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Mean and visit-to-visit variability of glycated hemoglobin, and the risk of non-alcoholic fatty liver disease

Jee Hee Yoo[1,2†], Mira Kang[3,4†], Gyuri Kim[1], Kyu Yeon Hur[1], Jae Hyeon Kim[1], Dong Hyun Sinn[5], Sang-Man Jin[1*]

[1]Division of Endocrinology and Metabolism, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, [2]Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea, [3]Department of Digital Health, SAIHST, Sungkyunkwan University, Seoul, Korea, [4]Center for Health Promotion, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, and [5]Division of Gastroenterology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

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***Correspondence**
Sang-Man Jin
Tel.: +82-2-3410-3849
Fax: +82-2-3410-0271
E-mail address:
sjin772@gmail.com

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ABSTRACT

Aims/Introduction: We aimed to determine whether mean and visit-to-visit glycated hemoglobin (HbA1c) variability independently increase the incidence of non-alcoholic fatty liver disease (NAFLD) across the diabetic continuum from normal glucose tolerance (NGT) to established diabetes.

Materials and Methods: In a longitudinal cohort study, 21,123 participants underwent five or more annual health screening checkups. Participants were categorized into diabetes (n = 1,635), prediabetes (n = 6,650) and NGT (n = 12,838) groups. Mean, standard deviation (SD) and coefficient of variation data on HbA1c were obtained from three consecutive measurements. The associations between those data and incident NAFLD were analyzed using Cox regressions.

Results: Over a median follow-up period of 57 months, 3,860 (18.3%) participants developed NAFLD. The risk of NAFLD increased continuously, with the mean HbA1c beginning at 4.9%, even in the NGT group. We found a significant association between increasing HbA1c variability and incident NAFLD (coefficient of variation, adjusted hazard ratio 1.14, 95% confidence interval 1.01–1.29; standard deviation, adjusted hazard ratio 1.19, 95% confidence interval 1.05–1.36) in the diabetes group, but not in the NGT or prediabetes group. Consistent findings were observed when NAFLD patients with a low possibility of fibrosis were excluded. The association between the coefficient of variation of HbA1c and incident NAFLD in the diabetes group was significant only in those with an increasing trend of post-baseline HbA1c (adjusted hazard ratio 1.24, 95% confidence interval 1.01–1.52).

Conclusions: Increased mean HbA1c levels elevated the risk of incident NAFLD, even with NGT. Increases in visit-to-visit variability of HbA1c independently elevated the risk of incident NAFLD, but only in the diabetes group.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most prevalent metabolic diseases worldwide[1]. It can progress to steatohepatitis, cirrhosis and even hepatocellular carcinoma, which are associated with high mortality rates[2].

In addition to the well-known association between NAFLD and established diabetes[3], several cross-sectional studies have shown an association between prediabetes and NAFLD[4]. A longitudinal study found an association between random plasma glucose levels and the risk of incident NAFLD, even in individuals without diabetes[5]. However, a threshold level at which glycated hemoglobin (HbA1c) increases that risk has not been determined by a longitudinal analysis.

Furthermore, the previous studies did not evaluate whether visit-to-visit HbA1c variability is a risk factor for the development of NAFLD, despite its increasingly accepted association with various complications of diabetes, such as diabetic

†These authors contributed equally to this work.
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retinopathy, diabetic kidney diseases and diabetic neuropathy[6–9]. Although a few studies have found an association between within-day glycemic variability and the severity of hepatic fibrosis in established NAFLD[10], the association between long-term, visit-to-visit HbA1c variability and the risk of incident NAFLD has not been explored.

It was recently reported that visit-to-visit HbA1c variability is significantly associated with major adverse cardiovascular events (MACEs) in people without diabetes[11] and with newly diagnosed diabetes[6]. Given the common pathophysiology of MACEs and NAFLD, such as insulin resistance and non-specific inflammation, it needs to be determined whether visit-to-visit HbA1c variability independently increases the incidence of NAFLD in individuals with various baseline glucose status, including normal glucose tolerance (NGT) and prediabetes.

Therefore, we aimed to determine whether the mean and visit-to-visit variability of HbA1c independently increase the incidence of NAFLD using a longitudinal analysis of a cohort of individuals on the diabetic continuum from NGT to established diabetes.

METHODS

Study population

The study population consisted of 30,354 people who participated in a medical health checkup program at the Health Promotion Center of Samsung Medical Center (Seoul, Korea) five or more times at 1- or 2-year intervals between July 2005 and July 2016. According to the Industrial Safety and Health Law of the Republic of Korea, all employees must complete free health screening examinations every 1 or 2 years. The study population thus consisted of employees and non-employed people who voluntarily participated in the health checkup program every 1 or 2 years[12].

Individuals who reported heavy alcohol consumption (daily consumption >20 g for women, 30 g for men, n = 2,212) and those with positive serological markers for hepatitis B (n = 1,230) or hepatitis C virus (n = 258) were excluded. Those who took >5 years to accumulate the three glycemic evaluations used to determine visit-to-visit glycemic variability (n = 455) were excluded. Participants with a hepatic steatosis index (HSI) of >36 at baseline (n = 5,076) were also excluded. A total of 21,123 participants were included in the final study population (Figure S1).

