JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Correlation between serum proinsulin levels and fatty liver: The Dynamics of Lifestyle and Neighborhood Community on Health Study Health Study

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Keywords
Fatty liver, Pancreatic β-cell dysfunction, Proinsulin

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J Diabetes Investig 2020; 11: 964–970

doi: 10.1111/jdi.13221

ABSTRACT

Aims/Introduction: We explored the association between fatty liver and pancreatic β-cell dysfunction in a general population.

Materials and Methods: This cross-sectional study included 489 (53.8% women) community-dwelling Japanese adults. The extent of fatty liver was estimated using the fatty liver index (FLI). After all participants were divided into three groups – low (FLI <30), moderate (30 ≤FLI <60) or high (FLI ≥ 60) degree of fatty liver – serum proinsulin levels transformed into natural logarithms were compared among the three groups. To determine whether obesity modified the association of interest, the participants were stratified into two groups according to the median body mass index. Next, to determine whether hyperinsulinemia modified the association of interest, a similar stratified analysis was carried out using the median serum insulin level.

Results: Logarithm (proinsulin) was significantly higher in the high FLI group than in the moderate and low groups, and it was significantly higher in the moderate group than in the low group after adjustment for age and sex ($P < 0.05$). Logarithm (proinsulin) was significantly higher in the high FLI group than in the low FLI group, regardless of body mass index, after adjustment for age and sex. A similar pattern was observed regardless of serum insulin levels.

Conclusions: The degree of fatty liver was positively associated with proinsulin level, regardless of the presence of obesity or hyperinsulinemia, suggesting that fatty liver reflects pancreatic β-cell dysfunction.

INTRODUCTION

Type 2 diabetes is characterized by two major features: insulin resistance and impaired insulin secretion from pancreatic β-cells[1]. Not only with type 2 diabetes, but also with prediabetes status, both higher insulin resistance and lower insulin secretion have already developed[2]. At the onset of type 2 diabetes, there is already a significant reduction in β-cell function[3]. With type 2 diabetes status, the insulin secretion from pancreatic β-cells is declined, but higher insulin resistance does not continue to worsen[4]. Therefore, impaired β-cell function plays a key role in the development of type 2 diabetes.

The prevalence of non-alcoholic fatty liver disease (NAFLD) is strikingly increasing[5]. Developing hepatic steatosis in NAFLD ranges from non-alcoholic fatty liver to non-alcoholic steatohepatitis. Non-alcoholic steatohepatitis frequently progresses to cirrhosis of the liver and hepatocellular carcinoma[6]. The prevalence of NAFLD also increases remarkably in patients with type 2 diabetes. A previous study found that 45% of patients

Received 9 December 2019; revised 24 January 2020; accepted 27 January 2020

with type 2 diabetes had a history of NAFLD[7]. NAFLD significantly increases the risk of incident type 2 diabetes and metabolic syndrome[8,9]. A number of studies have shown an association between the advance of NAFLD and insulin resistance[10–12]. NAFLD leads to insulin resistance through lipotoxicity, and hepatic steatosis in NAFLD is known to be independently correlated with insulin resistance[13–15]. However, it remains unclear whether pancreatic β-cell dysfunction is related to NAFLD in the general population.

Therefore, we utilized the fatty liver index (FLI) and fasting serum proinsulin (PI) level to explore the association between the advance of fatty liver and pancreatic β-cell dysfunction in a general Japanese population.

METHODS

Study design and population

This cross-sectional study was part of the Dynamics of Lifestyle and Neighborhood Community on Health Study (DOSANCO Health Study)[16,17]. Participants comprised residents of the town of Suttu, Hokkaido, Japan, aged 35–79 years. In 2015, a total of 545 residents, including 300 women, were enrolled, and their basic information (sex, age, anthropometric measurements, medical history and fasting blood samples) was collected. The research design was approved by the Ethical Board of Hokkaido University School of Medicine (15-002 and 17-015). Signed informed consent was obtained from all the participants. Of these 545 participants, three were excluded because of missing data on insulin levels, and 53 were excluded because they received insulin therapy, oral hypoglycemic agents or both. The remaining 489 participants (263 women) were considered eligible and were included in the subsequent analyses.

