


Impaired peripheral insulin sensitivity in non-obese Japanese patients with type 2 diabetes mellitus and fatty liver

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Keywords

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ABSTRACT

Aims/Introduction: Type two diabetes mellitus and fatty liver (FL) are not uncommon in Asians with normal body mass index. Previous studies reported a link between FL and insulin resistance. Thus, FL could coexist with insulin resistance in Asian type two diabetes mellitus patients with a normal body mass index. However, the clinical and metabolic features of such patients have not been characterized yet.

Materials and Methods: We recruited 29 non-obese (body mass index <25 kg/m²) Japanese patients with early type two diabetes mellitus. Based on intrahepatic lipid level measured by H-magnetic resonance spectroscopy, the participants were divided into the FL (intrahepatic lipid ≥5%, *n* = 7) and non-FL groups (intrahepatic lipid <5%, *n* = 22).

Results: Peripheral insulin sensitivity measured by hyperinsulinemic euglycemic clamp was ~25% lower in the FL group than in the non-FL group, whereas hepatic insulin sensitivity was comparable between the two groups. The subcutaneous fat area was larger, free fatty acid level was higher, C-reactive protein was higher and high molecular weight adiponectin was lower in the FL group than the non-FL group.

Conclusions: The present study showed that the metabolic features of non-obese Japanese type two diabetes patients with FL include impaired peripheral (mainly muscle) insulin sensitivity, fat accumulation and related metabolic disorders, such as elevated free fatty acid, low high molecular weight adiponectin and low-grade inflammation.

INTRODUCTION

The growing number of individuals with obesity and type 2 diabetes mellitus is currently a global health problem. The mean body mass index (BMI) of patients with type 2 diabetes in Western countries is approximately 30 kg/m^{2,1,2} and most patients are considered to have insulin resistance.¹ In comparison, it is not uncommon for Asian people with normal BMI (<25 kg/m²) to develop type 2 diabetes mellitus.^{2,3} Whereas this feature in Asian type 2 diabetes could be explained by decreased pancreatic β-cell function,³ it could also be due to substantial differences in insulin resistance between Asians and people of other ethnicities. Indeed, our group recently reported the presence of insulin resistance in muscle, but not in the liver, of non-obese non-diabetic men with even one cardiometabolic

risk factor.⁴ Thus, insulin resistance might be a common underlying pathogenic mechanism of various metabolic diseases, even in non-obese individuals.

Previous studies linked fatty liver to insulin resistance.^{4–10} Although the prevalence of non-alcoholic fatty liver disease, a condition caused by fat accumulation in the liver, is increased in obese individuals, previous data showed that Asians with low BMI can develop non-alcoholic fatty liver disease.^{11–13} These data suggest that in non-obese Asians, type 2 diabetes mellitus could be accompanied by liver fat accumulation, and that such individuals could perhaps have lower insulin sensitivity. To our knowledge, however, the metabolic features of Asian non-obese type 2 diabetes patients with fatty liver have not been investigated in detail.

Based on the aforementioned background, we designed the present study to evaluate fatty liver status and insulin sensitivity in non-obese Japanese patients with early type 2 diabetes. For

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this purpose, we evaluated fat accumulation in the liver by ^1H -magnetic resonance spectroscopy (MRS) and tissue-specific insulin resistance by the gold standard method – euglycemic hyperinsulinemic clamp – and compared the metabolic features of patients with and without fatty liver (FL).

METHODS

Participants

We screened patients with type 2 diabetes who attended Jun-endo University Hospital (Tokyo, Japan) on a regular basis between September 2013 and April 2015, and individuals who registered for clinical tests with the outsourcing company (SOUKEN, Tokyo, Japan). Among them, we included those who fulfilled all of the following criteria: (i) diagnosis of type 2 diabetes based on the criteria of the Japanese Diabetes Society^{14,15}; (ii) no obesity with BMI <25 kg/m²; (iii) glycosylated hemoglobin of 6.5–8.4%; (iv) age 40–65 years; and (v) no treatment with antidiabetic agents or α -glucosidase inhibitor alone. The following exclusion criteria were also applied: (i) type 1 diabetes; (ii) individuals who drink alcohol >20 g/day; (iii) serious liver disease or hepatitis B or C virus infection; (iv) chronic renal failure; (v) apparent heart failure or myocardial infarction; (vi) serious pancreatic disease; (vii) malignancy; (viii) serious diabetes complications, such as progressive nephropathy (urinary albumin excretion \geq 300 mg/g creatinine) and pre-proliferative retinopathy; (ix) significant infection or inflammation based on clinical symptoms and signs, such as fever, local pain, redness and swelling; (x) contraindication for magnetic resonance imaging; and (xii) refusal to give informed consent.