Participants were sorted into three groups – diabetes (n = 1,635), prediabetes (n = 6,650) and NGT (n = 12,838) – based on information provided at their third visit, which served as the baseline for the follow-up period. Diabetes was defined as fasting plasma glucose (FPG) ≥126 mg/dL, HbA1c ≥6.5%, or a self-reported history of the use of diabetes medication[13]. Prediabetes was defined as FPG ≥100 mg/dL but <126 mg/dL or HbA1c ≥ 5.7% but <6.5%, and no history of diabetes or receiving diabetes medication. NGT was defined as FPG <100 mg/dL and HbA1c <5.7% without a history of diabetes or diabetes medication. Associations between NAFLD and mean glucose and visit-to visit glycemic variability were analyzed separately in each group.

The present study was approved by the institutional review board of the Samsung Medical Center (2019-05-110). The requirement for informed consent was waived, because the study data were de-identified. The study was carried out in compliance with the recommendations of the Declaration of Helsinki.

Clinical and laboratory measurements

All participants completed questionnaires about their prior surgical and medical history, smoking and exercise habits, and prescribed medications. Smoking status was categorized as non-smoker, ex-smoker or current smoker. Regular exercise was defined as exercising three or more days per week.

Weight (kg) and height (cm) were measured in light clothing without shoes. Waist circumference was measured at the level of the umbilicus. Body mass index (BMI) was calculated by dividing bodyweight by the square of height (kg/m^2). Blood pressure was measured in a sitting position using a sphygmomanometer after a 5 min rest period and is expressed as the mean of two readings.

Detailed methods for the measurement of laboratory profiles are described in a previous study[14]. The homeostatic model assessment of insulin resistance (HOMA-IR) and the homeostatic model assessment of β-cell function (HOMA-β) was determined by the following formula: fasting plasma insulin (μIU/mL) × FPG (mg/dL) / 405, and fasting plasma insulin (μIU/mL) × 360 / (FPG-36), respectively[15]. A HOMA-IR of ≥2.5 was defined as increased insulin resistance. Hypertension was defined as blood pressure ≥140/90 mmHg or a clinical history of use of antihypertensive medication[16].

Assessment of visit-to-visit glycemic variability

Figure S2 provides the study design scheme, and illustrates how visit-to-visit variability was examined and how NAFLD incidence was followed up. Visit-to-visit variability of HbA1c was determined using three consecutive measurements before study entry. Evaluations of visit-to-visit glycemic variability lasted for up to 5 years, with a median duration of 25 months (interquartile range 25–31). The baseline of the follow-up period was set at the third HbA1c measurement, which was the last date for evaluating glycemic variability. The first visit >9 months after the baseline date was regarded as visit 1 during the follow-up period (Figure S2).

The standard deviation (SD) and coefficient of variation (CV) were used as indices of visit-to-visit glycemic variability. The SD was defined as a residual obtained from a linear regression analysis of the three HbA1c measurements for each individual. The mean and maximum HbA1c values during the three consecutive measurements were also determined. CV was defined as the SD divided by the mean.

Definition of NAFLD and metabolic dysfunction-associated fatty liver disease

NAFLD was defined as an HSI of >36, which has been validated as having a high correlation with liver biopsy, abdominal ultrasonography or proton magnetic resonance spectroscopy in various populations[17–19]. HSI values were calculated according to the following formula: 8 × (alanine aminotransferase/aspartate aminotransferase ratio) + BMI + 2 if female + 2 if diabetes mellitus is present[20]. To validate the reliability of the HSI in the participants of the current study, the correlation between the HSI and reports from 230,258 abdominal ultrasonography readings carried out in the Health Promotion Center of the Samsung Medical Center from July 2005 to July 2016 was analyzed. All participants in the current study underwent abdominal ultrasonography at least once from July 2005 to July 2016. Abdominal ultrasound exams were carried out by experienced radiologists unaware of the study aims using LogiQ E9 (GE Healthcare, Milwaukee, WI, USA), iU22 xMatrix (Philips Medical Systems, Cleveland, OH, USA) or ACUSON Sequoia 512 (Siemens, Issaquah, WA, USA) equipment. The ultrasonographic findings were classified as normal (n = 134,810; 58.5%), mild (n = 71,123; 30.9%) and moderate/severe (n = 24,326; 10.6%) fatty liver according to standard criteria, including parenchymal brightness, liver-to-kidney contrast, deep beam attenuation and bright vessel walls[21]. The mean HSI levels were 30.8 ± 3.4, 34.5 ± 3.9 and 36.9 ± 4.2 in the normal, mild and moderate/severe fatty liver groups, respectively (P-value <0.001). Among the 134,810 normal ultrasonographic findings, just 9,372 (7.0%) were associated with an HSI >36 (Figure S3).

An NAFLD fibrosis score (NFS) greater than −1.455 was used to exclude NAFLD patients with a low possibility of hepatic fibrosis[22,23]. The NFS is calculated according to the following formula: −1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet (10^9/L) − 0.66 × albumin (g/dL)[23].

Metabolic dysfunction-associated fatty liver disease (MAFLD) was defined using the criteria suggested by Eslam *et al.*[24] Hepatic steatosis was detected by ultrasonography carried out when incident NAFLD was diagnosed (when possible) or by the fatty liver index[25]. Among the participants with confirmed MAFLD in the present study, hepatic steatosis was detected by ultrasonography in 62.3% of them and by a fatty liver index >30[25] in the remaining 37.3%. When hepatic steatosis was detected, MAFLD was diagnosed when the participants were overweight/obese (BMI ≥23 kg/m^2) or had type 2 diabetes, or at least two of the following metabolic risk abnormalities (absence of overweight/obese and type 2 diabetes): (i) waist circumference ≥90 cm for men and ≥80 cm for women; (ii) blood pressure ≥130/85 mmHg or specific drug treatment; (iii) triglycerides (TGs) ≥150 mg/dL; (iv) high-density lipoprotein cholesterol <40 mg/dL for men and <50 mg/dL for women; (v) prediabetes (FPG 100–125 mg/dL or HbA1c 5.7–6.4%); (vi) HOMA-IR ≥2.5; and (vii) high-sensitivity C-reactive protein (hs-CRP) >2 mg/L.

