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Liver Fat Content Measured by Magnetic Resonance Spectroscopy at 3.0 Tesla Independently Correlates with Plasminogen Activator Inhibitor-1 and Body Mass Index in Type 2 Diabetic Subjects

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Ishii, M., Yoshioka, Y., Ishida, W., Kaneko, Y., Fujiwara, F., Taneichi, H., Miura, M., Toshihiro, M., Takebe, N., Iwai, M., Suzuki, K. and Satoh, J. *Liver Fat Content Measured by Magnetic Resonance Spectroscopy at 3.0 Tesla Independently Correlates with Plasminogen Activator Inhibitor-1 and Body Mass Index in Type 2 Diabetic Subjects*. Tohoku J. Exp. Med., 2005, **206** (1), 23-30 —— We measured liver fat content by 3-Tesla magnetic resonance spectroscopy (MRS) in 34 non- to mild obese Japanese subjects with type 2 diabetes, who were not complicated with any liver diseases including clinical fatty liver (liver/spleen ratio of computed tomography [CT] < 0.9) and were not being treated with oral hypoglycemic agents, insulin, or lipid-lowering agents, and analyzed the relationship between liver fat content and body composition and plasma metabolite. The liver fat content is significantly correlated with variables relating to obesity (body mass index [BMI], body weight, fat mass, waist to hip ratio, visceral fat area, subcutaneous fat area, and serum triglyceride), insulin resistance (fasting plasma insulin and homeostasis model assessment of insulin resistance [HOMA-IR]), adipocytokines (serum plasminogen activator inhibitor-1 [PAI-1] and leptin), and serum cholinesterase, but not CT liver/spleen ratio, which is correlated only with fasting plasma glucose, BMI, and HOMA-IR. Multiple regression analysis revealed that the liver fat content is independently associated with serum PAI-1 level ($p < 0.001$) and BMI ($p < 0.05$), but not visceral fat area. MRS is a more sensitive method for quantifying liver fat content than CT in type 2 diabetic subjects with non- to mild obesity and without clinical fatty liver. —— type 2 diabetes; fatty liver; magnetic resonance spectroscopy; plasminogen activator inhibitor-1; body mass index

Fatty liver, which is characterized by increased fat accumulation in the liver, is frequently associated with obesity and type 2 diabetes mellitus (Angulo 2002; Clark and Diehl 2002). Under these conditions, a large volume of free fatty acid was released to the portal vein and reached the

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liver from adipose tissues, utilized for triglyceride synthesis, and accumulated in the liver (Shulman 2004). Fatty liver increases hepatic insulin resistance (Seppala-Lindroos et al. 2002), which is one of the risk factors of type 2 diabetes. There have been a number of reports on the relationship between fatty liver and insulin resistance (Bugianesi et al. 2004; Reaven et al. 2004). However, the effects of a low liver fat content on insulin resistance and glucose metabolism has not well been known in subjects with type 2 diabetes. Therefore, we measured liver fat content using magnetic resonance spectroscopy (MRS) at 3.0 Tesla in subjects with type 2 diabetes and without clinical fatty liver detected by computed tomography (CT), and analyzed the relationship between liver fat content and variables such as body composition, glucose and lipid metabolism, and adipocytokines.

Patients and Methods

The subjects were 34 patients (17 males and 17 females) with type 2 diabetes, but without clinical fatty liver, who were treated in the outpatient clinic of the Department of Diabetes and Metabolism, Iwate Medical University Hospital. The inclusion criteria for the study were patients with type 2 diabetes who were treated only with diet or exercise therapy, and the exclusion criteria were patients who were treated with any oral hypoglycemic agents such as alpha-glucosidase inhibitors, glinides, sulfonylureas, biguanides and thiazolidinediones, or with lipid-lowering agents such as statins or fibrates, and who had any liver diseases such as viral hepatitis, autoimmune hepatitis, liver cirrhosis, alcoholic liver injury, and fatty liver. The study with human subjects or materials and its protocol were reviewed and approved by the Ethical Committee of Iwate Medical University, and all patients gave written, informed consent.