A total of 24 men and five women who fulfilled the aforementioned criteria were included in the present study. Four participants were treated with α -glucosidase inhibitor alone, whereas the other participants were not treated with any medication. All participants gave written informed consent to the study, which was approved by the ethics committee of Jun-endo University. This study was carried out in accordance with the principles outlined in the Declaration of Helsinki.

Study design

In the present cross-sectional study, all participants were prohibited to engage in regular exercise for 10 days before the first experimental day, and daily physical activity level was monitored by an ambulatory accelerometer (Lifecorder; Suzuken, Nagoya, Japan).⁴ The diet history of each patient was evaluated by a brief-type self-administered diet history questionnaire. The participants were instructed to follow a weight-maintaining standard diet (55% carbohydrate, 25% fat, 20% protein) for 3 days before the experimental day.⁴

On the day of the study, we measured intramyocellular lipid (IMCL) in the right tibialis anterior muscle and intrahepatic lipid (IHL) of segment six of the liver by 1.5 Tesla ^1H -MRS (VISART EX V4.40; Toshiba, Tokyo, Japan) after overnight fasting.^{16,17} We measured total body fat content by the bioimpedance method (InBody; BIOSPACE, Tokyo, Japan). Intra-

abdominal and subcutaneous fat areas were evaluated by using magnetic resonance imaging and specific software (AZE, Tokyo, Japan), as described previously.¹⁷ In addition, we carried out a hyperinsulinemic euglycemic clamp study to evaluate peripheral and hepatic insulin sensitivity.

Proton magnetic resonance spectroscopy and imaging

IHL and IMCL were measured as described previously.^{16,17} Briefly, IMCL and IHL were measured by 1.5 Tesla ^1H -MRS using specific coils (VISART EX V4.40; Toshiba, Tokyo, USA). After examination, resonance was quantified by reference to the methylene signal intensity (S-fat), with peaks being observed at \sim 1.3 ppm in the liver and at \sim 1.25 ppm in muscle. IMCL in right tibialis anterior was quantified by S-fat and the creatine signal at 3.0 ppm (Cre) was used as the reference, and was calculated as the ratio relative to Cre (S-fat/Cre). The IHL of liver segment 6 was quantified by S-fat and H₂O at \sim 4.7 ppm as the internal reference, and calculated as a percentage of H₂O + S-fat (S-fat \times 100 / [H₂O + S-fat]) as described previously.^{16,17}

Euglycemic hyperinsulinemic glucose clamp test

A euglycemic hyperinsulinemic glucose clamp study was carried out with an artificial endocrine pancreas as previously described (STG 55; Nikkiso, Shizuoka, Japan).⁴ Intravenous cannulas were placed in the forearms, and one cannula was used for infusion of the tracer and insulin. Another catheter placed in a vein on the contralateral hand was heated by a warming blanket for arterialized blood sampling. Then, we infused a priming dose of 6,6- $^2\text{H}_2$ -glucose intravenously (bolus 200 mg-FPG [mg/dL]/100-m⁻² body surface area), which was followed by constant infusion of 2 mg/m² body surface area per min for 3 h ($-$ 180 to 0 min). After a basal equilibration period, we infused primed insulin (160 mU/m² per min for 5 min followed by 80 mU/m² per min for 5 min) and started continuous insulin administration at 40 mU/m² per min for 3 h (0–180 min). Then, the plasma glucose level was maintained at \sim 95 mg/dL by variable 20% glucose infusion containing \sim 2.5% 6,6- $^2\text{H}_2$ -glucose. Blood samples were obtained for biochemical analysis at 10 min intervals during the last 30 min at baseline and steady state during the clamp study. The enrichment of 6,6- $^2\text{H}_2$ glucose in plasma was determined as previously described.¹⁸ The rates of endogenous glucose production (EGP) and glucose disposal (Rd)⁵ during the clamp study were evaluated by a steady-state equation.¹⁹ We used insulin-stimulated EGP suppression as an index of hepatic insulin sensitivity, and the Rd as an index of peripheral (mainly muscle)²⁰ insulin sensitivity.

