Hemoglobin Glycation Index Is Associated With Cardiovascular Diseases in People With Impaired Glucose Metabolism

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Context: There is a substantial interindividual variation in the association between glycated hemoglobin (HbA1c) and plasma glucose concentrations. Its impact on cardiovascular disease (CVD) has not been comprehensively evaluated.

Objective: We examined associations between interindividual variations in HbA1c, which was estimated as the hemoglobin glycation index (HGI), and CVD.

Design, Setting, and Participants: We performed a cross-sectional analysis with 1248 treatment-naïve subjects with prediabetes or diabetes. The HGI was defined as the measured HbA1c minus predicted HbA1c, which was calculated from the linear relationship between HbA1c and fasting plasma glucose levels.

Main Outcome Measures: The prevalence of composite and individual CVDs including coronary artery disease (CAD), stroke, and peripheral artery disease (PAD).

Results: The overall prevalence of composite CVD was 10.3% and individual prevalences of CAD, stroke, and PAD were 5.7%, 5.1%, and 1.3%, respectively. All prevalences significantly increased from the first to third tertile of HGI. In multivariate analysis, the highest HGI tertile was independently associated with composite CVD [odds ratio (95% confidence interval): 2.81 (1.59-4.98)], and individual CAD [2.30 (1.12-4.73)], stroke [3.40 (1.50-7.73)], and PAD [6.37 (1.18-34.33)] after adjustment for other CVD risk factors including HbA1c levels. Two consecutive measurements of HGI obtained on different days showed good correlation ($r = 0.651$, $P < 0.001$) and high concordance rate in the tertile classification (69.1%).

Conclusions: High HGI was independently associated with overall and individual CVDs. This result suggests that discrepancy between HbA1c and fasting glucose levels can reflect vascular health in subjects with impaired glucose metabolism. **(*J Clin Endocrinol Metab* 102: 2905–2913, 2017)**

Diabetes mellitus causes microvascular and macrovascular complications involving various organs and results in serious impairment to the quality of life. Susceptibility to developing such complications is considerably different between patients, even when they have similar degrees of hyperglycemia (1, 2). There have been numerous studies aiming to identify risk factors that can predict the development of diabetic complications (3–6).

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Abbreviations: AGE, advanced glycation end-product; CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disease; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HGI, hemoglobin glycation index; HOMA, homeostasis model assessment; OGTT, oral glucose tolerance test; OR, odds ratio; PAD, peripheral artery disease.

Stratifying patients with diabetes according to their risk of adverse outcomes is an essential part of individualized treatment (7), but known risk factors including genetic susceptibilities only partially explain individual variances in the development of such complications (8).

Glycated hemoglobin (HbA1c) is considered the gold standard method to evaluate glycemic control in patients with diabetes (9). However, in previous population-based studies, only 60% to 80% of the variance in HbA1c levels could be explained by the mean blood glucose levels (10, 11). The remaining variance in HbA1c might be influenced by interindividual differences in biological factors involving glucose metabolism and passive hemoglobin glycation (12). Several studies have reported that some individuals have higher or lower HbA1c levels compared with others with similar mean blood glucose levels, and this disparity persists over time (12–14). The hemoglobin glycation index (HGI) was introduced to quantify this variation (13). It is defined as the measured HbA1c levels minus predicted HbA1c calculated from a linear regression between blood glucose and HbA1c levels (13, 15). Patients with high or low HGI values have HbA1c levels that are higher or lower than those expected from their blood glucose levels, respectively.

Several studies have investigated the association between HGI and diabetic complications. In a *post hoc* analysis of the Diabetes Control and Complications Trial, subjects with higher HGI values calculated from the mean blood glucose level had higher incidences of diabetic retinopathy and nephropathy (15). In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, subjects with high HGI values calculated from fasting blood glucose levels had a higher incidence of cardiovascular disease (CVD) (16). The HGI was also reported to be associated with inflammatory markers in nondiabetic subjects (17), which suggests a possible association between HGI and CVD, mediated by chronic inflammation. Some studies used the glycation gap, which was calculated in the same way as the HGI, except using fructosamine to quantify the interindividual variations in HbA1c levels. They showed substantial associations between a high glycation gap and diabetic retinopathy, nephropathy, or macrovascular disease (18, 19). However, other studies failed to reproduce these associations of HGI or glycation gap with diabetic complications (20, 21). The properties of these studies were heterogeneous in terms of the diabetes status of the subjects: type 1 diabetes (15), type 2 diabetes (16, 19, 20) or nondiabetic subjects (17); the use of antidiabetic medications such as insulin (16) or oral antidiabetic medications; study design or setting such as a clinical trial (15, 16), a hospital-based cohort (18–20) or a national survey (17). This heterogeneity might have contributed to the inconsistent results in the literature.

In this study, we aimed to investigate the association between HGI and macrovascular complications of diabetes in a hospital-based cohort, with data collected prospectively. Multivariate analysis was performed to determine the impact of HGI on CVD, independent of traditional CVD risk factors.

Subjects and Methods

Study subjects

In 2005, we set up a prospective hospital-based cohort in which participants underwent a standardized 75 g oral glucose tolerance test (OGTT) for evaluation of their glucose metabolism in Seoul National University Bundang Hospital (SNUBH). Among the subjects who visited the outpatient clinic at SNUBH from June 2005 to December 2014, we exclusively enrolled drug-naïve patients with prediabetes or diabetes. We excluded those who had received antidiabetic agents within 6 months before enrollment and those with a history of malignancy or organ failure including end-stage renal disease, liver cirrhosis, chronic obstructive pulmonary disease, or symptomatic heart failure.