Body weight and height were measured, and body mass index (BMI) was calculated. Body water content, lean body mass, fat mass, and body fat mass were measured by a multi-frequency body composition analyzer (In body 3.0, BioSpace Japan Inc., Tokyo) based on tetrapolar bioelectrical impedance measurements.

Visceral and subcutaneous abdominal fat were measured by cross-sectional CT scan using Multislice CT (Aquilion 16, Toshiba, Tokyo), as reported elsewhere (Fujiwara et al. 2005). A single 10-mm thick slice at the navel level was used for the analysis. Visceral fat area (VFA) and subcutaneous fat area (SFA) were identified with threshold attenuation values between −150 to 0 Hounsfield unit (HU), and delineated by the Multislice CT. Liver CT attenuations were determined by the mean HU of two regions of interest (ROI) of 120 mm^2 in the liver (1 right lobe, 1 left lobe), and one in the spleen on a cross-sectional scan of 10-mm thickness at $T_{11\text{-}12}$ to image liver and spleen. ROI values in the liver and the spleen were selected in peripheral areas away from major portal, arterial, and venous vessels. Fatty liver was determined by CT attenuation, and clinical fatty liver was defined as < 0.9 of the ratio of liver/spleen CT number (L / S ratio), which correlated well with hepatic fat infiltration of more than 30% (Saitoh et al. 1988).

^{1}H-nuclear magnetic resonance (NMR) spectroscopy was performed at 3.0 Tesla (SIGNA Horizon LX VH / I 3.0 Tesla, GE Healthcare, Waukesha, WI, USA) in the non-fasting state in the morning or afternoon, using a stimulated echo acquisition method (Sawara et al. 2004). The NMR spectrum was obtained from the blocked region. The voxel size was 20 mm × 20 mm × 20 mm. The ROI was the center of the right lobe in the liver and did not contain vascular beds and bile ducts, which could be detected in the axial image. Acquisition parameters for ^{1}H-NMR spectroscopy were as follows: 3,000 milliseconds repetition time, 30 milliseconds echo time, 8 acquisitions (24 seconds), 2.5 kHz spectral width, 2 K data size. The raw data were transferred from a NMR scanner to a computer and processed (apodization, fast Fourier transform, and peak fitting). The typical NMR spectrum of the liver was shown in the Fig. 1. The signals of water and methylene in triglyceride (TG) appear at 4.7 and 1.2 parts per million (ppm), respectively. The NMR spectra were analyzed by an automatic peak-fitting procedure (GRAMS / AI; Galactic Industries, Salem, NH, USA) to determine the area of each peak. The ratio (%) of the areas under water and TG signals was used as a parameter corresponding to the content of TG. The standard deviations of the areas of water and methylene signals estimated repeatedly with the same patients' spectra were less than 1% and 5%, respectively. The deviation of the ratio of methylene/water was less than 5%.

Blood was obtained from patients who had fasted for more than 12 h. Fasting blood glucose (FPG), immunoreactive insulin (IRI), hemoglobin A1c (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), cholinesterase, total cholesterol (TC), triglyceride (TG), high densi-

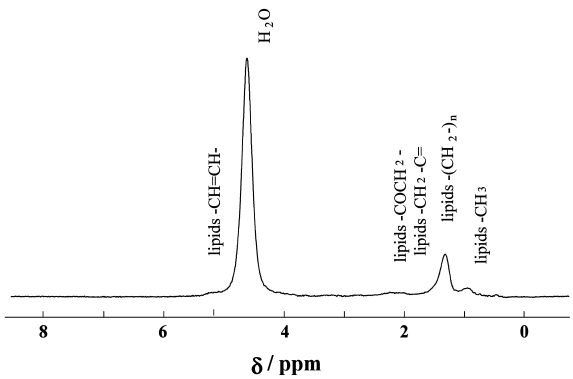

Fig. 1. Typical ^{1}H-NMR spectrum of human liver. The spectrum was obtained without water suppression. The chemical shift was calculated based on the shift of water (4.7 ppm from the DSS [dimethylsilapentane sodium sulfonate] methyl signal). Acquisition parameters were as follows: 3000 milliseconds repetition time, 30 milliseconds echo time, 2500 Hz spectral width, 8 aqusitions.
^{1}H-NMR, ^{1}H-nuclear magnetic resonance; ppm, parts per million.

ty lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and high-sensitive C-reactive protein (CRP) were measured by the Central Laboratory in our hospital. Plasminogen activator inhibitor-1 (PAI-1), leptin, tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) were measured by enzyme-linked immunosorbent assay (ELISA) by SRL Co. (Tokyo). Serum adiponectin level was measured with an ELISA kit (Otsuka Pharmaceutical Company, Tokyo).

