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Hemoglobin A1c Levels and Aortic Arterial Stiffness: The Cardiometabolic Risk in Chinese (CRC) Study

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Abstract

Objective: The American Diabetes Association (ADA) recently published new clinical guidelines in which hemoglobin A1c (HbA1c) was recommended as a diagnostic test for diabetes. The present study was to investigate the association between HbA1c and cardiovascular risk, and compare the associations with fasting glucose and 2-hour oral glucose tolerance test (2 h OGTT).

Research Design and Methods: The study samples are from a community-based health examination survey in central China. Carotid-to-femoral pulse wave velocity (cfPWV) and HbA1c were measured in 5,098 men and women.

Results: After adjustment for age, sex, and BMI, the levels of HbA1c were significantly associated with an increasing trend of cfPWV in a dose-dependent fashion (P for trend < 0.0001). The associations remained significant after further adjustment for blood pressure, heart rate, and lipids (P = 0.004), and the difference in cfPWV between the highest and the lowest quintiles of HbA1c was 0.31 m/s. Fasting glucose and 2 h OGTT were not associated with cfPWV in the multivariate analyses. HbA1c showed additive effects with fasting glucose or 2 h OGTT on cfPWV. In addition, age and blood pressure significantly modified the associations between HbA1c and cfPWV (P for interactions < 0.0001 for age; and = 0.019 for blood pressure). The associations were stronger in subjects who were older (>60 y; P for trend = 0.004) and had higher blood pressure (≥120 [systolic blood pressure]/80 mmHg [diastolic blood pressure]; P for trend = 0.028) than those who were younger and had lower blood pressure (P for trend > 0.05).

Conclusions: HbA1c was related to high cfPWV, independent of conventional cardiovascular risk factors. Senior age and high blood pressure might amplify the adverse effects of HbA1c on cardiovascular risk.

Introduction

Recently, the American Diabetes Association (ADA) published new clinical guidelines in which hemoglobin A1c (HbA1c) level, in addition to fasting glucose and 2-hour oral glucose tolerance test (2 h OGTT), was recommended as a diagnostic test for diabetes [1]. HbA1c is a marker of long-term glycaemic exposure, reflecting an average blood glucose level over 2–3 month period of time. The cut-points for the diagnosis of diabetes are based on the presence of long-term complications. However, the current evidence for the ADA recommendation is largely from the studies on the relations between HbA1c and microvascular diseases especially retinopathy [2]. Even though macrovascular disease complications are more prevalent and the primary causes for mortality in diabetes, the data about the associations between HbA1c and macrovascular risk are surprisingly lacking.

Arterial stiffness is an established marker for early-stage atherosclerosis. Among various approaches to assess early-stage arterial stiffness, carotid-to-femoral pulse wave velocity (cfPWV) has been widely recognized a gold standard method [3], and independently associated with cardiovascular outcomes such as myocardial infarction, heart failure, and mortality [4–7]. Some recent studies associated HbA1c with arterial stiffness measured by cfPWV in patients with type 2 diabetes [8] and hemodialysis [9]. Studies examining the relation between HbA1c and arterial stiffness in the general population are sparse. In addition, fasting and post-challenge glucose concentrations were also related to accelerated stiffening of the elastic arteries that contributes to the excessive cardiovascular risk [7,10,11]. The effects of various glucose exposures are not perfectly concordant and may be independent [12]. However, little is known about the relative influence of HbA1c and fasting and post-challenge glucose on cardiovascular risk.

The aim of this study was to examine the associations between HbA1c as the marker of long-term glucose exposure and cfPWV.
in a large sample of Chinese adults, and to compare with the associations of fasting glucose and 2 h OGTT. We also assessed the modification effects of age, sex, obesity and blood pressure on the relations between glucose exposures and aortic arterial stiffness.

Materials and Methods

Study population
In the Cardiometabolic Risk in Chinese (CRC) Study, we performed a community-based health examination survey for 6,431 individuals (18–93 y) who were randomly selected from residents living in the urban area of Xuzhou, China, in 2009. Written consents were obtained from all the participants. The study was reviewed and approved by the ethics committee of the Central Hospital of Xuzhou, China. Among the participants, 5,154 individuals were measured for both cfPWV and HbA1c. For the present study, we excluded subjects with history of diabetes, or fasting glucose ≥7.0 mmol/L, and/or 2 h OGTT ≥11.1 mmol/L, and/or HbA1c ≥6.5% [12,13]. In total 5,098 men and women were included in the final analyses. There was not significant difference in age and anthropometrics between individuals who were included and those who were not included in the analyses.