From a total of 2501 subjects in the SNUBH-OGTT registry, we included 1664 subjects aged ≥30 years and who were in a state of prediabetes [fasting plasma glucose (FPG) levels 100–125 mg/dL and/or 2-hour plasma glucose levels 140–199 mg/dL] or diabetes (FPG levels ≥126 mg/dL or 2-hour plasma glucose levels ≥200 mg/dL) for this study. The following types of subject were excluded: (1) patients with type 1 diabetes, which was diagnosed by the investigators' judgment based on the age of onset, C-peptide level (<0.2 nmol/L), presence of anti-glutamic acid decarboxylase antibody, and acute presentation of diabetes (n = 3); (2) chronic pancreatitis (n = 3); (3) those with medical conditions that could alter the process of hemoglobin glycation such as chronic kidney disease (serum creatinine, >1.5 mg/dL), anemia (hemoglobin levels for male subjects, <12 g/dL; female, <11 g/dL), recent transfusion within 3 months before enrollment, hemoglobinopathies, or a history of splenectomy (n = 39); and 4) those with insufficient anthropometric information, medical histories, or laboratory results (n = 371). Finally, 1248 subjects were included in analyses.

All participating subjects provided written informed consent. The study protocol was approved by the Institutional Review Board of SNUBH (institutional review board number: 15-2015-003) and conducted according to the Declaration of Helsinki (22).

Evaluation of subjects

All subjects performed a standardized 75 g OGTT with overnight fasting for 10 hours. The levels of plasma glucose, insulin, and C-peptide were measured at fasting and at 30 and 120 minutes after the OGTT. The HbA1c level was measured from the fasting blood sample. Medical histories of hypertension, dyslipidemia, and CVD and any family history of CVD were investigated. Hypertension was defined as having a history of physician-diagnosed hypertension, systolic blood pressure >140 mm Hg, or diastolic blood pressure >90 mm Hg. Dyslipidemia was defined as having a history of diagnosed

dyslipidemia by a physician or when the subject was taking lipid-lowering medications. Subjects were classified as having coronary artery disease (CAD) if they had a history of myocardial infarction, percutaneous coronary interventions, coronary artery bypass graft surgery, or physician-diagnosed angina. Subjects were classified as having stroke if they had a history of ischemic stroke diagnosed by a physician. Subjects were classified as having peripheral artery disease (PAD) if they had a history of percutaneous interventions or graft surgery of peripheral arteries, or peripheral artery occlusive disease diagnosed by a physician. The composite CVD was defined as any one of CAD, stroke, or PAD. A positive family history of CVD was defined as having any first-degree relatives with a history of CAD, stroke, or PAD. Smoking status was divided into three categories: current smokers (if the subjects had been smoking for more than 1 year), ex-smokers (if the subjects had quit), and never smokers.

Calculation of indices

To estimate the interindividual variance in HbA1c levels, the HGI was calculated using HbA1c and FPG levels (16, 17). The linear relationship between HbA1c and FPG was estimated from the linear regression analysis of the study subjects' data (HbA1c = 0.030 × FPG + 2.978, r = 0.859, and $P < 0.001$, Supplemental Fig. 1). A predicted HbA1c level was then calculated from this equation using each subject's FPG value. The HGI was defined as the difference between the measured HbA1c and the predicted HbA1c (HGI = measured HbA1c – predicted HbA1c). The mean ± standard deviation (range) of HGI was 0.05 ± 0.98 (–4.27 to +6.51). Subjects were divided into three groups according to the HGI tertile (first tertile, <–0.33; second tertile, ≥–0.33 and <0.18; and third tertile, ≥0.18).

In this study, various glucose metabolism indices were calculated from the OGTT measurements. The homeostasis model assessment (HOMA) was used to measure insulin resistance and pancreatic β-cell function (23). The formula for calculating HOMA-insulin resistance was as follows: fasting insulin (μU/mL) × [fasting glucose (mg/dL)/405]. HOMA-β cell function was calculated using the following formula: 360 × fasting insulin (μU/mL)/[fasting glucose (mg/dL) − 63]. The Matsuda index was calculated as $10{,}000/\sqrt{}$(fasting glucose × fasting insulin × mean glucose × mean insulin) (24). The insulinogenic index [(insulin at 30 minutes – insulin at 0 minutes)/(glucose at 30 minutes – glucose at 0 minutes)] and disposition index (Matsuda index × insulinogenic index) were also calculated (25).

Statistical analysis

Data are expressed as the means ± standard deviation or as the median ± interquartile range for continuous variables, or as percentages for categorical variables. Baseline characteristics were compared between the tertiles of HGI using analysis of variance, Kruskal-Wallis tests, or χ^2 tests as indicated. The association between HGI and prevalent composite CVD was analyzed using logistic regression models. We checked for any multicollinearity between HbA1c levels and HGI in the multivariate models with a cutoff of the variance inflation factor <5. Subgroup analysis was performed according to the HbA1c divided into <6.5% and ≥6.5%, age groups (<60 years vs ≥60 years), sex, smoking status, and glucose metabolism status (prediabetes vs diabetes).

Using the HbA1c and FPG data measured from different fasting blood samples within 6 months before or after the OGTT, an alternative HGI was calculated using the same equation as the original HGI. The original HGI values obtained from the OGTT set and the alternative HGI values were plotted to investigate any differences. The rate of agreement between the tertile classification of the original and alternative HGI values was also calculated. All the HGI values were calculated using FPG and HbA1c levels. Data are expressed as the odds ratio (OR) and 95% confidence interval (CI). Analyses were performed using SPSS Statistics for Windows (version 18.0; IBM SPSS Statistics, IBM Corp., Armonk, NY) and R (version 3.1.0; R Foundation for Statistical Computing, Vienna, Austria). A P value < 0.05 was considered significant.