Data collection

For this study, blood samples were collected by cubital venipuncture at rest in the morning after an overnight fast to measure levels of fasting plasma glucose, serum insulin, C-peptide (CPR), glycated hemoglobin, serum γ-glutamyl transferase (GGT) and triglyceride levels (TG). These parameters were measured using standard techniques. Serum samples were stored at −80°C until the measurement of PI. PI concentrations (pmol/L) were measured using a radioimmunoassay (Millipore Corporation Inc., Burlington, MA, USA). The extent of fatty liver was estimated using the FLI, which comprises body mass index (BMI), wait circumference (WC), GGT and TG[18]. This index was calculated using the following equation: FLI = {(exp (0.953 × log (TG) + 0.139 × BMI + 0.718 × log (GGT) + 0.053 × WC - 15.745) / 1 + exp (0.953 × log (TG) + 0.139 × BMI + 0.718 × log (GGT) + 0.053 × WC - 15.745)} × 100.

The weight, height and WC of the participants were measured using a calibrated scale. BMI was calculated as weight in kilograms divided by height in meters squared. Insulin sensitivity was estimated by homeostasis model assessment of insulin resistance[19]. Other data collected using the self-administered questionnaire included age, sex and medication for diabetes.

Statistical analysis

All participants were categorized into any of three groups: low (FLI <30), moderate (30 ≤ FLI < 60), or high (FLI ≥ 60) degree of fatty liver, based on a previous report showing that hepatic steatosis is ruled out in individuals with a FLI ≤30, and that a FLI ≥60 indicates fatty liver[20]. Biochemical and anthropometric characteristics were compared among the three FLI groups using one-way analysis of variance, the χ^2-test or the Kruskal–Wallis test, as appropriate. Because the data on PI showed a skewed distribution, the values of PI were transformed into natural logarithms (ln) and expressed as least squares means (95% confidence interval). Ln-transformed PI for the three FLI groups was compared using analysis of covariance, followed by Tukey's honest significant difference test for multiple post-hoc comparisons. The model incorporated sex (male or female) and age (in years, as a continuous variable) as covariates. To determine whether obesity modified the association of interest, the main analysis was carried out after the study population was stratified by the median BMI. In addition, to determine whether hyperinsulinemia affected PI, the study population was also stratified by the median fasting serum insulin level for the main analysis. Similar analyses were repeated for participants without diabetes after excluding 48 diabetes patients who had a previous history of diabetes, fasting plasma glucose ≥126 mg/dL or glycated hemoglobin ≥6.5%.

All tests were two-sided, and $P < 0.05$ was considered statistically significant. The statistical analyses were carried out using JMP 12 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Characteristics of the study population

A total of 489 participants (263 women) were categorized into three groups: low FLI (n = 303), moderate FLI (n = 106) and high FLI (n = 80). The biochemical and anthropometric characteristics of the full analytical sample and of each group are shown in Table 1. Male sex, BMI, WC, and levels of fasting plasma glucose, glycated hemoglobin, PI, insulin, CPR and homeostasis model assessment of insulin resistance were positively associated with the extent of fatty liver.

Association between pancreatic β-cell dysfunction evaluated by fasting serum proinsulin and the fatty liver index

Table 2 shows β-cell dysfunction evaluated by PI for each FLI group. In the crude analysis (model 1), ln(PI) was significantly higher in the high FLI group than in the low and moderate FLI groups, and it was also significantly higher in the moderate FLI group than in the low FLI group. Similar results were observed for this parameter after adjustment for age and sex (model 2). As shown in Table 3, ln(PI) was significantly higher in the high FLI group than in the low FLI group, and it was also higher in the moderate FLI group than in the low FLI group, regardless of BMI, after adjustment for age and sex. In addition, as shown in Table 4, ln(PI) was significantly higher in the high FLI group than in the low and moderate FLI groups