Statistical analysis

Data are presented as mean \pm standard deviation or median values (range 25–75%), or number of patients. To approximate normal distribution, natural log-transformed values for plasma γ -glutamyl transferase (GGT), insulin, high molecular weight (HMW)-adiponectin, the homeostasis model assessment of insulin resistance and C-reactive protein (CRP) were used in

the analysis. Differences between groups were evaluated by the χ^2 -test for non-continuous variables, or by Student's *t*-test or Mann–Whitney *U*-tests for continuous variables. Pearson's or Spearman's correlation coefficient was used to evaluate the correlation between variables as appropriate. All statistical tests were two-sided with a 5% significance level.

RESULTS

Anthropometric characteristics of non-obese type 2 diabetes with fatty liver

Table 1 summarizes the clinical characteristics of the study participants. The median IHL was 1.5%, and the mean or median liver enzyme levels were within the normal limits (aspartate aminotransferase [AST] 10–40 IU/L, alanine aminotransferase [ALT] 10–40 IU/L, GGT \leq 70 IU/L for men and \leq 30 IU/L for

women). Using the definition of fatty liver as IHL of $>5\%$,²¹ we divided the participants into those with IHL $\geq 5\%$ (FL group) and IHL $<5\%$ (non-FL group). Among the 29 study participants, seven patients (24%) had fatty liver. Table 1 shows the clinical characteristics of the two groups. Age, sex, glycated hemoglobin and FPG levels were comparable between the two groups. IHL levels were approximately 8.5-fold significantly higher in the FL group than the non-FL group. However, there were no differences in AST, ALT and GGT between the two groups, although the mean value of each liver enzyme was higher in the FL group. Ferritin level, another marker of liver damage,²² and type IV collagen level, a marker of liver fibrosis,²³ were also similar between the groups, respectively. BMI was higher, and subcutaneous fat area, but not visceral fat area, was larger in the FL group than the non-FL group. In addition,

Table 1 | Clinical characteristics of the 29 non-obese type 2 diabetes patients

	Total	Non-fatty liver	Fatty liver	<i>P</i> -value
<i>n</i>	29	22	7	–
Age (years)	55.1 \pm 6.9	53.8 \pm 6.4	59.3 \pm 7.0	0.06
Sex (male/female)	24/5	19/3	5/2	0.36
Duration (years)	5.5 \pm 3.0	5.4 \pm 2.9	5.9 \pm 3.7	0.71
BMI (kg/m ²)	21.7 \pm 2.2	21.2 \pm 2.1	23.5 \pm 1.4	$<0.05^*$
Total body fat content (%)	16.2 \pm 5.2	16.1 \pm 5.2	16.6 \pm 5.5	0.81
Systolic blood pressure (mmHg)	113.4 \pm 14.4	111.8 \pm 15.0	118.4 \pm 11.6	0.29
Diastolic blood pressure (mmHg)	72.6 \pm 7.6	71.6 \pm 7.6	75.9 \pm 6.9	0.20
Waist circumference (cm)	80.4 \pm 7.2	79.0 \pm 7.2	84.6 \pm 5.5	0.07
Alcohol intake (g/day)	5.3 \pm 6.6	5.5 \pm 7.0	4.8 \pm 5.8	0.82
Aspartate aminotransferase (IU/L)	21.4 \pm 7.2	20.3 \pm 6.4	25.1 \pm 8.9	0.12
Alanine aminotransferase (IU/L)	20.7 \pm 9.9	18.8 \pm 6.6	26.9 \pm 15.8	0.06
γ -Glutamyl transferase (IU/L)	25.0 (19.0–34.0)	24.0 (19.0–27.8)	50.0 (22.5–55.5)	0.10
Platelet (10 ⁴ / μ L)	22.3 \pm 5.5	22.2 \pm 5.5	22.7 \pm 6.1	0.84
Type IV collagen (ng/mL)	3.9 \pm 0.7	4.0 \pm 0.7	3.7 \pm 0.9	0.36
Ferritin (ng/mL)	111.9 \pm 83.8	107.1 \pm 58.5	127.0 \pm 143.1	0.60
Fasting plasma glucose (mg/dL)	130 \pm 21	130 \pm 21	130 \pm 22	0.99
Fasting serum insulin (μ U/mL)	2.8 (1.9–5.0)	2.3 (1.7–3.8)	5.1 (3.8–6.8)	$<0.05^*$
HOMA-IR	0.8 (0.6–1.7)	0.7 (0.5–1.4)	1.9 (1.1–2.4)	$<0.05^*$
HbA1c (%)	6.8 \pm 0.4	6.8 \pm 0.4	6.8 \pm 0.5	0.75
LDL cholesterol (mg/dL)	128.6 \pm 19.7	124.5 \pm 20.0	141.1 \pm 13.2	$<0.05^*$
HDL cholesterol (mg/dL)	55.9 \pm 15.6	59.0 \pm 16.1	46.1 \pm 8.5	0.05
Triglyceride (mg/dL)	110.9 \pm 48.6	110.1 \pm 52.0	113.1 \pm 39.3	0.89
Basal EGP (mg/m ² per min)	80.2 \pm 9.7	80.8 \pm 9.8	78.2 \pm 9.8	0.54
% Suppression of EGP (%)	87.1 \pm 8.6	87.9 \pm 7.5	84.3 \pm 11.9	0.34
Rd (mg/FFM kg per min)	6.7 \pm 2.0	7.1 \pm 1.8	5.3 \pm 2.1	$<0.05^*$
Free fatty acid (μ Eq/L)	573 \pm 156	539 \pm 160	681 \pm 76	$<0.05^*$
HMW-adiponectin (μ g/mL)	1.5 (0.7–2.2)	1.9 (1.0–2.4)	0.6 (0.3–0.8)	$<0.01^*$
C-reactive protein (ng/mL)	211 (132–769)	180 (131–309)	1120 (525–7240)	$<0.01^*$
Visceral fat area (cm ²)	82.0 \pm 31.1	77.0 \pm 33.0	97.0 \pm 19.0	0.14
Subcutaneous fat area (cm ²)	93.3 \pm 41.7	81.0 \pm 32.4	130.6 \pm 46.6	$<0.01^*$
Intrahepatic lipid (%)	1.5 (0.3–3.7)	1.2 (0.04–2.2)	10.3 (7.8–11.6)	$<0.01^*$
IMCL in tibialis anterior muscle (S-fat/Cre)	3.0 \pm 1.6	2.8 \pm 1.6	3.6 \pm 1.5	0.36