Assessment of Carotid-to-femoral PWV
Carotid-to-femoral PWV in subjects at rest was measured using Complior device (Artech-Medical, Pantin, France) that allows pulse wave recording and automatic calculation of cfPWV with 2 transducers. The operator recorded in succession the right carotid and femoral waveforms. cfPWV is calculated as the distance between the two recording sites divided by the time delay between the feet of the two waveforms at each site. Sixteen cfPWVs were measured for each participant. After removing 6 extreme values (3 maximum and 3 minimum), an average value of cfPWV was calculated.

Assessment of biomarkers and covariates
Venous blood sample was drawn from all subjects after an overnight fast (10 h). The blood was transferred into glass tubes and allowed to clot at room temperature. Immediately following clotting serum was separated by centrifugation for 15 min at 3,000 rpm. HbA1c was measured using high performance liquid chromatography (HPLC; HLC-723G7 hemoglobin HPLC analyzer, Tosoh Corp.) according to the standardized method. Participants with no history of diabetes underwent a community-based health examination survey for 5,514 individuals were measured for both cfPWV and HbA1c. For the present study, we excluded subjects with history of diabetes, or fasting glucose ≥7.0 mmol/L, and/or 2 h OGTT ≥11.1 mmol/L, and/or HbA1c ≥6.5% [12,13]. In total 5,098 men and women were included in the final analyses. There was not significant difference in age and anthropometrics between individuals who were included and those who were not included in the analyses.

Statistical analyses
A linear regression model was used to evaluate associations between glucose exposures (HbA1c, fasting glucose, and 2 h OGTT) and cfPWV, adjusting for covariates. Glucose exposures were analyzed in quintiles. cfPWV was analyzed as the dependent variable, and levels of HbA1c, fasting glucose, or 2 h OGTT were analyzed as the independent variables. Tests for linear trend were calculated by assigning median value for each quintile of intake and treated as continuous variables. We adjusted for the potential confounding variables: age, sex, BMI, MAP, heart rate and lipids (total cholesterol, triglyceride, HDL-C and LDL-C). The effect modifications of age (<40, 40–59, ≥60 y), BMI (<25, ≥25 kg/m²), sex, and blood pressure (low vs high by the cutoffs SBP ≥120 mmHg and DBP ≥80 mmHg) were tested by introduction of the cross-products of the tested variables and HbA1c into the models. We used the SAS statistical package for all analyses (Version 9.1, SAS Institute, Cary, NC). All P-values are two-sided.

Results
In total 5,098 men and women were included in the analyses. Table 1 presents the characteristics of the participants by the quintiles of HbA1c. Individuals with higher HbA1c had higher SBP, BMI, waist circumference, fasting glucose, 2 h OGTT, total cholesterol, triglyceride, and LDL-C, but lower HDL-C than those who were with lower HbA1c. We first examined the associations between three measures of glucose exposure (HbA1c, fasting glucose and 2 h OGTT) and cfPWV (Table 2). In the models adjusting for age, sex and BMI, all the three measures were significantly associated with increasing trend of cfPWV (P<0.0001). When MAP was further adjusted, the associations for fasting glucose (P = 0.01), 2 h OGTT (P = 0.04), and HbA1c (P = 0.005) remained significant. When other covariates including heart rate, total cholesterol, TG, HDL-C and LDL-C were further adjusted, the associations for fasting glucose and 2 h OGTT were attenuated to be not significant, while the association for HbA1c remained significant (P = 0.004). In the fully-adjusted model, the difference in cfPWV between the highest and the lowest quintiles of HbA1c was 0.31 m/s.

According to the ADA recommendation [12], we defined ‘prediabetes’ by the cutoffs of the three glucose exposures, i.e. impaired fasting glucose (IFG, fasting glucose 5.6–6.9 mmol/L), impaired glucose tolerance (IGT, 2 h OGTT 7.8–11.0 mmol/L), and high HbA1c (5.7–6.4%) to represent an increased risk of diabetes but without fulfilling the criteria of diagnosis. The prevalence of IFG, IGT, and high HbA1c in this non-diabetic population were 12%, 14.3%, and 14.8%; respectively. The differences in cfPWV between individuals with IFG, IGT and high HbA1c and those without these abnormalities were 0.97, 1.08 and 0.92 m/s (P<0.0001; Figure 1).

We then examined the additive effects of HbA1c with fasting glucose and 2 h OGTT on cfPWV (Figure 2). In the analyses adjusting for age, sex, and BMI, as compared with individuals with fasting glucose <5.6 mmol/L and HbA1c <5.7%, those with high HbA1c only, with IFG only, and with both IFG and high HbA1c had 0.28 (P = 0.03), 0.27 (P = 0.015) and 0.48 m/s (P = 0.0003) higher cfPWV. Similarly, as compared with individuals with 2 h OGTT <7.8 mmol/L and HbA1c <5.7%, those with high HbA1c only, with IGT only, and with both IGT and high HbA1c had 0.18 (P = 0.10), 0.26 (P = 0.008) and 0.44 m/s (P = 0.0001).
higher cPWV. Further adjustment for other covariates did not materially change the results. Our data also indicate that individuals of both high HbA1c and IFG or IGT had significantly higher levels of cPWV compared with those who only had high HbA1c (p = 0.036 and 0.03; respectively; or those only had IFG (p = 0.02); or those only had IGT (p = 0.04).