Results

The characteristics of the study participants including the OGTT results according to the tertiles of the HGI are summarized in Table 1. Most of the subjects with diabetes had been diagnosed less than 1 year before enrollment (Supplemental Table 1). The HbA1c levels and the increment of glucose (postload 2-hour glucose: FPG) showed an increasing trend according to the HGI tertile. The FPG level was lowest in the second HGI tertile and similar between the first and third HGI tertiles. Insulin sensitivity estimated by the Matsuda index decreased from the first to the third HGI tertile. Pancreatic β-cell function estimated by insulinogenic index and disposition index also decreased in the higher HGI tertile.

The prevalence of composite CVD, and individual CAD, stroke, and PAD were 10.3%, 5.7%, 5.1%, and 1.3%, respectively, and increased from the first to the third HGI tertiles (Fig. 1). To investigate any independent association between HGI and diabetic macrovascular complications, we performed multivariate analysis with typical CVD risk factors including the HbA1c level. Compared with the first HGI tertile, the second and third HGI tertiles were independently associated with composite CVD in the multivariate analysis adjusted for age, sex, body mass index, smoking, hypertension, dyslipidemia, family history of CVD, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and high-sensitivity C-reactive protein level with ORs of 1.77 (95% CI, 1.02 to 3.09) and 3.13 (1.83 to 5.34), respectively (Table 2). After further adjustment for HbA1c level, these associations were still significant, with ORs of 1.76 (1.01 to 3.07) and 2.82 (1.59 to 4.98), respectively. Among each component of composite CVD, CAD and PAD were significantly associated with the highest HGI tertile in the multivariate analysis. Stroke was significantly associated with both second and third HGI tertiles in the multivariate analysis. These associations remained

Table 1. Baseline Characteristics of Study Subjects According to Tertiles of the HGI

		HGI Category				
	Total (n = 1248)	First Tertile (n = 413)	Second Tertile (n = 421)	Third Tertile (n = 414)	*P* Value[a]	*P* for Trend[b]
HGI, mean ± standard deviation	0.05 ± 0.98	−0.80 ± 0.52	−0.07 ± 0.14	0.96 ± 0.92	<0.001	<0.001
HGI, ranges	−4.27 to 6.51	−4.27 to –0.33	−0.32 to 0.17	0.18-4.80		
Age (y)	55.3 ± 11.3	55.0 ± 10.6	56.3 ± 11.0	55.6 ± 11.8	0.010	0.346
Male (%)	743 (59.5)	279 (67.6)	238 (56.5)	226 (54.6)	<0.001	<0.001
Height (cm)	164.2 ± 8.8	165.4 ± 8.7	163.6 ± 9.0	163.3 ± 8.7	<0.001	<0.001
Weight (kg)	67.9 ± 11.7	68.7 ± 11.6	67.8 ± 12.0	67.5 ± 12.2	0.122	0.144
Body mass index (kg/m^2)	25.1 ± 3.3	25.0 ± 3.0	25.2 ± 3.5	25.2 ± 3.5	0.602	0.317
SBP (mm Hg)	126.9 ± 15.8	127.5 ± 15.2	127.4 ± 17.1	125.7 ± 15.3	0.142	0.072
DBP (mm Hg)	78.2 ± 10.6	79.1 ± 10.3	78.3 ± 10.7	77.0 ± 10.8	0.013	0.004
Hypertension (%)	544 (43.6)	172 (41.6)	196 (46.6)	176 (42.5)	0.311	0.803
Dyslipidemia (%)	537 (43.0)	159 (38.5)	175 (41.6)	203 (49.0)	0.007	0.002
FHx of CVD (%)	217 (17.4)	59 (14.3)	84 (20.0)	74 (17.9)	0.092	0.174
Duration of diabetes (y)[c]	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.3)	0.025	0.006
Smoking status						
Ex-smoker (%)	223 (17.9)	78 (18.9)	91 (21.6)	54 (13.0)	0.006	0.014
Current smoker (%)	266 (21.3)	98 (23.7)	78 (18.5)	90 (21.7)	0.224	0.21
Prediabetes	491 (39.3)	188 (45.5)	211 (50.1)	92 (22.2)	<0.001	<0.001
DM	757 (60.7)	225 (54.5)	210 (49.9)	322 (77.8)	<0.001	<0.001
HbA1c (%)	7.0 ± 1.7	6.4 ± 1.4	6.5 ± 1.0	8.0 ± 1.9	<0.001	<0.001
Fasting glucose (mmol/L)	7.3 ± 2.5	7.8 ± 3.0	6.7 ± 1.8	7.5 ± 2.6	<0.001	0.629
Postload 2-h glucose (mmol/L)	12.7 ± 5.7	11.9 ± 6.0	11.1 ± 4.4	15.3 ± 5.6	<0.001	<0.001
Glucose increment (mmol/L)	5.4 ± 4.2	4.0 ± 4.0	4.4 ± 3.4	7.8 ± 4.2	<0.001	<0.001
Fasting insulin (pmol/L)	84.7 ± 67.4	84.0 ± 70.1	86.1 ± 62.5	86.8 ± 84.0	0.885	0.628
Fasting C-peptide (nmol/L)	0.66 ± 0.33	0.66 ± 0.33	0.66 ± 0.33	0.70 ± 0.33	0.889	0.939
Creatinine (μmol/L)	79.6 ± 17.7	88.4 ± 17.7	79.6 ± 17.7	79.6 ± 17.7	0.240	0.092
Total cholesterol (mmol/L)	5.1 ± 1.0	5.1 ± 1.0	5.0 ± 0.9	5.1 ± 1.1	0.508	0.844
Triglyceride (mmol/L)	1.8 ± 1.1	1.8 ± 1.3	1.7 ± 0.9	1.7 ± 1.0	0.250	0.455
HDL-cholesterol (mmol/L)	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	0.457	0.278
LDL-cholesterol (mmol/L)	2.8 ± 0.8	2.8 ± 0.8	2.8 ± 0.8	2.9 ± 0.9	0.248	0.344
Hemoglobin (g/L)	147 ± 41	147 ± 13	145 ± 13	146 ± 15	0.110	0.097
hsCRP (nmol/L)[d]	0.19 (0.10–1.62)	0.10 (0.10–1.43)	0.10 (0.10–1.62)	0.95 (0.10–1.91)	<0.001	<0.001
HOMA-IR[d]	3.2 (2.3–4.5)	3.1 (2.2–4.4)	3.1 (2.2–4.3)	3.4 (2.4–4.8)	0.013	0.566
HOMA-β[d]	66.6 (44.1–97.9)	61.5 (41.0–85.7)	73.3 (52.5–106.7)	64.5 (40.9–100.3)	<0.001	0.111
Matsuda index[d]	3.1 (2.2–4.5)	3.6 (2.3–5.1)	3.2 (2.2–4.6)	2.9 (2.1–3.8)	<0.001	<0.001
Insulinogenic index[d]	0.19 (0.09–0.36)	0.23 (0.13–0.42)	0.21 (0.12–0.37)	0.12 (0.06–0.27)	<0.001	<0.001
Disposition index[d]	0.57 (0.27–1.16)	0.79 (0.41–1.73)	0.63 (0.31–1.23)	0.36 (0.18–0.74)	<0.001	<0.001

