$ was considered statistically significant. All data were analyzed by SPSS 11.0 software.

RESULTS

Animal model and liver histology

Compared to saline group and leptin group, the mice in TAA group and TAA plus leptin group were thin and less haired. The surface of liver was rough and formation of small nodules was observed, indicating that liver fibrosis developed as expected. The degree of type I collagen deposition was confirmed by liver histology stained with picric acid-Sirius red. As anticipated, liver tissue specimens from both the saline group and leptin group showed no significant picric acid-Sirius red staining, indicating a lack of type I collagen deposition outside of central and portal blood vessels. Apparent hepatocyte degeneration, necrosis, infiltration of inflammatory cells, expanded portal tracts and collagen deposition were found in TAA group and TAA plus leptin group. The degree of liver fibrosis in these two groups significantly increased compared to that in saline group or leptin group ($P<0.01$). But compared to TAA group, the deposition of type I collagen in TAA plus leptin group was more apparent and the degree of liver fibrosis also increased ($P<0.05$). The details about the degree of liver fibrosis in each group are shown in Table 1. Type I collagen deposition in each group is shown in Figure 1.

Serum ALT and AST level

At the end of the study, there was no difference in serum ALT and AST levels between saline group and leptin group. But serum ALT and AST levels in TAA group and TAA plus leptin group significantly increased compared to those in saline group and leptin group ($P<0.01$). Furthermore, treatment with TAA plus leptin increased the ALT and AST levels compared to treatment with TAA ($P<0.05$, Table 2).

Table 1 Degree of liver fibrosis in each group of mice

Group	*n*	Grade of liver fibrosis					
		0	I	II	III	IV	Average
Saline	6	6	0	0	0	0	0
Leptin	6	6	0	0	0	0	0
TAA	6	0	1	4	1	0	2.00[b,d]
TAA+leptin	6	0	0	0	5	1	3.17[a,b,d]

[a]$P<0.05$ *vs* TAA group; [b]$P<0.01$ *vs* saline group; [d]$P<0.01$ *vs* leptin group.

