The Association Between Glycemia and Endothelial Function in Nondiabetic Individuals: The Importance of Body Weight

Paraskevi T. Voidonikola[1], Kimon S. Stamatelopoulos[1], Maria Alevizaki[1], George E. Kollias[1], Nikolaos A. Zakopoulos[1], John P. Lekakis[2], Eleni Anastasiou[1], Michael J. Theodorakis[1], Anastassios G. Pittas[3] and Christos M. Papamichael[1]

The aim of this study was to examine the association between glycemia and markers of early atherosclerosis in healthy nondiabetic individuals. In 309 individuals without diabetes or symptomatic cardiovascular disease, we assessed long-term glycemia by glycosylated hemoglobin (HbA1c) and endothelial function by flow-mediated dilatation (FMD) in the brachial artery. HbA1c was negatively associated with FMD ($r = –0.162$, $P = 0.004$). Multivariate linear regression analysis after adjusting for common risk factors of cardiovascular disease showed that BMI was an effect modifier of the association between HbA1c and FMD ($P = 0.034$ for the HbA1c × BMI interaction). We stratified the FMD outcome data into two groups separated by the median BMI (group 1: BMI ≤ 26.1 kg/m^2 and group 2: BMI > 26.1 kg/m^2). In the lower BMI group, HbA1c was an independent predictor of FMD even when adjusted for confounding factors associated with impaired glucose metabolism ($r = –0.215$, $P = 0.009$), but in the higher BMI group HbA1c was not associated with FMD ($r = –0.051$, $P = 0.5$). In a nondiabetic population, long-term glycemia was associated with endothelial dysfunction only in lean individuals. In the overweight individuals, this association was not apparent, possibly because some of the mechanisms that mediate the effect of glycemia on vascular function are shared by obesity.

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INTRODUCTION

Glycemia may be associated with macrovascular disease and adverse cardiovascular events even in individuals without overt diabetes (1–6). However, the relevant mechanisms in individuals without diabetes have not yet been established. Whereas in most studies high glucose levels in individuals without diabetes positively correlated with endothelial dysfunction and other markers of early atherosclerosis (7–14); however, in others no such associations were observed (15,16). Moreover, hyperglycemia, even below the threshold required for the diagnosis of diabetes, is frequently accompanied by obesity, insulin resistance, and hyperlipidemia, all of which are also risk factors for endothelial dysfunction (17–19) and adverse cardiovascular outcomes (20,21). Therefore, the association between glycemia and cardiovascular outcomes may be confounded by such factors. Furthermore, in most studies where the association between glycemia and vascular disease in persons without diabetes was examined, the level of glycemia was estimated based on a single glucose measurement (7,8,11,15,16). A more reliable measure to use for studying the association between glycemia and early cardiovascular damage is glycosylated hemoglobin (HbA1c), which reflects recent mean glucose concentration (22). HbA1c was an independent predictor of endothelial function in a mixed population comprising both diabetic and nondiabetic patients (23). However, in the Hoorn study, diabetes was associated with impaired endothelial function, but impaired glucose metabolism was not (14). Thus, current data are inconclusive about the association between glycemia and endothelial function in nondiabetic individuals.

The aims of this study were to determine the association between glycemia, as assessed by HbA1c concentration, and endothelial function in individuals without diabetes and to identify metabolic characteristics of participants, which may modify the impact of glycemia on endothelial function. Identifying such subgroups, would contribute to better risk stratification of nondiabetic populations and, therefore, in the improvement of strategies for primary prevention of cardiovascular disease.

[1]Vascular Laboratory and Endocrine Unit, Department of Clinical Therapeutics, Alexandra Hospital, Medical School at the National University, Athens, Greece; [2]2nd Department of Cardiology, Attikon Hospital, Medical School, National University of Athens, Athens, Greece; [3]Division of Endocrinology, Diabetes and Metabolism, Tufts Medical Center, Boston, Massachusetts, USA. Correspondence: Kimon Stamatelopoulos (stamatelopoulosk@yahoo.gr)

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METHODS AND PROCEDURES

Participants

Participants were recruited among the outpatient population, staff, and visitors of an academic hospital, who responded to an announcement that detailed vascular health screening was to be performed. Exclusion criteria included age <25 and >60 years, diabetes mellitus (defined as taking hypoglycemic medication or as fasting glucose ≥7 mmol/l and/or 2-h postload glucose ≥11.1 mmol/l during screening), symptomatic cardiovascular disease, onsite diagnosis of obstructive carotid, peripheral arterial disease, and other concomitant systemic diseases such as autoimmune disease, cancer, or active infection. The cohort was recruited over a period of 12 months. After excluding eight participants due to diagnosis of diabetes mellitus and five due to diagnosis of obstructive arterial disease, a total of 309 participants were included in the cohort. The study was approved by the institutional ethical committee, and all participants gave informed consent.