Abbreviations: β, β cell function; DM, diabetes mellitus; DBP, diastolic blood pressure; FHx, family history; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IR, insulin resistance; LDL, low-density lipoprotein; SBP, systolic blood pressure.

[a] *P* value for comparison between groups of three tertiles using analysis of variance, Kruskal-Wallis test, or χ^2 tests.

[b] *P* value for linear trend from the first to the third HGI tertiles.

[c] Duration of diabetes was surveyed only in patients with type 2 DM. Because of its skewed distribution, the data were expressed as median (interquartile range) and compared using Kruskal-Wallis test.

[d] Data are showed as median (interquartile range). Log-transformed values were used for statistical comparisons.

significant after further adjustment for HbA1c level (Table 2, Supplemental Table 2).

To investigate potential interactions affecting the association between HGI and composite CVD, we performed subgroup analyses according to age group, sex, smoking status, glucose metabolism status, and HbA1c levels (Fig. 2). There were no noteworthy interactions between these covariates and HGI. The highest HGI tertile had similar trend of increased risk of composite CVD in all subgroups. The association was stronger in age ≥60 years, female (Supplemental Table 3), nonsmoker, diabetes (Supplemental Table 4), and HbA1c ≥6.5% than the counterparts of the subgroups.

To confirm these findings, we calculated an alternative HGI from FPG and HbA1c values obtained on a different day and compared this with the original data. A total of 1198 (96.0%) of the subjects had repeated measures of FPG and HbA1c data. Among them, 69.1% had concordance between the tertile classifications of the two HGI estimates (Supplemental Table 5) and the correlation coefficient (r) between the two measured HGI values was 0.651 ($P < 0.001$). We classed those subjects who

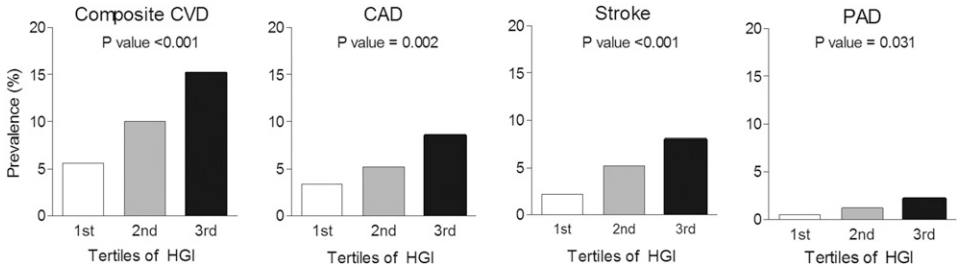

Figure 1. Prevalence of composite CVD and individual CAD, stroke, and PAD according to HGI tertiles. *P* values are shown for linear trends.

were consistently classified as having the highest HGI tertile in the two consecutive measurements as a consistently high HGI group and compared the prevalence of macrovascular complications in this group with the remaining subjects. A consistently high HGI was significantly associated with composite CVD (OR, 2.80; 95% CI, 1.75 to 4.48), CAD (OR, 2.84; 95% CI, 1.57 to 5.15), stroke (OR, 2.12; 95% CI, 1.14 to 3.93), and PAD (OR, 4.97; 95% CI, 1.44 to 17.19) after adjustment for the same CVD risk factors and HbA1c levels (Table 3).

Discussion

In this cross-sectional study of subjects with impaired glucose metabolism, the HGI reflecting interindividual variation in hemoglobin glycation was significantly associated with prevalent macrovascular diseases after adjusting for typical CVD risk factors including HbA1c levels.