Insulin resistance was evaluated by a homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated by FPG (mg / 100 ml) × fasting immunoreactive insulin (F-IRI) (μU / ml) / 405 (Matthews et al. 1985). Patients with FPG of more than 170 mg / 100 ml were excluded.

Data are presented as means ± S.D., unless otherwise indicated. Spearman's rank correlation coefficient was used to examine associations between liver fat content and variables. A p value < 0.05 was considered significant. Statistical analysis was performed by StatView Ver. 5.0 for Macintosh (SAS Institute Inc., Berkeley, CA, USA).

Results

Table 1 shows the characteristics of subjects with type 2 diabetes including liver fat contents, body composition, glucose and lipid metabolism, and cytokines. The data indicate that the majority of subjects were non- to mild obese (BMI < 30), normotensive, and normolipidemia, and had no abnormal serum liver enzyme or cytokine levels. HOMA-IR of most of the subjects was normal and they were under good glycemic control.

The simple linear regression analysis of the relationship between the liver fat content measured by MRS and various variables of the patients is also shown in Table 1. The liver fat content was significantly correlated with BMI ($p < 0.005$), body weight ($p < 0.05$), fat mass ($p < 0.05$), waist to hip ratio ($p < 0.05$), visceral fat area ($p < 0.05$), subcutaneous fat area ($p < 0.05$), fasting plasma insulin ($p < 0.01$), HOMA-IR ($p < 0.005$), and serum levels of triglicerides ($p < 0.01$), cholinesterase ($p < 0.05$), leptin ($p < 0.01$), and PAI-1 ($p < 0.005$), but not with the CT L / S ratio (Fig. 2). Figs. 3 and 4 show the relationship of liver fat content with PAI-1 and BMI, respectively, which have the greatest correlation coefficient among the variables. Moreover there is no sex difference in these relationships (Figs. 2, 3 and 4).

A comparison of the correlation coefficient between the variables and the liver fat content by MRS or the CT L / S ratio shows that the liver fat content is more closely correlated with the variables than the CT L / S ratio (Table 2).

Multiple regression analysis adjusted for the variables, which have a significant simple relationship with liver fat content in Table 1, indicates that liver fat content is independently associated with serum total PAI-1 level ($p < 0.001$) and BMI ($p = 0.036$) (Table 3).

Discussion

The current study shows the clinical significance of the liver fat content at relatively low levels measured by the 3 Tesla MRS. The MRS could quantitatively measure liver fat contents in diabetic patients without clinical fatty liver. The liver fat content obtained by MRS is not correlated with L / S ratio of CT in these patients without clinical fatty liver (Fig. 2). Nevertheless, the liver fat content is significantly correlated with various

TABLE 1. *Clinical characteristics of subjects and correlation of liver fat content with variables*

