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Clinical Research Article

Hepatic Steatosis and High-Normal Fasting Glucose as Risk Factors for Incident Prediabetes

Toru Aizawa,[1] Yasuto Nakasone,[1] Norimitsu Murai,[2] Rie Oka,[3] Shoichiro Nagasaka,[2] Koh Yamashita,[1] Takahiro Sakuma,[4] and Kendo Kiyosawa[5]

[1]Diabetes Center, Aizawa Hospital, Matsumoto 390-8510, Japan
[2]Division of Diabetes, Metabolism and Endocrinology, Showa University Fujigaoka Hospital, Yokohama 227-8501, Japan
[3]Department of Internal Medicine, Hokuriku Central Hospital, Toyama 932-8503, Japan
[4]Department of Internal Medicine, Ina Central Hospital, Ina 396-8555, Japan
[5]Department of Gastroenterology, Aizawa Hospital, Matsumoto 390-8510, Japan

Correspondence: Toru Aizawa, MD, PhD, Diabetes Center, Aizawa Hospital, 2-5-1 Honjo, Matsumoto, 390-8510, Japan. Email taizawax@ai-hosp.or.jp

Abstract

Context: The role of hepatic steatosis (HS) in the initial stages of developing type 2 diabetes remains unclear.

Objective: We aimed to clarify the impact of HS indexed by Fatty Liver Index (FLI) and high-normal fasting plasma glucose (FPG) as risk factors for incident prediabetes in a nonobese cohort.

Methods: Data from 1125 participants with ADA-defined normal glucose metabolism (median age 52 years; BMI 23.1 kg/m^2) were used for retrospective analysis. In the entire population, correlation between normal FPG and FLI was evaluated by multiple regression adjusted for age and sex. Follow-up data from 599 participants in whom 75-g OGTT was repeated 3.7 years later showed that 169 developed prediabetes. This was analyzed by the multivariate Cox proportional hazards model.

Results: In the entire population, FLI was positively correlated with FPG ($P < 0.01$): mean FLI increased from 15.8 at FPG 4.2 mmol/L to 31.6 at FPG 5.5 mmol/L. Analysis of the 599 participants (2061 person-years) by Cox model, adjusted for sex, age, family history of diabetes, $ISI_{MATSUDA}$, and Stumvoll-1, clarified an increased risk of prediabetes with high-normal FPG and FLI. Risk was increased 2.2 times with FLI ≥ 16.5 vs FLI < 16.5, $P < 0.001$, and increased 2.1 times in participants with FPG ≥ 5.3 mmol/L, $P < 0.001$. Cutoff values (unadjusted) were obtained by ROC at the point of the largest Youden's index using the entire range of the variables.

Conclusion: Even among nonobese individuals, HS indexed by FLI and a high-normal FPG (≥ 5.3 mmol/L) are risk factors for prediabetes, independently from insulin.

Key Words: fasting plasma glucose, insulin secretion, insulin sensitivity, hepatic steatosis, prediabetes

Abbreviations: ADA, American Diabetes Association; AUC, area under the curve; BMI, body mass index; FLI, Fatty Liver Index; FPG, fasting plasma glucose; HOMAbeta, homeostatic model assessment for beta-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; HR, hazard ratio; HS, hepatic steatosis; *Isec*, insulin secretion; $ISI_{MATSUDA}$, Matsuda insulin sensitivity index; NAFLD, nonalcoholic fatty liver disease; NGM, normal glucose metabolism; OGTT, oral glucose tolerance test; PG, plasma glucose; ROC, receiver operating characteristics curve; *Si*, insulin sensitivity.

Patients with established type 2 diabetes show both insulin insensitivity and insulin deficiency, and overt hyperglycemia is sustained mainly due to these 2 pathological processes [1]. The existence of 2 major components in diabetes, insulin insensitivity and insulin deficiency, has been recognized since in the early 1900s (2). Despite the accumulation of an enormous amount of knowledge regarding the epidemiology, endocrinology, pathophysiology, and genetics of diabetes, the temporal profile or natural course of altered insulin sensitivity (S_i) and insulin secretion (I_{sec}) remain unclear. In particular, the risk factors for type 2 diabetes and their contributions in the initial phases of the disease remain uncertain [1, 3-11]. Specifically, the trajectory of plasma glucose level before clinical diagnosis of diabetes and prediabetes reveal that a stable, long-lasting, slow elevation is followed by an accelerated rise for several years before the diagnosis [7, 12-15]. This so-called multistage model strongly suggests unfavorable interactions between minimally elevated glucose per se and the glucose regulatory mechanism. However, the possibility of such a deleterious effect of high-normal range glycemia has not been discussed [16-18].

