TNF-α Induces Hepatic Steatosis in Mice by Enhancing Gene Expression of Sterol Regulatory Element Binding Protein-1c (SREBP-1c)

MIZUKI ENDO, TAKAYUKI MASAKI, MASATAKA SEIKE, AND HIRONOBU YOSHIMATSU[1]

Department of Internal Medicine, Faculty of Medicine, Oita University, Hasama, Oita, 879-5593 Japan

We investigated the effect of tumor necrosis factor-α (TNF-α), a member of the proinflammatory cytokine family, on steatosis of the mouse liver by analyzing morphological changes and hepatic triglyceride content in response to TNF-α. We also examined expression of the sterol regulatory element binding protein-1c gene. Intraperitoneal injection of TNF-α acutely and dramatically accelerated the accumulation of fat in the liver, as evidenced by histological analysis and hepatic triglyceride content. This treatment increased liver weight, increased serum levels of free fatty acids, and increased fatty acid synthase and sterol regulatory element binding protein-1c mRNA expression. Furthermore, intraperitoneal injection of lipopolysaccaride (LPS) to induce TNF-α expression also accelerated hepatic fat accumulation. Pretreatment with anti-TNF-α antibody attenuated the development of LPS-induced fatty change in the liver. Antibody pretreatment not only decreased sterol regulatory element binding protein-1c expression in LPS-treated mice but also attenuated the expression of suppressors of cytokine signaling-3 mRNA. This study suggests that TNF-α, acting downstream of LPS, increases intrahepatic fat deposition by affecting hepatic lipogenetic metabolism involving sterol regulatory element binding protein-1c. Exp Biol Med 232:614–621, 2007

Key words: TNF-α; LPS; liver; SREBP-1c

Introduction

Proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), have been suggested to cause obesity-related metabolic disorders, including insulin resistance (1). In addition to direct impairment of insulin signaling (2–4), lipotoxicity induced by fat accumulation in the liver also leads to hepatic insulin resistance (5–7). TNF-α may thus affect hepatic lipid metabolism. TNF-α treatment has been shown to accelerate hepatic triglyceride production and hyperlipidemia (8–11). However, the molecular mechanisms underlying TNF-α–induced fatty liver disease are not clear. A previous study suggested that TNF increases triglyceride (TG) production in the liver by providing an increased amount of fatty acids as substrate, since TNF did not increase the activity of triglyceride synthesis enzymes (10).

Fat accumulation in the liver is regulated by a number of lipogenetic and lipolytic factors. In particular, fatty acid synthase (FAS) and sterol regulatory element binding protein-1c (SREBP-1c) play important roles in lipogenetic processes in the liver (12–14). FAS is an enzyme necessary for *de novo* synthesis of fatty acids, which is regulated both transcriptionally and post-transcriptionally in response to nutrients and hormones (14). SREBP-1c, a transcription factor integral to maintaining lipid homeostasis, regulates gene expression related to fatty acid metabolism, including expression of FAS (12, 13, 15). Several studies have demonstrated that these lipogenetic processes are influenced by obesity and insulin-resistant conditions (16–18). Recently, suppressors of cytokine signalling proteins (SOCS) have been suggested to play pathogenetic roles in obesity-related metabolic disorders, including insulin resistance and fatty liver disease, by affecting cytokine signaling (19, 20). Overexpression of SOCS-1 and SOCS-3 in the liver has been shown to induce insulin resistance accompanied by elevation of SREBP-1c (19). This study suggests that proinflammatory cytokines, including TNF-α, may affect steatosis of the liver by modulating SOCS and SREBP-1c.

This work was supported by Grants-in-Aid 136770067 from the Japanese Ministry of Education, Science and Culture, and by Research Grants for Intractable Diseases from the Japanese Ministry of Health and Welfare. This work was also supported in part by a grant from the Smoking Research Foundation, venture business laboratory in Oita University.

[1] To whom correspondence should be addressed at Department of Internal Medicine, Faculty of Medicine, Oita University, 1-1 Idaigaoka,Yufu-Hasama, Oita, 879-5593 Japan. E-mail: EMIZUKI@med.oita-u.ac.jp

Received October 23, 2006.
Accepted January 2, 2007.

1535-3702/07/2325-0614$15.00

However, it is still not clear how TNF-α actually affects the development of fatty liver *in vivo*. To address this issue, we examined the acute effects of TNF-α on hepatic lipid metabolism by analyzing lipogenetic and lipolytic markers in the liver. In addition, we examined the effect of TNF-α inhibition on lipopolysaccaride (LPS)-induced fatty changes in the liver.

Materials and Methods

Animals. Mice (C57BL/6, Seac Yoshitomi Ltd, Fukuoka, Japan) weighing 20–27 g at 10–11 weeks of age, were housed in a room illuminated daily from 0700–1900 hrs (12:12-hr light:dark cycle) at constant temperature (21°C ± 1°C) and humidity (55% ± 5%). The mice were allowed free access to standard mice chow pellets (CLEA Japan Ltd., Tokyo, Japan) and tap water. The animals were treated humanely in accordance with the Oita Medical University Guidelines for the Care and Use of Laboratory Animals.

Reagents. Recombinant human TNF-α (Dainippon Pharmacy Co. Ltd., Tokyo, Japan) was dissolved in saline to a final concentration of 50 μg/ml, anti-TNF-α antibody (Merck & Co., Whitehouse Station, NJ) was dissolved in saline to a final concentration of 1 mg/ml, and the LPS (Sigma Chemical Co., St. Louis, MO) was dissolved in saline to a final concentration of 1 mg/ml. The pH of each solution was adjusted to 6.8–7.4, and each solution was freshly prepared on the day of use.