Statistical analysis

Data are expressed as the mean ± SD for normally distributed continuous variables, the median (interquartile range 25th–75th percentile) for continuous variables with a skewed distribution and frequency with a percentage for categorical variables. Comparisons between baseline characteristics according to glycemic status (diabetes, prediabetes and NGT) were made using analysis of variance (ANOVA) with Bonferroni's method for continuous variables, and the χ^2-test with linear-by-linear analysis for categorical variables. The Kruskal–Wallis test or Mann–Whitney U-test was used to analyze data with a skewed distribution. Changes in HbA1c when evaluating visit-to visit glycemic variability and post-baseline HbA1c changes were categorized as stable (0.3% < HbA1c change < 0.3%), decreasing (HbA1c change ≤−0.3%) or increasing (HbA1c change ≥0.3%)[9]. The distribution of HbA1c variability is shown in Figure S4.

A Cox proportional hazards analysis was carried out to determine independent associations between the development of NAFLD and the mean and variability (SD and CV) of HbA1c in each glucose status group. Hazard ratios (HRs) are reported for a 1-SD increase in variability (Figure S4). For multivariable-adjusted analyses, model 1 was non-adjusted; model 2 was adjusted for age, sex, systolic blood pressure (SBP), BMI, TGs, HOMA-IR, exercise status and smoking status; and model 3 was further adjusted for mean HbA1c. The confounding factors were selected with clinically important variables with a P-value <0.1 in the univariate analyses. Among the lipid parameters, we selected TGs as a covariate, because elevated plasma TG levels are hallmarks of the NAFLD progression[26,27]. We assumed collinearity in the model when the variance inflation factor was greater than five without weak correlation (r < 0.25)[28]. Interaction analyses were carried out to identify interactions between groups divided by age, sex, obesity, HOMA-IR, use of glucose-lowering agents or lipid-modifying agents and changes in HbA1c. To validate the robustness of the analysis, sensitivity analyses were carried out excluding either NAFLD (HSI >36) patients with a low possibility of fibrosis (NFS >−1.455) or NAFLD (HSI >36) patients that failed to meet the criteria for MAFLD[24]. Potential non-linearity was further examined by a non-parametric spline-smoothing method and presented as an HR plot. HRs were computed with the mean HbA1c at which the HR was minimal as the reference value[29]. Cubic splines were used to determine the threshold of HbA1c levels that contributed to NAFLD incidence.

All P-values were two-tailed, and those <0.05 were considered statistically significant. Statistical analyses were carried out using IBM SPSS version 26.0 for Windows (Armonk, NY, USA) or R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics and visit-to-visit glycemic variability according to baseline glucose status

The baseline characteristics and visit-to-visit glycemic variability of the 21,123 study participants stratified by glucose status are summarized in Table 1. Among the total study population, 51.1% were men, the mean age was 52.9 ± 8.3 years, and the mean BMI was 22.8 ± 2.3 kg/m^2. During a median follow-up period of 57 months (interquartile range 35–80) or 100,125 person-years, 3,860 participants (18.3%) developed NAFLD. The incidence of NAFLD by group was 1,920 events (15.0%) in the NGT group (n = 12,838; 62,266 person-years), 1,507 events (22.7%) in the prediabetes group (n = 6,650; 30,495 person-years) and 433 events (26.5%) in the diabetes group (n = 1,635; 7,365 person-years).

The diabetes group had higher SD and CV of HbA1c than the prediabetes and NGT groups (P for trend < 0.001). A total of 23% of participants in the NGT group, 40.9% in the prediabetes group and 44.4% in the diabetes group had an increasing trend in HbA1c over the three consecutive HbA1c measurements made during the visit-to-visit glycemic variability measurements.

Effect of mean HbA1c and visit-to-visit HbA1c variability on the risk of incident NAFLD

In the univariate analyses, high BMI, waist circumference, HOMA-IR, hs-CRP, current smoker, high mean and maximum HbA1c, and high SD and CV of HbA1c at baseline were associated with incident NAFLD (Table S1). Incident NAFLD was also associated with an increasing trend of post-baseline HbA1c and new-onset prediabetes/diabetes during the study period (Table S1).

The independent associations between the risk of incident NAFLD and mean HbA1c and visit-to-visit HbA1c variability were explored using separate Cox proportional hazards regression analyses of data from the diabetes, prediabetes and NGT groups (Table 2).