Experimental protocol

Participants came to the Vascular Laboratory once in the morning after a 12-h fast and abstinence from smoking (for smokers, $n = 130$). Participants were advised not to take their prescription medications in the morning of the examination. Women of reproductive age were examined during the early follicular phase of the menstrual cycle. Anthropometric indices (weight (kg) and height (m)) were measured and BMI (weight (kg)/height^2 (m^2)) was computed. Waist circumference (cm) was defined as the maximal abdominal circumference between the xiphoid process and the iliac crest. Vascular studies were performed after 10 min of rest in the supine position in a quiet room with controlled temperature at 20–25 °C. Subsequently, a fasting blood draw and 75-g oral glucose-tolerance test with glucose measurement after 2 h were performed.

Blood assays

Plasma glucose was measured using the hexokinase method. HbA1c was measured by HPLC method (HiAuto A1C Analyser HA-8140 Menarini). Insulin was measured by ELISA (Boehringer Mannheim). Total cholesterol, triglycerides, and high-density lipoprotein-cholesterol were measured using standard analyzers. low-density lipoprotein-cholesterol was calculated by the Friedwald equation (24). Insulin resistance was estimated by homeostasis model assessment basal insulin resistance (HOMA-IR = (fasting glucose (mmol/l) × insulin (mU/l)/22) (ref. 25).

Vascular function assessment

Brachial artery reactivity was measured both by endothelium-dependent and endothelium-independent vasodilatation as previously described (26,27). Flow-mediated dilatation (FMD) is induced in response to reactive hyperemia and is expressed as the percentage change of internal diameter of the brachial artery from baseline. FMD has been previously shown to be nitric oxide dependent in humans, and it is considered as a marker of endothelial function (26,27). Assessment of FMD was performed using a 7.0–14.0 MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Vivid 7 Pro, GE). Ultrasound analysis was performed in each case by two independent observers. The inter- and intra-observer variability for brachial artery diameter measurements in our laboratory is 0.1 ± 0.12 and 0.08 ± 0.19 mm, respectively, whereas the FMD variability measured in the same patient on two different days varied by 1.1 ± 1% (mean ± s.d.).

Statistical analysis

Pearson's χ^2 was used to compare distribution across ordinal variables. Univariate linear regression analysis was used to identify significant correlations between continuous variables. Multivariate regression analysis was used to identify possible effect modifiers of the correlation between HbA1c and FMD by entering one traditional risk factor in each step (age, gender, smoking, hypertension, hyperlipidemia, BMI), including interaction items. Because BMI was an effect modifier, we divided participants in two subgroups according to the median BMI of the entire cohort (group 1 with BMI ≤26.1 kg/m^2 and group 2 with BMI >26.1 kg/m^2) to examine the association between HbA1c and FMD in each subgroup. Subsequently, we performed univariate linear regression analysis in each subgroup to identify predictors of FMD. Multivariate analysis was performed when HbA1c was significantly correlated with FMD. The regression lines of HbA1c (adjusting for hemodynamic and demographic parameters) vs. FMD (dependent) were compared between the two subgroups of BMI (28). Also, for descriptive purposes, we divided participants into four subgroups according to the median HbA1c and median BMI (Group 1: individuals with BMI ≤26.1 kg/m^2 and HbA1c ≤5%, group 2 individuals with BMI ≤26 kg/m^2 and HbA1c >5%, group 3 individuals with BMI >26.1 kg/m^2 and HbA1c ≤5%, and group 4 individuals with BMI >26.1 kg/m^2 and HbA1c >5%. Analysis of variance with Bonferroni *post hoc* tests was used to compare means of different parameters across the four groups. Statistical significance was at $P < 0.05$. Data are presented as mean ± s.d. unless otherwise noted.