Pulse wave velocity is strongly associated with age and blood pressure, and is also related to sex and BMI [14–16]. Therefore, we examined whether these variables modified the relation between HbA1c and cPWV. We found significant interactions between HbA1c and age ($\leq 40$, $40–59$, $\geq 60$ y; $P\leq 0.0001$) and blood pressure (low vs high by the cutoffs SBP $\leq 120$ mmHg and/...
or DBP \( \geq 80 \) mmHg [17]; \( P = 0.019 \). The associations between HbA1c were stronger in participants with age \( \geq 60 \) y (\( P \) for trend \( = 0.004 \)) than in those who were younger (\( P \) for trend \( > 0.05 \)) (Table 3); and were stronger in participants with high MAP (\( P \) for trend \( = 0.028 \)) than those with low MAP (\( P \) for trend \( > 0.05 \)) (Figure 3). Although the associations between HbA1c and cfPWV appeared to be more significant in women and in subjects with BMI \( \geq 25 \) kg/m\(^2\) than in men and in those with BMI \( < 25 \) kg/m\(^2\), the tests for interactions with sex and BMI were not significant (Table 3).

**Discussion**

In this study of a large sample of Chinese adults, long-term glycemic exposure, measured by HbA1c, was significantly associated with higher cfPWV, independent of other cardiovascular risk factors. We found that fasting glucose and 2 h OGTT showed additive effects with HbA1c on cfPWV. In addition, age and blood pressure significantly modified the associations between HbA1c and cfPWV.

Carotid-femoral pulse wave velocity, a gold-standard measure of intrinsic stiffness of the aortic wall, is an important predictor of cardiovascular disease risk [4,18,19]. Our findings are consistent with some previous studies. Matsumae et al. reported that HbA1c level was an independent determinant of cfPWV in hemodialysis patients with and without diabetes [9]. The association between HbA1c levels and increased arterial stiffness (measured by brachial-ankle pulse wave velocity [baPWV]) was recently observed in patients with type 2 diabetes [8]. Our data indicate that the long-term glucose exposure HbA1c may lead to increased arterial stiffness in non-diabetic individuals. The precise mechanisms how chronic glucose exposure may affect arterial stiffening are not fully understood. It was documented that BMI, blood pressure, heart rate, total cholesterol, LDL-C, HDL-C and triglyceride were related to cfPWV [15,16,20,21]. In our study, adjustment for these potential risk factors did not change the associations of HbA1c with cfPWV, suggesting that the effects of HbA1c are not mediated by these metabolic changes and likely to be through other independent pathways.

Individuals with prediabetes defined by the three measures of glucose exposure HbA1c, fasting glucose and 2 h OGTT all had significantly higher cfPWV. Our data indicate that, however, the changes in arterial stiffness were better associated with long-term glycemic exposure HbA1c than single measures of fasting glucose and 2 h OGTT on the continuous scales. Though highly correlated, these various measures for glucose exposure may convey different information regarding their relations with cardiovascular risk. Our data are consistent with previous studies in which HbA1c was a better predictor of cardiovascular disease than fasting glucose [22] and post-challenge glucose levels [23]. In addition, HbA1c has shown more consistent associations with retinopathy than fasting glucose level [2]. HbA1c value is a more stable biological index for long-term glycemia exposure than fasting glucose, which fluctuate within and between days and is therefore not a clear indicator of general glycemia.

Various mechanisms have been proposed to link glucose exposure with development of atherosclerosis. Long-term high

**Figure 1.** Geometric means of carotid-to-femoral pulse wave velocity (cfPWV, in m/s) by the presence of prediabetes status defined by IFG, IGT, and high HbA1c (5.7–6.4%). The analyses were adjusted for age, sex, BMI, MAP, heart rate and lipids (total cholesterol, triglyceride, HDL-C and LDL-C). Symbol ‘*’ represents significant difference (\( p < 0.05 \)) between the ‘absence’ and ‘presence’ groups of each marker.