TNF-α and Anti-TNF-α Antibody Treatment. After being matched according to body mass and food intake during a pre-experimental adaptation period, the mice were divided into two groups. One group was treated with recombinant human TNF-α, and the other group (control) was treated with saline. TNF-α was injected ip at 0.166 mg/kg, and the controls received the same volume of saline at the same time of day (1500 hrs). This TNF-α dose, determined based on a preliminary dosage study, causes necroinflammatory changes in the liver and is relevant to clinical conditions such as sepsis. Peak serum TNF-α levels were reached 3 hrs following treatment during our experimental period (3, 6, 9, 24 hrs after treatment). To avoid any influence of food consumption on lipid metabolism, food was withdrawn after the injection. Mice were decapitated after their body weights were measured at 6 hrs (2100 hrs) or 24 hrs (1500 hrs, the next day) following TNF-α injection.

Another set of mice was divided into four groups. The animals were pretreated with either 3.3 mg/kg anti-TNF-α antibody or the same volume of physiological saline by ip injection 30 mins before (1430 hrs) the administration of either 3.3 mg/kg LPS or the same volume of saline (1500 hrs). Food was withdrawn after the second injection. Mice were decapitated after their body weights were measured 6 hrs (2100 hrs) or 24 hrs (1500 hrs, the next day) following LPS injection. As with TNF-α, the doses of LPS and anti-TNF-α antibody were based on our preliminary dosage study (data not shown).

Tissue and Blood Sampling. Blood samples were obtained following decapitation at 3 hrs, 6 hrs, 9 hrs, and 24 hrs post-TNF-α treatment. Plasma was isolated, immediately frozen at −20°C, and stored until analysis. The livers were surgically removed and weighed, and the samples were immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction. Levels of serum glucose, insulin, TG, and free fatty acids were measured by using commercially available assay kits (WAKO Chemical, Tokyo, Japan).

Histological Analysis. Liver samples were fixed with 10% formalin and embedded in paraffin. Sections measuring 5 μm were cut and stained with hematoxylin/eosin (HE) and Sudan III. Liver histology was examined using the analysis system (Olympus, Tokyo, Japan).

Liver Triglycerides. Liver (100 mg) was homogenized in 2 ml of solution containing 150 m*M* NaCl, 0.1% Triton X-100, and 10 m*M* Tris using a polytron homogenizer (NS-310E; Micro Tech Nichion, Chiba, Japan) for 1 min. The TG content of this 100-μl solution was determined by using a commercially available kit (WAKO). To normalize the samples by protein content, the protein concentration of each sample was determined using a protein assay kit (Bio-Rad Laboratories, Hercules, CA).

Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction. Liver FAS, SREBP-1c, PPAR-α, and SOCS3 mRNAs were detected by polymerase chain reaction (PCR) amplification and quantified by real-time quantitative PCR. Total cellular RNA was prepared from mouse tissue using TRIzol (Lifetech, Tokyo, Japan) according to the manufacturer's protocol. Total RNA (20 μg) was separated by electrophoresis on 1.2% formaldehyde-agarose gels. RNA quality and quantity were assessed by EtBr staining and by measuring the relative absorbance at 260 nm versus 280 nm. cDNA was synthesized from 150 ng of total RNA in a volume of 20 μl with the ReverTra Dash reverse transcriptase kit (Toyobo, Tokyo, Japan) using random hexamer primers. Reactions were diluted to 50 μl with sterile distilled H_2O and stored at −20°C. Primers for genes were designed, synthesized, optimized, and provided as preoptimized kits: FAS (Cat No. Mm00662319m1), SREBP1-c (primers generated by Nihon Gene Research Laboratory, Sedai, Miyagi, Japan), PPAR-α (Catalog No. Mm00440939m1), SOCS3 (Catalog No. Mm00545913-s1). Primers for ribosomal RNA as internal controls were also provided as a preoptimized kit (Catalog No. Hs99999901). Using an ABI PRISM 7000 sequence detector, PCR amplifications were performed in 50-μl volumes containing 100 ng cDNA template in 2× PCR Master Mix (Roche, Branchburg, NJ) according to the following program: 50°C for 2 mins; 95°C for 10 mins; 40 cycles at 95°C for 15 secs, and 60°C for 1 min. Duplicate samples were processed. Results were analyzed with Sequence Detection Software (ABI), and expression levels of FAS, SREBP-1c, and SOCS3 mRNAs

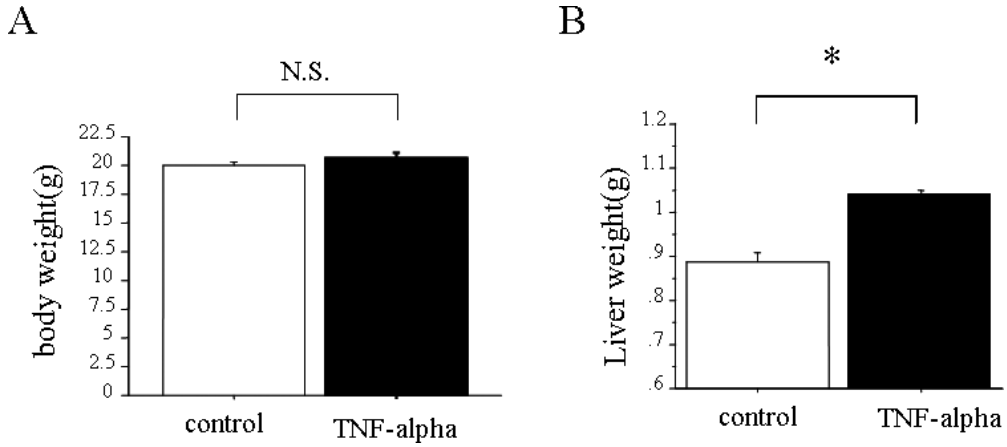

Figure 1. Body weight (A) and liver weight (B) 24 hrs after intraperitoneal injection of TNF-α at a dose of 0.166 mg/kg (TNF-α) or injection with the same volume of saline (control). Values and vertical bars: mean ± SEM ($n = 4$–8 for each). * $P < 0.05$ versus the corresponding controls.