We have also been interested in the recent studies showing that an index of hepatic steatosis (HS) [19] predicted worsening glucose metabolism [20, 21]. The involvement of hepatic insulin resistance in the evolution of diabetes has been firmly established [22], but HS leading to hyperglycemia *independent of insulin resistance* [23] is a new idea that warrants further investigation. HS as a risk factor for prediabetes has only recently been examined.

The evolution of diabetes may show ethnic disparities. In Caucasians and Pima Indians, insulin resistance that evolves during the development of type 2 diabetes induces insulin hypersecretion and increased insulin synthesis [1, 4-6]. However, such compensatory hyperinsulinemia

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during the development of type 2 diabetes has not been shown in Japanese people who are generally nonobese and insulin-sensitive [3, 8-12].

In previous studies, the possible heterogeneity in the potential to develop future prediabetes or diabetes in individuals with normal glucose metabolism (NGM) has not been fully clarified [4, 5, 7, 11, 15]. Additionally, subjects with NGM were often treated as homogeneous, with little derangement in glucose regulation [1, 2, 4-6]. This assumption may be incorrect, since S_i and/or I_{sec} may certainly be abnormal at the stage of NGM, and diabetogenic change is likely taking place while individuals are still euglycemic [7, 8, 11].

In this context, we critically re-analyzed the data of Japanese adults with NGM. Here, we aimed to identify the earliest risk factors for diabetes and their quantitative contributions. In this process, we paid special attention to the mechanism underlying the multistage model and insulin-independent risk of HS to prediabetes.

Methods

Study Participants

Data from health examinees at Hokuriku Central Hospital, Toyama, Japan were retrospectively analyzed. The detailed characteristics of the study population are described elsewhere [10]. Briefly, out of 2340 health examinees who visited the hospital between April 2006 and March 2010, those with American Diabetes Association (ADA)-defined NGM [24], that is, fasting plasma glucose (FPG) level < 5.5 mmol/L and plasma glucose (PG) level at 2 hours after 75-g oral glucose loading (2hPG) < 7.8 mmol/L were selected (n = 1125), and their data were analyzed in the present study. Eight participants positive for hepatitis B virus surface antigen, and 3 participants positive for hepatitis C were excluded. All included participants were born, raised, and still currently living in Japan. The characteristics of the study participants were comparable to members of the Japanese general population with normal glucose tolerance [25]. Written informed consent was obtained from all participants, and the study was approved by the ethics committees of Hokuriku Central Hospital and Aizawa Hospital. Correlation between the level of Fatty Liver Index (FLI) and the degree of HS was analyzed in 766 health examinees with NGM who visited Ina Central Hospital. The study was conducted in accordance with the guidelines of the Declaration of Helsinki [26]. The former population has been previously analyzed for different purposes by some of the authors (R.O. and T.A.) and their colleagues [10, 27].

Measurements

Screening of insulin sensitivity (S_i), insulin secretion (I_{sec}), and HS

The equations and features of each index are summarized in Table 1. Initially, 15 indices were evaluated: 5 indices of S_i, 1/homeostasis model assessment of insulin resistance (HOMA-IR) [28, 29], $ISI_{Matsuda}$ [30, 31], Gutt index [32], Avignon's SiM [33], and the reciprocal of hepatic insulin resistance (HIR) proposed by Abdul-Gahni [34], 1/HIR; 5 indices of I_{sec}, HOMAbeta [28, 29], Stumvoll's first and second phase indices (Stumvoll-1 and Stumvoll-2, respectively) [35], insulinogenic index [36], and immunoreactive insulin at 30 minutes divided by PG at 30 minutes after 75-g oral glucose tolerance test (OGTT) (I_{30}/G_{30}) [37]; and 5 surrogate markers of HS, the FLI [19], nonalcoholic fatty liver disease (NAFLD)-liver fat score (NAFLD-LFS) [38], HS index (HIS) [39], Visceral Adiposity Index (VAI) [40], and Triglyceride times Glucose index (TyG index) [41]. The most significant risk for incident prediabetes among the indices were $ISI_{Matsuda}$ and Avignon's SiM for S_i, Stumvoll-1 for I_{sec}, and FLI for HS. $ISI_{MATSUDA}$ was strongly correlated with Avignon's SiM (r = 0.92), and $ISI_{MATSUDA}$ is commonly used worldwide. Therefore, it was adopted as the index of S_i in the next step. Stumvoll-1 and FLI were also highly significant risk for incident prediabetes at the preliminary screening. FLI has been well-validated as a marker of fatty liver [19] and has been shown to be able to predict diabetes in the Japanese population [42, 43]. Accordingly, Stumvoll-1, $ISI_{MATSUDA}$ and FLI were utilized in the following analysis, except for the stratification analysis where Gutt and Avignon's SiM were also evaluated (see below).