Table 1 | Participant characteristics overall and by the extent of fatty liver

	Total participants	Extent of fatty liver			*P*-value
		Low FLI group	Moderate FLI group	High FLI group	
n	489	303	106	80	
Age (years)	58.0 ± 12.5	57.6 ± 12.8	58.5 ± 12.3	58.4 ± 11.5	0.77
No. women (%)	263 (53.8)	204 (67.3)	43 (40.6)	16 (20.0)	<0.05
BMI (kg/m^2)	23.7 ± 3.6	22.0 ± 2.5	25.1 ± 2.8	28.3 ± 3.4	<0.05
Waist circumference (cm)	81.6 ± 10.4	76.0 ± 7.4	86.8 ± 6.1	95.9 ± 7.1	<0.05
FPG (mmol/L)	5.2 (4.8, 5.6)	5.0 (4.7, 5.4)	5.3 (5.0, 5.7)	5.5 (5.1, 6.2)	<0.05
HbA1c (%)	5.4 (5.2, 5.7)	5.4 (5.1, 5.6)	5.6 (5.3, 5.9)	5.6 (5.3, 6.0)	<0.05
Proinsulin (pmol/L)	8.9 (6.7, 14.2)	7.8 (5.8, 10.4)	11.4 (7.6, 16.6)	17.7 (13.1, 29.5)	<0.05
Insulin (pmol/L)	30.9 (20.1, 46.6)	25.8 (17.9, 33.0)	42.3 (29.2, 58.8)	59.2 (42.3, 86.1)	<0.05
C-peptide (ng/mL)	1.2 (0.9, 1.7)	1.0 (0.8, 1.3)	1.5 (1.2, 1.9)	2.1 (1.5, 2.6)	<0.05
HOMA-IR	1.0 (0.6, 1.6)	0.8 (0.5, 1.1)	1.4 (0.9, 2.0)	2.1 (1.5, 3.2)	<0.05

Total $n = 489$. Values are expressed as the mean ± standard deviation, median (interquartile range) or number (%) of participants in each category. One-way analysis of variance, the Kruskal–Wallis test or the χ^2-test was used to compare each parameter among the three fatty liver index (FLI) groups. BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance.

Table 2 | β-Cell dysfunction evaluated by proinsulin by fatty liver index category

	Extent of fatty liver			*P*-value		
	Low FLI group	Moderate FLI group	High FLI group	Low vs moderate	Low vs high	Moderate vs high
n	303	106	80			
Model 1 ln (PI)†	2.05 (2.00, 2.11)	2.46 (2.36, 2.56)	2.94 (2.83, 3.06)	*	*	*
Model 2 ln (PI)†	2.07 (2.01, 2.13)	2.45 (2.35, 2.55)	2.91 (2.79, 3.03)	*	*	*

Model 1: crude model; model 2: adjustment for age and sex. PI, proinsulin. *$P < 0.05$. †Values are normalized by natural logarithmic transformation and expressed as least squares means (95% confidence interval). Analysis of covariance and Tukey's honest significant difference test were used to compare ln(proinsulin) among the three fatty liver index (FLI groups).

in both of the fasting serum insulin strata after adjustment for age and sex.

Table 5 shows the characteristics of the 441 participants without diabetes (248 women). For this group, the characteristics differed among the three FLI groups, and the patterns were similar to those observed for the full group of participants (Table 1). Table 6 shows β-cell dysfunction evaluated by PI for each FLI group in the participants without diabetes. In the crude analysis (model 1), ln(PI) was significantly higher in the high FLI group than in the low and moderate FLI groups, and this parameter was significantly higher in the moderate FLI group than in the low FLI group. After adjusting for age and sex, similar results were observed for this parameter (model 2).

DISCUSSION

To the best of our knowledge, this is the first study to show that the degree of fatty liver is positively associated with PI level. The correlations remained significant when stratifying participants into two groups according to the median BMI and serum insulin levels. In the present study, we examined this relationship using PI, which has served as a marker of pancreatic β-cell dysfunction[21]. In a recent study, we showed that, among several estimation methods of β-cell function, fasting PI was the most sensitive to glucose intolerance[17]. Therefore, PI was used as an indicator of β-cell dysfunction in the present study. Recognizing the limitations related with this cross-sectional study design, the present findings suggest that fatty liver could affect β-cell dysfunction. The existence of reciprocal cross-talk between the pancreas and fatty liver has been suggested. Activation of pancreatic fat cells and islet-resident macrophages by fatty liver-derived fetuin-A induces the impairment of glucose-induced insulin secretion, as well as the increase of β-cell apoptosis[22]. Considered together with these reports, our findings might suggest that the exacerbation of hepatic steatosis is positively associated with pancreatic β-cell dysfunction.

