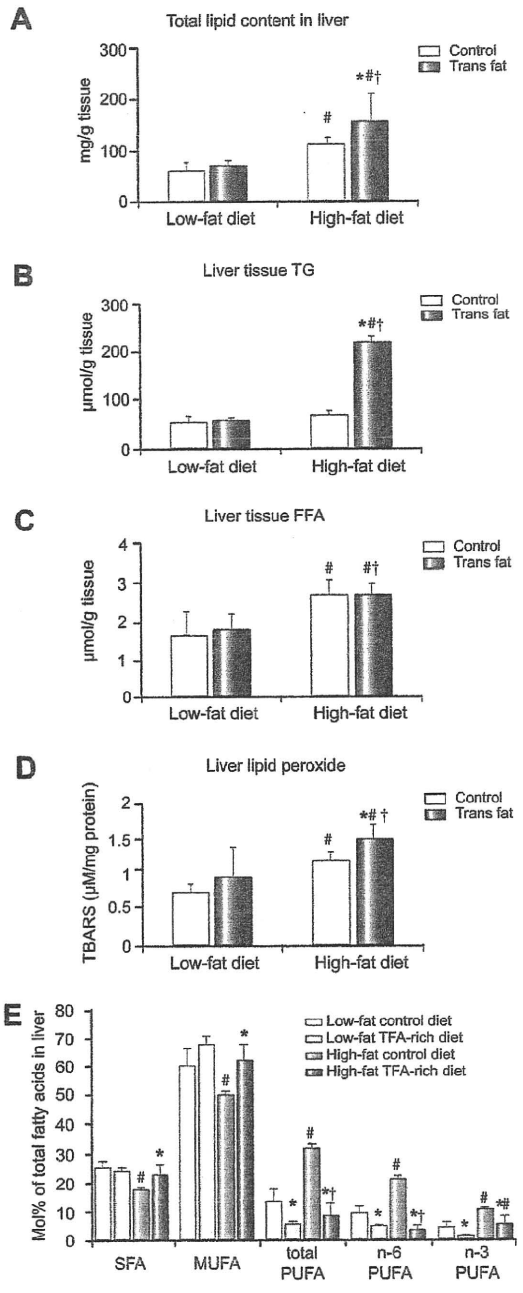

Fig. 2. Lipid accumulation in the liver. (A) Total lipid, (B) TG, and (C) FFA in the liver were measured and normalized to the tissue weight. (D) Lipid peroxide in the liver was measured and normalized by each amount of protein. (E) The hepatic fatty acid composition analyzed by gas chromatography was organized as follows: SFA, saturated fatty acid; MUFA, *cis*-monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; *n*-6, *n*-6 PUFA; and *n*-3, *n*-3 PUFA ($n = 6$ for each group). *Significantly different from the control group with the same dietary composition; ‡significantly different from the low-fat diet with same dietary oil; †significantly different from LF-C-fed and HF-T-fed group.

terms of linoleic acid (18:2*n*-6), the precursor of arachidonic acid. Except for elaidic acid and arachidonic acid, there were no specific alterations for the HF-T group.

Cytokine-, adipokine- and lipid metabolism-related gene expression in liver

Real-time RCR showed that TNFα and inducible nitric oxide synthase (iNOS) mRNA expression increased in the HF groups compared to the LF groups by approximately 2-fold when evaluated for each C or T group (Table 4), while no difference was seen in IL-6 and transforming growth factor-β (TGF-β) mRNA expression in liver among all groups. In addition, adiponectin receptor 1 and 2 gene expression was measured as adipokine related genes, but they did not differ among all groups.