Insulin and glucose assay

Serum insulin concentration was determined using a chemiluminescence immunoassay (Siemens Healthcare Diagnostics, Tokyo, Japan) at a commercial laboratory (BML, Inc. Tokyo, Japan). The antibody used in the insulin assay did not cross-react with proinsulin. Fasting blood samples were obtained after at least 10 hours of fasting in the morning, and post-glucose samples were obtained at 30, 60, and 120 minutes after 75-g oral glucose loading. PG level was determined using the glucose oxidase method (Automatic Glucose Analyzer ADAMS Glucose GA-1160, Arkray, Kyoto). The error in the glucose measurement was ± 2%.

Statistical Analyses

Cross-sectional analysis

Using the data from the entire Hokuriku cohort (N = 1125), multiple regression of FLI against FPG was performed with adjustments for age and sex. Correlation between FLI and HS was confirmed in a separate population using the data obtained at Ina Central Hospital. The participants of this part of study were 766 health examinees with NGM: male/female 396/370, median (interquartile range [IQR]) age 53 (42-65), body mass index (BMI) 22 (20.0-24.2), FPG 5.2 (5.1-5.4), and FLI 10.2 (4.5-28.5). HS was graded as reported previously [44].

Analysis of the relationship of the baseline variables and the progression to prediabetes

As a preliminary step, the hazard ratios (HRs) and confidence intervals of each index for incident prediabetes were examined individually with adjustments for FPG, age, sex, and BMI. BMI was omitted from the covariates adjusted for analyses with the Gutt index, SiM, and FLI, which were body weight–adjusted figures. $ISI_{MATSUDA}$ and Stumvoll-1 were selected as representative indices of S_i and I_{sec}, respectively, because they showed the highest level of significance of the HRs among each group. FLI was also the most significant risk factor for incident prediabetes. Accordingly, the main analysis was conducted by utilizing FLI and FPG with $ISI_{Matsuda}$ and Stumvoll-1, in the model. The entire range of each variable was evaluated as a possible risk factor in the Cox proportional hazard model. The cutoff values maximizing the separation of progressors and nonprogressors were derived from receiver operating characteristics (ROC) curves as the value with the maximum Youden's index (unadjusted).

Table 1. Indices of insulin sensitivity, insulin secretion, and hepatic steatosis used in this study

Indices	Characteristics	Equation	Ref.
Indices of insulin sensitivity (S_i)			
1/HOMA-IR	Index of basal insulin sensitivity in which hepatic and muscle insulin sensitivity are assumed to be equal	1/HOMA-IR*= 1/([IRI_0 · PG_0]/405)	[28, 29]
$ISI_{Matsuda}$	Index of 0- to 2h postload insulin sensitivity reflecting both hepatic and muscle insulin sensitivity	$ISI_{Matsuda}$*= 10^4/SQRT (IRI_0 · PG_0 · IRI_{120} · PG_{120})	[30, 31]
Gutt	Index of basal and 2h (0-120 min) postload insulin sensitivity taking into account glucose volume based on the BW and correcting for the skewness of insulin	$ISI_{0,120}$ = MCR/log MSI = m/MPG/log MSI	[32]
Avignon's SiM	Index of basal and 2h (0-120 min) postload insulin sensitivity taking into account glucose volume based on the BW	SiM* = [(0.137 · Sib) + Si2h]/2`	[33]
1/HIR	Index of post-glucose-load hepatic insulin sensitivity	1/hepatic insulin resistance (HIR) = 1/[$PG_{0\text{-}30}$ (AUC) · $IRI_{0\text{-}30}$ (AUC)]	[34]
Indices of insulin secretion (I_{sec})			
HOMAbeta	Index of basal, unstimulated insulin secretion	HOMAbeta*= ([IRI_0 · 360]/[PG_0- 63])	[28, 29]
Stumvoll-1	Index of acute (10 min) insulin response to iv glucose	Stumvoll-1 = 1283 + 1.829 · IRI_{30} – 138.7 · PG_{30} + 3.772 · IRI_0	[35]
Stumvoll-2	Index of late-phase (120-180 min) insulin secretion at glucose-clamp	Stumvoll-2 = 287 + 0.4164 · IRI_{30} – 26.07 · PG_{30} + 0.9226 · IRI_0	[35]
Insulinogenic index	Index of glucose-stimulated early-phase (30 min) insulin secretion	I.I.*= $\delta IRI_{0\text{-}30}/\delta PG_{0\text{-}30}$	[36]
I_{30}/G_{30}	Index of glucose-stimulated early-phase (30 min) insulin secretion	I_{30}/G_{30}*= IRI_{30}/PG_{30}	[37]
Indices of hepatic steatosis (HS)			
Fatty Liver Index (FLI)	Index of fatty liver	FLI = ($e^{0.952 \cdot \log(TG) + 0.139 \cdot BMI + 0.718 \cdot \log(\gamma GTP) + 0.052 \cdot WC - 15.745}$)/ ($1 + e^{0.952 \cdot \log(TG) + 0.139 \cdot BMI + 0.718 \cdot \log(\gamma GTP) + 0.052 \cdot WC - 15.745}$) · 100	[19]
NAFLD liver fat score (LFS)	Index of fatty liver	NAFLD-LFS = -2.89 + 1.182 · Metabolic Sx (yes = 1/no = 0) + 0.45 · type 2 diabetes (yes = 2/no = 0) + 0.15 · IRI (mU/L) + 0.04 · AST (U/L) – 0.94 · AST/ALT	[38]
Hepatic steatosis index (HIS)	Index of fatty liver	HIS = 8·ALT/AST + BMI (+2, if type 2 diabetes; +2, if female)	[39]
Visceral adiposity index (VAI)	Index of fatty liver	VAI = [WC/39:68 + 1.88 · BMI] · (triglycerides/1.03) · (1.31/HDL-c), for males; [WC/36.58 + 1.89 · BMI]·(triglycerides/0.81)·(1.52/HDL-c), for females	[40]
TyG index	Index of fatty liver	TyG index = ln [triglycerides × glucose/2]	[41]