Several previous studies have investigated possible correlations between CVD and interindividual variation in HbA1c levels. In those studies that used the glycation gap, the higher glycation gap was associated with prevalent macrovascular diseases defined as any previous cardiac, cerebral, or peripheral vascular event (18), but not with silent myocardial ischemia evaluated by stress electrocardiography and myocardial scintigraphy (20). In a recent study analyzing the results of the ACCORD trial, it was suggested that HGI could be a guide for individualizing treatment goals for achieving better CVD outcomes (16). Overall, the ACCORD trial failed to show the benefit of intensive glucose-lowering treatment on CVD outcome (16). However, when the subjects were divided into low, moderate, and high HGI groups, intensive treatment lowered the incidence of CVD events by 25% and 23% without any increase in total mortality in the low and moderate HGI groups, respectively. In contrast, the intensive treatment did not lower the incidence of CVD events but rather increased the total mortality by 41% in the high HGI group (16). The incidence of CVD also increased from the low to the high HGI groups (8.7%, 9.5%, and 12.4% in the low, moderate, and high HGI groups, respectively; statistical significance was not reported) (16).

These data suggest that the discrepancy between HbA1c and glucose levels is a possible predictor of poor vascular health; however, there are several confounding issues to be considered. First, the HbA1c and FPG levels were measured in patients who were taking different types of antidiabetic treatments (15, 16, 18–20, 26). Different classes of medications can differentially affect

Table 2. Associations Between HGI and Macrovascular Complication

	First HGI Tertile	Second HGI Tertile		Third HGI Tertile	
	OR (95% CI)	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Composite CVD					
Multivariate[a]	1.00 (reference)	1.77 (1.02–3.09)	0.044	3.13 (1.83–5.34)	<0.001
Multivariate + HbA1c	1.00 (reference)	1.76 (1.01–3.07)	0.047	2.82 (1.59–4.98)	<0.001
CAD					
Multivariate[a]	1.00 (reference)	1.46 (0.72–2.95)	0.292	2.47 (1.27–4.81)	0.008
Multivariate + HbA1c	1.00 (reference)	1.45 (0.72–2.94)	0.300	2.30 (1.12–4.73)	0.023
Stroke					
Multivariate[a]	1.00 (reference)	2.32 (1.03–5.23)	0.043	3.78 (1.73–8.27)	<0.001
Multivariate + HbA1c	1.00 (reference)	2.31 (1.02–5.21)	0.044	3.40 (1.50–7.73)	0.003
PAD					
Multivariate[a]	1.00 (reference)	2.22 (0.42–11.84)	0.351	4.46 (0.92–21.67)	0.063
Multivariate + HbA1c	1.00 (reference)	2.31 (0.43–12.42)	0.328	6.37 (1.18–34.33)	0.031

[a] Adjusted for age, sex, body mass index, smoking, hypertension, dyslipidemia, family history of CVD, high-density lipoprotein cholesterol, LDL-cholesterol, and hsCRP level.

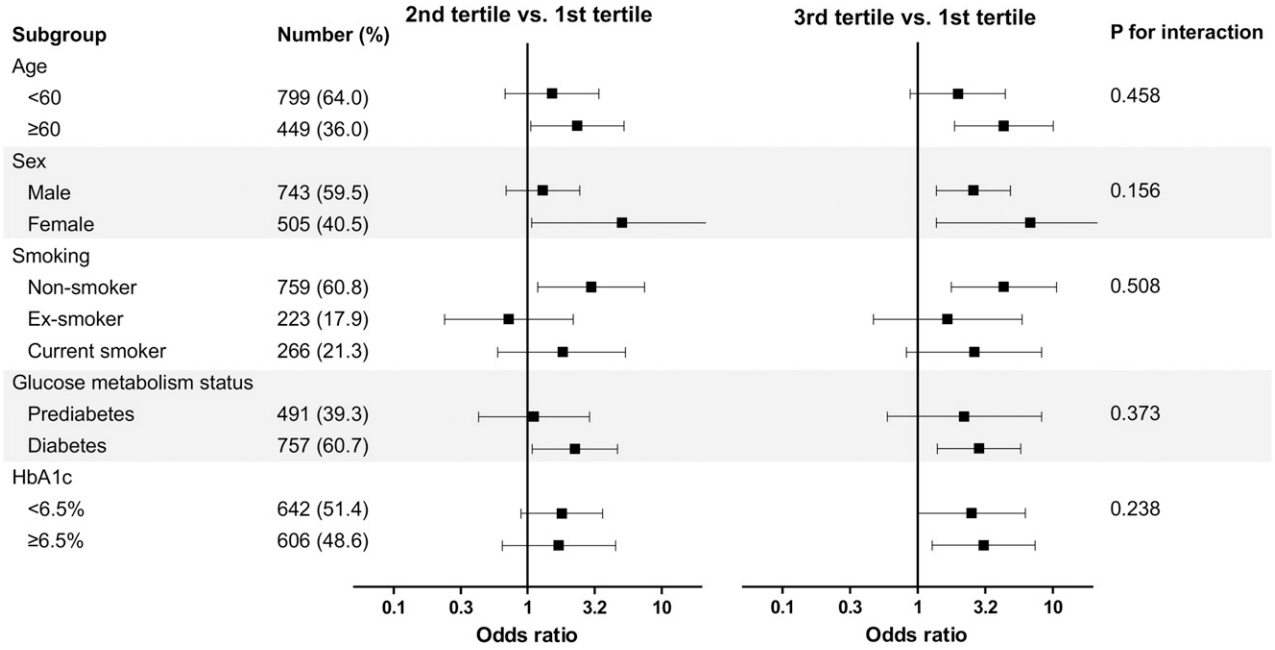

Figure 2. Subgroup analyses of association between HGI and composite CVD stratified by age, sex, smoking, glucose metabolism status, and HbA1c levels. The ORs for composite CVD in different subgroups are presented in logarithmic scale.

fasting or postprandial glucose levels. For example, basal long-acting insulin treatment lowers fasting blood glucose levels more than HbA1c levels (27). Accordingly, patients with basal insulin treatment might have a higher HGI regardless of their intrinsic glycation phenotype. In contrast, dipeptidyl peptidase 4 inhibitors reduce postprandial glucose levels greater than fasting glucose levels because of their glucose-dependent action (28, 29). Second, the HGI in previous studies was calculated with single measurements of HbA1c and blood glucose levels (16, 17, 26). Considerable day-to-day variations in blood glucose levels might limit the reliability of the HGI estimate. Third, the HbA1c level was not adjusted in the regression models in several studies (15, 16, 18, 20, 26), and correlation between HbA1c and HGI values might confound the results of previous studies (21). To overcome those problems, we enrolled only drug-naïve individuals, validated the HGI with repeated blood samples, and performed multivariate analyses including the HbA1c level as a covariate.