Regardless of glucose status at baseline, mean HbA1c was independently associated with an increased risk of incident NAFLD. The HRs were 1.24 (95% confidence interval [CI] 1.13–1.37; P for trend <0.001), 2.01 (95% CI 1.68–2.52, P for trend < 0.001) and 1.52 (95% CI 1.23–1.88, P for trend <0.001) in the diabetes, prediabetes and NGT groups, respectively, after adjusting for age, sex, SBP, BMI, TGs, HOMA-IR, exercise status and smoking status (model 2). In the HR plots constructed

Table 1 | Baseline characteristics according to glucose status

	NGT (n = 12,838)	Prediabetes (n = 6,650)	Diabetes† (n = 1,635)	P-value
Age (years)	51.2 ± 7.8	54.9 ± 8.2	57.5 ± 8.86	<0.001
Sex, n (%)				
Males	5,931 (46.2)	3,778 (56.8)	1,095 (67.0)	<0.001
Females	6,907 (53.8)	2,872 (43.2)	540 (33.0)	
Bodyweight, kg	61.3 ± 9.59	63.7 ± 9.6	63.3 ± 8.7	<0.001
BMI (kg/m^2)	22.5 ± 2.3	23.2 ± 2.2	22.9 ± 2.0	<0.001
Waist circumference (cm)	79.7 ± 7.8	82.6 ± 7.5	82.8 ± 7.2	<0.001
Current smoker	1,663 (13.0)	1,080 (16.3)	318 (19.5)	<0.001
Regular exercise	3,676 (30.8)	1,929 (30.9)	520 (34.2)	0.001
SBP (mmHg)	114.5 ± 14.8	118.7 ± 15.3	119.2 ± 15.6	<0.001
DBP (mmHg)	71.9 ± 10.8	74.4 ± 10.6	73.8 ± 9.9	<0.001
Total cholesterol (mg/dL	)193.8 ± 31.8	200.6 ± 33.5	185.0 ± 35.4	<0.001
HDL-C (mg/dL)	58.8 ± 14.8	55.9 ± 14.3	53.6 ± 13.5	<0.001
Triglycerides (mg/dL)	104.5 ± 60.7	125.1 ± 73.8	123.9 ± 85.2	<0.001
LDL-C (mg/dL)	119.4 ± 28.0	125.4 ± 29.5	112.1 ± 30.9	<0.001
AST (IU/L)	21.5 ± 11.0	22.8 ± 8.2	23.4 ± 11.6	<0.001
ALT (IU/L)	18.4 ± 9.7	20.7 ± 9.5	20.6 ± 10.9	<0.001
Baseline HSI	30.4 ± 2.8	31.3 ± 2.7	32.6 ± 2.3	<0.001
Lipid-modifying agents, n (%)	1,600 (12.5)	1,528 (23.0)	584 (35.7)	<0.001
HbA1c				
Mean (%)	5.2 ± 0.2	5.7 ± 0.2	6.5 ± 0.9	<0.001
Maximum (%)	5.4 ± 0.2	5.8 ± 0.3	6.9 ± 1.2	<0.001
SD (%)	0.17 ± 0.15	0.18 ± 0.16	0.37 ± 0.44	<0.001
CV (%)	3.3 ± 2.8	3.2 ± 2.9	5.5 ± 5.8	<0.001

Values shown are the mean ± standard deviation (SD) or n (%). †In this group, 925 (56.6%) participants took glucose-lowering medications. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CV, coefficient of variation; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; HbA1c, glycated hemoglobin; HSI, hepatic steatosis index; LDL-C, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; NGT, normal glucose tolerance; SBP, systolic blood pressure; SD, standard deviation.

Table 2 | Effect of mean glycated hemoglobin and visit-to-visit glycated hemoglobin variability on the risk of incident non-alcoholic fatty liver disease

	Model 1		Model 2		Model 3	
	HR (95% CI)	*P* Trend	HR (95% CI)	*P* Trend	HR (95% CI)	*P* Trend
Diabetes						
Mean†	1.28 (1.18–1.39)	<0.001	1.24 (1.13–1.37)	<0.001	1.24 (1.13–1.37)	<0.001
Maximum	1.23 (1.15–1.31)	<0.001	1.23 (1.14–1.33)	<0.001	1.52 (1.20 –1.93)	0.001
SD	1.24 (1.12–1.38)	<0.001	1.29 (1.15–1.45)	<0.001	1.19 (1.05–1.36)	0.007
CV	1.17 (1.04–1.30)	0.006	1.22 (1.08–1.37)	0.001	1.14 (1.01–1.29)	0.039
Prediabetes						
Mean†	2.10 (1.75–2.54)	<0.001	2.01 (1.68–2.52)	<0.001	2.01 (1.68–2.52)	<0.001
Maximum	1.72 (1.50–1.97)	<0.001	1.79 (1.52–2.11)	<0.001	1.20 (0.81–1.76)	0.363
SD	1.01 (0.95–1.07)	0.752	1.00 (0.94–1.07)	0.933	1.01 (0.94–1.07)	0.888
CV	1.01 (0.95–1.07)	0.735	1.01 (0.94–1.08)	0.820	1.02 (0.95–1.09)	0.583
NGT						
Mean†	2.12 (1.74–2.58)	<0.001	1.52 (1.23–1.88)	<0.001	1.52 (1.23–1.88)	<0.001
Maximum	1.80 (1.55–2.09)	<0.001	1.50 (1.25–1.81)	<0.001	1.44 (0.94–2.21)	0.098
SD	0.97 (0.92–1.02)	0.176	1.00 (0.95–1.06)	0.911	1.01 (0.96–1.07)	0.736
CV	0.96 (0.91–1.01)	0.097	0.99 (0.94–1.05)	0.836	1.01 (0.96–1.07)	0.755

Model 1: crude. Model 2: adjusted for age, sex, systolic blood pressure (SBP), body mass index, triglycerides, homeostatic model assessment for insulin resistance, exercise status and smoking status. Model 3: adjusted for variables in model 2 plus mean glycated hemoglobin. †Mean glycated hemoglobin was analyzed for model 2. Hazard ratios (HRs) represent per 1 standard deviation (SD) increase in SD and coefficient of variation (CV). HRs represent per 1% increase in mean and maximum. CI, confidence interval; NAFLD, non-alcoholic fatty liver disease; NGT, normal glucose tolerance; SBP, systolic blood pressure; SD, standard deviation; TGs, triglycerides.

using a non-parametric spline-smoothing method, the HRs for mean HbA1c increased continuously without definite thresholds from the lowest levels. The lower limit of the HR was >1.0 at HbA1c levels below the current lower limit for diagnosis of prediabetes (4.9%; Figure 1).