The subscripts indicate the sampling time at 75-g OGTT. The unit for measurements was mmol/L for glucose and pmol/L for insulin unless marked by *, where the unit was conventional, that is, mg/dL for glucose and μU/mL for insulin. Sib, 10^8/(IRI_0 · FPG · VD); Si2h, 10^8/(IRI_{120} · PG_{120} · VD); VD (glucose volume), 150 ml/kg.BW, MCR; m/MPG, MPG = (FPG + PG_{120})/2 (unit of PG is mg/L); MSI, (IRI_0 + IRI_{120})/2; ln, natural log; Metabolic syndrome was diagnosed based on the Japanese criteria.
Abbreviations: AUC, area under the curve; BW, body weight; HDL-c, high-density lipoprotein cholesterol; HOMAbeta, homeostatic model assessment for beta-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; NAFLD, nonalcoholic fatty liver disease.

The interaction between FPG and $ISI_{Matsuda}$ was significant ($P = 0.02$); therefore, the modifying effect of FPG was assessed by stratifying the 599 participants based on the median baseline FPG (5.2 mmol/L). The interactions between FPG and Gutt index ($P = 0.02$) and Avignon's SiM ($P = 0.049$) were also significant. Therefore, to prove that the interaction did not only have an impact on $ISI_{MATSUDA}$ but also on Gutt and Avignon's SiM, stratification analysis was carried out 3 times each with different indices of S_i (Table 2).

Efficiency of prediction of prediabetes was evaluated from ROC curves which was obtained based on the probability from the Cox model. To this end, the 599 participants (the entire follow-up minus those who developed diabetes) followed for the mean of 3.7 years were randomly divided into derivation ($N = 302$) and validation cohort ($N = 299$). Sensitivity and specificity and area under the receiver operating characteristic curve (AUC) in the validation cohort was taken as the final prediction efficiency.

HRs and CIs obtained by Cox proportional hazard model were scaled to the IQR (computed by subtracting the 25th percentile from the 75th percentile and defined as the unit for HR) to facilitate the comparison of relative risk strength for each predictor. The Wilcoxon and chi-squared tests were used for descriptive statistics. JMP Pro 15.0, and SPSS 21.0 were used for statistical calculations. Statistical significance was set at $P < 0.05$.

Results

The baseline characteristics of the study participants are presented in Table 3. The participants were health examinees with ADA-defined NGM [24], so their metabolic profile was comparable to the general Japanese population with NGM [25]. This was also the case in the subpopulation used for the analysis of correlation between FLI and HS, at Ina Central Hospital.

Cross-sectional Analysis

As shown in Fig. 1A, the mean FLI exhibited an excellent correlation ($P < 0.001$) with FPG, 4.2 to 5.5 mmol/L. No index of I_{sec} exhibited a significant elevation in association with FPG rise (data not shown). FLI showed a good correlation with the degree of HS (Fig. 1B). The best cutoff FLI value for presence of fatty liver (grade 2 or over) was 14.3.