were normalized to ribosomal RNA, as outlined in Perkin-Elmer's User Bulletin No. 2.

Statistical Analysis. All data are expressed as mean ± SEM. We used ANOVA to analyze differences for multiple comparisons (StatView 4.0, Abacus Concepts, Berkeley, CA); when appropriate, we used the Mann-Whitney *U* test.

Results

Effect of TNF-α on Body and Liver Weight. As shown in Figure 1A, administration of TNF-α at a dose of 0.166 mg/kg did not induce changes in body weight compared with the controls (Fig. 1A, $P > 0.1$). In contrast, this dose of TNF-α acutely increased liver weight, even under fasting conditions ($P < 0.05$) (Fig. 1B).

Effect of TNF-α on Serum Levels of Blood Glucose (BG), Insulin, TG, and Free Fatty Acids (FFA). We investigated the effect of TNF-α on serum levels of BG, insulin, TG, and FFA at 3 hrs, 6 hrs, 9 hrs, and 24 hrs after treatment. TNF-α treatment increased serum FFA levels compared with controls 6 hrs after treatment ($P < 0.05$) (Fig. 2D). However, serum levels of BG, insulin, and TG showed no remarkable change 6 hrs after TNF-α treatment (Fig. 2A, B, and C). In addition, no significant change in serum levels of BG, insulin, TG, and FFA were found at 3 hrs, 9 hrs, and 24 hrs after treatment.

Effect of TNF-α on Intrahepatic TG Content and Liver Histology. TNF-α treatment significantly increased liver TG content compared with controls ($P < 0.05$) (Fig. 3B). Histological analysis using HE and SUDAN-III staining also showed fat accumulation in the liver induced by TNF-α administration (Fig. 3A).

Effect of TNF-α on Hepatic FAS, PPAR-α, and SREBP-1c mRNA Expression. After TNF-α treatment, liver SREBP-1c, and FAS mRNA expression increased 1.4-fold and 1.8-fold, respectively, compared with controls ($P < 0.05$ for each) (Fig. 4). Peroxisome proliferator–activated receptor mRNA-α expression was not significantly changed after TNF-α treatment (control, 100.0% ± 4.1%; TNF-α, 109% ± 5.9%) ($P > 0.1$).

Effect of Anti-TNF-α Antibody on LPS-Induced Fatty Change in the Liver. Administration of LPS, similar to administration of TNF-α, accelerated fat accumulation in the liver as assessed by liver TG content ($P < 0.05$). This LPS-induced change in the liver was attenuated by pretreatment with anti-TNF-α antibody (control, 9.6 mg/dl ± 1.8 mg/dl; LPS, 29.5 mg/dl ± 6.9 mg/dl; LPS–anti-TNF-α, 12 mg/dl ± 4.0 mg/dl [control vs. LPS, $P < 0.05$; LPS vs. LPS–anti-TNF-α, $P < 0.05$]) (Fig. 5B). The attenuating effect of anti-TNF-α antibody was confirmed by histological analysis (Fig. 5A). SREBP1-c mRNA expression in LPS-treated mice decreased 0.8-fold after pretreatment with anti-TNF-α antibody (control, 100.0% ± 8.0%; LPS, 102.0% ± 8.1%; LPS–anti-TNF-α, 76.8% ± 1.8% [LPS vs. LPS–anti-TNF-α, $P < 0.05$]). FAS mRNA expression was not significantly changed. In addition, SOCS3 mRNA expression was attenuated by pretreatment with anti-TNF-α antibody (control, 100.0% ± 9.8%; LPS, 139% ± 11.7%; LPS–anti-TNF-α, 101% ± 8.6% [control vs. LPS, $P < 0.05$; LPS vs. LPS–anti-TNF-α, $P < 0.05$]) (Fig. 6).

Discussion

The present study demonstrates that TNF-α treatment in mice increases liver weight and hepatic TG levels. Histological analysis of TNF-α–treated mice by SUDAN-III staining also showed fatty changes in the liver tissue. These findings indicate that TNF-α treatment acutely accelerates the development of fatty liver in mice. Hypertriglyceridemia is often observed in patients or animals with fatty livers, reflecting the increased secretion of TG-rich lipoprotein from the liver (21–23). In fact, a previous study showed that TNF-α induced an increase in hepatic TG production, causing hyperlipidemia (24). However, this TNF-α–induced response was not observed in fasted animals, indicating that TNF-α effects are influenced by

