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Liver steatosis, but not fibrosis, is associated with insulin resistance in nonalcoholic fatty liver disease

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Background. To address the hypothesis that liver steatosis causes systemic insulin resistance, we sought to determine the liver histological feature that most strongly contributes to insulin resistance in patients with nonalcoholic fatty liver disease (NAFLD). ***Methods.*** Liver biopsy specimens were obtained from 131 patients with clinically suspected NAFLD. The stage, grade of nonalcoholic steatohepatitis (NASH), and level of steatosis were scored and analyzed in relation to the homeostasis model assessment of insulin resistance (HOMA-IR) and the metabolic clearance rate (MCR), measured using the glucose clamp method. ***Results.*** In the univariate analysis, the degree of hepatic steatosis ($r = 0.458$, $P < 0.001$), stage ($r = 0.360$, $P < 0.001$), and grade ($r = 0.349$, $P < 0.01$) of NASH were significantly correlated with the HOMA-IR. Multiple regression analysis adjusting for age, sex, body mass index, and each histological score showed that steatosis was significantly and independently associated with HOMA-IR (coefficient = 1.42, $P < 0.001$), but not with the stage (coefficient = 0.33, $P = 0.307$) or grade (coefficient = 0.67, $P = 0.134$) of NASH. Similar independent relationships were observed between steatosis and MCR, but the relationship was weaker (coefficient = –0.98, $P = 0.076$). ***Conclusions.*** Steatosis of the liver, but not the stage or the grade of NASH, is associated with insulin resistance in patients with NAFLD.

Key words: nonalcoholic fatty liver disease, insulin resistance, steatosis, liver histology, nonalcoholic steatohepatitis

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is often associated with various metabolic abnormalities, including obesity,[1,2] type 2 diabetes,[1–4] and dyslipidemia,[3–7] all of which are closely associated with insulin resistance and are important risk factors for cardiovascular diseases. Insulin resistance, which can be accurately measured using the glucose clamp method,[8] is a condition in which the cells of the body become resistant to the effects of insulin, resulting in a decreased response to a given amount of insulin. This condition is associated with various underlying metabolic abnormalities, including obesity, type 2 diabetes, dyslipidemia, and hypertension.[9,10] Insulin resistance is also associated with NAFLD,[11–15] which can range from a simple fatty liver to nonalcoholic steatohepatitis (NASH).[16–18] This latter condition can progress to cirrhosis of the liver and hepatocellular carcinoma.[19–24]

The particular histological findings of NAFLD that are associated with the metabolic abnormalities have not been elucidated fully.[11,12,25–31] Steatosis of the liver has been reported to be associated with body mass index (BMI),[25] waist circumference,[11] hypertension,[11,25] glucose intolerance,[11,25] dyslipidemia,[11,25] and insulin resistance.[11,12] Furthermore, experiments have demonstrated that hepatic steatosis per se causes insulin resistance in vivo.[32–35] Nevertheless, the association between the degree of hepatic fibrosis and metabolic abnormalities is still controversial. Some reports suggest that, in NAFLD patients, fibrosis is associated with insulin resistance and higher rates of diabetes, dyslipidemia, and hypertension,[11,12,25–29] whereas other reports suggest that the stage of fibrosis is not significantly associated with metabolic abnormalities.[30,31] These discrepant findings may be due to differences in the severity of obesity or the complicated metabolic abnormalities of the patients in these studies.

To test the hypothesis that liver steatosis causes systemic insulin resistance, we sought to determine the liver histological feature that most strongly contributes to insulin resistance in patients with NAFLD.

Methods

Patients

The study subjects were 131 patients with clinically suspected NAFLD, who were recruited consecutively between 1997 and 2004 at Kanazawa University Hospital, Japan. Twenty of the patients were referred to our hospital for evaluation of liver injury, while in the remaining 111 patients, the liver injury was identified during the treatment of other metabolic disorders, such as diabetes mellitus and obesity. Fatty liver was clinically diagnosed based on ultrasound examination showing an increase in hepatorenal contrast, defined as a ratio of hepatic to kidney echo levels of >1.0, also known as a "bright liver." In each patient, all other liver disorders were excluded, including viral hepatitis B and C, primary biliary cirrhosis, autoimmune hepatitis, sclerosing cholangitis, hemochromatosis, Wilson's disease, drug-induced liver injury, and biliary obstruction. All patients reported drinking less than 20 g/day of ethanol.