Longitudinal Analysis

Relationship of the baseline variables with prediabetes

A total of 604 (54%) study participants underwent follow-up 75-g OGTTs after a mean period of 3.7 years, of whom 174 participants (174/604, 29%) showed abnormal glucose metabolism (isolated impaired fasting glucose, 102 participants; isolated impaired glucose tolerance, 39 participants; impaired fasting glucose and impaired glucose tolerance, 28 participants; and diabetes, 5 participants), while 430 (430/604, 71%) remained as NGM (Table 3) [24]. The 5 participants who developed diabetes were excluded; thus, the remaining 169 participants who developed nondiabetic hyperglycemia were collectively treated as "progressors" and the rest ($N = 430$) were labeled as "nonprogressors."

Multivariate analysis revealed that higher FLI and FPG were significant risk factors for incident prediabetes additionally to, and independent from, lowered $ISI_{MATSUDA}$ and Stumvoll-1 (Table 4). The best cutoff values discriminating progressors from nonprogressors were ≥ 16.5 for FLI, ≥5.3 mmol/L (95 mg/dL) for FPG, ≤11.99 for $ISI_{MATSUDA}$ and ≤ 486.3 for Stumvoll-1. The multivariate model clarified an increased risk (2.1 times) of prediabetes in participants with FPG ≥ 5.3 mmol/L, as compared with those whose FPG was < 5.3 mmol/L, $P < 0.0001$. Accordingly, the high-normal FPG was defined as ≥ 5.3 mmol/L in this communication. The risk was also increased (2.2 times) in participants with FLI ≥ 16.5, as compared with those with FLI < 16.5, $P < 0.0001$. Incidence of prediabetes was progressively higher at 6/95 (6%), 523/146 (16%), 56/206 (27%), 68/128(53%), and 16/24(57%) among the participants with 0, 1, 2, 3, and 4 unfavorable values, respectively.

The FPG cutoff value with the best discrimination of progressors and nonprogressors was very close to the baseline median FPG used for the stratification analysis (see below). Quantitatively, the risk from $ISI_{Matsuda}$, Stumvoll-1, FLI, and FPG were not significantly different from each other. AUC of

Table 2. Stratified analysis

Model	Strata	Index of S_i	HR	95% CI	*P* value
1	High FPG (≥5.2 mmol/L)	$ISI_{Matsuda}$	0.781	0.647-0.945	0.01
	Low FPG (<5.2 mmol/L)		0.867	0.523-1.480	0.56
2	High FPG (≥5.2 mmol/L)	Gutt	0.785	0.627-0.93	0.02
	Low FPG (<5.2 mmol/L)		1.057	0.891-1.254	0.52
3	High FPG (≥5.2 mmol/L)	Avignon's SiM	0.801	0.660-0.973	0.03
	Low FPG (<5.2 mmol/L)		0.812	0.381-1.731	0.51

Interaction between FPG was significant for $ISI_{MATSUDA}$, Gutt, and Avignon's SiM so that effect of stratification was examined for the 3 indices. After stratification, by Cox proportional hazards model, the HR and CI of each index were calculated with age, sex, Stumvoll-1 and FLI as covariates. HR was scaled to the $IQR_{increase}$ (computed by subtracting the 25th percentile from the 75th percentile and defined as the unit for HR) for comparison. Note that $HR/IQR_{increase}$ so that protection was shown. N/Cases was 121/326 and 47/273 for the high- and low-FPG groups, respectively.
Abbreviations: FPG, fasting plasma glucose; HR, hazard ratio.