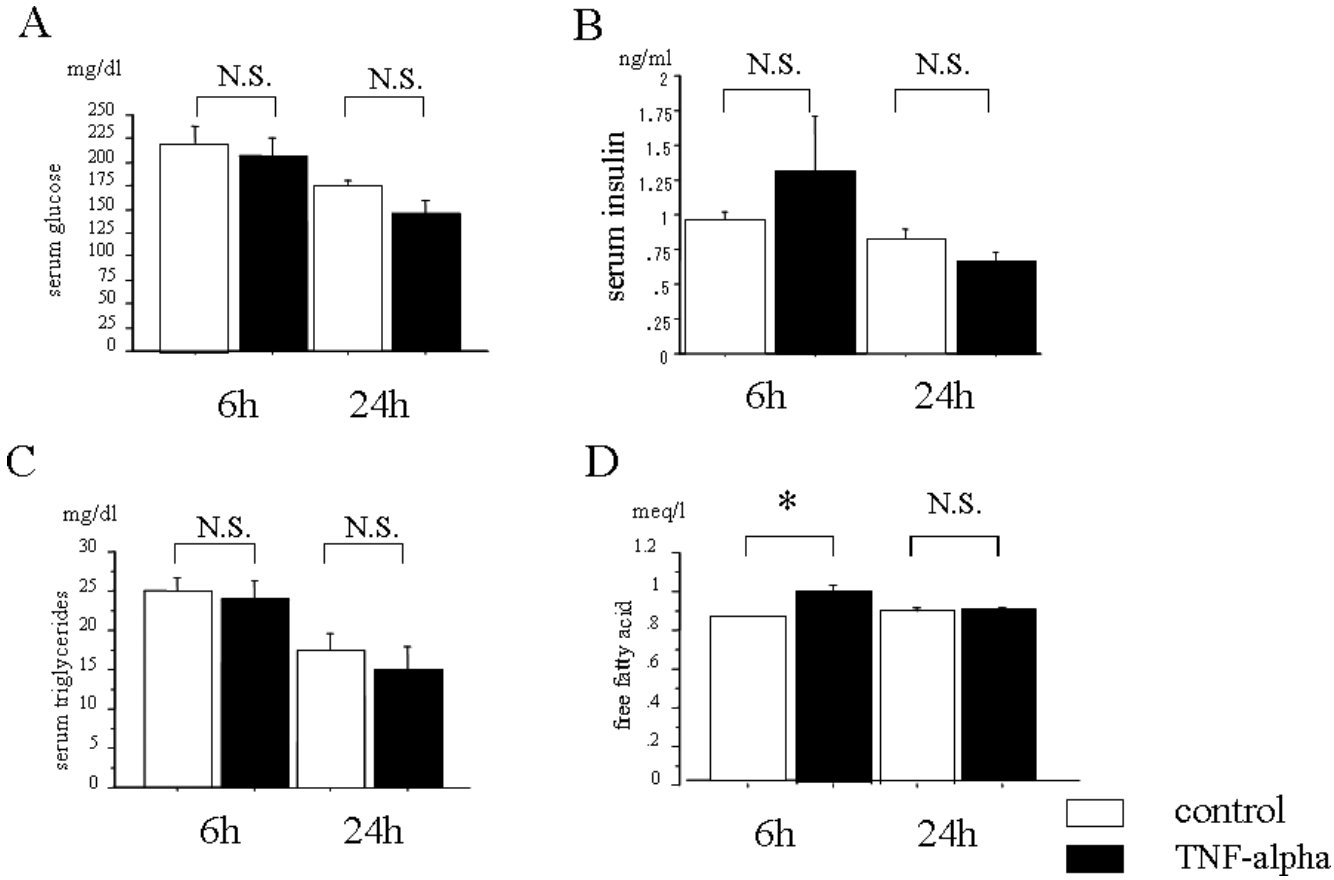

Figure 2. Serum glucose (A), serum insulin (B), serum triglycerides (C), and free fatty acid (D) levels at 6 hrs and 24 hrs after ip injection of TNF-α at a dose of 0.166 mg/kg (TNF-α) or injection with the same volume of saline (control). Values and vertical bars: mean ± SEM ($n = 4$ for each). * $P < 0.05$ versus the corresponding controls.

dietary factors (9). In the present study, serum TG content showed no response to acute TNF-α treatment. It is difficult to explain the difference in the serum TG responses. We speculate that the acute effect of the TNF-α treatment in our study may be restricted to fatty acid synthesis and TG production and may not influence very-low-density-lipoprotein secretion or insulin resistance.

We observed that serum FFA levels increased after TNF-α treatment. This result is consistent with a previous study showing that TNF-α treatment increases serum FFA level by activation of lipolysis in adipose tissue (25–27). It was also demonstrated that pretreatment with an antilipolytic drug prevented the TNF-induced increase in serum FFA (10). Taken together, these data indicate that TNF-α treatment induces lipolysis in adipose tissue and lipogenesis in the liver. However, in the present study, only large doses of TNF-α were used, and the results were similar to severe sepsis. Thus, further studies are needed to investigate the involvement of plasma and intrahepatic TNF-α in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis (NASH). Previous studies demonstrated that obese patients with nonalcoholic steatohepatitis presented with increased expression of TNF-α mRNA in the liver (28). In addition, plasma TNF-α concentration was also elevated in patients with NASH (29). Furthermore, genetically obese mice with NASH enhanced intestinal permeability leading to increased portal endotoxemia (30). These findings suggest that the LPS and TNF-α system may be involved in the pathogenesis of NASH in human and rodent.