In our study, a higher HGI was significantly associated with macrovascular complications even after adjusting for traditional CVD risk factors and HbA1c levels. This suggests that the HGI might have an additional impact on macrovascular complications beyond the HbA1c level. Several mechanisms might mediate this association between high HGI and macrovascular complications. Individuals with a high HGI have higher HbA1c levels at the same blood glucose level than those with a low HGI; thus, the HGI might reflect the propensity of nonenzymatic glycation processes. Advanced glycation end-products (AGEs) are well known to contribute to the development of atherosclerosis by several mechanisms (30). AGEs enhance low-density lipoprotein modification and induce oxidative stress and Toll-like receptor 4–mediated proinflammatory signaling (31). AGEs also cause endothelial dysfunction and stimulate the production of reactive oxygen species (32). Serum concentration of AGE was an independent determinant factor of circulating CRP levels in patients with diabetes (33). In addition, AGEs induce insulin resistance and β-cell dysfunction, leading to the deterioration of glucose metabolism (34, 35). A study on young patients

Table 3. Associations Between Consistently High HGI and CVDs

	Consistently High HGI	
	OR (95% CI)	***P* Value**
Composite CVD		
Multivariate[a]	3.58 (2.38–5.39)	<0.001
Multivariate + HbA1c	3.52 (2.24–5.51)	<0.001
CAD		
Multivariate[a]	3.37 (2.01–5.63)	<0.001
Multivariate + HbA1c	3.53 (1.99–6.23)	<0.001
Stroke		
Multivariate[a]	2.94 (1.72–5.01)	<0.001
Multivariate + HbA1c	2.80 (1.57–5.01)	<0.001
PAD		
Multivariate[a]	3.05 (1.10–8.42)	0.032
Multivariate + HbA1c	4.13 (1.36–12.51)	0.012

[a] Adjusted for age, sex, body mass index, smoking, hypertension, dyslipidemia, any family history of CVD, high-density lipoprotein cholesterol, LDL-cholesterol, and hsCRP levels.

with type 1 diabetes reported that the levels of AGEs measured by skin intrinsic fluorescence were significantly associated with the HGI (36). Taken together, the high CRP levels in the high HGI group in our study might reflect high AGEs in this group, although we did not measure these factors. Both insulin sensitivity and pancreatic β-cell function were also deteriorated in the subgroups with high HGI in our study.

Another issue is that the HGI might be affected by postprandial glycemic excursion (27). A high HGI might reflect higher daytime or postprandial glucose levels. Although the FPG level did not increase from the first to the third HGI tertiles in our study, the glucose increment, which was calculated as postload 2-hour glucose minus the FPG level, increased significantly (Table 1). Because a high postprandial glucose concentration is known to be a predictor of cardiovascular events (37, 38), it is conceivable that the relatively high postprandial glucose levels in our high HGI groups mediated the increased risk of macrovascular complications. We have summarized the possible factors determining the level of HGI in Supplemental Table 6.

In subgroup analyses, the association between high HGI and macrovascular complications had generally similar trends in all subgroups. However, there were several distinct features. Although the *P* value for interaction was not significant, the OR of the highest HGI tertile for composite CVD was numerically higher in women than men. Because women have a lower risk of CVD than men, the greater impact of HGI in women suggests that HGI might be more useful to stratify the risk of CVD in this subgroup. In contrast, the association between HGI and macrovascular disease was weaker and more attenuated by HbA1c levels in the prediabetes group than in the diabetes mellitus group. In prediabetes states, HbA1c level itself might have more impact on CVD risk than it does in diabetes states. According to smoking status, the impact of HGI was stronger in nonsmokers than in ex- or current smokers. Although we do not have additional data to explain this differential impact of HGI, one possible explanation is that because endothelial function is already impaired in smokers, the impact of high HGI on the progression of CVD may be less prominent in current or ex-smokers than in nonsmokers.

In this study, there was a significant correlation between HbA1c and HGI levels ($r = 0.575$, $P < 0.001$), which might raise a concern about multicollinearity. However, the variance inflation factor of the HbA1c level for estimating the HGI was 1.495, which was below the predefined cutoff. In addition, we also provide results with and without HbA1c adjustment to compare the data. The independent association of HGI with CVD changed slightly after HbA1c adjustment. These results suggest that there was no multicollinearity issue in our multivariate analyses.

There were several limitations in our study. First, in this study, the information on the presence of CVD was checked from each subject's medical record and questionnaire, instead of investigating CVD directly. This could have led to an underestimation of the prevalence of CVD because of missing undiagnosed cases. Second, because of its cross-sectional nature, we could not address any causal link between the HGI and diabetic complications. Longitudinal studies with sufficient follow-up periods are necessary. Third, the HGI could not be evaluated in patients with a long duration of diabetes because only drug-naïve subjects were enrolled. From a different perspective, because we only included subjects who were not taking any antidiabetic medications, future studies investigating whether a specific antidiabetic medication can affect HGI and eventually reduce CVD events would be interesting. Last, the calculation of HGI used in our study cannot be generalized to other populations. New regression models should be derived for each population.