Table 3. Baseline characteristics of the study participants

Variable	All (*N* =1125)	Followed-up		
		Nonprogressors (*N* = 430)	Progressors (*N* = 169)	*P* value*
Age (years)	52 (47-59)	52 (46-58)	52 (48-57)	0.591
Male (%)	62%	61%	80%	<0.001
Body mass index (kg/m^2)	23.1 (21.4-25.0)	23.0 (21.2-24.8)	24.3 (22.9-25.8)	<0.001
Waist circumference (cm)	82 (77-87)	82 (77-86)	85 (81-89)	<0.001
Glycosylated hemoglobin A1c (%) (mmol/mol)	5.1 (4.9-5.2) 32 (30-34)	5.1 (4.9-5.2) 32 (30-32)	5.2 (5.0-5.4) 33 (32-36)	<0.001
Triglycerides (mmol/L)	1.12(0 81-1.55)	1.07 (0.77-1.49)	1.29 (0.98-1.74)	<0.001
γGTP (IU/L)	26 (18-44)	26 (18-43)	40 (25-57)	<0.001
Drinking everyday	26%	31%	26%	0.394
Drinking 1-6 days a week	35%	35%	34%	0.780
Fasting plasma glucose (mmol/L)	5.2 (4.9-5.2)	5.1 (4.9-5.3)	5.3 (5.1-5.4)	<0.001
30 min postload glucose (mmol/L)	7.8 (6.9-8.8)	7.6 (6.8-8.5)	8.2 (7.2-9.2)	<0.001
2h postload glucose (mmol/L)	5.8 (5.1-6.5)	5.7 (5.0-6.3)	6.2 (5.5-6.9)	<0.001
Fasting insulin (pmol/L)	25.3 (18.9-34.3)	24.5 (18.2-33.6)	26.6 (20.3-37.8)	0.037
30 min postload insulin (pmol/L)	216.3 (145.6-325.5)	217 (148.4-317.8)	205.8 (130.2-319.2)	0.301
2h postload insulin (pmol/L)	146.3 (96.6-226.1)	136.5 (91-205.8)	170.1 (108.5-258.3)	<0.001
Indices of insulin sensitivity, insulin secretion, or hepatic steatosis				
1/HOMA-IR	1.21 (0.89-1.64)	1.25 (0.91-1.69)	1.13 (1.81-1.52)	0.008
$ISI_{Matsuda}$	11.99 (8.23-17.42)	12.6 (9.5-18.9)	3.8 (2.9-5.4)	<0.001
HOMAbeta	45.3 (33.8-61.2)	44.7 (33.8-62.4)	29.4 (18.6-45.6)	0.995
Insulinogenic index (I.I.)	0.59 (0.35-1.07)	0.60 (0.38-1.09)	0.50 (0.28-0.85)	<0.001
I_{30}/G_{30}	0.22 (0.15-0.33)	0.23 (0.15-0.33)	0.20 (0.13-0.30)	0.024
Stumvoll-1	656 (481-874)	656.6 (506.6-882.4)	613.2 (411.3-804.7)	0.003
Stumvoll-2	183.3 (144.5.329.1)	183.4 (150.3-230.1)	172.7 (133.5.310.3)	0.008
Fatty Liver Index	21.3 (10.1-41.8)	17.3 (8.3-36.4)	31.5 (18.7-55.2)	<0.001

604 participants were re-evaluated after a mean of 3.7 years. Five persons who developed diabetes were excluded. Data are the median (IQR) or percent. I_{30}/G_{30}, immunoreactive insulin 30 min postload/plasma glucose 30 min postload. *, nonprogressors vs progressors. The subscripts indicate sampling time at 75 g OGTT.