Table 3 | β-Cell dysfunction evaluated by proinsulin by fatty liver index category after stratification according to median body mass index

	Extent of fatty liver			*P*-value		
	Low FLI group	Moderate FLI group	High FLI group	Low vs moderate	Low vs high	Moderate vs high
High BMI group						
n	91	80	74			
ln (PI)†	2.27 (2.15, 2.38)	2.52 (2.41, 2.64)	2.92 (2.79, 3.04)	*	*	*
Low BMI group						
n	212	26	6			
ln (PI)†	1.98 (1.91, 2.05)	2.26 (2.06, 2.46)	2.61 (2.20, 3.02)	*	*	

BMI, body mass index; High BMI group, participants with high body mass index, adjusted for age and sex; Low BMI group, participants with low body mass index, adjusted for age and sex; PI, proinsulin. *$P < 0.05$. †Values are normalized by natural logarithmic transformation and expressed as least squares means (95% confidence interval). Analysis of covariance and Tukey's honest significant difference test were used to compare ln(proinsulin) among the three fatty liver index (FLI) groups.

Table 4 | β-Cell dysfunction evaluated by proinsulin by fatty liver index category after stratification according to median fasting serum insulin level

	Extent of fatty liver			*P*-value		
	Low FLI group	Moderate FLI group	High FLI group	Low vs moderate	Low vs high	Moderate vs High
High insulin group						
n	100	77	69			
ln (PI)†	2.34 (2.24, 2.45)	2.63 (2.51, 2.74)	2.98 (2.86, 3.11)	*	*	*
Low insulin group						
n	203	29	11			
ln (PI)†	1.93 (1.87, 1.99)	1.98 (1.82, 2.14)	2.40 (2.13, 2.66)		*	*

High insulin group, participants with high fasting serum insulin levels adjusted for age and sex; Low insulin group, participants with low fasting serum insulin levels adjusted for age and sex; PI, proinsulin. *$P < 0.05$. †Values are normalized by natural logarithmic transformation and expressed as least squares means (95% confidence interval). Analysis of covariance and Tukey's honest significant difference test were used to compare ln (proinsulin) among the three fatty liver index (FLI) groups.

Table 5 | Characteristics of participants without diabetes overall and by extent of fatty liver

	Total participants	Extent of fatty liver			*P*-value
		Low FLI group	Moderate FLI group	High FLI group	
n	441	286	90	65	
Age (years)	57.4 ± 12.6	57.2 ± 12.9	57.5 ± 12.5	57.7 ± 11.6	0.95
Number of female (%)	248 (56.2)	197 (68.9)	37 (41.1)	14 (21.5)	<0.05
BMI (kg/m^2)	23.6 ± 3.6	22.0 ± 2.5	25.2 ± 2.8	28.4 ± 3.4	<0.05
Waist circumference (cm)	81.6 ± 10.2	75.9 ± 7.2	86.9 ± 6.0	95.7 ± 7.0	<0.05
FPG (mmol/L)	5.1 (4.7, 5.4)	4.9 (4.7, 5.3)	5.3 (5.0, 5.6)	5.4 (5.0, 5.9)	<0.05
HbA1c (%)	5.4 (5.2, 5.6)	5.4 (5.1, 5.6)	5.4 (5.2, 5.7)	5.5 (5.3, 5.9)	<0.05
Proinsulin (pmol/L)	8.5 (6.4, 13.2)	7.7 (5.6, 10.1)	11.1 (7.3, 15.5)	16.3 (11.2, 23.5)	<0.05
Insulin (pmol/L)	29.4 (20.1, 44.1)	25.1 (17.2, 33.0)	41.6 (26.9, 56.7)	58.1 (40.9, 85.7)	<0.05
C-peptide (ng/mL)	1.1 (0.9, 1.6)	1.0 (0.8, 1.3)	1.4 (1.1, 1.9)	2.0 (1.5, 2.5)	<0.05
HOMA-IR	0.9 (0.6, 1.5)	0.8 (0.5, 1.1)	1.4 (0.9, 1.8)	2.0 (1.4, 3.0)	<0.05

Total $n = 441$. Values are expressed as the mean ± standard deviation, median (interquartile range) or number (%) of participants in each category. One-way analysis of variance, the Kruskal–Wallis test or the χ^2-test was used to compare each parameter among the three fatty liver index (FLI) groups. BMI, body mass index, FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance.