There were several strengths to our study. The levels of HbA1c might be affected by determinants other than blood glucose levels. To avoid this confounding effect, we excluded individuals with medical conditions that could alter the process of hemoglobin glycation, such as chronic kidney disease, anemia, hemoglobinopathies, and a history of splenectomy or recent transfusion within 3 months from enrollment. We also adjusted for most CVD risk factors in the final regression models. Using two measurements of HGI at different time points, we found that a consistently high HGI was strongly associated with CVD. Although single measurements of HGI have limited accuracy, this can be improved with repeated measurements. In addition, this has been one of the largest studies to investigate the association between HGI and diabetic complications.

In conclusion, we have demonstrated a substantial association between high HGI and macrovascular complications. This was independent of HbA1c levels as well as conventional cardiovascular risk factors. HGI is a simple derivative of FPG and HbA1c, and this simplicity is the strength of HGI as a clinical index; however, we cannot determine whether HGI is a dominant factor contributing to the development of CVD in people with impaired glucose metabolism. The clinical implications of HGI should be investigated prospectively.

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Author contributions: C.H.A. designed the study, analyzed the data, and drafted the manuscript. S.H.M. collected, organized, and analyzed the data. D.H.L. and T.J.O. collected and analyzed the data. K.M.K. recruited the subjects in the Seoul National University Bundang Hospital Oral Glucose Tolerance Test (SNUBH-OGTT) registry and collected the data. J.H.M. recruited the subjects in the SNUBH-OGTT registry and collected the data. S.H.C., K.S.P., and H.S.J. designed and initiated the SNUBH-OGTT registry and collected the data. J.H. and A.S. reviewed the data and contributed to the critical revision of the manuscript. S.L. designed and initiated the SNUBH-OGTT registry, and conceptualized the study, assisted data analysis and revised the manuscript. All authors approved the final manuscript as submitted. S.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C; Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;**329**(14):977–986.
2. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*. 1998;**352**(9131):837–853.
3. Donnan PT, Donnelly L, New JP, Morris AD. Derivation and validation of a prediction score for major coronary heart disease events in a U.K. type 2 diabetic population. *Diabetes Care*. 2006;**29**(6):1231–1236.
4. Knuiman MW, Welborn TA, McCann VJ, Stanton KG, Constable IJ. Prevalence of diabetic complications in relation to risk factors. *Diabetes*. 1986;**35**(12):1332–1339.
5. Stevens RJ, Kothari V, Adler AI, Stratton IM; United Kingdom Prospective Diabetes Study (UKPDS) Group. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). *Clin Sci (Lond)*. 2001;**101**(6):671–679.
6. Tanaka S, Tanaka S, Iimuro S, Yamashita H, Katayama S, Akanuma Y, Yamada N, Araki A, Ito H, Sone H, Ohashi Y; Japan Diabetes Complications Study Group; Japanese Elderly Diabetes Intervention Trial Group. Predicting macro- and microvascular complications in type 2 diabetes: the Japan Diabetes Complications Study/the Japanese Elderly Diabetes Intervention Trial risk engine. *Diabetes Care*. 2013;**36**(5):1193–1199.
7. Raz I, Riddle MC, Rosenstock J, Buse JB, Inzucchi SE, Home PD, Del Prato S, Ferrannini E, Chan JC, Leiter LA, Leroith D, Defronzo R, Cefalu WT. Personalized management of hyperglycemia in type 2 diabetes: reflections from a Diabetes Care Editors' Expert Forum. *Diabetes Care*. 2013;**36**(6):1779–1788.
8. Ahlqvist E, van Zuydam NR, Groop LC, McCarthy MI. The genetics of diabetic complications. *Nat Rev Nephrol*. 2015;**11**(5):277–287.
9. American Diabetes Association. (6) Glycemic targets. *Diabetes Care*. 2015;**38**(Suppl):S33–S40.
10. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ; A1c-Derived Average Glucose Study Group. Translating the A1C assay into estimated average glucose values [published correction appears in *Diabetes Care*. 2009;32(1):207]. *Diabetes Care*. 2008;**31**(8):1473–1478.
11. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE. Defining the relationship between plasma glucose and HbA(1c): analysis of glucose profiles and HbA(1c) in the Diabetes Control and Complications Trial. *Diabetes Care*. 2002;**25**(2):275–278.
12. Soros AA, Chalew SA, McCarter RJ, Shepard R, Hempe JM. Hemoglobin glycation index: a robust measure of hemoglobin A1c bias in pediatric type 1 diabetes patients. *Pediatr Diabetes*. 2010;**11**(7):455–461.
13. Hempe JM, Gomez R, McCarter RJ Jr, Chalew SA. High and low hemoglobin glycation phenotypes in type 1 diabetes: a challenge for interpretation of glycemic control. *J Diabetes Complications*. 2002;**16**(5):313–320.
14. Wilson DM, Xing D, Cheng J, Beck RW, Hirsch I, Kollman C, Laffel L, Lawrence JM, Mauras N, Ruedy KJ, Tsalikian E, Wolpert H; Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. Persistence of individual variations in glycated hemoglobin: analysis of data from the Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Randomized Trial. *Diabetes Care*. 2011;**34**(6):1315–1317.
15. McCarter RJ, Hempe JM, Gomez R, Chalew SA. Biological variation in HbA1c predicts risk of retinopathy and nephropathy in type 1 diabetes. *Diabetes Care*. 2004;**27**(6):1259–1264.
16. Hempe JM, Liu S, Myers L, McCarter RJ, Buse JB, Fonseca V. The hemoglobin glycation index identifies subpopulations with harms or benefits from intensive treatment in the ACCORD trial. *Diabetes Care*. 2015;**38**(6):1067–1074.
17. Liu S, Hempe JM, McCarter RJ, Li S, Fonseca VA. Association between Inflammation and biological variation in hemoglobin A1c in U.S. nondiabetic adults. *J Clin Endocrinol Metab*. 2015;**100**(6):2364–2371.
18. Nayak AU, Nevill AM, Bassett P, Singh BM. Association of glycation gap with mortality and vascular complications in diabetes. *Diabetes Care*. 2013;**36**(10):3247–3253.
19. Rodríguez-Segade S, Rodríguez J, Cabezas-Agricola JM, Casanueva FF, Camiña F. Progression of nephropathy in type 2 diabetes: the glycation gap is a significant predictor after adjustment for glycohemoglobin (Hb A1c). *Clin Chem*. 2011;**57**(2):264–271.
20. Cosson E, Banu I, Cussac-Pillegand C, Chen Q, Chiheb S, Jaber Y, Nguyen MT, Charnaux N, Valensi P. Glycation gap is associated with macroproteinuria but not with other complications in patients with type 2 diabetes. *Diabetes Care*. 2013;**36**(7):2070–2076.
21. Lachin JM, Genuth S, Nathan DM, Rutledge BN. The hemoglobin glycation index is not an independent predictor of the risk of microvascular complications in the Diabetes Control and Complications Trial. *Diabetes*. 2007;**56**(7):1913–1921.
22. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;**310**(20):2191–2194.
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;**28**(7):412–419.
24. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999;**22**(9):1462–1470.
25. Retnakaran R, Qi Y, Goran MI, Hamilton JK. Evaluation of proposed oral disposition index measures in relation to the actual disposition index. *Diabet Med*. 2009;**26**(12):1198–1203.
26. Cohen RM, Holmes YR, Chenier TC, Joiner CH. Discordance between HbA1c and fructosamine: evidence for a glycosylation gap