To examine the potential mechanisms of hepatic steatosis by the TFA-rich diet, we determined the expression of known mediators of lipogenesis, fatty acid oxidation and TG excretion in liver, the imbalance of which is thought to lead to steatosis. Sterol regulatory element-binding protein-1 (SREBP-1) induces fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC), and is implicated in steatosis [27]. In relation to hepatic fatty acid synthesis, the mRNA expression of SREBP-1 was significantly elevated in the LF-T, HF-C and HF-T groups when compared to the LF-C group, whereas FAS and ACC were elevated significantly only in the HF-T group (Table 4). Peroxisome proliferator activated receptor γ (PPARγ) is also implicated in steatosis [33]. The expression of PPARγ1 did not differ among the groups, while PPARγ2 was significantly elevated 13-fold in the HF-C group and, remarkably, 50-fold in the HF-T group. Although, the expression of PPARγ coactivator-1β (PGC-1β), known to coactivate the SREBP-1 and stimulate lipogenic gene expression [19], was decreased in HF-fed mouse livers. The fatty acid oxidation-related genes of PPARα and carnitine palmitoyl transferase-1, and the TG excretion-related genes of microsomal triglyceride transfer protein and apolipoprotein B did not differ among the groups; fatty acid oxidation-related genes showed a tendency to be decreased in the LF-T group, but without statistical significance.

Concerning cholesterol metabolism-related gene expression in liver, SREBP-2 was increased in the HF groups, but was not affected by the dietary lipid sources. Hydroxymethylglutaryl-CoA synthase-1 and reductase were significantly increased and apolipoprotein A-1, a component of HDL, was decreased only in the HF-T group, while they did not change in the LF-T group, which showed an alteration of plasma cholesterol fraction (Table 4).

Phosphorylation status of AKT in high-fat diet-fed mice livers

As Koppe et al. suggested that TFA feeding increased insulin resistance in mice [17], and to determine if the exacerbating effects of TFA intake on liver were associated with increased insulin resistance, we evaluated the hepatic phosphorylation status of AKT (Fig. 3A). The phospho-AKT(Thr308) level was significantly decreased (Fig. 3B) and the phospho-AKT(Ser473) level was also decreased, but without statistical significance (Fig. 3C) as determined by densitometrical analysis.

TFA increases TNFα production and alters phagocytotic ability of KCs

The *cis*- or *trans*-fatty acid-containing medium showed no cytotoxicity towards KCs when compared to the fatty acid-free control medium (Fig. 4A). TNFα production of KCs induced by LPS was increased in both the C18:1 and C18:2 TFA-containing medium compared to that of *cis*-structural isomer-containing

Table 3. Individual fatty acid composition of the liver.

		Low-fat diet		High-fat diet	
		Control oil (LF-C)	TFA-rich oil (LF-T)	Control oil (HF-C)	TFA-rich oil (HF-T)
SFA					
Myristic	14:0	0.6 ± 0.1	0.5 ± 0.0	0.3 ± 0.1[‡]	0.4 ± 0.1[*†]
Palmitic	16:0	21.0 ± 1.6	19.2 ± 1.1	12.1 ± 0.6[‡]	18.7 ± 3.6[*]
Stearic	18:0	3.7 ± 0.8	3.6 ± 0.6	4.6 ± 0.6	3.3 ± 1.2
Arachidic	20:0	0.6 ± 0.3	1.0 ± 0.1[*]	0.6 ± 0.3	0.9 ± 0.3
MUFA					
Palmitoleic	16:1 *n*-7	6.9 ± 4.9	7.9 ± 4.8	2.5 ± 0.2	4.9 ± 1.0
Oleic	18:1	53.4 ± 6.7	59.8 ± 2.3	47.4 ± 1.3	57.2 ± 4.8[*†‡]
Elaidic	18:1 (9-*trans*)	0.0 ± 0.0	2.2 ± 0.5[*]	0.3 ± 0.2	6.2 ± 2.4[*#†]
PUFA					
Linoleic	18:2 *n*-6	6.5 ± 2.1	2.1 ± 0.4[*]	18.1 ± 1.6[‡]	2.6 ± 1.4[*†]
α-Linolenic	18:3 *n*-3	0.6 ± 0.4	0.0 ± 0.0[*]	3.2 ± 0.5[‡]	0.2 ± 0.2[*]
Dihomo-γ-linolenic	20:3 *n*-6	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.2[‡]	0.2 ± 0.2
Arachidonic	20:4 *n*-6	2.5 ± 0.9	2.2 ± 0.5	2.6 ± 0.6	0.6 ± 0.3[*#†]
Eicosapentaenoic	20:5 *n*-3	0.4 ± 0.2	0.1 ± 0.0	1.4 ± 0.1[‡]	1.2 ± 0.9[#†]
Docosapentaenoic	22:5 *n*-3	0.2 ± 0.1	0.0 ± 0.0	0.8 ± 0.0[‡]	0.4 ± 0.3[*#†]
Docosahexaenoic	22:6 *n*-3	3.0 ± 1.1	1.0 ± 0.3	5.2 ± 0.4	3.2 ± 2.4