		Correlation with liver fat content	
		r	*p*
No. of subjects (M / F)	34 (17/17)	-	-
Liver fat content (%)	4.7 ± 3.6	-	-
CT L / S ratio	1.2 ± 0.1	- 0.121	0.486
Age (years)	65 ± 12	- 0.174	0.368
Diabetes duration (years)	5.7 ± 4.3	- 0.112	0.528
Systolic BP (mmHg)	133 ± 13	0.157	0.368
Diastolic BP (mmHg)	77 ± 9	0.109	0.533
Body mass index (kg / m^2)	24.2 ± 2.1	0.523	0.003
Body weight (kg)	61.5 ± 8.1	0.391	0.025
Fat mass (kg)	15.2 ± 4.2	0.416	0.018
Body fat (%)	24.2 ± 6.8	0.297	0.093
Waist to hip ratio	0.9 ± 0.0	0.390	0.027
Visceral fat area (cm^2)	143.2 ± 54.2	0.429	0.014
Subcutaneous fat area (cm^2)	177.6 ± 73.8	0.344	0.048
Fasting plasma glucose (mg / 100 ml)	121.7 ± 18.2	0.323	0.064
HbA1c (%)	6.1 ± 0.7	0.313	0.072
Fasting insulin (μU / ml)	6.16 ± 3.84	0.475	0.006
HOMA-IR	1.89 ± 1.28	0.503	0.004
HOMA-β	39.1 ± 23.1	0.286	0.099
Total cholesterol (mg / 100 ml)	221 ± 31	- 0.057	0.745
HDL cholesterol (mg / 100 ml)	62 ± 17	- 0.159	0.361
LDL cholesterol (mg / 100 ml)	136 ± 28	0.031	0.858
Triglycerides (mg / 100 ml)	113 ± 48	0.460	0.008
Non-esterified fatty acid (mEq / liter)	0.56 ± 0.23	- 0.257	0.159
AST (IU / liter)	21 ± 6	- 0.121	0.488
ALT (IU / liter)	21 ± 8	0.145	0.405
γ GTP (IU / liter)	33 ± 26	0.109	0.530
Cholinesterase (IU / liter)	346 ± 61	0.366	0.035
Adiponectin (μg / ml)	1.22 ± 0.69	- 0.344	0.079
Leptin (ng / ml)	7.2 ± 5.5	0.459	0.008
Plasminogen activator inhibitor-1 (ng / ml)	28.1 ± 12.1	0.536	0.002
Tumor necrosis factor-α (pg / ml)	< 5	-	-
Interleukin-1β (pg / ml)	< 10	-	-
Interleukin-6 (pg / ml)	1.7 ± 1.8	- 0.045	0.800
High sensitive CRP (mg / 100 ml)	0.117 ± 0.109	0.179	0.304

Data are means ± S.D.

CT L / S ratio, computed tomography liver / spleen ratio; BP, blood pressure; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of beta cell; HDL, high density lipoprotein; LDL, low density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ GTP, γ-glutamyl transpeptidase; CRP, C-reactive protein.

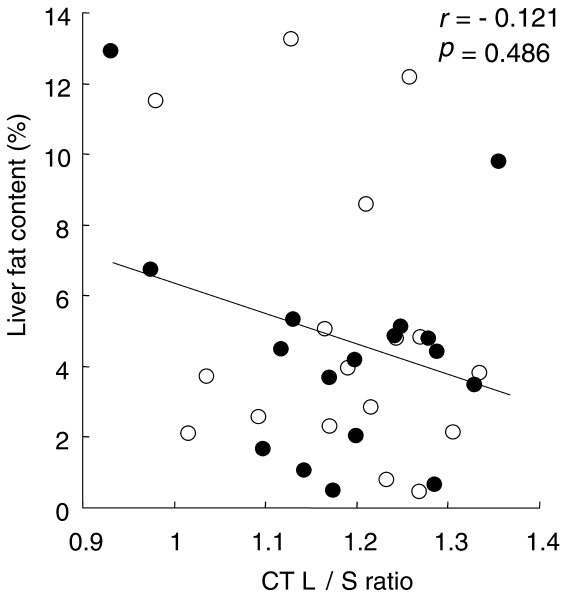

Fig. 2. No relationship between liver fat content measured by MRS and CT L/S ratio.
●, male; ○, female.
MRS, magnetic resonance spectroscopy; CT L / S, computed tomography liver / spleen ratio.