The 131 patients had a mean age of 48 years and a mean BMI of 27.4 kg/m^2 (Table 1). Of the 131 patients, 108 (82%) had type 2 diabetes according to the American Diabetes Association criteria.[36] Among these patients, 54 were treated with diet alone; the remainder were treated with an α-glucosidase inhibitor (n = 14), a rapid-acting insulin secretion agent (nateglinide, n = 11), or a premeal rapid-acting insulin analog (n = 40). Hypertension and dyslipidemia were defined according to the metabolic syndrome definitions of the National Cholesterol Education Program—Adult Treatment Panel III.[37] Hypertension was observed in 65 patients (50%), 36 of whom had been treated with antihypertensive agents. High triglyceride levels were observed in 47 (36%) patients, and low high-density lipoprotein (HDL) levels were observed in 33 (25.2%), 30 of whom had been treated with statins. None of the patients were on medication, such as sulfonylureas, thiazolidinediones, metformins, vitamin E, or ursodeoxycholic acid, which could influence the fasting insulin/glucose levels or induce histological changes in the liver. The patients gave their written informed consent for this study, which was approved by the hospital ethics board.

Laboratory studies

After an overnight fast, venous blood samples were withdrawn from each patient. Serum samples were assayed for total cholesterol, triglycerides, HDL cholesterol, and insulin. Plasma samples were assayed for glucose.

Evaluation of insulin sensitivity

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula, HOMA-IR = [fasting insulin (μU/ml) × fasting plasma glucose (mmol/l)]/22.5.[38]

Insulin sensitivity was also evaluated using the glucose clamp method[8] in 46 patients (35 diabetic and 11

Table 1. Clinical characteristics of the study subjects[a]

	Mean ± SD	Normal range
Age (years)	48 ± 14	
BMI (kg/m^2)	27.4 ± 5.5	18–25
AST (IU/l)	39.4 ± 38.5	10–48
ALT (IU/l)	62.1 ± 74.6	3–50
Fasting plasma glucose (mg/dl)	127 ± 40	70–110
Basal insulin (μU/ml)	12.0 ± 11.7	<20
HbA_{1C} (%)	6.9 ± 1.8	4.3–5.8
Total cholesterol (mg/dl)	205 ± 44	132–220
Triglycerides (mg/dl)	139 ± 77	32–150
HDL cholesterol (mg/dl)	48 ± 13	40–97
HOMA-IR	3.4 ± 3.2	<2.0
Metabolic clearance rate (mg/kg per minute)[b]	6.1 ± 2.7	9.9–16.9
Histological scores		
Stage (0/1/2/3/4)	22/45/38/23/3	
Grade (0/1/2/3)	48/61/19/3	
Steatosis (0/1/2/3)	7/82/27/15	

BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance

[a] Male, n = 84; female, n = 47; total, n = 131

[b] Metabolic clearance rate was measured in only a part of the subjects (n = 46)

nondiabetic patients). These patients did not receive any medication on the morning of the examination. At approximately 9 a.m., after an overnight fast of at least 10 h, an intravenous catheter was placed in an antecubital vein of each subject for infusion, while a second catheter was placed in the contralateral hand for blood sampling. The euglycemic hyperinsulinemic clamp technique was done using an artificial pancreas (model STG-22; Nikkiso, Tokyo, Japan), as described previously.[39] A solution of 0.8 U/ml insulin (Novolin R: Novo Nordisk, Copenhagen, Denmark) in normal saline was allowed to remain in the intravenous lines for at least 15 min, and the lines were then flushed before starting the insulin infusion. Insulin was infused at a rate of 3.0 mU/kg per minute, resulting in a steady-state insulin concentration of 290.3 ± 61.7 μU/ml (mean ± SD). Blood glucose levels were determined continuously during the clamp study and maintained by variable-rate infusion of 20% glucose at fasting levels or at 100 mg/dl, whichever was higher. The steady-state period was maintained for 30 min or longer, during which the coefficients of variation for blood glucose and the glucose infusion rate were each less than 5%. Glucose levels reached during the clamp study were 91.3 ± 15.5 mg/dl. Insulin sensitivity was expressed as the glucose metabolic clearance rate (MCR) in mg/kg per minute. The mean MCR in healthy subjects (n = 9; age, 26.6 ± 2.9 years; BMI, 22.3 ± 2.1 kg/m^2) was 13.5 ± 3.4 mg/kg per minute.