The relative percentage (mean ± SD) of each fatty acid to the total fatty acids is presented ($n = 6$ per each group).
[*] Significantly different from the corresponding control group with the same dietary composition; $p < 0.05$.
[‡] Significantly different from the low-fat diet with the same dietary lipid as a source; $p < 0.05$.
[†] Significantly different from low-fat control diet group; $p < 0.05$.

medium (Fig. 4B). However, IL-6 production by KCs did not differ between *cis*- and *trans*-fatty acid-containing medium for both C18:1 and C18:2, respectively (Fig. 4C). The phagocytotic ability of KCs incubated in *trans*-C18:1-containing medium was lower than that in *cis*-C18:1-containing medium (Fig. 4D), while the influence of the structural difference of C18:2 fatty acid was small (Fig. 4E).

Discussion

Both of the dietary lipid species [3] and their amounts [6,23] are known to affect hepatic steatosis and inflammation. TFAs have been mainly linked with coronary heart disease, possibly by decreasing HDL-cholesterol and increasing LDL-cholesterol [20,29]; while little attention has been paid to liver disease, even

Table 4. Cytokine-, adipokine- and lipid metabolism-related gene expression in liver.

		Low-fat TFA-rich (LF-T)	High-fat control (HF-C)	High-fat TFA-rich (HF-T)
Cytokine and adipokine				
Tumor necrosis factor	TNF	0.98 ± 0.20	2.11 ± 0.73[‡]	1.94 ± 0.77[#†]
Interleukin-6	IL-6	1.30 ± 0.28	1.16 ± 0.14	1.10 ± 0.25
Transforming growth factor-β	TGB-β	0.86 ± 0.17	1.35 ± 0.50	0.98 ± 0.22
Nitric oxide synthase 2, inducible	iNOS	1.29 ± 0.34	2.23 ± 0.75[‡]	2.69 ± 0.74[#†]
Adiponectin receptor 1	AdipoR1	1.01 ± 0.17	1.29 ± 0.29	1.21 ± 0.25
Adiponectin receptor 2	AdipoR2	0.92 ± 0.16	1.20 ± 0.22	1.28 ± 0.26
Lipogenesis				
Fatty acid synthase	FAS	1.36 ± 0.21	0.79 ± 0.12	1.69 ± 0.46[*†]
Acetyl-CoA carboxylase	ACC	1.27 ± 0.10	0.80 ± 0.05	1.49 ± 0.37[*†]
Sterol regulatory element-binding protein-1	SREBP-1	3.76 ± 0.51[*]	1.93 ± 0.23[‡]	4.69 ± 0.17[*#†]
Peroxisome proliferator activated receptor γ1	PPARγ1	1.45 ± 0.42	1.58 ± 0.48	1.29 ± 0.26
Peroxisome proliferator activated receptor γ2	PPARγ2	1.88 ± 0.33	12.92 ± 6.04[‡]	50.18 ± 3.61[*#†]
PPARγ coactivator-1β	PGC-1β	0.81 ± 0.10	0.61 ± 0.15[‡]	0.59 ± 0.12[#†]
Fatty acid oxidation				
Peroxisome proliferator activated receptor α	PPARα	0.51 ± 0.23	1.25 ± 0.48	1.28 ± 0.41
Carnitine palmitoyl transferase-1	CPT-1	0.63 ± 0.11	1.03 ± 0.34	1.08 ± 0.57
Triglyceride excretion				
Microsomal triglyceride transfer protein	MTP	1.04 ± 0.16	0.92 ± 0.11	0.91 ± 0.11
Apolipoprotein B	ApoB	1.11 ± 0.15	1.20 ± 0.13	1.15 ± 0.09
Cholesterol metabolism				
Sterol regulatory element-binding protein-2	SREBP-2	0.87 ± 0.15	1.66 ± 0.25	1.69 ± 0.34
Hydroxymethylglutaryl-CoA synthase-1	HMGCs1	1.03 ± 0.11	1.60 ± 0.26	4.56 ± 0.73[*#†]
Hydroxymethylglutaryl-CoA reductase	HMGCr	0.99 ± 0.13	1.22 ± 0.32	2.93 ± 0.44[*#†]
Apolipoprotein A-1	ApoA1	0.93 ± 0.09	1.26 ± 0.21	0.61 ± 0.11[*#†]