The next important question we addressed was how TNF-α accelerates the development of fatty liver. A previous study showed that TG production was increased by the delivery of fatty acids from adipose tissue to the liver but not by activation of TG synthetic enzymes, such as diacylglycerol acyltransferase (10). In the study using sucrose-fed rats, TNF-α treatment was shown to stimulate hepatic *de novo* FFA synthesis, which provides FFA. Thus, the mechanism by which TNF-α stimulates hepatic triglyceride production seems to depend on dietary conditions (9, 10). However, the present study demonstrated that TNF-α treatment caused an upregulation of SREBP-1c *in vivo*, a key regulator of fatty acid synthesis in the liver, and FAS, an enzyme that catalyzes long-chain fatty acid biosynthesis through the condensation of acetyl-CoA and malonyl-CoA (14). The SREBP family is a family of transcription factors that activate genes encoding enzymes that regulate cholesterol and fatty acid biosynthesis. In particular, SREBP-1c preferentially enhances transcription

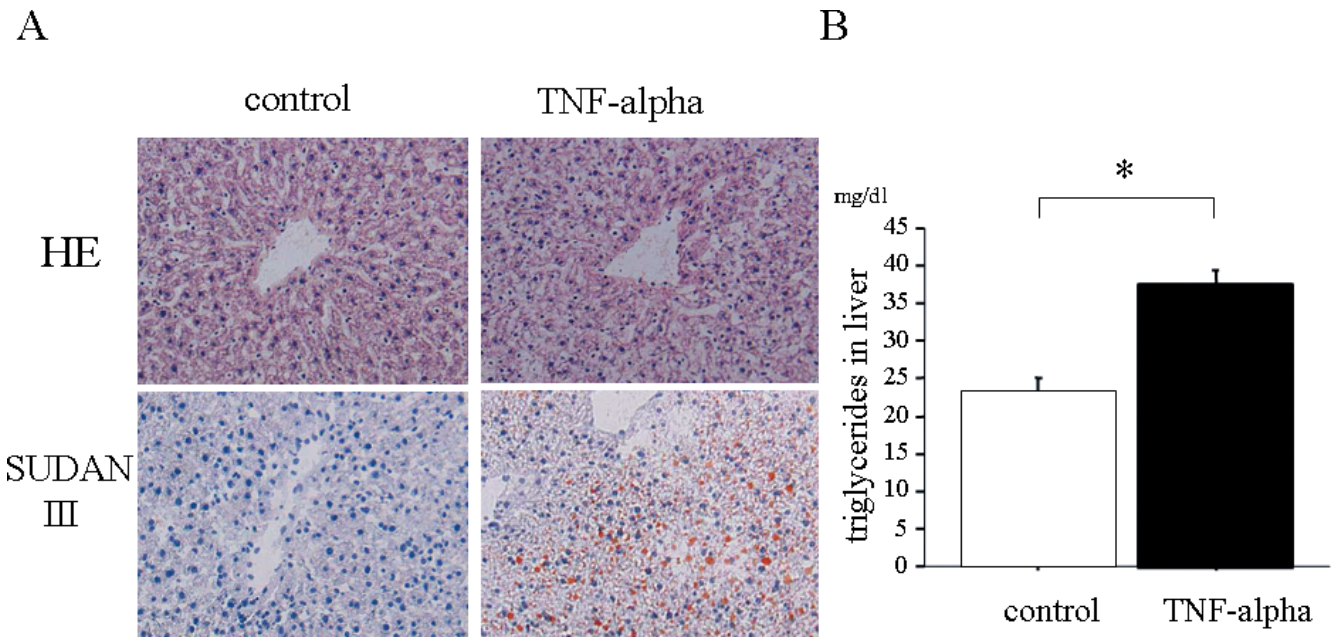

Figure 3. Histological changes (A) assessed by HE and SUDAN III (×200 magnification), and liver triglyceride levels (B) in the liver 24 hrs after ip injection of TNF-α at a dose of 0.166 mg/kg (TNF-α) or injection with the same volume of saline (control). Values and vertical bars: mean ± SEM ($n = 4$ for each). * $P < 0.05$ versus the corresponding controls.

of genes associated with fatty acid synthesis, including FAS (12, 13). TNF-α–induced activation of FAS has also been investigated in a previous study using a binding-activity assay (31).

Considering these data, a picture of the mechanism by which TNF-α induces fatty liver emerges. First, TNF-α treatment up-regulates SREBP-1c and FAS; second, these lipogenetic factors increase synthesis of fatty acids; and third, the synthesized fatty acids may be used as substrates for TG production in the liver.

How does cytokine signaling contribute to the functional linkage between TNF-α and SREBP-1c expression? Recently, suppressors of cytokine signaling (SOCS) proteins have been shown to play an important role in cytokine signaling in the regulation of insulin resistance and the development of fatty change of liver (32). First, increased expression of SOCS-1 and SOCS-3 was observed in livers of obese, insulin-resistant animals (33). Second, overexpression of SOCS-1 or SOCS-3 in the liver caused insulin resistance. Third, the inhibition of SOCS-1 and SOCS-3 in livers of obese mice by antisense treatment normalizes the increased expression of SREBP-1 (33). Finally, proinflammatory cytokines have been shown to stimulate production of SOCS family proteins (19, 32). With these considerations, TNF-α treatment in our study may be increasing SREBP-1c expression and fatty acid