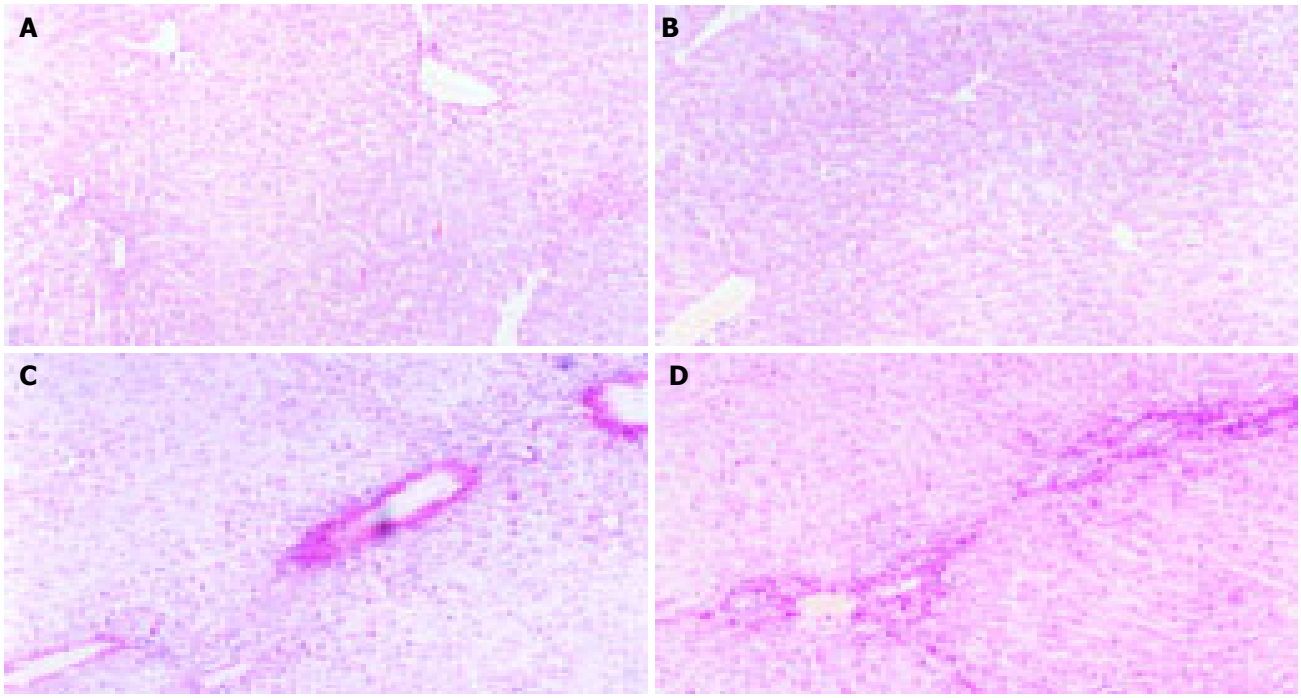

Figure 1 Type I collagen deposition in saline group (**A**), leptin group (**B**), TAA (**C**), and TAA plus leptin group (**D**). 200× (red color indicates picric acid-Sirius red staining for collagen).

Table 2 Serum ALT and AST level (mean±SD)

Group	*n*	ALT (U/L)	AST (U/L)
Saline	6	50.67±10.46	76.33±12.27
Leptin	6	47.50±11.78	71.67±14.39
TAA	6	205.67±27.69[b,d]	177.50±23.65[b,d]
TAA+leptin	6	256.17±22.50[a,b,d]	234.17±27.37[a,b,d]

[a]*P*<0.05 *vs* TAA group; [b]*P*<0.01 *vs* saline group; [d]*P*<0.01 *vs* leptin group.

Table 4 Level of α1(I) procollagen mRNA in relation to β-actin (mean±SD)

Group	*n*	Ratio
Saline	6	0.11±0.02
Leptin	6	0.13±0.02
TAA	6	0.28±0.04[b,d]
TAA+leptin	6	0.54±0.07[b,d,f]

[b]*P*<0.01 *vs* saline group; [d]*P*<0.01 *vs* leptin group; [f]*P*<0.01 *vs* TAA group.

Changes of MDA and SOD in liver tissue

The amount of MDA and SOD did not obviously change in leptin group, compared to that in the saline group. The amount of MDA in TAA group and TAA plus leptin group was significantly higher than that in saline group (*P*<0.01), while SOD in the two groups was significantly lower than that in saline group (*P*<0.01). But MDA was higher in TAA plus leptin group than in TAA group (*P*<0.05), while SOD was lower in TAA plus leptin group than in TAA group (*P*<0.05, Table 3).

Table 3 Changes of MDA and SOD in liver tissue (mean±SD)

Group	*n*	SOD (U/mg pro)	MDA (nmol/mg pro)
Saline	6	81.52±11.40	1.91±0.14
Leptin	6	83.63±10.68	1.93±0.13
TAA	6	51.80±8.36[b,d]	2.60±0.18[b,d]
TAA+leptin	6	35.78±6.11[a,b,d]	2.97±0.19[a,b,d]

[a]*P*<0.05 *vs* TAA group; [b]*P*<0.01 *vs* saline group; [d]*P*<0.01 *vs* leptin group.

α1(I) procollagen mRNA expression

To determine the expressions of collagen, mRNA transcripts for α1(I) procollagen were assessed. RNA extracts from livers were analyzed by reverse transcription PCR. Furthermore, the band intensity ratio of α1(I) procollagen to β-actin was evaluated and presented as percentage of β-actin. As shown in Table 4 and Figure 2, the expression of α1(I) procollagen in leptin group did not obviously change, compared to that in the saline group, but significantly increased in TAA group and TAA plus leptin group (*P*<0.01). The expression of α1(I) procollagen in TAA plus leptin group was much higher than that in TAA group (*P*<0.01, Table 4 and Figure 2).