All results are expressed as the relative fold change compared to the low-fat control diet group ± SD ($n = 6$ per each group).
[*] Significantly different from the corresponding control group with the same dietary composition; $p < 0.05$.
[‡] Significantly different from the low-fat diet with the same dietary lipid as a source; $p < 0.05$.
[†] Significantly different from low-fat control diet group; $p < 0.05$.

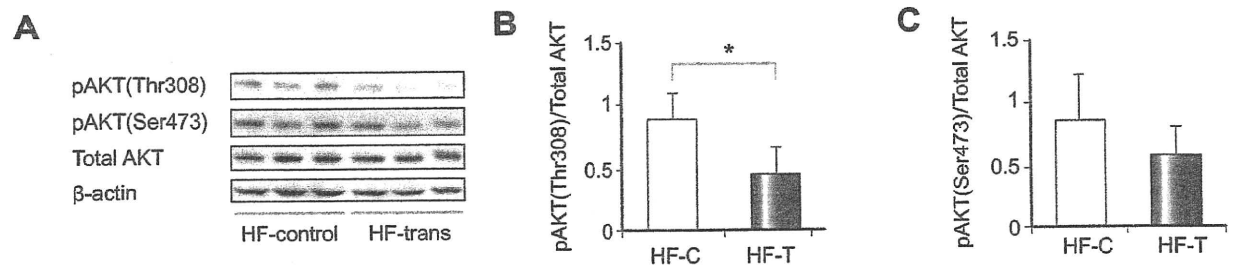

Fig. 3. Effect of excessive TFA consumption of AKT in the high-fat diet-fed mice liver. (A) Representative pictures of phospho-AKT (Thr308 and Ser473), total AKT and β-actin Western blots as well as densitometric analysis of the (B) pAKT(Thr308) or (C) pAKT(Thr473)/total AKT ratio. *p <0.05.

though a few studies have reported that hepatic steatosis [30] and ALT elevation [17] were induced by a TFA-rich diet in mice, the mechanisms remain to be clarified. In agreement with this, the HF-T group showed severe steatosis with a significant transaminase elevation, while HF-C-fed mice only showed moderate steatosis without liver injury, in addition we showed that relatively small amounts of TFA-rich oil intake do not induce severe steatosis and liver injury in the current study. Interestingly, the plasma cholesterol fraction was significantly altered even in the LF-T group in association with the elevation of the total: HDL-cholesterol ratio, a risk factor index of coronary artery disease [22]. The alteration of the plasma cholesterol fraction might be partially explained by changes in the cholesterol metabolism-related gene expressions in the HF-T group, but not in the LF-T group. We could not determine why the cholesterol fraction was altered in the LF-T group, but it might be partially due to the modified membrane fluidity induced by TFA intake [14]. That a relatively small TFA intake could affect the cholesterol fraction but not the liver might be the reason why less attention has been paid to the liver until recently.