RESULTS

HbA1c was negatively associated with FMD ($r = -0.162$, $P = 0.004$). By multivariate linear regression analysis adjusting for common risk factors of cardiovascular disease, BMI was the only effect modifier of the association between HbA1c and FMD ($P = 0.034$ for the HbA1c × BMI interaction). When BMI was inserted in a multivariate model including age, gender, smoking, hypertension, hyperlipidemia, and HbA1c no other variable was statistically significant except for BMI ($P = 0.02$). Therefore, we divided the cohort into two subgroups by median BMI (subgroup 1: BMI ≤26.1 kg/m^2 and subgroup 2: BMI >26.1 kg/m^2). Participant characteristics and differences in variables between the two subgroups are shown in **Table 1**. As expected, several variables were different between the two subgroups. Univariate correlations between FMD and other parameters in each subgroup are shown in **Table 2**. Significant associations were found between classical cardiovascular risk factors and FMD. In subgroup 1 (lower BMI), HbA1c was the only independent predictor of FMD ($r^2 = 0.035$, $P = 0.021$, B = –1.39, 95% CI –2.58, –0.21) after adjusting for confounding factors associated with impaired glucose metabolism (age, gender, smoking, blood pressure, cholesterol, and HOMA-IR). In subgroup 2 (higher BMI), HbA1c did not correlate with FMD by univariate linear regression (**Table 2**). By multivariate regression analysis adjusting for age, gender, smoking, blood pressure, cholesterol, and HOMA-IR, both the slope ($P = 0.034$, B = 1.52, 95% CI 0.11, 2.93) and intercept ($P = 0.015$, B = –8.36, 95% CI: –15.11 – (–1.62)) of the regression lines for the associations between HbA1c and FMD were significantly different (**Figure 1**). Participants with BMI ≤26.1 kg/m^2 and HbA1c ≤5% (group 1) had significantly higher FMD compared to either lean subjects with high HbA1c (group 2, BMI ≤26.1 kg/m^2 and HbA1c >5%) or overweight subjects (BMI >26.1 kg/m^2, groups 3 and 4) irrespective of HbA1c (FMD: 4.5 ± 3.3% for group 1 vs. 2.95±2.5% for group 2 ($P < 0.05$), vs. 3.0 ± 2.7% for group 3 ($P < 0.01$), vs. 2.9 ± 2.7% for group 4 ($P < 0.001$), Bonferroni *post hoc*, $P = 0.0001$); FMD did not differ among the latter three groups. Because measures of central

Table 1 Participant characteristics

	Total population (n = 309)	BMI ≤ 26.1 kg/m² (n = 154)	BMI > 26.1 kg/m² (n = 155)	P value
Age (years)	46.7 ± 8.9	44.9 ± 9.0	48.6 ± 8.5	0.0001
Sex (no (%) women)	186 (61)	107 (69)	79 (51)	0.001
BMI (kg/m²)	27.0 ± 4.8	23.2 ± 1.9	30.7 ± 3.7	<0.0001
Waist circumference (cm)	90.5 ± 14.8	81.9 ± 9.3	99.6 ± 14.1	<0.0001
Hemoglobin A1c (%)	4.85 ± 0.51	4.7 ± 0.4	5.0 ± 0.6	<0.0001
Fasting glucose (mmol/l)	4.9 ± 0.55	4.74 ± 0.45	5.05 ± 0.62	<0.0001
2-h postload glucose (mmol/l)	5.5 ± 1.7	5.2 ± 1.3	5.9 ± 1.9	0.001
HOMA-IR	1.81 ± 1.3	1.2 ± 0.7	2.4 ± 1.5	<0.0001
Hyperlipidemia (%)	126 (41)	52 (34)	74 (48)	0.009
Total cholesterol (mmol/l)	11 ± 1.9	11.2 ± 2.2	12.0 ± 2.1	0.001
Triglycerides (mmol/l)	4.2 ± 1.8	4.5 ± 2.0	6.3 ± 3.0	<0.0001
HDL cholesterol (mmol/l)	3.6 ± 0.9	3.6 ± 0.9	3.0 ± 0.8	<0.0001
LDL cholesterol (mmol/l)	7.2 ± 1.7	7.4 ± 2.2	8.5 ± 1.9	<0.0001
Smoking (%)	130 (42)	75 (49)	55 (36)	0.02
Hypertension (%)	60 (19)	17 (11)	43 (28)	<0.001
Systolic BP (mm Hg)	119.7 ± 20.3	112.5 ± 17.8	127.1 ± 20.2	<0.001
Diastolic BP (mm Hg)	77.2 ± 12.3	72.5 ± 10.8	82.0 ± 11.9	<0.0001
FMD (%)	3.5 ± 3.0	4.21 ± 3.22	2.8 ± 2.5	<0.0001

Data are presented as mean ± s.d. unless otherwise indicated. P values correspond to differences in means or proportions for categorical variables by t-test or χ^2-test respectively between BMI groups.
BP, blood pressure; FMD, flow-mediated dilatation; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment basal insulin resistance, LDL, low-density lipoprotein.