We found significant independent associations between visit-to-visit variability of HbA1c and the risk of incident NAFLD (SD: HR 1.29 per 1 SD [0.45%], 95% CI 1.15–1.45, *P* for trend < 0.001; and CV: HR 1.22 per 1 SD [5.77%], 95% CI 1.08–1.37, *P* for trend = 0.001) in the diabetes group after adjusting for age, sex, SBP, BMI, TG, HOMA-IR, exercise status, and smoking status (model 2), and after further adjustment for mean HbA1c (model 3; SD: HR 1.19 per 1 SD, 95% CI 1.05–1.36, *P* for trend = 0.007; and CV: HR 1.14 per 1 SD, 95% CI 1.01–1.29, *P* for trend = 0.039). The results were similar when high-density lipoprotein cholesterol was included as a covariate instead of TGs in models 2 and 3 (data not shown). In subgroup analyses, an association between the CV of HbA1c and the risk of incident NAFLD was observed regardless of age, but only in men, participants with a BMI ≥23 kg/m^2, those whose trend of HbA1c was stable (change of −0.3 to 0.3%) during the period of evaluating visit-to-visit HbA1c variability, those who used glucose-lowering agents and those whose trend of HbA1c increased (change of more than 0.3%) during the follow-up period. However, significant interactions among those factors were not observed (Table S2, all *P*-values >0.10). The association between visit-to-visit variability in HbA1c levels and the risk of NAFLD was not significant in the prediabetes and NGT groups (Table 2).

Inflammation markers, indices of insulin resistance and insulin secretion and HbA1c variability

We further explored a potential association between markers of subclinical inflammation (hs-CRP and ferritin), indices of insulin resistance (HOMA-IR) and insulin secretion (HOMA-β), and the CV or SD of HbA1c in the diabetes group (Figure 2). hs-CRP (*P* for trend = 0.038), ferritin (*P* for trend = 0.029) and HOMA-IR (*P* for trend = 0.001) increased with the CV and SD of HbA1c. HOMA-β decreased with the CV and SD of HbA1c (*P* for trend <0.001). Those associations were not observed in the prediabetes and NGT groups (not shown).

Sensitivity analysis

Among the incident NAFLD patients (n = 3,860), 3,075 (79.7%) met the MAFLD criteria (Table S3). In sensitivity analyses including only the patients meeting the MAFLD criteria, the association between visit-to-visit variability of HbA1c and incident NAFLD remained significant in the diabetes group (SD: HR 1.22 per 1 SD [0.45%], 95% CI 1.06–1.41, *P* for trend = 0.007; and CV: HR 1.17 per 1 SD [5.77%], 95% CI 1.02–1.34, *P* for trend = 0.024; Table 3).

When 2,358 NAFLD patients with a low possibility of fibrosis (NFS >−1.455) were excluded, the incidence of NAFLD over the median follow-up period of 57 months was 534 events

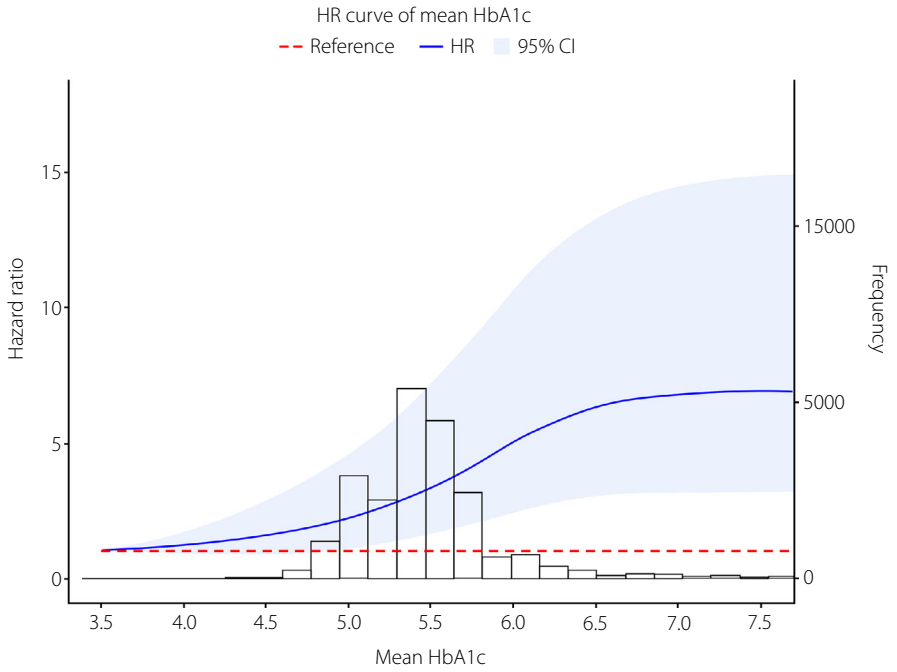

Figure 1 | Smoothing splines and 95% confidence intervals (CI) for hazard ratios (HR) according to mean glycated hemoglobin (HbA1c). Broken lines indicate a hazard ratio of 1, solid lines indicate the hazard ratios and the grey zone indicates the 95% confidence intervals. Columns indicate the relative proportion of participants.