Pathology

After obtaining informed consent, liver biopsy specimens were obtained from all 131 patients; 59 had elevated aminotransferase levels on admission for the liver biopsy, and 72 had normal aminotransferase levels at the time of biopsy but a history of elevated aminotransferase levels before admission. Each specimen was stained with hematoxylin-eosin and silver reticulin stains and histologically examined by one pathologist who was blinded to the patient's clinical condition and biochemical data. Each biopsy was scored according to the standard criteria for grading and staging of NASH proposed by Brunt et al.[40,41] Steatosis of the liver was also scored from 0 to 3 (0, none; 1, <33%; 2, 33%–66%; 3, >66%) according to the modified criteria of Brunt et al.[40,41]

Statistical analyses

The correlation coefficients between pairs of indices were calculated, with $P < 0.05$ considered statistically significant. Multiple linear regression analysis was used to calculate age-, sex-, and BMI-adjusted coefficients between each histological score and insulin resistance. The t-statistic was used to compare the strength of the relationship.

Results

The characteristics of the study subjects and the number of patients with each histological score are shown in Table 1. We found that the mean HOMA-IR was higher and the mean MCR was lower in NAFLD patients than in normal subjects. Scores of the stage and grade of NASH were strongly correlated (r = 0.570, $P < 0.001$), and each was weakly correlated with the hepatic steatosis score (stage, r = 0.389, $P < 0.001$; grade, r = 0.397, $P < 0.001$), suggesting that steatosis and inflammation or fibrosis are pathological attributes of patients with NAFLD.

We evaluated the association between markers for insulin resistance and histological scores of the liver. In a univariate analysis (Table 2), the degree of hepatic steatosis, stage, and grade of NASH were significantly correlated with HOMA-IR. The degree of hepatic steatosis and grade of NASH were significantly correlated with MCR.

Multiple linear regression models were computed to assess the age-, sex-, and BMI-adjusted relative influence of each histological score on insulin resistance (Table 3). Steatosis, as well as the stage and grade of NASH, was associated with HOMA-IR, even after adjusting for age, sex, and BMI in the multiple regression analysis. When these three histological scores were adjusted for each other in a model that included all three simultaneously, only the steatosis score was associated with HOMA-IR, and the associations of the stage and grade of NASH with HOMA-IR were not significant. A similar independent relationship was observed between steatosis of the liver and MCR, but the relationship was weaker.

Discussion

Patients with NAFLD have features of the insulin resistance-associated metabolic syndrome, which puts

Table 2. Univariate correlation between histological scores and insulin resistance

	HOMA-IR (n = 131)	Metabolic clearance rate (n = 46)
Steatosis	0.46***	–0.42**
Stage	0.36***	–0.17
Grade	0.35***	–0.30*

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

Table 3. Age-, sex-, and BMI-adjusted association between insulin resistance and histological changes of the liver

	HOMA-IR (n = 131)			Metabolic clearance rate (n = 46)		
	Coefficient	t-statistic	P	Coefficient	t-statistic	P
Steatosis	1.81	4.59	<0.001	–1.23	–2.62	0.012
Stage	0.90	3.15	0.002	–0.23	–0.62	0.540
Grade	1.37	3.51	0.001	–1.07	–2.26	0.029
Steatosis[a]	1.42	3.40	<0.001	–0.98	–1.82	0.076
Stage[a]	0.33	1.03	0.307	0.35	0.85	0.401
Grade[a]	0.67	1.51	0.134	–0.86	–1.46	0.151

All models were adjusted for age, sex, and BMI by multiple linear regression
[a]Three histological scores are included in the same model

them at increased risk for developing cardiovascular diseases.[13–15] Patients with advanced NASH have also been reported to have a higher intra-abdominal fat mass, a higher prevalence of hypertriglyceridemia, low HDL cholesterol, and more severe insulin resistance than healthy subjects matched for age, sex, race, and BMI.[27] However, the association between the degree of the histological changes, especially liver fibrosis, and metabolic abnormalities is still controversial in NAFLD patients.[11,12,25–31] When we investigated the HOMA-IR and MCR calculated by using the glucose clamp method, which is the gold standard for evaluating insulin resistance,[8] we found that insulin resistance was increased in NAFLD patients. On examining the relationship between insulin resistance and NAFLD histological scores, we found that insulin resistance was associated with the severity of hepatic steatosis, but not with the severity of inflammation or fibrosis after adjusting for the hepatic steatosis score.