Table 2 Univariate regression analysis showing β values between cardiovascular risk factors and flow-mediated dilation (FMD) in the study population

	Flow-mediated dilatation (FMD) (%)		
Dependent	Total population (n = 309)	BMI ≤ 26.1 kg/m² (n = 154)	BMI > 26.1 kg/m² (n = 155)
Age (years)	–0.065	–0.021	–0.006
Gender (female)	0.043	0.088	–0.108
BMI (kg/m²)	–0.208***	0.033	–0.077
Waist circumference (cm)	–0.218***	0.019	–0.178*
Hemoglobin A1c (%)	–0.162**	–0.203*	–0.014
Fasting glucose (mmol/l)	–0.114*	–0.165*	0.058
2-h postload glucose (mmol/l)	–0.104	–0.076	–0.042
HOMA-IR	–0.120*	–0.066	–0.020
Total cholesterol (mmol/l)	–0.089	–0.018	–0.096
Triglycerides (mmol/l)	–0.141*	–0.065	–0.071
HDL cholesterol (mmol/l)	0.133*	0.125	–0.045
LDL cholesterol (mmol/l)	–0.136*	–0.079	–0.089
Smoking (yes/no)	0.067	0.077	0.032
Systolic blood pressure (mm Hg)	–0.149**	–0.121	–0.005
Diastolic blood pressure (mm Hg)	–0.163**	–0.108	–0.039

HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment basal insulin resistance; LDL, low-density lipoprotein.
*P < 0.05, **P < 0.01, ***P < 0.001.

obesity may be a stronger predictor of cardiometabolic risk than BMI, we repeated our analyses using waist circumference as the predictor variable, and the results did not change. In subjects with normal waist (men ≤102 cm; women ≤88 cm), waist circumference was the only independent predictor of FMD (r^2 = 0.042, P = 0.005, B = –1.41, 95% CI –2.4, –0.42) after adjusting for confounding factors associated with impaired glucose metabolism (age, gender, smoking, blood pressure, cholesterol, and

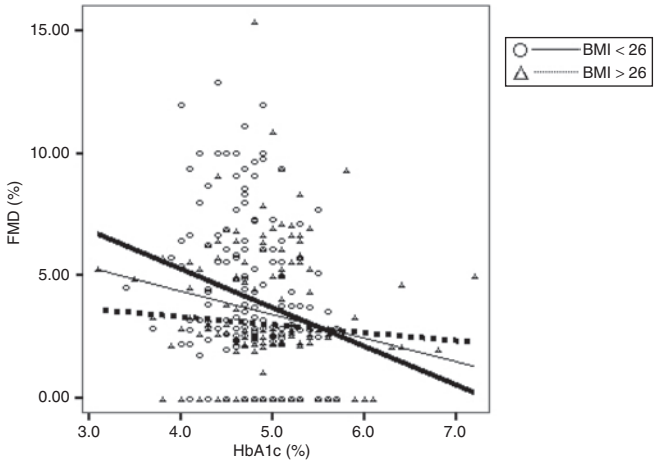

Figure 1 Correlations between flow-mediated dilatation (FMD) and Hemoglobin A1c (HbA1c) in the entire cohort (continuous thin line) and by each subgroup (bold continuous line, BMI ≤ 26.1 kg/m^2; bold dotted line, BMI > 26.1 kg/m^2). The figure shows a significantly ($P = 0.034$) steeper regression line extracted from the correlation between FMD and HbA1c in the lean group (bold continuous line) as compared to the overweight group (bold dotted line).

HOMA-IR). In participants with increased waist circumference (men ≥102 cm; women ≥88 cm), HbA1c did not correlate with FMD. As with BMI analysis, the regression lines for the associations between HbA1c and FMD were significantly different between those with or without increased waist circumference.

DISCUSSION

In the present study, we found a significant correlation between HbA1c and FMD in the entire study population, which was due to a stronger correlation in the subgroup of participants with low BMI. Multivariate analysis showed that this association was independent of other established predisposing factors.