Data are mean \pm standard deviation or median (range 25–75%), or number of participants. *P*-values are for comparisons of non-fatty liver group versus the fatty liver group. *Statistically significant. BMI, body mass index; Cre, creatine; EGP, endogenous glucose production; FFM, fat-free mass; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; HMW, high molecular weight; IMCL, intramyocellular lipid; Rd, rate of glucose disposal; S-fat, methylene signal intensity.

free fatty acid (FFA), CRP and insulin levels were significantly higher, and HMW-adiponectin level was significantly lower in the FL group than the non-FL group.

Steady-state insulin levels during glucose clamp were comparable between the groups (non-FL group $76.9 \pm 25.8 \mu\text{U/mL}$, FL group $85.4 \pm 10.4 \mu\text{U/mL}$, $P = 0.41$). Peripheral insulin sensitivity, determined by Rd during the clamp study, was significantly lower in the FL group than the non-FL group. Consistently, homeostasis model assessment of insulin resistance was significantly higher in the FL group. In contrast, hepatic insulin sensitivity, determined by insulin-stimulated suppression of EGP during clamp, was similar in the two groups. As well as fasting state, FFA level at steady state during glucose clamp was relatively higher in the FL group compared with non-FL group, but the difference was not statistically significant (non-FL group $53.9 \pm 94.9 \mu\text{Eq/L}$, FL group $153 \pm 153 \mu\text{Eq/L}$, $P = 0.15$). Physical activity level and dietary composition were similar between the two groups (data not shown).

Factors associated with insulin sensitivity

Next, correlation analysis was carried out to further investigate the association between IHL and insulin sensitivity. IHL level correlated only weakly with peripheral insulin sensitivity (Table 2), and it did not correlate significantly with hepatic insulin sensitivity (Table 2). Univariate analysis that included data of all participants showed a close and significant relationship between peripheral insulin sensitivity and liver insulin sensitivity ($r = 0.45$, $P < 0.05$).

Next, to further search for factors associated with tissue-specific insulin resistance in this population, we carried out preliminary analysis using the parameters listed in Table 1. Homeostasis model assessment of insulin resistance (an index of insulin sensitivity) and ALT correlated significantly with both hepatic and peripheral insulin sensitivity (Table 2). In addition, total body fat content and fasting serum insulin correlated significantly with insulin sensitivity in peripheral tissues, but not in the liver. In contrast, only GGT (Table 2) and ferritin (Table 2) levels correlated significantly with insulin sensitivity in the liver, but not in the peripheral tissues. None of the other factors analyzed in the study correlated with hepatic or peripheral insulin sensitivity (Table 2).