Table 6 | β-Cell dysfunction evaluated by proinsulin in participants without diabetes by their fatty liver index category

	Extent of fatty liver			*P*-value		
	Low FLI group	Moderate FLI group	High FLI group	Low vs moderate	Low vs high	Moderate vs high
n	286	90	65			
Model 1 ln (PI)†	2.03 (1.97, 2.08)	2.36 (2.26, 2.46)	2.78 (2.67, 2.90)	*	*	*
Model 2 ln (PI)†	2.04 (1.98, 2.10)	2.35 (2.26, 2.36)	2.76 (2.65, 2.88)	*	*	*

Model 1: crude model; model 2: adjustment for age and sex. PI, proinsulin. *$P < 0.05$. †Values are normalized by natural logarithmic transformation and expressed as least squares means (95% confidence interval). Analysis of covariance and Tukey's honest significant difference test were used to compare ln(proinsulin) among the three fatty liver index (FLI) groups.

Obesity and hyperinsulinemia, which are important risk factors for insulin resistance as well, are the most commonly complicated with fatty liver[15,23]. However, previous studies have found that lean patients with NAFLD were characterized by severe histological features similar to those of obese patients[24,25]. These reports suggest that NAFLD develops and progresses regardless of the presence of obesity or hyperinsulinemia. Feldman *et al.*[26] reported that lean patients with NAFLD had fasting insulin levels similar to lean healthy patients, but had markedly impaired glucose tolerance. This previous work might provide further support for the present results showing that the degree of fatty liver is associated with pancreatic β-cell dysfunction regardless of the presence of obesity or hyperinsulinemia (Tables 3,4). Furthermore, it has been shown that not only participants who were obese, but also non-overweight participants with NAFLD had a high risk of incident type 2 diabetes in a population-based retrospective cohort study of Japanese patients. The incidence rate of type 2 diabetes has been reported to be significantly higher in the non-overweight patients with NAFLD than in overweight or non-overweight patients without NAFLD[27]. This result might also support the present study's findings.

As it has been reported that the PI : insulin ratio and PI : CPR ratio are biomarkers of pancreatic β-cell dysfunction[28,29], we also examined the association between these ratios and the extent of fatty liver. Although there was no statistically significant difference in the PI : insulin ratio among the three FLI groups, ln(PI : CPR) and ln(PI) were significantly higher in the high FLI group than in the low FLI group after adjustment for age and sex (Table S1). It should be noted that the PI : insulin ratio might not be an accurate measure in fatty liver or hepatic insulin resistance, because fasting insulin levels are affected by hepatic clearance of insulin[29]. In contrast, the PI : CPR ratio is a known biomarker of pancreatic β-cell dysfunction and is unaffected by hepatic insulin clearance[30]. These results provide further support for the present results showing that the degree of fatty liver is associated with pancreatic β-cell dysfunction.

Based on the positive association between the FLI and PI in participants without diabetes, the present data might suggest the possibility of pancreatic β-cell function recovery after improvement in liver steatosis. Diet, exercise and medication might improve liver steatosis, ultimately playing an important role in the prevention of the development and progression of type 2 diabetes, as well as liver cirrhosis and hepatocellular carcinoma.

The main strength of the present study was that we showed the relationship between the exacerbation of fatty liver and pancreatic β-cell dysfunction in a community-based general population rather than a hospital-based population.

The present study also had several limitations. First, the extent of fatty liver was estimated using the FLI, an indirect index not using ultrasound, magnetic resonance spectroscopy, computed tomography or liver biopsy. The FLI is a simpler and less expensive method compared with magnetic resonance spectroscopy, and a strong correlation has been reported between the FLI and hepatocellular lipid content[31,32]. In addition, imaging and liver biopsy are inappropriate because of invasive and expensive tests in a community-based study, such as this. Second, although the proper use of biomarkers of pancreatic β-cell dysfunction including fasting PI levels and the PI : insulin ratio remains to be discussed, we recently showed that fasting PI was the index most sensitive to glucose intolerance in the general population[17]. Third, because of its cross-sectional design, the present study was unable to prove causal relationships, or examine the time course of the link between the exacerbation of fatty liver and pancreatic β-cell dysfunction.

This community-based cross-sectional study showed a positive correlation between the degree of liver steatosis and pancreatic β-cell dysfunction, regardless of the presence of obesity or hyperinsulinemia. The present findings suggest that fatty liver reflects pancreatic β-cell dysfunction.

ACKNOWLEDGMENTS

We express special gratitude to all the participants from Suttu, Hokkaido, Japan. We thank Jennifer Barrett, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this

manuscript. We presented a part of the result at the 55th European Association for the Study of Diabetes annual meeting. This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number JP26670322, and Integration Research for Agriculture and Interdisciplinary Fields (No. 14538261).

DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | β-Cell dysfunction evaluated by each parameter by their fatty liver index category.