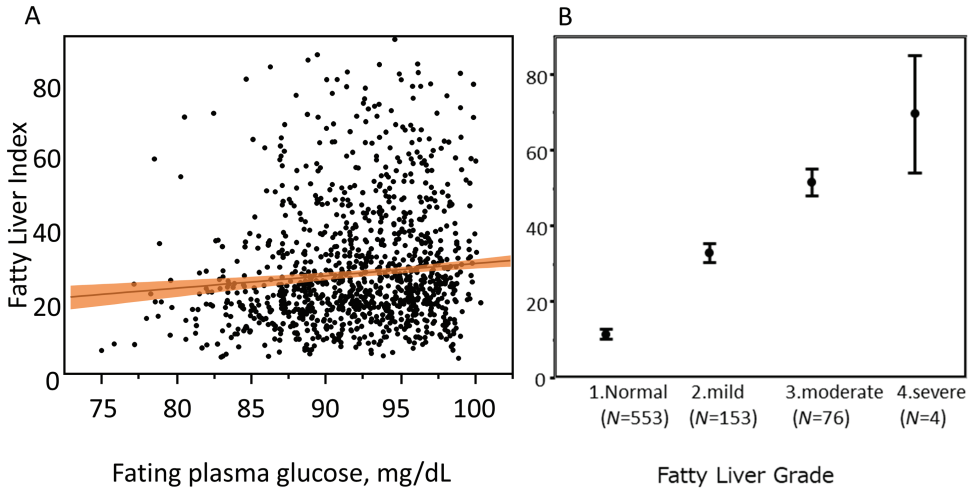

Figure 1. A, Multiple regression of Fatty Liver Index (FLI) to FPG. Data are presented as mean (red line) and 95% CI (red broken lines) adjusted for age and sex. The conversion factor for glucose was 0.055 (mg/dL to mmol/L). Regression was $y = -35.0 + [0.63 \cdot \text{glucose (mg/dL)}] - [0.12 \cdot \text{age (years)}] + [15.6 \cdot \text{sex (1 for male, 0 for female)}]$: $P < 0.0001$ for glucose and sex and $P = 0.139$ for age. B, Correlation of serum Fatty Liver Index values and hepatic steatosis evaluated by ultrasonography. Data were obtained from 766 health examinees in Ina Central Hospital. Hepatic steatosis was graded as reported previously (44). FLI values, adjusted for age and sex, in participants with fatty liver grades 2, 3, and 4 were significantly greater than values in participants with grade 1 (normal). The best cutoff GLI value for presence of fatty liver (grade 2 or over) was 14.3.

Table 4. HR (95% CI) of $ISI_{MATSUDA}$, Stumvoll-1, Fatty Liver Index, and FPG for incident prediabetes

Variable	HR (95% CI)	*P* value
$ISI_{Matsuda}$	0.838 (0.718-0.977)	0.03
Stumvoll-1	0.785 (0.640-0.964)	0.02
FLI	1.307 (1.033-1.654)	0.02
FPG	1.397 (1.069-1.826)	0.01

HR was adjusted for age, sex, and family history, and scaled to the IQR (computed by subtracting the 25th percentile from the 75th percentile and defined as the unit for HR). Note that $HR/IQR_{increase}$ so that protection was shown for $ISI_{MATSUDA}$ and Stumvoll-1, and $HR/IQR_{increase}$, which was risk, was shown for FLI and FPG. The interaction term for attenuated $ISI_{MATSUDA}$ and elevated FPG levels was also significant in this analysis ($P = 0.02$). The model fitness: Akaike Information Criterion corrected (AICc), 1730.5; x^2, 49.40; $P < 0.0001$. HRs of $ISI_{Matsuda}$, Stumvoll-1, and FPG did not substantially change if body weight and BMI were included as covariates. Such procedure erased significance of GLI as a risk.

ROC (95% CI) obtained by a combination of lower $ISI_{MATSUDA}$ and Stumvoll-1 for the prediction of incident prediabetes was 0.682 (0.627-0.732) with 61.5% sensitivity and 67.7% specificity. Addition of high FLI and high-normal FPG as predictors yielded significantly better ($P < 0.001$) results: AUC of ROC 0.747 (0.694-0.795), with 70.4% sensitivity and 67.0% specificity.

Stratification performed based on the baseline median FPG revealed that attenuated $ISI_{Matsuda}$ was a significant and independent risk factor for progression to prediabetes exclusively among participants with FPG levels ≥ 5.2 mmol/L (Table 2, Model 1). Additionally, attenuated Gutt index and Avignon's SiM, if used in place of $ISI_{MATSUDA}$, were significant risk factors for incident prediabetes exclusively among participants with FPG levels ≥ 5.2 mmol/L (Table 2, Model 2 and 3).

Discussion

In this study, we characterized recently identified risk factors for incident prediabetes among middle-aged individuals with NGM. Also, WE observed in our cohort that an increase in FLI, a reliable surrogate marker of HS ([19] and present study) was a risk factor for incident prediabetes. Fatty liver reportedly occurs in Japanese individuals with lower FLI values, compared with Caucasian individuals. In line with such findings, the best FLI cutoff value predicting incident prediabetes was low, comparable to previous reports [42, 43].

Quantitatively, the risk attributable to HS was as large as the risk from attenuated S_i or I_{sec}. In a previous study [23], HS was a significant risk factor for diabetes independently from HOMA-IR, an index of basal insulin sensitivity at an unstimulated state. We found that FLI was a risk factor for incident prediabetes independently from $ISI_{MATSUDA}$, which is a more robust index of insulin sensitivity than HOMA-IR [29]. Even under this condition, risk of HS independent from S_i was clearly demonstrated. This indicated that HS contributed directly to prediabetes, not via attenuated insulin sensitivity. However, we must consider that HS might be associated with worsening of glucose metabolism, without directly contributing to it. In fact, HS is strongly correlated with FPG (Fig. 1), and the high-normal FPG is a strong risk factor for incident prediabetes. Evidence for insulin-independent lipogenesis by hepatocytes has been demonstrated under experimental conditions [45]. However, this phenomenon has not been observed in humans under physiological conditions. Further studies are needed to prove the direct causal relationship of HS to incident prediabetes or diabetes beyond insulin sensitivity.