To further investigate the impact of IHL on glucose metabolism and insulin resistance, we finally evaluated the correlation between IHL level and factors raised in Table 1. We found that only AST ($r = 0.44$, $P < 0.05$), HMW-adiponectin ($r = -0.48$, $P < 0.05$) and CRP ($r = 0.51$, $P < 0.05$) were significantly correlated to IHL, respectively.

DISCUSSION

Although type 2 diabetes mellitus is not uncommon in Asian people with normal BMI ($<25 \text{ kg/m}^2$),^{2,3} little is known about the metabolic features of non-obese Asian diabetes patients. The present study focused on the presence of FL, and evaluated

Table 2 | Results of univariate regression analysis of hepatic and muscle insulin sensitivity using data of 29 non-obese type 2 diabetes patients

	% Reduction of EGP		Rd	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
Age	0.33	0.08	-0.002	0.99
Duration	0.06	0.75	0.003	0.99
BMI	-0.29	0.13	-0.36	0.06
Total body fat content	-0.10	0.60	-0.44*	<0.05*
Systolic blood pressure	0.08	0.68	0.16	0.40
Diastolic blood pressure	-0.02	0.94	0.03	0.90
Waist	-0.16	0.42	-0.01	0.62
Alcohol intake	-0.07	0.74	0.06	0.77
Aspartate aminotransferase	-0.31	0.10	-0.30	0.12
Alanine aminotransferase	-0.58*	<0.01*	-0.67*	<0.01*
γ -Glutamyl transferase	-0.41*	<0.05*	-0.33	0.09
Platelet	-0.04	0.84	-0.02	0.93
Type 4 collagen	-0.19	0.33	0.20	0.29
Ferritin	-0.43*	<0.05*	-0.34	0.07
Fasting plasma glucose	-0.37	0.05	-0.36	0.06
Fasting serum insulin	-0.22	0.25	-0.64*	<0.01*
HbA1c	-0.21	0.28	-0.35	0.06
HOMA-IR	-0.40*	<0.05*	-0.67*	<0.01*
LDL cholesterol	0.12	0.53	-0.18	0.35
HDL cholesterol	-0.01	0.94	0.25	0.19
Triglyceride	-0.30	0.11	-0.27	0.15
Basal EGP	0.02	0.91	0.24	0.22
Free fatty acid	0.16	0.42	-0.20	0.31
HMW-adiponectin	0.26	0.18	0.30	0.12
CRP	-0.33	0.08	-0.08	0.68
Visceral fat area	-0.13	0.51	-0.23	0.23
Subcutaneous fat area	-0.08	0.68	-0.16	0.41
Intrahepatic lipid	-0.02	0.92	-0.32	0.09
IMCL in tibialis anterior muscle	0.01	0.95	0.11	0.60

*Statistically significant. BMI, body mass index; CRP, C-reactive protein; EGP, endogenous glucose production; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; HMW, high molecular weight; IMCL, intramyocellular lipid; Rd, rate of glucose disposal.

insulin resistance in non-obese Japanese patients with early type 2 diabetes by euglycemic hyperinsulinemic clamp, which is considered to be the most accurate method for measurement of insulin resistance. In Japan, FL is not rare among non-obese diabetes patients. The present results showed that such patients have impaired insulin sensitivity in peripheral tissues, compared with those without FL. In addition, the results also showed that non-obese type 2 diabetes patients with FL had various metabolic abnormalities, such as a larger subcutaneous fat area, and higher FFA and CRP levels, in addition to lower HMW-adiponectin levels, compared with those without FL.

The relationships between FL and various metabolic abnormalities have already been reported in other ethnic groups, in studies that mainly included obese participants.^{10,24,25} The present analysis showed that similar relationships are also observed

even in non-obese Japanese with type 2 diabetes. In this regard, although the main FFA supply to the liver in humans is from endogenous rather than exogenous sources,²⁶ dysfunction of adipose tissue is characterized by enhanced FFA release. Furthermore, decreased adiponectin secretion from adipocytes and low-grade inflammation are other features of adipose tissue dysfunction. Adiponectin is known to increase fat oxidation in muscle and the liver,²⁷ and low adiponectin levels are associated with the development of FL.²⁸ Low-grade inflammation was also associated with the development of non-alcoholic fatty liver disease in healthy Korean male workers.²⁹ In addition, all these factors, including FFA, inflammation and low adiponectin, are known to induce insulin resistance. Therefore, it is possible that adipocyte dysfunction could induce fatty liver and muscle insulin resistance, simultaneously, thus these metabolic abnormalities were accumulated in non-obese type 2 diabetes patients with FL. In contrast, muscle insulin resistance has been reported to promote fatty liver by altering the pattern of post-prandial carbohydrate storage away from muscle glycogen and into hepatic de novo lipogenesis.^{30,31} These data suggest another possibility that adipocyte dysfunction and muscle insulin resistance could be primarily present in non-obese Japanese type 2 diabetes with FL, and that IHL accumulation might be a secondary change associated with such metabolic abnormalities.