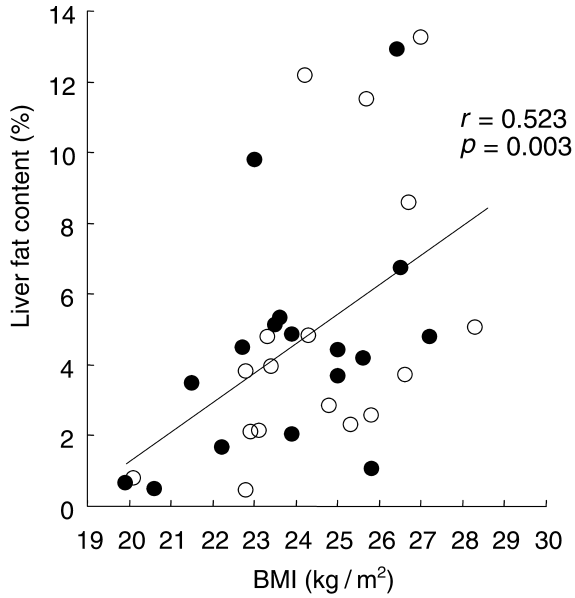

Fig. 4. Relationship between liver fat content measured by MRS and BMI.
●, male; ○, female.
MRS, magnetic resonance spectroscopy; BMI, body mass index.

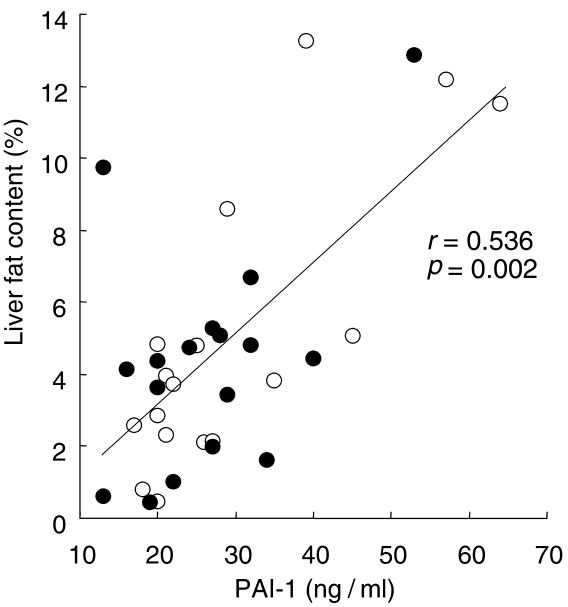

Fig. 3. Relationship between liver fat content measured by MRS and serum level of PAI-1.
●, male; ○, female.
MRS, magnetic resonance spectroscopy; PAI-1, plasminogen activator inhibitor-1.

factors associated with body fat accumulation (BMI, body weight, fat mass, W-H ratio, VFA, SFA, and TG), factors related with insulin resistance (fasting serum IRI and HOMA-IR), adipocytokines (leptin and PAI-1), and cholinesterase, a liver enzyme that increases in fatty liver (Table 1). There is also a tendency of a correlation between liver fat content and other metabolic and adipocyte factors such as FPG, HbA1c, HOMA-β, body fat, and serum adiponectin. However, the multiple regression analysis suggests that independent variables relating to liver fat content are serum PAI-1 and BMI (Table 3).

Independent correlation of BMI with liver fat content indicates that an increase of body fat promotes liver fat accumulation even in non- to mild obese subjects. The liver fat accumulation is associated with hepatic insulin resistance evaluated by HOMA-IR. The link among obesity and liver fat accumulation and insulin resistance could be explained by the release of FFA to the portal vein from the splanchnic bed, accounting for fat accumulation in the liver (Frayn 2000). The current data indicate that liver fat content is more

TABLE 2. *Comparison of correlation of liver fat content with Variables between MRS and L / S ratio*

Variables	MRS		CT L / S ratio	
	r	*p*	*r*	*p*
BMI	0.523	0.003	- 0.400	0.021
Body weight	0.391	0.025	- 0.187	0.281
Fat mass	0.416	0.018	- 0.340	0.054
Waist to hip ratio	0.390	0.027	- 0.318	0.071
Visceral fat area1	0.429	0.014	- 0.180	0.302
Subcutaneous fat area	0.344	0.048	- 0.121	0.488
Cholinesterase	0.366	0.035	- 0.143	0.412
Triglycerides	0.460	0.008	- 0.127	0.479
Fasting plasma glucose	0.323	0.064	- 0.442	0.011
Fasting insulin	0.475	0.006	- 0.239	0.170
HOMA-IR	0.503	0.004	- 0.360	0.038
Leptin	0.459	0.008	- 0.246	0.158
PAI-1	0.536	0.002	- 0.314	0.071

MRS, magnetic resonance spectroscopy; CT L / S ratio, computed tomography liver / spleen ratio; HOMA-IR, homeostasis model assessment of insulin resistance; PAI-1, plasminogen activator inhibitor-1.