**Figure 2.** Geometric means of cfPWV (in m/s) by the combinations of high HbA1c (5.7–6.4%) with IFG and IGT. Symbol ‘*’ represents the presence of the corresponding glucose exposures. The analyses were adjusted for age, sex, BMI, MAP, heart rate and lipids (total cholesterol, triglyceride, HDL-C and LDL-C). ‘**’ represents significant difference (\( p < 0.05 \)) comparing individuals with high HbA1c and IFG/IGT with the normal subjects (without any of these abnormalities); ‘***’ represents significant difference (\( p < 0.05 \)) comparing individuals with high HbA1c and IFG/IGT with those who had only high HbA1c and ‘****’ represents significant difference (\( p < 0.05 \)) comparing individuals with high HbA1c and IFG/IGT with those who had only IFG or IGT.

**Figure 3.** The geometric means of cfPWV (in m/s) by according to HbA1c (in quintiles) by the presence/absence of high blood pressure defined by SBP \( \geq 120 \) mmHg and DBP \( \geq 80 \) mmHg. The analyses were adjusted for age, sex, BMI, heart rate and lipids.

![Image](https://www.plosone.org/doi/10.1371/journal.pone.0038485.g002)
though the mechanisms underlying such an additive

levels of circulating glucose lead to formation of advanced
glycation endproducts (AGE), which result from non-enzymatic
protein glycation forming irreversible cross-links in stable tissue
proteins [24]. Matrix in the blood vessel wall is steadily reduced
from exposure to AGE [25]. In addition, high AGE is known to
impact endothelial function by quenching NO, enhancing the
generation of reactive oxygen species (ROS), and induction of
inflammation. All these alterations may contribute to development
and progression of atherosclerosis [24,26]. It has been acknowl-
dged that various glucose exposures are not perfectly concordant
regarding their relations with disease outcomes [12]. Our study
suggests that different measures of glucose exposure might have
additive effects on arterial stiffness. Although these measures have
been individually related to cardiovascular risk [8,10], the
mechanisms underlying their joint effects are not unequivocally
clarified. These various measures may reflect distinct abnormal-
ities in glucose metabolism. High fasting glucose detects fasting
hyperglycaemia, which is more typical of pancreatic β cell
dysfunction; whereas high 2 h OGTT reveals post-prandial
hyperglycaemia that is more closely associated with insulin
dysfunction; whereas high 2 h OGTT reveals post-prandial
hyperglycaemia that is more closely associated with insulin
function. These various measures may reflect recent changes in diet or treatment [28]. We postulate that at least parts of the pathways linking these different markers to cardiovascular conditions are not overlapped. Therefore, when different markers are considered jointly, their effects would appear additive. Our findings of the additive effects of different measures of glucose exposures highlight the importance of measuring long-term glucose exposure in addition to fasting glucose and 2 h OGTT in characterizing individuals at high risk of diabetes and cardiovascular complications.

Arterial stiffness is strongly associated with age and blood
pressure [21]. The data from the present study showed that these
two factors significantly modified the associations between HbA1c
and cfPWV, and stronger associations were observed in the
individuals who were older and had higher blood pressure. A
synergistic effect between raised blood pressure and raise plasma
glucose on arterial stiffening was reported in middle-aged Japanese
men [29]. Though the mechanisms underlying such an additive
effect remain not clear, some studies have shown that the
cocurrence of hyperglycemia and high blood pressure might
augment the production of advanced glycation end products and
deteriorate endothelial dysfunction [29,30]. Future studies are
needed to investigate the mechanisms underlying the additive
effects between HbA1c and blood pressure and aging.

The sample size of this study is large, which ensures sufficient
power to detect the moderate effects of glucose exposures on
arterial stiffness and interactions between HbA1c and other risk
factors. Our study is cross-sectional in design. Therefore, a causal
relation between HbA1c and arterial stiffness could not be derived.
In addition, the study was performed in a Chinese population.
Further studies in other populations of different ethnicities are
warranted to verify our findings. Moreover, we used the
standardized method in measurement of HbA1c. However, it
has been argued that the use of HbA1c for diagnosing diabetes has
some limitations [31]. For example, the measurement of HbA1c
level might be influenced by various medical conditions, such as
kidney failure, chronic excessive alcohol intake, acute or chronic
blood loss and liver failure. Although we have carefully excluded
patients with chronic diseases from the analysis, it is still possible
some conditions that were not assessed in our study might
influence the associations.

In summary, in the present study of non-diabetic Chinese
adults, the marker of long-term glucose exposure HbA1c showed
stronger association with aortic arterial stiffness than fasting
glucose and 2 h OGTT. Various measures for glucose exposures
might additively affect arterial stiffness. In addition, we found that
senior age and high blood pressure might amplify the effects of
chronic glucose exposure on the cardiovascular risk.

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Author Contributions

Conceived and designed the experiments: JL LQ. Performed the
experiments: JL NZ FT CZ YX MY HS LQ. Analyzed the data: JL
LQ. Contributed reagents/materials/analysis tools: JL LQ. Wrote the
paper: JL LQ. Submitted the revised version of the manuscript and
answered queries from editor and readers: JL LQ.
References