The etiology of hepatic insulin resistance in non-obese individuals remains unclear. Petersen *et al.*³² used the same glucose clamp protocol (insulin infusion rate; 40 mU/m² per min) and ¹H-MRS method to evaluate hepatic insulin resistance and IHL level in obese type 2 diabetes patients (mean FPG 158 mg/dL) before and after moderate weight reduction (~8%). Interestingly, although the IHL level in their study (~12%) was similar to that in the participants of the FL group of the present study (10.3%), the insulin-stimulated EGP suppression during glucose clamp was only ~30%. In addition, after moderate weight reduction, the IHL level approached normality (2.2%) and the EGP level during glucose clamp was completely suppressed (99%). In parallel with these changes, the FPG level decreased significantly (mean FPG 115 mg/dL). These data highlight the pathological role of FL in hepatic insulin resistance and glycemic control in obese type 2 diabetes patients. In contrast, we included patients who were not treated with antidiabetes drugs and those treated with α -glucosidase inhibitor alone to avoid the effect of other antihyperglycemic agents on the results. Thus, compared with previous studies, blood glucose level was well controlled (mean FPG 113 mg/dL). In addition, all study participants were non-obese Japanese, and their mean BMI was much lower than previous studies.^{6,32–35} Thus, at least in non-obese diabetes patients with moderate FL and well-controlled blood glucose levels, IHL accumulation does not seem to be a strong determinant of hepatic insulin resistance.

Previous studies showed that plasma ALT and/or GGT levels correlated with insulin resistance in Caucasians, American Africans,³⁶ Pima Indians³⁷ and Asians.³⁸ It has been reported that liver enzymes are often elevated in non-obese Asians, and that

mildly high ALT and GGT levels can predict the development of metabolic syndrome and type 2 diabetes in Asians.^{39–42} These data suggest that ALT and GGT levels could be potentially used as surrogate markers of insulin resistance, and predictors of future metabolic syndrome and type 2 diabetes mellitus. Consistently, our preliminary correlation analysis in the present study also showed a significant correlation between liver enzymes and insulin resistance in non-obese Japanese type 2 diabetes patients, and similar correlations were also observed in non-obese non-diabetes men in our recent study.⁴ However, these correlation analyses were based on a small number of participants, therefore, further large studies with multiple regression analysis are required to confirm whether elevated liver enzyme levels can be used to predict impaired insulin sensitivity in non-obese individuals.

The present study had several limitations. First, we infused insulin at a relatively higher rate (40 mU/m² per min) during glucose clamp, and used the insulin-stimulated EGP suppression as an index of hepatic insulin sensitivity. Several reports using the same insulin infusion rate (40 mU/m² per min) showed that the EGP levels were weakly (~30%)³² to moderately suppressed (~60%)^{33–35} in diabetes patients, whereas the EGP level was almost completely suppressed in healthy individuals.³² In the present study, the EGP level was highly suppressed (80–90%), thus the study participants had moderate hepatic insulin resistance compared with those of previous studies. In such case, a small difference in hepatic insulin resistance can be detected by glucose clamp only with lower insulin infusion protocol (20 mU/m² per min), but not with higher insulin infusion protocol (40 mU/m² per min).⁴³ Thus, our glucose clamp protocol could not detect a small difference in hepatic insulin resistance between the two groups. In fact, although fasting serum insulin levels were higher in the FL group compared with the non-FL group, basal EGP levels were comparable between the two groups. Accordingly, we cannot exclude the possible presence of very modest impaired hepatic insulin sensitivity in the FL group.³³ Second, the study included mainly male patients. This is important, because metabolism and fat distribution are different in women compared with men.⁴⁴ Thus, care should be exercised when extrapolating our data to female patients. Third, the study included a relatively small number of participants. This limitation was related to the application of the time-consuming clamp study in each participant. Surrogate markers of muscle insulin sensitivity do not always accurately predict insulin sensitivity.^{45,46} Thus, despite the small number of participants, our data provide useful information. Finally, the present study was cross-sectional, therefore, it could not address causality.