(4.2%) in the NGT group (n = 12,838; 65,845 person-years), 696 events (10.5%) in the prediabetes group (n = 6,650; 32,456 person-years) and 272 events (16.6%) in the diabetes group (n = 1,635; 7,765 person-years). In sensitivity analyses that excluded incident NAFLD with a low possibility of hepatic fibrosis (NFS >−1.455; Table 3), the association of visit-to-visit variability of HbA1c and incident NAFLD remained significant in the diabetes group (SD: HR 1.26 per 1 SD [0.45%], 95% CI 1.06–1.48, P for trend = 0.008; and CV: HR 1.18 per 1 SD [5.77%], 95% CI 1.01–1.38, P for trend = 0.036; Table 3).

DISCUSSION

In a cohort of individuals with various glucose statuses at baseline (n = 21,123), an independent association between the mean HbA1c level and risk of incident NAFLD was observed, even in individuals with NGT, with a continuously increasing risk beginning at 4.9%. Furthermore, in patients with established diabetes, visit-to-visit HbA1c variability significantly increased the risk of incident NAFLD, even after adjusting for various confounding factors, including mean HbA1c. The results were consistent, even after excluding incident NAFLD cases that did not meet the MAFLD criteria[24] or had a low possibility of hepatic fibrosis. In contrast to the association between mean HbA1c and the risk of incident NAFLD, which was found even in the NGT group, the association between HbA1c variability and the risk of incident NAFLD was not significant in the NGT and prediabetes groups.

To the best of our knowledge, the present study is the first to show an independent association between HbA1c variability and the risk of incident NAFLD in people with diabetes. This is consistent with previous studies that showed that an increase in HbA1c variability affected the risk of MACEs, including ischemic stroke, heart failure, coronary artery disease and micro-complications, such as retinopathy, neuropathy and all-cause mortality in type 2 diabetes patients[6,9,30]. No association between HbA1c variability and the risk of incident NAFLD was observed in the prediabetes and NGT groups, which is in contrast to the results of recent studies involving 6,756 individuals that showed significant associations between HbA1c variability and MACEs, even in individuals with NGT and prediabetes[11].

In the present study, increases in hs-CRP, ferritin and HOMA-IR were observed in participants in the diabetes group whose CV and SD of HbA1c were high. Several mechanistic studies that focused on short-term, within-day or day-to-day glycemic variability reported an increase in reactive oxidative stress and insulin resistance, which induce inflammatory cytokines and the inflammation of liver cells, caused by glucose fluctuations rather than sustained hyperglycemia[31–34]. However, no dedicated studies have examined the mechanisms underlying long-term, visit-to-visit glycemic variability and the various complications of diabetes. For this reason, we analyzed whether the mechanisms reported in studies of within-day glycemic variability could be extrapolated to an association between visit-

Table 3 | Sensitivity analyses excluding incident non-alcoholic fatty liver disease cases that did not meet the criteria for metabolic dysfunction-associated fatty liver disease or had a low possibility of fibrosis

	Model 1		Model 2		Model 3	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Excluding incident NAFLD cases that did not meet the MAFLD criteria						
Diabetes						
Mean†	1.25 (1.12–1.39)	<0.001	1.20 (1.07–1.34)	0.001		
Maximum	1.23 (1.14–1.32)	<0.001	1.19 (1.09–1.30)	<0.001	1.42 (1.07–1.89)	0.015
SD	1.28 (1.13–1.44)	<0.001	1.30 (1.14–1.48)	<0.001	1.22 (1.06–1.41)	0.007
CV	1.20 (1.06–1.35)	0.004	1.23 (1.08–1.40)	0.002	1.17(1.02–1.34)	0.024
Prediabetes						
Mean†	3.06 (2.33–4.02)	<0.001	2.13 (1.71–2.66)	<0.001		
Maximum	1.77 (1.51–2.06)	<0.001	1.85 (1.55–2.21)	<0.001	1.20 (0.78–1.84)	0.400
SD	0.99 (0.92–1.06)	0.727	0.98 (0.91–1.05)	0.588	0.98 (0.91–1.05)	0.592
CV	0.98 (0.92–1.05)	0.614	0.98 (0.91–1.05)	0.513	0.99 (0.92–1.06)	0.686
Normal glucose tolerance						
Mean†	2.80 (1.92–4.09)	<0.001	1.94 (1.51–2.50)	<0.001		
Maximum	2.06 (1.76–2.40)	<0.001	1.74 (1.41–2.15)	<0.001	1.21 (0.73–2.03)	0.462
SD	0.94 (0.88–0.99)	0.046	1.01 (0.94–1.07)	0.938	1.01 (0.95–1.08)	0.790
CV	0.93 (0.87–0.98)	0.009	0.98 (0.92–1.04)	0.453	1.00 (0.93–1.06)	0.875
Excluding incident NAFLD cases with a low possibility of fibrosis						
Diabetes						
Mean†	1.25 (1.12–1.39)	<0.001	1.18 (1.04–1.35)	<0.001		
Maximum	1.21 (1.11–1.31)	<0.001	1.18 (1.07–1.32)	0.001	1.59 (1.15–2.20)	0.005
SD	1.21 (1.05–1.39)	0.009	1.31 (1.13–1.52)	<0.001	1.26 (1.06–1.48)	0.008
CV	1.12 (0.97–1.30)	0.118	1.23 (1.06–1.43)	0.001	1.18(1.01–1.38)	0.036
Prediabetes						
Mean†	3.06 (2.33–4.02)	<0.001	2.41 (1.78–3.27)	<0.001		
Maximum	2.17 (1.82–2.58)	<0.001	2.16 (1.73–2.70)	<0.001	1.78 (1.09–2.90)	0.020
SD	1.00 (0.92–1.10)	0.940	1.01 (0.94–1.09)	0.871	1.01 (0.91–1.11)	0.915
CV	1.00 (0.92–1.09)	0.992	1.01 (0.91–1.11)	0.892	1.02 (0.92–1.12)	0.774
Normal glucose tolerance						
Mean†	2.80 (1.92–4.09)	<0.001	1.07 (0.72–1.61)	0.731		
Maximum	2.03 (1.47–2.62)	<0.001	1.09 (0.75–1.59)	0.657	1.17 (0.45–3.06)	0.743
SD	0.95 (0.86–1.05)	0.353	1.02 (0.91–1.13)	0.752	1.02 (0.91–1.13)	0.740
CV	0.95 (0.86–1.04)	0.279	1.02 (0.92–1.13)	0.773	1.02 (0.92–1.13)	0.737