Our findings differ from those of a recent report showing that metabolic syndrome is associated with advanced NASH.[26] In that report, patients with advanced NASH had a higher BMI than patients who had a simple fatty liver. Since a higher BMI itself is strongly associated with insulin resistance and metabolic abnormalities, metabolic abnormalities associated with liver pathology should be determined only after adjusting for BMI. In fact, our study showed that BMI was independently associated with both liver steatosis and the stage of NASH. When we evaluated the association between the histological scores of the liver and insulin sensitivity using multiple regression models, the hepatic steatosis score was associated with insulin sensitivity, even after adjusting for age, sex, BMI, and the other histological scores, whereas the association between the stage or grade of NASH and insulin resistance was no longer significant. These findings clearly indicate that only the degree of hepatic steatosis is an independent predictor of insulin resistance in patients with NAFLD.

We evaluated the insulin sensitivity using two methods: HOMA-IR and MCR. In our results, steatosis of the liver was more strongly associated with HOMA-IR than MCR. In this study, MCR was measured only in 46 of 131 NAFLD patients; therefore, the statistical power would not be sufficient to detect a significant relationship. Furthermore, organ-specific insulin resistance may also influence the results. MCR is a marker for splanchnic insulin resistance, mainly muscle insulin resistance.[8] By contrast, HOMA-IR is considered a marker for hepatic insulin resistance because HOMA-IR is calculated using fasting glucose and insulin levels, which are determined by fasting hepatic glucose production. Therefore, hepatic steatosis may be associated more strongly with the index of "hepatic" insulin resistance.

Recently, we used cDNA microarrays to characterize the changes that occur in the moderate fatty liver of type 2 diabetic patients.[42,43] In the livers of patients with type 2 diabetes, the genes for mitochondrial oxidative phosphorylation and gluconeogenesis are upregulated, and are associated with fasting hyperglycemia.[43] These findings, together with our results, suggest that hepatic steatosis per se causes insulin resistance in patients with NAFLD.

The main limitation of this study is that most of the study subjects were diabetic patients. Therefore, insulin resistance may be greater in these study subjects than in the general population, and this may have influenced the results. A large-scale prospective study is needed to confirm our conclusion and to clarify the prognosis of patients with NAFLD.

In this paper, we showed that, steatosis of the liver per se is an independent predictor for insulin resistance in patients with NAFLD. Simple fatty liver has been considered as benign condition compared with advanced NASH, but we should regard patients with liver steatosis, irrespective with the degree of inflammation and fibrosis, as high-risk group for cardiovascular disease because they have higher insulin resistance. To prevent

cardiovascular disease, patients with severe fatty liver should be evaluated for insulin resistance-associated metabolic abnormalities and monitored intensively to reduce the occurrence of risk factors for atherosclerosis.