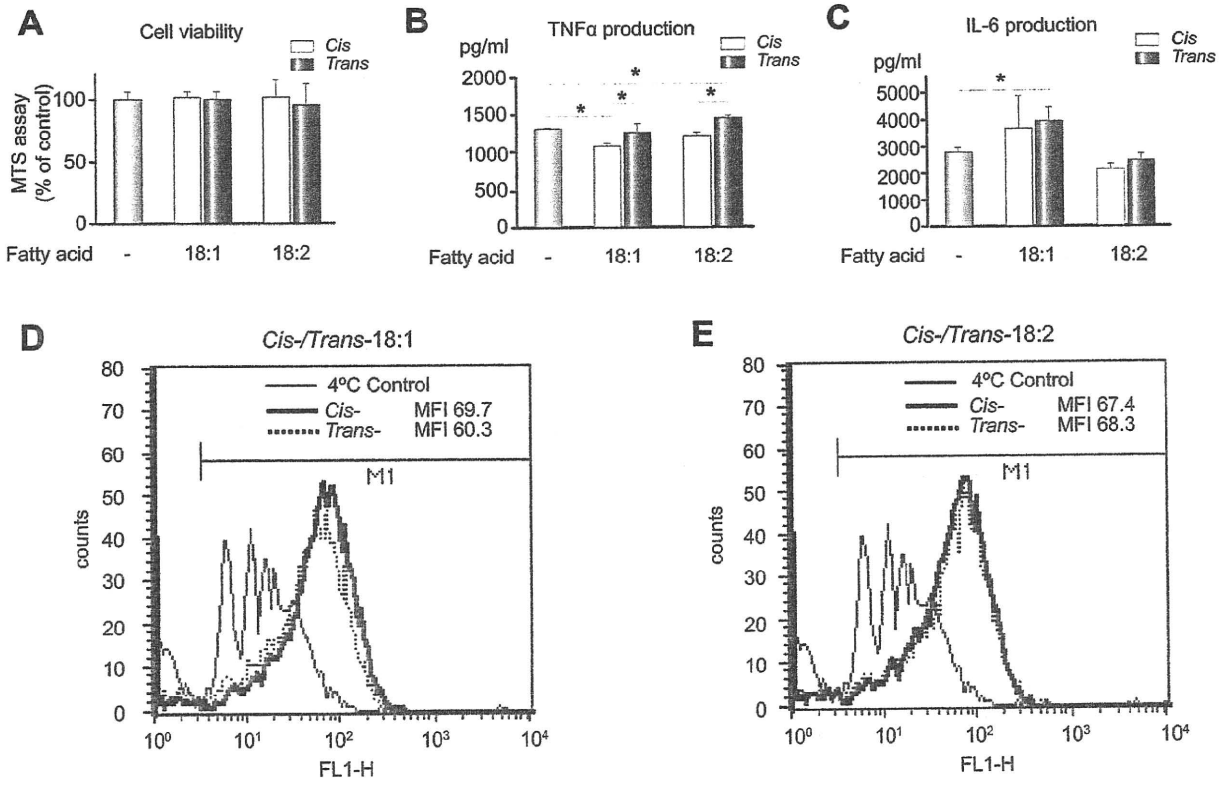

Fig. 4. The impact of *cis-/trans*-fatty acid on the cytokine production and phagocytotic ability of KCs. (A) No fatty acids (200 μM) shows cytotoxicity for primary KCs after 24 h incubation, but (B) increases TNFα production in both C18:1 and C18:2-TFA-containing medium compared to its *cis*-structural isomer. (D) IL-6 production increases in C18:1-containing medium and remains unchanged in C18:2-containing medium, whereas the *cis*- or *trans*-structural difference does affect the results. The influence of the C18:1 (D) and C18:2 (E) *cis-/trans*-fatty acid on the phagocytotic ability of KCs is measured by flow cytometry analysis. MFI: mean fluorescence intensity (n = 8 for each group, A–C). *p <0.05.

One of the noted biochemical changes seen in the HF-T group was the elevation of plasma FFA, almost all of which is derived from adipose tissue and accumulates in the liver as TG in a dose-dependent fashion [7]. The increase of plasma FFA might be due to the TFA incorporation in the adipocyte plasma membrane resulting in decreased membrane fluidity, accompanied by increased adipose tissue insulin resistance as evidenced by increased lipolysis, decreased antilipolysis and decreased glucose uptake in rat adipocytes [14]. In addition, in contrast to TG, the circulating FFAs [21] and accumulation of FFAs in liver [32] are known to exert lipotoxicity to hepatocytes, so the significant increase of the plasma FFA in the HF-T group might affect the pathophysiology. On the other hand, hepatic FFA was higher in HF than LF irrespective of the dietary lipid sources, and it was reported that forced high-fat feeding induced NASH in mice, while usual high-fat intake does not [6]. Hepatic FFA accumulation remained unaltered among healthy, NAFLD and NASH subjects liver [24], so it might not be necessary for the progression to NASH but could be a exacerbating factor in the case of excess fat consumption. Therefore, elevated plasma FFA and accumulated hepatic lipid peroxide [23] would contribute synergistically to the liver injury in this model.