and its relation to diabetic nephropathy. *Diabetes Care*. 2003; **26**(1):163–167.
27. Riddle MC, Gerstein HC. Comment on Hempe et al. The hemoglobin glycation index identifies subpopulations with harms or benefits from intensive treatment in the ACCORD trial. Diabetes Care 2015;38: 1067-1074. *Diabetes Care*. 2015;**38**(10):e170–e171.
28. Aschner P, Kipnes MS, Lunceford JK, Sanchez M, Mickel C, Williams-Herman DE; Sitagliptin Study 021 Group. Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. *Diabetes Care*. 2006;**29**(12):2632–2637.
29. Gerich J. Pathogenesis and management of postprandial hyperglycemia: role of incretin-based therapies. *Int J Gen Med*. 2013;**6**:877–895.
30. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation*. 2006;**114**(6):597–605.
31. Hodgkinson CP, Laxton RC, Patel K, Ye S. Advanced glycation end-product of low density lipoprotein activates the toll-like 4 receptor pathway implications for diabetic atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2008;**28**(12):2275–2281.
32. Linden E, Cai W, He JC, Xue C, Li Z, Winston J, Vlassara H, Uribarri J. Endothelial dysfunction in patients with chronic kidney disease results from advanced glycation end products (AGE)-mediated inhibition of endothelial nitric oxide synthase through RAGE activation. *Clin J Am Soc Nephrol*. 2008;**3**(3):691–698.
33. Tan KC, Chow WS, Tam S, Bucala R, Betteridge J. Association between acute-phase reactants and advanced glycation end products in type 2 diabetes. *Diabetes Care*. 2004;**27**(1): 223–228.
34. Guan SS, Sheu ML, Yang RS, Chan DC, Wu CT, Yang TH, Chiang CK, Liu SH. The pathological role of advanced glycation end products-downregulated heat shock protein 60 in islet β-cell hypertrophy and dysfunction. *Oncotarget*. 2016;**7**(17):23072–23087.
35. Hunter SJ, Boyd AC, O'Harte FP, McKillop AM, Wiggam MI, Mooney MH, McCluskey JT, Lindsay JR, Ennis CN, Gamble R, Sheridan B, Barnett CR, McNulty H, Bell PM, Flatt PR. Demonstration of glycated insulin in human diabetic plasma and decreased biological activity assessed by euglycemic-hyperinsulinemic clamp technique in humans. *Diabetes*. 2003;**52**(2):492–498.
36. Felipe DL, Hempe JM, Liu S, Matter N, Maynard J, Linares C, Chalew SA. Skin intrinsic fluorescence is associated with hemoglobin A(1c)and hemoglobin glycation index but not mean blood glucose in children with type 1 diabetes [published correction appears in *Diabetes Care*. 2013;36(4):1056]. *Diabetes Care*. 2011;**34**(8):1816–1820.
37. Cavalot F, Petrelli A, Traversa M, Bonomo K, Fiora E, Conti M, Anfossi G, Costa G, Trovati M. Postprandial blood glucose is a stronger predictor of cardiovascular events than fasting blood glucose in type 2 diabetes mellitus, particularly in women: lessons from the San Luigi Gonzaga Diabetes Study. *J Clin Endocrinol Metab*. 2006;**91**(3):813–819.
38. Meigs JB, Nathan DM, D'Agostino RB Sr, Wilson PW; Framingham Offspring Study. Fasting and postchallenge glycemia and cardiovascular disease risk: the Framingham Offspring Study. *Diabetes Care*. 2002;**25**(10):1845–1850.