References

1. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 1990;12:1106–10.
2. Eriksson S, Eriksson KF, Bondesson L. Nonalcoholic steatohepatitis in obesity: a reversible condition. Acta Med Scand 1986;220: 83–8.
3. Powell EE, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow up study of forty-two patients for up to 21 years. Hepatology 1990;11:74–80.
4. Bacon BR, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. Gastroenterology 1994;107:1103–9.
5. Itoh S, Yougel T, Kawagoe K. Comparison between nonalcoholic steatohepatitis and alcoholic hepatitis. Am J Gastroenterol 1987; 82:650–4.
6. Lee RG. Nonalcoholic steatohepatitis: a study of 49 patients. Hum Pathol 1987;20:594–8.
7. Diehl AM, Goodman Z, Ishak KG. Alcohol-like liver disease in non-alcoholics. A clinical and histologic comparison with alcohol-induced liver injury. Gastroenterology 1988;95:1056–62.
8. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–23.
9. Reaven GM. Role of insulin resistance in human disease. Diabetes 1988;37:1595–607.
10. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991;14:173–94.
11. Marceau P, Biron S, Hould FS, Marceau S, Simard S, Thung SN, et al. Liver pathology and the metabolic syndrome X in severe obesity. J Clin Endocrinol Metab 1999;84:1513–7.
12. Schwimmer JB, Deutsch R, Rauch JB, Behling C, Newbury R, Lavine JE. Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. J Pediatr 2003;143:500–5.
13. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, et al. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 1999;107: 450–5.
14. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, et al. NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 2002;35:373–9.
15. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001;50:1844–50.
16. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434–8.
17. Ludwig J, McGill DB, Lindor KD. Review: nonalcoholic steatohepatitis. J Gastroenterol Hepatol 1997;12:398–403.
18. Brunt EM, Janney CG, DiBisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467–74.
19. Cotrim HP, Parana R, Braga E, Lyra L. Nonalcoholic steatohepatitis and hepatocellular carcinoma: natural history? Am J Gastroenterol 2000;95:3018–9.
20. Zen Y, Katayanagi K, Tsuneyama K, Harada K, Araki I, Nakanuma Y. Hepatocellular carcinoma arising in non-alcoholic steatohepatitis. Pathol Int 2001;51:127–31.
21. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123:134–40.
22. Shimada M, Hashimoto E, Taniai M, Hasegawa K, Okuda H, Hayashi N, et al. Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. J Hepatol 2002;37:154–60.
23. Mori S, Yamasaki T, Sakaida I, Takami T, Sakaguchi E, Kimuta T, et al. Hepatocellular carcinoma with nonalcoholic steatohepatitis. J Gastroenterol 2004;39:391–6.
24. Yoshioka Y, Hashimoto E, Yatsuji S, Kaneda H, Taniai M, Tokushige K, et al. Nonalcoholic steatohepatitis: cirrhosis, hepatocellular carcinoma, and burn-out NASH. J Gastroenterol 2004; 39:1215–8.
25. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 2001;121:91–100.
26. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917–23.
27. Chalasani N, Deeg MA, Persohn S, Crabb DW. Metabolic and anthropometric evaluation of insulin resistance in non-diabetic patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2003;98:1849–55.
28. Shimada M, Hashimoto E, Kaneda H, Noguchi S, Hayashi N. Nonalcoholic steatohepatitis: risk factor for liver fibrosis. Hepatol Res 2002;24:429–38.
29. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183–92.
30. Laine F, Bendavid C, Moirand R, Tessier S, Perrin M, Guillygomarch A, et al. Prediction of liver fibrosis in patients with features of the metabolic syndrome regardless of alcoholic consumption. Hepatology 2004;39:1639–46.
31. Garcia-Monzon C, Martin-Perez E, Iacono OL, Fernandez-Bermejo M, Majano PL, Apolinario A, et al. Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. J Hepatol 2000;33:716–24.
32. Ibdah JA, Perlages P, Zhao Y, Angdisen J, Borgerink H, Shadoan MK, et al. Mice heterozygous for a defect in mitochondrial trifunctional protein develop hepatic steatosis and insulin resistance. Gastroenterology 2005;128:1381–90.
33. Ide T, Shimano H, Yahagi N, Matsuzaka T, Nakakuki M, Yamamoto T, et al. SREBPs suppress IRS-2-mediated insulin signaling in the liver. Nat Cell Biol 2004;6:351–7.
34. Takahashi A, Shimano H, Nakagawa Y, Yamamoto T, Motomura K, Matsuzaka T, et al. Transgenic mice overexpressing SREBP-1a under the control of the PEPCK promoter exhibit insulin resistance, but not diabetes. Biochim Biophys Acta 2005;1740: 427–33.
35. Schatternberg JM, Wang Y, Singh R, Rigoli RM, Czaja MJ. Hepatocyte CYP2E1 overexpression and steatohepatitis lead to impaired hepatic insulin signaling. J Biol Chem 2005;280:9887–94.
36. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20: 1183–97.
37. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High

Blood Cholesterol in Adults (Adult Treatment Panel III) JAMA 2001;285:2486–97.
38. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
39. Nagai Y, Takamura T, Nohara E, Yamashita H, Kobayashi K. Acute hyperinsulinemia reduces plasma concentrations of homocysteine in healthy men. Diabetes Care 1999;22:1004.
40. Brunt EM, Janny CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999:9: 2467–74.
41. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of AASLD Single Topic Conference. Hepatology 2003:5:1202–19.
42. Takamura T, Sakurai M, Ota T, Ando H, Honda M, Kaneko S. Genes for systemic vascular complications are differentially expressed in the livers of Type 2 diabetic patients. Diabetologia 2004:47:638–47.
43. Misu H, Takamura T, Matsuzawa N, Shimizu A, Ota T, Sakurai M, et al. Genes for oxidative phosphorylation are coordinately up-regulated with fasting hyperglycemia in livers of patients with type 2 diabetes. Diabetologia 2007;50:268–77.