A previous study reported that TFA intake decreased the arachidonic acid level and induced insulin resistance in adipose tissue, probably due to the decreased membrane fluidity [14], and the present study also revealed a decrease of the arachidonic acid level in the liver and lower phosphorylation status of AKT which could reflect the hepatic insulin resistant status in the HF-T group. In addition, a human lipidomic analysis of NAFLD/NASH liver described a decrease of arachidonic acid and unaltered levels of precursor linoleic acid, but the study did not address TFA consumption [24]. Although, the mechanisms of the arachidonic acid decrease and involvement in the insulin resistance and progression to NASH remain to be clarified, these common findings might suggest that TFA intake influences both the liver and adipose tissue in a somewhat similar fashion in NASH patients and people who consume excess TFAs.

Typical pathological findings in human NAFLD/NASH patients such as macrovesicular lipid deposit and inflammation are usually identified around zone 3, though the mice liver showed lipid deposits around zone 1 even in the control lipid-fed group. Microvesicular lipid droplets were remarkable in the HF-T group, so it might be difficult to assess the pathological changes using a human scoring system such as NAS. The pathological differences might be due to a specific problem, since the pediatric NAFLD is known to show histological findings around zone 1 [26], which might be related to the dietary habit of consuming excess TFAs from snacks and first food.

Although the expression of proinflammatory cytokines such as TNFα [30] and IL-1β [17] have been shown to be induced in the mice liver by TFA-rich diets, this was not the case in the current study. However, a previous study reported that, among hypercholesterolemic subjects, the production of TNFα by cultured mononuclear cells was increased by a TFA-rich soy bean margarine diet compared with a natural soybean oil diet [12], and we showed KCs increased TNFα production in TFA-containing medium compared to that of *cis*-structural isomer-containing medium. Accordingly, pathophysiological conditions induced by TFA consumption could be partially due to alterations in the monocyte/macrophage ability in proinflammatory cytokine production and phagocytosis, and KCs in particular may play important roles in the local circumstances.

With regard to the adipokines, the adiponectin levels were not changed between the HF-C and HF-T group, but plasma leptin was significantly increased in the HF-T group. Leptin is an appetite-suppressing and body weight-regulating adipokine, and is even related to liver regeneration and fibrosis [13]; hyperleptinemia might be related directly to the elevation of type1 collagen α1 mRNA expression in the liver.

In terms of the severe steatosis of the model, the lipogenetic genes such as FAS, ACC, SREBP-1 and PPARγ2 were coordinately induced, but PGC-1β was not. PGC-1β is known to be induced in the liver by short term high-fat feeding, to coactivate SREBP-1, but to reduce hepatic fat accumulation. However, since it was not investigated in this study, the decrease of PGC-1β gene expression might be due to long term feeding or any other factors, such as the relatively low carbohydrate diet. It was reported that a TFA-rich diet suppressed the PPARγ gene expression in rat adipose tissue [8], and that lipogenic gene expressions in the liver and the adipose tissue are reciprocal modulations [4], which might reflect the core mechanisms of the heterotopic fat accumulation *in vivo*.

In summary, excess TFA consumption induces significant hepatic steatosis accompanied by augmentation of the hepatic lipogenic gene expressions, FFA influx into the liver, and the hepatic accumulation of lipid peroxide. The hepatic accumulation of TFA and the reduction of the arachidonic acid content were the lipidomic properties in this model. Together with their potential induction of local cytokines by KCs, lipid species including TFA may play a pivotal role in the development of non-alcoholic fatty liver diseases.

Acknowledgements

This study was supported in part from Health and Labour Sciences Research Grants for the Research on Measures for Intractable Diseases (from the Ministry of Health, Labour and Welfare of Japan), from Grant-in-Aid for Scientific Research C (20590755 to KF) from Japanese Society of Promotion of Science (JSPS).

Conflicts of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2010.02.029.

References

[1] Babior BM. Oxygen-dependent microbial killing by phagocytes (first of two parts). N Engl J Med 1978;298:659–668.
[2] Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387–1395.
[3] Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Scholmerich J, et al. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. J Mol Endocrinol 2006;36:485–501.
[4] Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell 2008;134:933–944.