The short-term impact of a high-normal FPG—especially in nonobese, middle-aged participants for incident prediabetes/diabetes—has not been reported. Our data clearly indicate that having high-normal FPG for a period as short as 3.7 years is a risk factor for incident prediabetes in nonobese, middle-aged adults. A high-normal FPG among young men (mean age, 32-33 years) [17] and school-age children (mean age, 12-13 years) [18], was a risk factor for future diabetes. This was also observed in relatively young, obese participants [46, 47]. The best cutoff glucose values were significantly higher in our study than in previous studies [17, 18]. This was to be well expected due to the fact that we analyzed an elder population with lower glycemic targets, that is, prediabetes rather than diabetes.

In a sense, the issue is semantics regarding "what is the normal range?" If we permit the inclusion of people with the possibility of developing diabetes or prediabetes in the future [7, 11, 12] as "normal," the upper limit of "the normal range" naturally goes up. However, if we completely exclude such people from the normal, the upper limit of "the normal range" goes down.

We also established the interaction between FPG and $ISI_{Matsuda}$, which was compatible with the interaction between FPG and BMI reported by Tirosh et al [17]. They performed stratification with BMI and observed a drastic rise in incident diabetes with an increase in BMI. The result was qualitatively similar to our stratification study. Specifically, we also found the incidence of prediabetes among participants with NGM was dichotomous, with the incidence being more than twice as high in those with FPG levels higher than 5.2 mmol/L. Notably, this level of glycemia, 5.2 mmol/L, was close to the level where an upward shift was observed in the clinical trajectories [7, 8, 11-14]. The FPG cutoff that most effectively differentiated progressors from nonprogressors, 5.3 mM, was also in the vicinity of 5.2 mmol/L. Taken together, we hypothesize that the interaction between FPG and attenuated S_i starts to operate around this level of glucose, 5.2 to 5.3 mmol/L, leading to accelerated worsening of glucose metabolism. The possibility of so-called glucose toxicity taking place at around this low level of glucose was recently hypothesized by Weir et al [48].

Sensitivity of prediction of incident prediabetes by attenuated $ISI_{MATSUDA}$ and Stumvoll-1 was 61.5%, which was significantly increased to 70.4% by incorporation of FLI and FPG. The improved prediction by incorporation of the newer risk factors is meaningful in clinical practice. Regardless of the mechanism, more caution should be given to the development of prediabetes/diabetes in participants with HS and a high-normal FPG. In this study, FLI as a surrogate marker of HS was confirmed in a different cohort (at Ina Central Hospital). Within this limitation, FLI was a reliable marker for HS. If FLI was not sensitive enough to detect HS, it may not be detected as a risk factor for incident prediabetes, especially for insulin-sensitivity-independent cases.

In the context of the study, the participants were well-characterized and balanced and can be considered representative of the general population of Japanese adults with NGM. Thus, conclusions can be generalized to nonobese, middle-aged individuals in Japan. The data were analyzed with clear targets and novel viewpoints although the study protocol was retrospective.

Despite the study's strengths, they must be considered within its limitations. As the study population was limited to Japanese individuals, the conclusions obtained may not be relevant to other ethnic groups and must be further studied in other groups. The bias leading to repeated health examinations cannot be ruled out, although the extremely basic biological data such as BMI and FPG level of the study participants was not significantly different from the expected values from Japanese adults with NGM [25]. We could not trace the conversion from NGM to diabetes due to the relatively short follow-up period. As such, only 5 participants developed diabetes during the observation period, which prevented us from performing statistical analysis. Finally, as the study was purely retrospective, cause-effect relationships are difficult to derive from these findings. For instance, direct proof that HS is causal for prediabetes, independent from attenuated insulin action was not obtainable in this study.

In conclusion, among Japanese adults with NGM, HS and normal range high glucose are substantial risk factors for incident prediabetes. HS might be directly contributing to prediabetes beyond attenuated insulin sensitivity. The interaction between glucose and insulin sensitivity may be contributing to the accelerated glucose rise seen at the higher end of the normal range glucose. Further studies are needed before definitively lowering the current upper limit of the normal range.

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Prior Presentation

Different aspects of the study population have been reported in the following previous publications which are cited in this communication as References 10 and 27: Oka R, Yagi K, Sakurai M, et al. Insulin secretion and insulin sensitivity on the oral glucose tolerance test (OGTT) in middle-aged Japanese. *Endocr J*. 2012;59:55-64; and Oka R, Yagi K, Hayashi K, et al. The evolution of non-diabetic hyperglycemia: a longitudinal study *Endocr J*. 2014;61:91-99.

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Disclosure Summary

The authors have nothing to disclose.

Data Availability

Some or all data sets generated during and/ or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

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