Model 1: unadjusted. Model 2: adjusted for age, sex, systolic blood pressure, body mass index, triglycerides, homeostatic model assessment for insulin resistance, exercise status and smoking status. Model 3: adjusted for variables in model 2 plus mean glycated hemoglobin. †Mean glycated hemoglobin was analyzed for model 2. Hazard ratios (HR) represent per 1 standard deviation (SD) increase in SD and coefficient of variation (CV). CI, confidence interval; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease.

to-visit HbA1c variability and the risk of incident NAFLD. The present results suggest that subclinical inflammation and resulting increases in insulin resistance are also associated with visit-to-visit HbA1c variability.

The lack of an association between inflammatory markers and visit-to-visit HbA1c variability in the NGT and prediabetes groups could explain the lack of association between HbA1c variability and risk of incident NAFLD in those groups, which was in contrast to the association between mean HbA1c and the risk of incident NAFLD found in the present study and the findings of the previous study on the association between HbA1c variability and MACEs in individuals without diabetes[11]. It has been suggested that NAFLD in established diabetes patients could have a distinct pathophysiology. For example, the presence of diabetes cancels the sexual dimorphism of NAFLD, although men are more prone to developing NAFLD in the general population[35,36]. Our current findings might indicate that increased HbA1c variability, along with subclinical inflammation and insulin resistance, could be one of the distinct characteristics of NAFLD associated with established diabetes, especially in patients already using glucose-lowering agents at baseline. Notably, increased CV of HbA1c at baseline in the context of a stable trend of pre-baseline HbA1c predicted incident NAFLD, and the association was significant in those with increasing trend of post-baseline HbA1c during the follow-up period, but not in those with an increasing trend