[5] Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842–845.
[6] Deng QG, She H, Cheng JH, French SW, Koop DR, Xiong S, et al. Steatohepatitis induced by intragastric overfeeding in mice. Hepatology 2005;42:905–914.
[7] Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 2005;115:1343–1351.
[8] Duque-Guimaraes DE, de Castro J, Martinez-Botas J, Sardinha FL, Ramos MP, Herrera E, et al. Early and prolonged intake of partially hydrogenated fat alters the expression of genes in rat adipose tissue. Nutrition 2009;25: 782–789.
[9] Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006;43:S99–S112.
[10] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226: 497–509.
[11] Gonzalez-Periz A, Horrillo R, Ferre N, Gronert K, Dong B, Moran-Salvador E, et al. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. FASEB J 2009;23:1946–1957.
[12] Han SN, Leka LS, Lichtenstein AH, Ausman LM, Schaefer EJ, Meydani SN. Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. J Lipid Res 2002;43:445–452.
[13] Honda H, Ikejima K, Hirose M, Yoshikawa M, Lang T, Enomoto N, et al. Leptin is required for fibrogenic responses induced by thioacetamide in the murine liver. Hepatology 2002;36:12–21.
[14] Ibrahim A, Natrajan S, Ghafoorunissa R. Dietary trans-fatty acids alter adipocyte plasma membrane fatty acid composition and insulin sensitivity in rats. Metabolism 2005;54:240–246.
[15] Kechagias S, Ernersson A, Dahlqvist O, Lundberg P, Lindstrom T, Nystrom FH. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. Gut 2008;57:649–654.
[16] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
[17] Koppe SW, Elias M, Moseley RH, Green RM. Trans fat feeding results in higher serum alanine aminotransferase and increased insulin resistance compared with a standard murine high-fat diet. Am J Physiol Gastrointest Liver Physiol 2009;297:G378–G384.
[18] Larter CZ, Yeh MM, Haigh WG, Williams J, Brown S, Bell-Anderson KS, et al. Hepatic free fatty acids accumulate in experimental steatohepatitis: role of adaptive pathways. J Hepatol 2008;48:638–647.
[19] Lin J, Yang R, Tarr PT, Wu PH, Handschin C, Li S, et al. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. Cell 2005;120:261–273.
[20] Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ, et al. Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. J Nutr 2005;135:562–566.
[21] Malhi H, Gores GJ. Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. Semin Liver Dis 2008;28:360–369.
[22] Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr 2003;77:1146–1155.
[23] Milagro FI, Campion J, Martinez JA. Weight gain induced by high-fat feeding involves increased liver oxidative stress. Obesity (Silver Spring) 2006;14: 1118–1123.
[24] Puri P, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, et al. A lipidomic analysis of nonalcoholic fatty liver disease. Hepatology 2007;46:1081–1090.
[25] Reid AE. Nonalcoholic steatohepatitis. Gastroenterology 2001;121:710–723.
[26] Schwimmer JB, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology 2005;42:641–649.
[27] Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS, Goldstein JL. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J Clin Invest 1996;98:1575–1584.
[28] Su GL, Klein RD, Aminlari A, Zhang HY, Steinstraesser L, Alarcon WH, et al. Kupffer cell activation by lipopolysaccharide in rats: role for lipopolysaccharide binding protein and toll-like receptor 4. Hepatology 2000;31: 932–936.
[29] Sun Q, Ma J, Campos H, Hankinson SE, Manson JE, Stampfer MJ, et al. A prospective study of trans fatty acids in erythrocytes and risk of coronary heart disease. Circulation 2007;115:1858–1865.
[30] Tetri LH, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. Am J Physiol Gastrointest Liver Physiol 2008;295:G987–G995.
[31] Tsuzuki T, Tokuyama Y, Igarashi M, Nakagawa K, Ohsaki Y, Komai M, et al. Alpha-eleostearic acid (9Z11E13E-18:3) is quickly converted to conjugated linoleic acid (9Z11E-18:2) in rats. J Nutr 2004;134:2634–2639.
[32] Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 2007;45:1366–1374.
[33] Zhang YL, Hernandez-Ono A, Siri P, Weisberg S, Conlon D, Graham MJ, et al. Aberrant hepatic expression of PPARgamma2 stimulates hepatic lipogenesis in a mouse model of obesity, insulin resistance, dyslipidemia, and hepatic steatosis. J Biol Chem 2006;281:37603–37615.