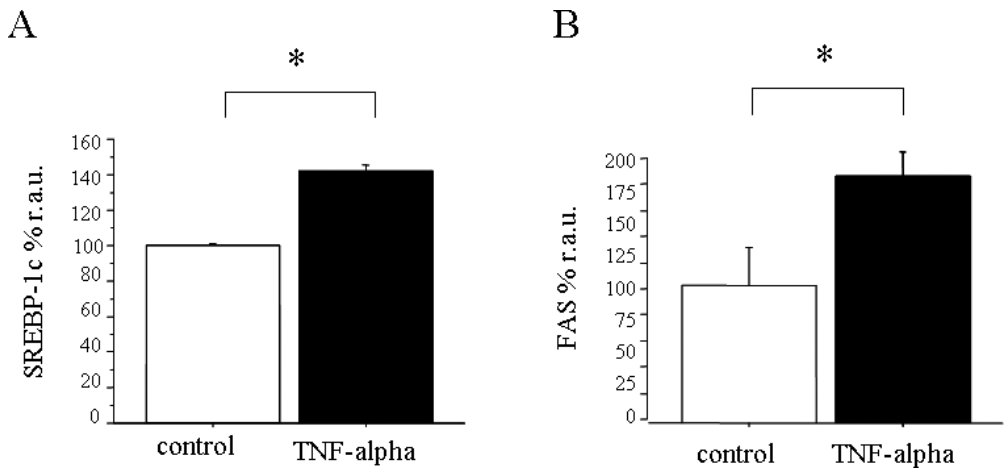

Figure 4. Expression of SREBP-1c mRNA (A) and FAS mRNA (B) in the liver 24 hrs after ip injection of TNF-α at a dose of 0.166 mg/kg (TNF-α) or injection with the same volume of saline (control). Real-time PCR was performed with total RNA from the liver samples. Values and vertical bars: mean ± SEM ($n = 4$ for each). * $P < 0.05$ versus the corresponding controls.

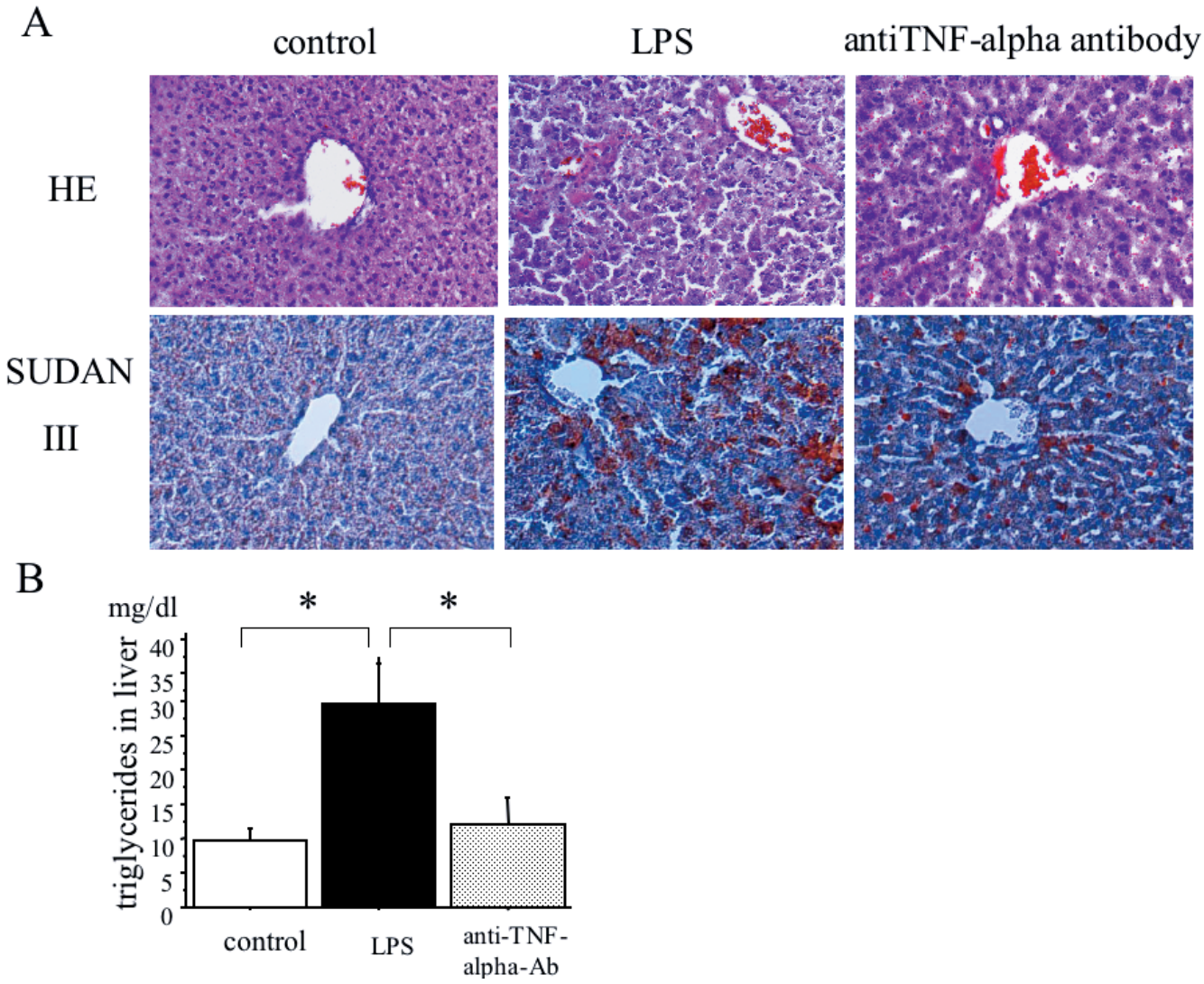

Figure 5. Effect of anti-TNF-α antibody on LPS-induced development of fatty liver 24 hrs after treatment. (A) Histological changes in the liver assessed by HE and SUDAN III staining (×200 magnification). (B) Liver triglyceride levels. Mice were injected with ip anti-TNF-α antibody at a dose of 3.3 mg/kg (anti-TNF-α antibody) or saline (LPS) before the administration of LPS at a dose of 3.3 mg/kg. Alternatively, both injections contained saline (control). Values and vertical bars: mean ± SEM ($n = 4$ for each). * $P < 0.05$ versus the corresponding controls.