In conclusion, the present study showed that the metabolic features of non-obese Japanese type 2 diabetes patients with FL include impaired muscle insulin sensitivity, fat accumulation and related metabolic disorders, such as elevated FFA, low HMW-adiponectin and low-grade inflammation. In other words, muscle insulin resistance and various metabolic

abnormalities seem common in non-obese Japanese type 2 diabetes patients with FL.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. DeFronzo RA. Banting lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 2009; 58: 773–795.
2. Hsu WC, Araneta MRG, Kanaya AM, *et al.* BMI cut points to identify at-risk Asian Americans for type 2 diabetes screening. *Diabetes Care* 2015; 38: 150–158.
3. Chan JC, Malik V, Jia W, *et al.* Diabetes in Asia: epidemiology, risk factors, and pathophysiology. *JAMA* 2009; 301: 2129–2140.
4. Takeno K, Tamura Y, Kawaguchi M, *et al.* Relation between insulin sensitivity and metabolic abnormalities in Japanese men with BMI of 23–25 kg/m². *J Clin Endocrinol Metab* 2016; 101: 3676–3684.
5. Kelley DE, McKolanis TM, Hegazi RA, *et al.* Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. *Am J Physiol* 2003; 285: E906–E916.
6. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, *et al.* Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002; 87: 3023–3028.
7. Gastaldelli A, Cusi K, Pettiti M, *et al.* Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology* 2007; 133: 496–506.
8. Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* Baltimore, Md. 2010; 51: 679–689.
9. Bugianesi E, Gastaldelli A, Vanni E, *et al.* Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* 2005; 48: 634–642.
10. Yki-Jarvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol* 2014; 2: 901–910.
11. Farrell GC, Wong VW, Chitturi S. NAFLD in Asia—as common and important as in the West. *Nat Rev Gastroenterol Hepatol* 2013; 10: 307–318.
12. Azuma K, Kadowaki T, Cetinel C, *et al.* Higher liver fat content among Japanese in Japan compared with non-Hispanic whites in the United States. *Metabolism* 2009; 58: 1200–1207.
13. Petersen KF, Dufour S, Feng J, *et al.* Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci USA* 2006; 103: 18273–18277.
14. Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus. Seino Y, Nanjo K, *et al.* Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Investig* 2010; 1: 212–228.
15. Kuzuya T, Nakagawa S, Satoh J, *et al.* Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Res Clin Pract* 2002; 55: 65–85.
16. Tamura Y, Tanaka Y, Sato F, *et al.* Effects of diet and exercise on muscle and liver intracellular lipid contents and insulin sensitivity in type 2 diabetic patients. *J Clin Endocrinol Metab* 2005; 90: 3191–3196.
17. Sato F, Tamura Y, Watada H, *et al.* Effects of diet-induced moderate weight reduction on intrahepatic and intramyocellular triglycerides and glucose metabolism in obese subjects. *J Clin Endocrinol Metab* 2007; 92: 3326–3329.
18. Watanabe T, Tamura Y, Kakehi S, *et al.* Effects of sitagliptin on ectopic fat contents and glucose metabolism in type 2 diabetic patients with fatty liver: a pilot study. *J Diabetes Investig* 2015; 6: 164–172.
19. Maggs DG, Buchanan TA, Burant CF, *et al.* Metabolic effects of troglitazone monotherapy in type 2 diabetes mellitus. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1998; 128: 176–185.
20. DeFronzo RA, Jacot E, Jequier E, *et al.* The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 1981; 30: 1000–1007.
21. Wei JL, Leung JC, Loong TC, *et al.* Prevalence and severity of nonalcoholic fatty liver disease in non-obese patients: a population study using proton-magnetic resonance spectroscopy. *Am J Gastroenterol* 2015; 110: 1306–1314.
22. Lombardi R, Pisano G, Fargion S. Role of serum uric acid and ferritin in the development and progression of NAFLD. *Int J Mol Sci* 2016; 17: 548.
23. Murawaki Y, Ikuta Y, Koda M, *et al.* Serum type III procollagen peptide, type IV collagen 7S domain, central triple-helix of type IV collagen and tissue inhibitor of metalloproteinases in patients with chronic viral liver disease: relationship to liver histology. *Hepatology* Baltimore, Md. 1994; 20: 780–787.
24. Kotronen A, Westerbacka J, Bergholm R, *et al.* Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* 2007; 92: 3490–3497.
25. Maximos M, Bril F, Portillo Sanchez P, *et al.* The role of liver fat and insulin resistance as determinants of plasma aminotransferase elevation in nonalcoholic fatty liver disease. *Hepatology* Baltimore, Md. 2015; 61: 153–160.