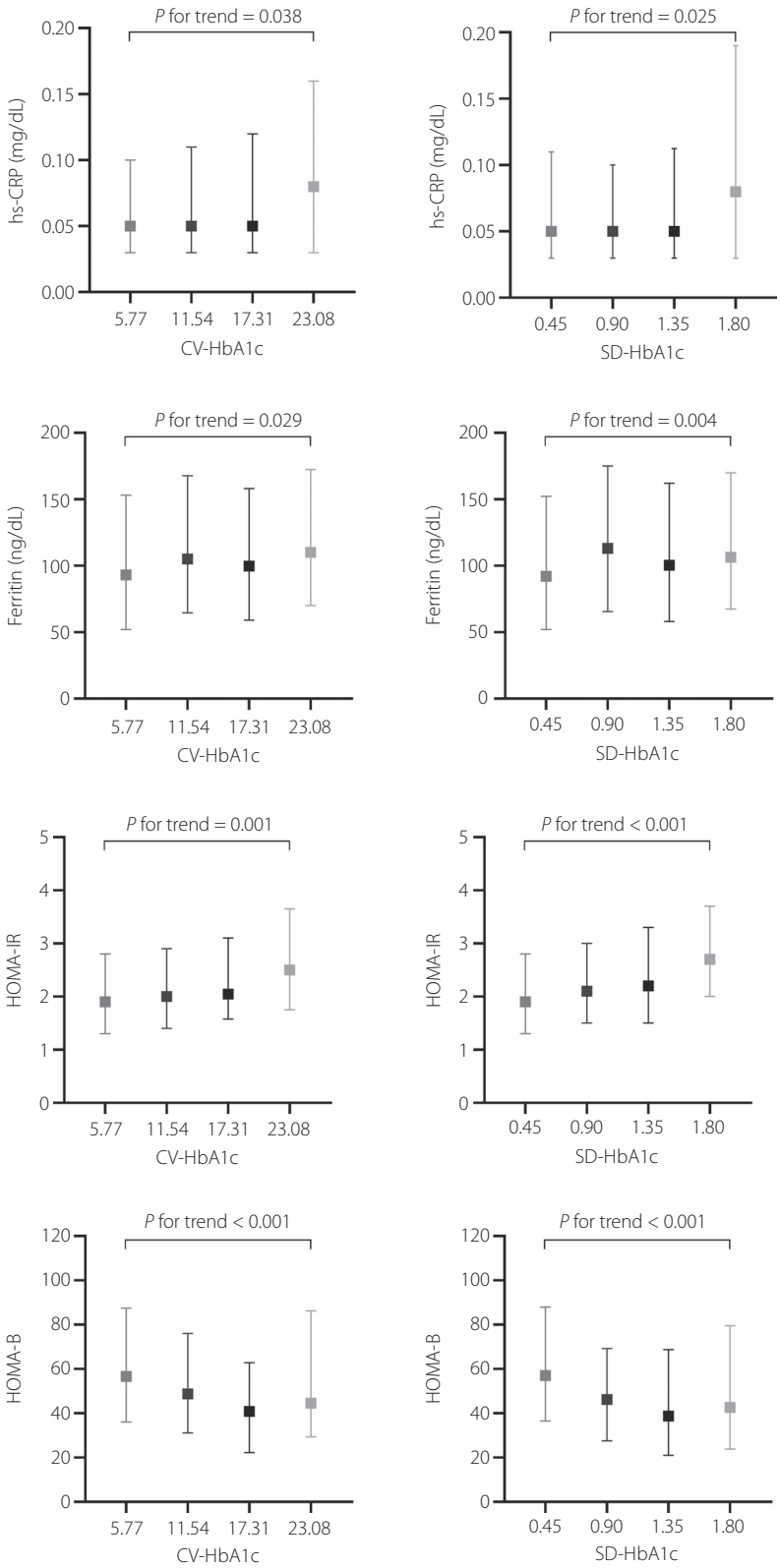

Figure 2 | Trends in high-sensitivity C-reactive protein (hs-CRP), ferritin, homeostatic model assessment for insulin resistance (HOMA-IR) and homeostatic model assessment of β-cell function (HOMA-β) according to the standard deviation (SD) and coefficient of variation (CV) of glycated hemoglobin (HbA1c) in participants with diabetes.

of HbA1c before the baseline (Table S2). It is possible that increased CV of HbA1c, preceding the worsening of HbA1c in established diabetes patients, would be a risk factor for NAFLD in established diabetes patients.

Another notable finding of the present study was a continuously increasing risk of NAFLD as the mean HbA1c increased from 4.9%, which is below the current lower limit for the diagnosis of prediabetes. In the NGT group (n = 12,838), the incidence of NAFLD was 1,920 events (15.0%). Among them, 74.5% met the criteria for MAFLD[24]. Although most of the MAFLD patients in the NGT group were associated with overweight/obesity (68.9%) or new-onset type 2 diabetes (7.5%), 5.1% of the patients were lean MAFLD without type 2 diabetes (Table S3). This proportion was similar to that in the prediabetes group in the present study (6.0%), and was consistent with previous findings that 6–20% of patients with MAFLD are neither overweight nor obese[24]. The development of incident NAFLD, even in a considerable proportion of the participants in the NGT group in the present study, indicates the diversity of clinical characteristics found in NAFLD, which is a basis for the recently advocated MAFLD criteria[24].

A limitation of the present study was a lack of histological confirmation of NAFLD. Although the HSI has been validated as a non-invasive metric for diagnosing NAFLD[20], and we confirmed the correlation between the HSI and the findings of 230,258 abdominal ultrasonography reports, abdominal ultrasonography was not regularly followed after the baseline for some of the study participants. For this reason, the detection of steatosis, which is a requisite for the diagnosis of MAFLD, depended on a serum biomarker alone in 37.3% of the MAFLD patients in the sensitivity analysis, although it was based on the abdominal ultrasonography after the baseline in the remaining 62.7% of the study participants. In addition, participants were recruited during health examinations at a single hospital, and therefore might not be representative of the general Korean population or foreign populations and could be subject to inherent selection bias. Finally, the details of the lipid-modifying and glucose-lowering medications used by participants, which could potentially affect incident NAFLD, could not be reliably identified, because medication history was given only in response to a questionnaire.

In conclusion, an independent association between the mean HbA1c level and the risk of incident NAFLD was observed, even in participants with NGT. Increases in visit-to-visit variability of HbA1c independently elevated the risk of incident NAFLD, subclinical inflammation and insulin resistance in established diabetes patients, but not in the NGT and prediabetes groups. Such an association was significant only in those with an increasing trend of post-baseline HbA1c. Increased visit-to-visit HbA1c variability at baseline, especially in those with an increasing trend of post-baseline HbA1c, could thus be a novel risk factor for NAFLD associated with established diabetes.

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.

Figure S1 | Flow diagram of the study population.

Figure S2 | Study design scheme.

Figure S3 | Frequency distribution of hepatic steatosis index (HSI) according to the degree of fatty liver disease measured by abdominal ultrasound.

Figure S4 | Distribution of glycated hemoglobin (HbA1c) variability by percent rescaled to the standard deviations.

Table S1 | Univariate comparison between cases with and without incident non-alcoholic fatty liver disease during the study period.

Table S2 | Subgroup analysis of how the coefficient of variation of glycated hemoglobin affects the risk of incident non-alcoholic fatty liver disease in participants with diabetes.

Table S3 | Prevalence of metabolic dysfunction-associated fatty liver disease among participants with incident hepatic steatosis index-defined non-alcoholic fatty liver disease according to baseline glucose status.