TABLE 3. *Multiple regression analysis (Dependent variable: Liver fat content)*

Independent variables	β	t	*p*
PAI-1	0.576	4.191	< 0.001
BMI	0.349	2.196	0.036
Triglycerides	0.253	1.628	0.115
Fasting insulin	- 0.169	- 0.929	0.361
Visceral fat area	- 0.054	- 0.370	0.714

Adjusted $R^2 = 0.502$.
PAI-1, plasminogen activator inhibitor-1; BMI, body mass index.

closely associated with BMI than the visceral fat area. This result is consistent with a recent report that hepatic FFA delivery from visceral adipose tissue is not so much (less than 20%) in subjects with a visceral fat area of less than 200 cm^2 (Nielsen et al. 2004), as our subjects with average visceral fat area of 143 cm^2. It has been reported that suppression with insulin of lipolysis and gluconeogenesis in the liver is impaired in the patients with fatty liver (Marchesini et al. 2001; Sanyal et al. 2001; Seppala-Lindroos et al. 2002), and impaired suppression of gluconeogenesis with insulin is correlated with liver fat content (Ryysy et al. 2000). Recently, a molecular mechanism of liver insulin resistance by fatty liver has been reported, i.e., hepatic fat accumulation stimulates gluconeogenesis and activates PKC-epsilon and JNK1, which may interfere with tyrosine phosphorylation of IRS-1 and IRS-2, and impair the ability of insulin to activate glycogen synthase (Samuel et al. 2004). Our results indicate that a metabolic link among obesity, fatty liver, and hepatic insulin resistance is also present in diabetic patients without severe obesity and apparent fatty liver.

Thus, a quantitative measurement of liver fat content is important for evaluating the role of liver fat in insulin resistance and type 2 diabetes. Histological examination of liver specimen obtained by liver biopsy is most reliable, but the method is invasive and the specimen is too small for evaluation. CT and ultrasonographic imaging of the liver are feasible. However, these are not

accurate for a quantitative evaluation of fatty liver, although they are widely used in the clinical diagnosis of the fatty liver (Saadeh et al. 2002). Therefore, we utilized a 3-Tesla MRS with high sensitivity and high resolution (Fischbach et al. 2004; Sawara et al. 2004), which needs shorter breath-holding time than previous 1.5 Tesla MRS. In our subjects without apparent fatty liver, liver fat content by MRS does not correlate with L / S ratio of CT. Furthermore, a total of 12 variables are significantly correlated with liver fat content by MRS, whereas only 2 out of 12 variables were correlated with the L / S ratio, and the correlation coefficient of these 2 is much lower than the fat contents obtained by MRS. Thus, MRS is superior for studying liver fat content at relatively low levels.

In the current study, we also show that liver fat content is most significantly and independently correlated with serum PAI-1 concentration. It has already been reported that hepatocytes produce PAI-1 (Anfosso et al. 1993), and plasma PAI-1 levels are more strongly related to liver steatosis than to adipose tissue accumulation (Alessi et al. 2003). Our results may indicate that even a low liver fat content stimulates hepatocytes to produce PAI-1, which may be involved in cardiovascular risk (Bastard et al. 2000).

In summary, the results indicate that the 3.0 Testa MRS is a sensitive method for a quantitative measurement of liver fat content in subjects without clinical fatty liver, that fat accumulation of the liver is associated with an increase of BMI and insulin resistance in non- to mild obese diabetic patients without clinical fatty liver, and that liver fat content is also significantly and independently correlated with PAI-1. It is suggested that an evaluation of liver fat content is important for understanding pathophysiology of insulin resistance in non- to mild obese diabetic patients without clinical fatty liver.

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