DISCUSSION

In the present study, chronic administration of TAA or TAA plus leptin caused liver fibrosis as indicated by the changes of serum markers, histopathological changes, and molecular biological changes. But more overt liver fibrosis was observed, when leptin was used in combination with TAA. Our results are in line with other findings that leptin-deficient *ob/ob* mice do not develop fibrosis during steatohepatitis or in response to chronic toxic liver injury[12,13], indicating that leptin can exacerbate the degree of fibrosis in mouse liver induced by TAA, and may be an important

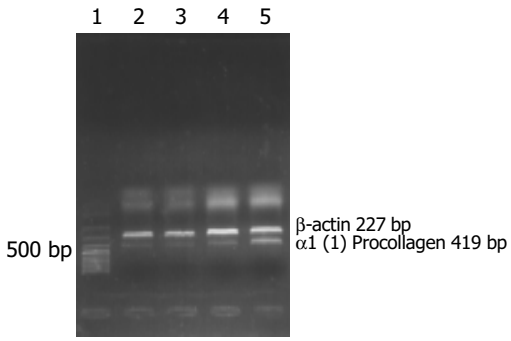

Figure 2 Expression of α1(I) procollagen mRNA. M: 100-bp DNA marker; lane 1: saline group; lane 2: leptin group; lane 3: TAA group; and lane 4: TAA plus leptin group.

factor in the development of liver fibrosis.

The activities of serum fibrosis-associated enzymes, namely ALT and AST, significantly increased along with the increased expression of α1(I) procollagen mRNA in TAA group and TAA plus leptin group. But leptin could augment TAA-induced inflammatory response and increase the expression of α1(I) procollagen mRNA. The histological examination also found that leptin increased the TAA-induced deposition of type I collagen. Specific increase in procollagen mRNAs in liver fibrosis parallels increase in tissue collagen content. Since increased synthesis of procollagen contributes to the increase of collagen content in fibrotic liver, the expression of α1(I) procollagen mRNA can reflect the degree of hepatic fibrosis[14,15]. Activation of HSCs is the essential event in hepatic fibrogenesis, because HSCs transactivate to myofibroblast-like cells that produce a large, excess amount of matrix proteins (e.g., fibrillar collagen, fibronectin, laminin, and proteoglycans) in injured liver[16]. It was reported that HSCs express short forms of the leptin receptor (Ob-Ra), but not the functional long form of the leptin receptor Ob-Rb. Since the sinusoidal endothelial cells and Kupffer cells in the liver express the long form of the leptin receptor Ob-Rb[17,18], leptin may exert an indirect effect on the activation of HSCs mediated by TGF-β_1. As Ob-Rb leptin can increase TGF-β_1 expression in the sinusoidal endothelial cells and Kupffer cells, TGF-β_1 activates type I collagen promoters, and upregulates the extracellular matrix such as collagen and fibronectin from HSCs[19]. Recently, it was reported that leptin also can activate HSCs directly[20]. However, the role of leptin in the activation of HSCs needs to be further analyzed.

TAA is a typical hepatotoxin and causes centrilobular necrosis by generation of ROS. ROS triggers lipid peroxidation chain reaction. MDA is the production of lipid peroxidation, the amount of MDA can express the degree of lipid peroxidation. SOD is a ubiquitous chain breaking anti-oxidant found in all aerobic organisms. It is a metalloprotein widely distributed in all cells and protects cells against ROS-induced oxidative damage. Oxidative stress contributes to the pathogenesis of hepatic fibrosis induced by alcohol and virus infection[21,22]. Furthermore, oxidative stress is involved in activation of HSCs and promotion of their proliferation, collagen synthesis, and migration[23]. The activation of quiescent HSCs plays an important role in the process of liver fibrosis[16,24]. In our experiment, MDA was higher in TAA plus leptin group than in TAA group ($P<0.05$), while SOD was lower in TAA plus leptin group than in TAA group. The results suggest leptin can augment TAA-induced lipid peroxidation by lowering the level of enzymic antioxidant. But leptin administration alone has no apparent effect on lipid peroxidation. Whether leptin is just a co-factor needs to be further studied. On the other hand, leptin can regulate macrophage function[25] and augment endotoxin-stimulated TNF production by macrophages[3]. Hematopoietic cells including macrophages and lymphocytes contain a functional leptin receptor[26], through which leptin regulates proliferation and maturation of immune cells[27,28] and elicits release of pro-inflammatory cytokines from macrophages[3]. These pro-inflammatory cytokines and immune responses play a critical role in the development of liver fibrosis. The above findings may explain, at least in part, why leptin increases the degree of liver fibrosis in mice induced by TAA.