synthesis in the liver through the action of SOCS proteins. We used LPS and anti-TNF-α antibody to examine the contribution of this cytokine signaling system and the specificity of TNF-α treatment in the development of cytokine-induced fatty change of liver. Similar to TNF-α, LPS induced fatty changes in the liver as assessed by TG content and histological observation of fat accumulation. The LPS-induced increase in liver TG content was partially attenuated by pretreatment with anti-TNF antibody. Histological analysis also demonstrated attenuation of hepatic fat accumulation by pretreatment with anti-TNF-α antibody. In addition, as expected by the functional role of SOCS proteins, expression of SREBP-1c and SOCS3 mRNA in LPS-treated mice was also attenuated by pretreatment with anti-TNF-α antibody. These results indicate that TNF-α may partially mediate LPS action to induce the development of fatty liver disease, and that SOCS3 and SREBP-1c may be involved in this signaling mechanism. LPS is known to activate not only TNF-α but also other cytokines (34). A previous study showed that LPS induced fatty liver through cytokines such as TNF-α and interleukin-1 (11, 35). Thus, it is highly probable that TNF-α is one of the mediators of LPS-induced acceleration of fatty liver development as well as increased SREBP-1c expression.

The present study has some limitations. In the present study, hepatic SREBP-1c expression was not increased by LPS treatment compared with vehicle-treated controls although the anti-TNF-α antibody did down-regulate hepatic SREBP-1c expression. It may be due to the sensitivity or timing of our analysis system. In addition, it is also possible that other lipogenetic markers besides SREBP-1c in the liver may be involved in LPS and TNF-α–induced fatty liver. In anyway, further study would be necessary to clarify that point.

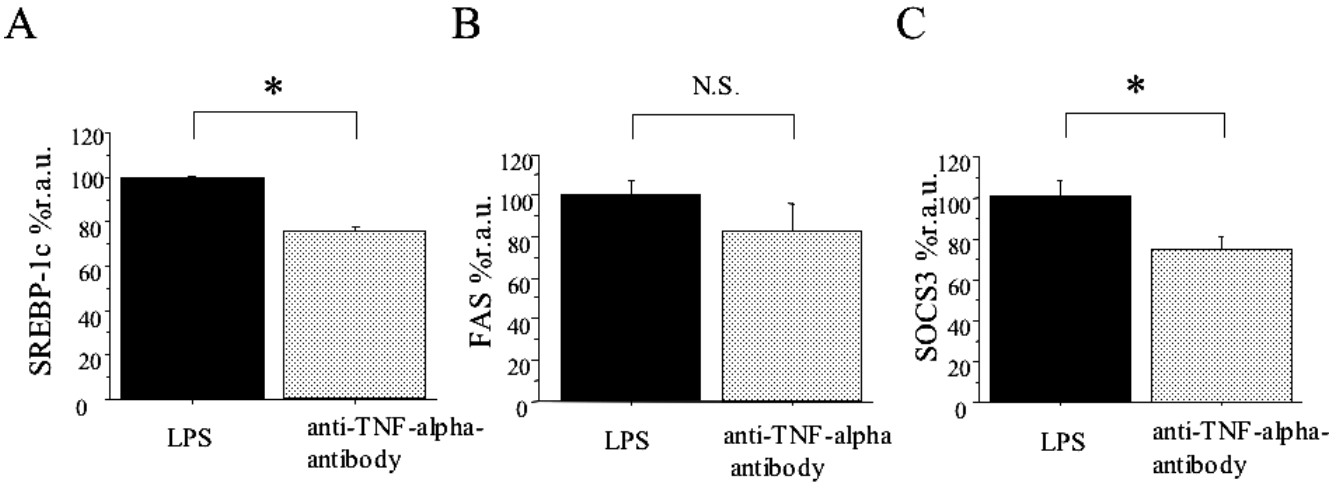

Figure 6. Expression of SREBP-1c mRNA (A), FAS mRNA (B), and SOCS3 (C) in the liver 24 hrs after treatment with anti-TNF-α antibody at a dose of 3.3 mg/kg (anti-TNF-α antibody) or saline (LPS) 30 mins before the administration of LPS at a dose of 3.3 mg/kg. Real-time PCR was performed with total RNA from the liver samples. Values and vertical bars: mean ± SEM ($n = 4$ for each). * $P < 0.05$ versus the corresponding controls.

In summary, the present study indicates that TNF-α enhances the expression of SREBP-1c and FAS *in vivo* can acutely increase intrahepatic fat deposition. It can be expected that further studies of interactions among cytokine signaling and hepatic lipid metabolism involving SREBP-1c may provide a novel strategy for the treatment of inflammation-related disorders.

1. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87–91, 1993.
2. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. J Clin Invest 94:1543–1549, 1994.
3. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proc Natl Acad Sci U S A 91:4854–4858, 1994.
4. Stephens JM, Pekala PH. Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. J Biol Chem 266:21839–21845, 1991.
5. den Boer M, Voshol PJ, Kuipers F, Havekes LM, Romijn JA. Hepatic steatosis: a mediator of the metabolic syndrome. Lessons from animal models. Arterioscler Thromb Vasc Biol 24:644–649, 2004.
6. Diehl AM. Fatty liver, hypertension, and the metabolic syndrome. Gut 53:923–924, 2004.
7. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 37:917–923, 2003.
8. Feingold KR, Grunfeld C. Tumor necrosis factor-alpha stimulates hepatic lipogenesis in the rat *in vivo*. J Clin Invest 80:184–190, 1987.
9. Feingold KR, Soued M, Serio MK, Adi S, Moser AH, Grunfeld C. The effect of diet on tumor necrosis factor stimulation of hepatic lipogenesis. Metabolism 39:623–632, 1990.
10. Feingold KR, Adi S, Staprans I, Moser AH, Neese R, Verdier JA, Doerrler W, Grunfeld C. Diet affects the mechanisms by which TNF stimulates hepatic triglyceride production. Am J Physiol 259:E177–E184, 1990.
11. Memon RA, Grunfeld C, Moser AH, Feingold KR. Tumor necrosis factor mediates the effects of endotoxin on cholesterol and triglyceride metabolism in mice. Endocrinology 132:2246–2253, 1993.
12. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109:1125–1131, 2002.
13. Shimomura I, Bashmakov Y, Horton JD. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. J Biol Chem 274:30028–30032, 1999.
14. Semenkovich CF. Regulation of fatty acid synthase (FAS). Prog Lipid Res 36:43–53, 1997.
15. Bennett MK, Lopez JM, Sanchez HB, Osborne TF. Sterol regulation of fatty acid synthase promoter. Coordinate feedback regulation of two major lipid pathways. J Biol Chem 270:25578–25583, 1995.
16. Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc Natl Acad Sci U S A 96:13656–13661, 1999.
17. Boizard M, Le Liepvre X, Lemarchand P, Foufelle F, Ferre P, Dugail I. Obesity-related overexpression of fatty-acid synthase gene in adipose tissue involves sterol regulatory element-binding protein transcription factors. J Biol Chem 273:29164–29171, 1998.
18. Shimomura I, Bashmakov Y, Horton JD. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. J Biol Chem 274:30028–30032, 1999.
19. Ueki K, Kondo T, Tseng YH, Kahn CR. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. Proc Natl Acad Sci U S A 101: 10422–10427, 2004.
20. Shi H, Cave B, Inouye K, Bjorbaek C, Flier JS. Overexpression of suppressor of cytokine signaling 3 in adipose tissue causes local but not systemic insulin resistance. Diabetes 55:699–707, 2006.
21. Ginsberg HN. Diabetic dyslipidemia: basic mechanisms underlying the common hypertriglyceridemia and low HDL cholesterol levels. Diabetes 45:S27–S30, 1996.
22. Fungwe TV, Cagen L, Wilcox HG, Heimberg M. Regulation of hepatic secretion of very low density lipoprotein by dietary cholesterol. J Lipid Res 33:179–191, 1992.
23. Wang CS, Fukuda N, Ontko JA. Studies on the mechanism of hypertriglyceridemia in the genetically obese Zucker rat. J Lipid Res 25:571–579, 1984.

24. Feingold KR, Serio MK, Adi S, Moser AH, Grunfeld C. Tumor necrosis factor stimulates hepatic lipid synthesis and secretion. Endocrinology 124:2336–2342, 1989.
25. Feingold KR, Doerrler W, Dinarello CA, Fiers W, Grunfeld C. Stimulation of lipolysis in cultured fat cells by tumor necrosis factor, interleukin-1, and the interferons is blocked by inhibition of prostaglandin synthesis. Endocrinology 130:10–16, 1992.
26. Green A, Dobias SB, Walters DJ, Brasier AR: Tumor necrosis factor increases the rate of lipolysis in primary cultures of adipocytes without altering levels of hormone-sensitive lipase. Endocrinology 134:2581–2588, 1994.
27. Ruan H, Miles PD, Ladd CM, Ross K, Golub TR, Olefsky JM, Lodish HF. Profiling gene transcription in vivo reveals adipose tissue as an immediate target of tumor necrosis factor-alpha: implications for insulin resistance. Diabetes 51:3176–3188, 2002.
28. Crespo J, Cayon A, Fernandez-Gil P, Hernandez-Guerra M, Mayorga M, Dominguez-Diez A, Fernandez-Escalante JC, Pons-Romero F. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. Hepatology 34:1158–1163, 2001.
29. 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 38:413–419, 2003.
30. Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palú G, Martines D. Increased intestinal permeability in obese mice: new evidences in the pathogenesis of nonalcoholic steatohepatitis. Am J Physiol Gastrointest Liver Physiol 292:G518–525, 2007.
31. Grunfeld C, Verdier JA, Neese R, Moser AH, Feingold KR. Mechanisms by which tumor necrosis factor stimulates hepatic fatty acid synthesis in vivo. J Lipid Res 29:1327–1335, 1988.
32. Ueki K, Kondo T, Kahn CR. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. Mol Cell Biol 24:5434–5446, 2004.
33. Ueki K, Kadowaki T, Kahn CR. Role of suppressors of cytokine signaling SOCS-1 and SOCS-3 in hepatic steatosis and the metabolic syndrome. Hepatol Res 33:185–192, 2005.
34. Hessle CC, Andersson B, Wold AE. Gram-positive and Gram-negative bacteria elicit different patterns of pro-inflammatory cytokines in human monocytes. Cytokine 30:311–318, 2005.
35. Adi S, Pollock AS, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. Role for monokines in the metabolic effects of endotoxin. Interferon-gamma restores responsiveness of C3H/HeJ mice in vivo. J Clin Invest 89:1603–1609, 1992.