26. Barrows BR, Parks EJ. Contributions of different fatty acid sources to very low-density lipoprotein-triacylglycerol in the fasted and fed states. *J Clin Endocrinol Metab* 2006; 91: 1446–1452.
27. Yamauchi T, Kadowaki T. Adiponectin receptor as a key player in healthy longevity and obesity-related diseases. *Cell Metab* 2013; 17: 185–196.
28. Polyzos SA, Toulis KA, Goulis DG, *et al.* Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Metabolism* 2011; 60: 313–326.
29. Lee J, Yoon K, Ryu S, *et al.* High-normal levels of hs-CRP predict the development of non-alcoholic fatty liver in healthy men. *PLoS ONE* 2017; 12: e0172666.
30. Rabol R, Petersen KF, Dufour S, *et al.* Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals. *Proc Natl Acad Sci USA* 2011; 108: 13705–13709.
31. Flannery C, Dufour S, Rabol R, *et al.* Skeletal muscle insulin resistance promotes increased hepatic de novo lipogenesis, hyperlipidemia, and hepatic steatosis in the elderly. *Diabetes* 2012; 61: 2711–2717.
32. Petersen KF, Dufour S, Befroy D, *et al.* Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 2005; 54: 603–608.
33. Miyazaki Y, Glass L, Triplitt C, *et al.* Abdominal fat distribution and peripheral and hepatic insulin resistance in type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab* 2002; 283: E1135–E1143.
34. Miyazaki Y, Mahankali A, Matsuda M, *et al.* Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. *Diabetes Care* 2001; 24: 710–719.
35. Gastaldelli A, Miyazaki Y, Pettiti M, *et al.* Separate contribution of diabetes, total fat mass, and fat topography to glucose production, gluconeogenesis, and glycogenolysis. *J Clin Endocrinol Metab* 2004; 89: 3914–3921.
36. Hanley AJ, Williams K, Festa A, *et al.* Elevations in markers of liver injury and risk of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 2004; 53: 2623–2632.
37. Vozarova B, Stefan N, Lindsay RS, *et al.* High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 2002; 51: 1889–1895.
38. Sato KK, Hayashi T, Nakamura Y, *et al.* Liver enzymes compared with alcohol consumption in predicting the risk of type 2 diabetes: the Kansai Healthcare Study. *Diabetes Care* 2008; 31: 1230–1236.
39. Nakanishi N, Suzuki K, Tataru K. Serum gamma-glutamyltransferase and risk of metabolic syndrome and type 2 diabetes in middle-aged Japanese men. *Diabetes Care* 2004; 27: 1427–1432.
40. Oka R, Aizawa T, Yagi K, *et al.* Elevated liver enzymes are related to progression to impaired glucose tolerance in Japanese men. *Diabet Med* 2014; 31: 552–558.
41. Kim CH, Park JY, Lee KU, *et al.* Association of serum gamma-glutamyltransferase and alanine aminotransferase activities with risk of type 2 diabetes mellitus independent of fatty liver. *Diabetes Metab Res Rev* 2009; 25: 64–69.
42. Cho NH, Jang HC, Choi SH, *et al.* Abnormal liver function test predicts type 2 diabetes: a community-based prospective study. *Diabetes Care* 2007; 30: 2566–2568.
43. Staehr P, Hother-Nielsen O, Levin K, *et al.* Assessment of hepatic insulin action in obese type 2 diabetic patients. *Diabetes* 2001; 50: 1363–1370.
44. Ishibashi E, Eguchi Y, Eguchi T, *et al.* Waist circumference correlates with hepatic fat accumulation in male Japanese patients with non-alcoholic fatty liver disease, but not in females. *J Gastroenterol Hepatol* 2008; 23: 908–913.
45. Lorenzo C, Haffner SM, Stancakova A, *et al.* Relation of direct and surrogate measures of insulin resistance to cardiovascular risk factors in nondiabetic finnish offspring of type 2 diabetic individuals. *J Clin Endocrinol Metab* 2010; 95: 5082–5090.
46. Yudkin JS. Insulin resistance and the metabolic syndrome—or the pitfalls of epidemiology. *Diabetologia* 2007; 50: 1576–1586.