FMD is a well-recognized index of endothelial function (26). Impaired FMD may be detected even in apparently healthy individuals (29), it may be present prior to the angiographic evidence of disease (30) and is predictive of future cardiovascular events (30,31). Previous studies have shown glucose levels in the upper-normal range or increased BMI to correlate with impaired endothelial function (7,8,32,33). The novel finding of our study is that glycemia in nondiabetic individuals, as a contributing factor for endothelial dysfunction, may be more important in lean individuals, whereas increasing BMI attenuates this association. It is interesting that in the group of individuals with high-normal HbA1c but low BMI, endothelial function, as measured by FMD, is impaired to the same degree as in overweight individuals with either low- or high-normal glycemia. In subjects who were both overweight and had high-normal glycemia, no significant further deterioration in FMD was found probably because some of the mechanisms involved in the impairment of endothelial function are common between glycemia and overweight.

The mechanisms responsible for glycemia-induced endothelial dysfunction have not been fully elucidated. Several factors may be involved such as increased free oxygen radicals and circulating advanced glycosylation end products, changes in prostaglandin levels, leading to attenuation of endothelial derived NO production (34). Acute hyperglycemia may also induce endothelial dysfunction in humans (35). Moreover, hyperglycemia often coexists with other metabolic abnormalities such as insulin resistance, hyperlipidemia, hypertension, and obesity associated with endothelial dysfunction (17–19). Although the current study was not designed to examine mediating mechanisms, the observation that the association of HbA1c with FMD was independent of basal insulin resistance and other metabolic disorders implies a direct effect of elevated glucose on the endothelium in lean individuals. In contrast, in overweight subjects, metabolic abnormalities are heavily clustered and may, therefore, mediate most of the endothelial damage undermining the effects of glycemia. Another point of interest is that HbA1c was correlated with FMD in lean individuals despite the fact that the former was significantly lower in this group as compared to HbA1c levels in overweight patients; this suggests that long-term glycemia may be detrimental even when ranging within low concentrations suggesting that HbA1c may be used as a marker to identify lean individuals at high risk for cardiovascular disease.

In the Hoorn study, FMD was not associated with impaired glucose metabolism in nondiabetic individuals (14). Furthermore, although HbA1c was an independent predictor of FMD in a mixed population of diabetics and nondiabetics (23), it did not correlate with FMD in diabetic or nondiabetic individuals separately possibly due to weak associations not revealed in smaller sample sizes. The current study confirms the previously observed weak association between glycemia and FMD in a nondiabetic population. Importantly, however, it identifies a significant interaction between body weight and glycemia on endothelial function which may provide an explanation for the statistically weak association seen previously between glycemia and vascular function in nondiabetic individuals. Lean individuals without traditional risk factors for cardiovascular disease are considered to be at very low risk for cardiovascular disease. Our results suggest that an elevated HbA1c within the "normal" range (e.g., >5%) might stratify these individuals into a higher risk category.

A limitation of the study is its cross-sectional design and, therefore, the directionality of the association cannot be determined. Additionally, our results may be confounded by medications, which may have an independent effect on FMD. However, we asked participants to hold their medications on the morning of the examination, and we adjusted for relevant conditions such as hypertension and hypercholesterolemia. Physical activity or sedentary lifestyle may also have an independent effect on FMD; however, because we have incomplete data on these variables, they were not included as potential confounders. Our findings may also have been related to power issues because there were more participants with lower/normal HbA1c in the lean group. However, this is unlikely as the range of HbA1c values was wide in both groups (3.4–7.2%), whereas almost one-third of the lean individuals had an HbA1c >5%. Also, given that the correlation coefficient in

the higher BMI group was so low, it would be unlikely to reach statistical significance with increasing numbers of participants. Finally, because our analyses were *post hoc*, our findings need to be confirmed in prospective studies.

In conclusion, the current study indicates that in individuals without diabetes the level of glycemia, as reflected by HbA1c concentration, is an independent factor for impaired endothelial function only in lean individuals. Specifically, in lean individuals with HbA1c in the high-normal range, endothelial function was impaired to the same degree as in overweight individuals with either low- or high-normal glycemia, suggesting a direct effect of elevated glucose on the endothelium. Finally, the combination of increased adiposity and high-normal glycemia does not appear to have any additive effects in regard to endothelial function, possibly because some of the mechanisms involved are common.

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DISCLOSURE

The authors declared no conflict of interest.

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