It has been reported that hepatic cirrhosis is six fold more prevalent in obese individuals than in the general population[29,30], and obesity is an independent risk factor for the development of chronic liver diseases caused by alcohol, chronic hepatitis C, and NASH[31-34]. These observations indicate that there could be a pathogenic link between obesity and liver fibrosis. But the reason why obesity accelerates the development of hepatic fibrosis has not been elucidated. Circulating levels of leptin correlate well with body fat mass[35]. Obese people have remarkably high serum leptin levels. Increased serum leptin presumably enhances pro-inflammatory and pro-fibrogenic responses in the liver, and generate oxidative stress. Leptin may be a determinant of hepatic inflammation and fibrosis in obese individuals, especially in those with NASH. Our findings support the hypothesis that leptin is involved in the progression of liver fibrosis. However, additional studies are needed to elucidate the role of leptin in the progression of chronic liver fibrosis in obese patients.

REFERENCES

1 **Zhang Y,** Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425-432

2 **Halaas JL,** Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995; **269**: 543-546

3 **Loffreda S,** Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD, Diehl AM. Leptin regulates proinflammatory immune responses. *FASEB J* 1998; **12**: 57-65

4 **Goren I,** Kampfer H, Podda M, Pfeilschifter J, Frank S. Leptin and wound inflammation in diabetic ob/ob mice: differential regulation of neutrophil and macrophage influx and a potential role for the scab as a sink for inflammatory cells and mediators. *Diabetes* 2003; **52**: 2821-2832

5 **Murad A,** Nath AK, Cha ST, Demir E, Flores-Riveros J, Sierra-Honigmann MR. Leptin is an autocrine/paracrine regulator of wound healing. *FASEB J* 2003; **17**: 1895-1897

6 **Frankenberry KA,** Somasundar P, McFadden DW, Vona-Davis LC. Leptin induces cell migration and the expression of growth factors in human prostate cancer cells. *Am J Surg*

2004; **188**: 560-565
7 **Potter JJ,** Womack L, Mezey E, Anania FA. Transdifferentiation of rat hepatic stellate cells results in leptin expression. *Biochem Biophys Res Commun* 1998; **244**: 178-182
8 **Henriksen JH,** Holst JJ, Moller S, Brinch K, Bendtsen F. Increased circulating leptin in alcoholic cirrhosis: relation to release and disposal. *Hepatology* 1999; **29**: 1818-1824
9 **Testa R,** Franceschini R, Giannini E, Cataldi A, Botta F, Fasoli A, Tenerelli P, Rolandi E, Barreca T. Serum leptin levels in patients with viral chronic hepatitis or liver cirrhosis. *J Hepatol* 2000; **33**: 33-37
10 **Wang XQ,** Zhang WW, Li Y. Serum leptin levels in patients with liver cirrhosis. *Shijie Huaren Xiaohua Zazhi* 2002; **10**: 1177-1179
11 **Chalasani N,** Crabb DW, Cummings OW, Kwo PY, Asghar A, Pandya PK, Considine RV. Does leptin play a role in the pathogenesis of human nonalcoholic steatohepatitis? *Am J Gastroenterol* 2003; **98**: 2771-2776
12 **Leclercq IA,** Farrell GC, Schriemer R, Robertson GR. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. *J Hepatol* 2002; **37**: 206-213
13 **Honda H,** Ikejima K, Hirose M, Yoshikawa M, Lang T, Enomoto N, Kitamura T, Takei Y, Sato N. Leptin is required for fibrogenic responses induced by thioacetamide in the murine liver. *Hepatology* 2002; **36**: 12-21
14 **Goddard CJ,** Smith A, Hoyland JA, Baird P, McMahon RF, Freemont AJ, Shomaf M, Haboubi NY, Warnes TW. Localisation and semiquantitative assessment of hepatic procollagen mRNA in primary biliary cirrhosis. *Gut* 1998; **43**: 433-440
15 **Pierce RA,** Glaug MR, Greco RS, Mackenzie JW, Boyd CD, Deak SB. Increased procollagen mRNA levels in carbon tetrachloride-induced liver fibrosis in rats. *J Biol Chem* 1987; **262**: 1652-1658
16 **Friedman SL.** Seminars in medicine of the Beth Israel Hospital, Boston. The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. *N Engl J Med* 1993; **328**: 1828-1835
17 **Ikejima K,** Takei Y, Honda H, Hirose M, Yoshikawa M, Zhang YJ, Lang T, Fukuda T, Yamashina S, Kitamura T, Sato N. Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat. *Gastroenterology* 2002; **122**: 1399-1410
18 **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**: 906-911
19 **Penttinen RP,** Kobayashi S, Bornstein P. Transforming growth factor ß increases mRNA for matrix proteins both in the presence and in the absence of changes in mRNA stability. *Proc Natl Acad Sci USA* 1988; **85**: 1105-1108
20 **Saxena NK,** Saliba G, Floyd JJ, Anania FA. Leptin induces increased alpha2(I) collagen gene expression in cultured rat hepatic stellate cells. *J Cell Biochem* 2003; **89**: 311-320
21 **Kurose I,** Higuchi H, Miura S, Saito H, Watanabe N, Hokari R, Hirokawa M, Takaishi M, Zeki S, Nakamura T, Ebinuma H, Kato S, Ishii H. Oxidative stress-mediated apoptosis of hepatocytes exposed to acute ethanol intoxication. *Hepatology* 1997; **25**: 368-378
22 **Lieber CS.** Alcohol and hepatitis C. *Alcohol Res Health* 2001; **25**: 245-254
23 **Lee KS,** Lee SJ, Park HJ, Chung JP, Han KH, Chon CY, Lee SI, Moon YM. Oxidative stress effect on the activation of hepatic stellate cells. *Yonsei Med J* 2001; **42**: 1-8
24 **Okuyama H,** Shimahara Y, Kawada N. The hepatic stellate cell in the post-genomic era. *Histol Histopathol* 2002; **17**: 487-495
25 **Lee FY,** Li Y, Yang EK, Yang SQ, Lin HZ, Trush MA, Dannenberg AJ, Diehl AM. Phenotypic abnormalities in macrophages from leptin-deficient, obese mice. *Am J Physiol* 1999; **276**: C386-394
26 **Bennett BD,** Solar GP, Yuan JQ, Mathias J, Thomas GR, Matthews W. A role for leptin and its cognate receptor in hematopoiesis. *Curr Biol* 1996; **6**: 1170-1180
27 **Gainsford T,** Willson TA, Metcalf D, Handman E, McFarlane C, Ng A, Nicola NA, Alexander WS, Hilton DJ. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. *Proc Natl Acad Sci USA* 1996; **93**: 14564-14568
28 **Santos-Alvarez J,** Goberna R, Sanchez-Margalet V. Human leptin stimulates proliferation and activation of human circulating monocytes. *Cell Immunol* 1999; **194**: 6-11
29 **Ratziu V,** Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 2000; **118**: 1117-1123
30 **McCullough AJ,** Falck-Ytter Y. Body composition and hepatic steatosis as precursors for fibrotic liver disease. *Hepatology* 1999; **29**: 1328-1330
31 **Chitturi S,** Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 2001; **21**: 27-41
32 **Naveau S,** Giraud V, Borotto E, Aubert A, Capron F, Chaput JC. Excess weight risk factor for alcoholic liver disease. *Hepatology* 1997; **25**: 108-111
33 **Hourigan LF,** Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, Powell EE. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 1999; **29**: 1215-1219
34 **Adinolfi LE,** Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; **33**: 1358-1364
35 **Frederich RC,** Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* 1995; **1**: 1311-1314

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