Deleterious Impact of “High Normal” Glucose Levels and Other Metabolic Syndrome Components on Arterial Endothelial Function and Intima-Media Thickness in Apparently Healthy Chinese Subjects: The CATHAY Study

G. Neil Thomas, Ping Chook, Mu Qiao, Xin S. Huang, Hok C. Leong, David S. Celermajer, Kam S. Woo

Objective—Endothelial vasodilator dysfunction and carotid intima-media thickening are useful surrogate markers of cardiovascular disease, a major cause of morbidity and mortality in type 2 diabetic patients. However, because most studies reporting the relationships between endothelial function, intima-media thickness (IMT), and hyperglycemia have compared diabetic patients with healthy controls, we report their relationship with glycemia as a continuum.

Methods and Results—Brachial artery endothelial function (flow-mediated dilatation [FMD]) and carotid IMT were measured noninvasively by high-resolution ultrasound B-mode imaging in 228 apparently healthy Chinese subjects recruited from Hong Kong and Macau. FMD and IMT were significantly associated with increasing levels of glycemia, particularly in the “high normal” glycemic range, with IMT increasing and endothelium-independent dilatation decreasing linearly across the glucose tertiles, and endothelium-dependent dilatation significantly lower in the upper glucose tertile compared with the other 2 groups (P<0.01). Using multiple linear regression, fasting glucose level was identified as an independent predictor of each of these markers of vascular function (P<0.004). Additionally, other conventional cardiovascular risk factors, including obesity, blood pressure, and an adverse lipid profile, were also related to levels of glycemia (P<0.05), further contributing to impaired vascular function.

Conclusion—Increasing levels of glycemia and the coexistence of other cardiovascular risk factors in apparently healthy subjects are adversely associated with arterial endothelial dysfunction and intima-media thickening. (Arterioscler Thromb Vasc Biol. 2004;24:739-743.)

Key Words: epidemiology ■ atherosclerosis ■ diabetes mellitus ■ hypertension ■ lipids

The endothelium plays a major role in the regulation of vascular tone and in the prevention of atherogenesis. Endothelial damage and thickening of the intima-media layers are early events in the atherosclerotic process.¹ These useful surrogate markers of cardiovascular disease can now be measured noninvasively by high-resolution B-mode ultrasound.²-¹⁰ Reports have shown close associations between these parameters and conventional cardiovascular risk factors, including type 2 diabetes, hypertension, obesity, dyslipidemia, and smoking.¹¹-¹⁷ Impaired endothelium-mediated regulation of vascular tone has been associated with the presence of coronary atherosclerotic lesions²,³ and has been observed in patients with traditional cardiovascular risk factors, even in the absence of atherosclerotic lesions.⁴ Recent studies have reported that endothelial dysfunction is a predictor of cardiovascular events.⁵,⁶ Similarly, increases in the carotid intima-media thickness (IMT) are closely associated with cardiovascular disease⁷,⁸ and predictive of cardiovascular risk.⁹

Type 2 diabetes is closely associated with microvascular and macrovascular disease, the major causes of morbidity and mortality in these patients.¹⁸ However, interventions that lower glycemia reduce the development of the microvascular, but not necessarily macrovascular disease.¹⁹ Most studies reporting the relationships between arterial endothelial function or carotid IMT and hyperglycemia have compared diabetic patients with healthy controls. However, as with most biochemical parameters, these risk factors and the markers of vascular function are continuous rather than dichotomous variables. In the current study, we report the relationship between arterial endothelial function, carotid IMT, and glycemia in apparently healthy normoglycemic Chinese subjects.

Methods

Subjects
The study was approved by the appropriate institutional ethics committees, and all subjects gave written informed consent in their

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native language. To minimize any selection bias resulting from the use of community volunteers, we applied similar strategies for subject recruitment in the different localities, as described previously.

Subjects were recruited from Macau (n = 164) and Hong Kong (n = 78) in southern China, as part of the ongoing Chinese Atherosclerosis in the Aged and Young (CATHAY) study, and were included if they had been living in the relevant location for at least 10 years and were originally of southern Chinese heritage. All 242 subjects (age 18 to 75 years) were recruited from the community and were apparently healthy, with none receiving medication for the treatment for any vascular disease or risk factors, including hypertension, hyperglycemia, or dyslipidemia, or specific antioxidant supplements. A total of 14 asymptomatic diabetic subjects were identified. Because diabetes mellitus is already known to be associated with endothelial dysfunction and thickened carotid IMT,14,21,22 these subjects were excluded from further analysis.

Measurements
Each subject had one visit to the study center, during which a medical history was taken, including an assessment of smoking history (‘never’/‘ever’). Measurement of seated blood pressure, anthropometrics (waist circumference and body mass index [BMI]), and fasting plasma lipids and glucose were taken as previously described.20–23

Subjects were defined as hypertensive if, after 5 minutes rest, their seated systolic blood pressure (SBP) was ≥140 mm Hg and/or diastolic blood pressure (DBP) ≥90 mm Hg. Impaired fasting glucose (IFG) and diabetes were diagnosed based on fasting plasma glucose levels of 6.1 to 7.0 mmol/L and ≥7.0 mmol/L, respectively.24 General obesity was defined as a BMI, weight (kg)/height (m)2, of ≥25.0 kg/m2 and central obesity as a waist/hip ratio (WHR) ≥0.85 or ≥0.90 in females and males, respectively.25 Dyslipidemia was defined as a fasting total plasma cholesterol level ≥6.2 mmol/L or total cholesterol level between 5.2 and 6.1 mmol/L and total cholesterol to HDL/cholesterol ratio ≥5.0, or if plasma triglyceride was ≥2.3 mmol/L.

Ultrasound Imaging: IMT Measurement
B-mode ultrasound examinations were performed with an Acuson 128XP/10 mainframe with a 7-MHz scanning frequency linear array transducer or an ATL 3000 mainframe with a high-resolution linear array scanner (medium frequency 7.5MHz). All ultrasound systems used similar gain and depth settings, as previously described.20–26 All scans were performed by operators after a predetermined standardized scanning protocol for the right and left carotid arteries using images of the far wall of the distal 10 mm of the common carotid arteries. Three scanning angles were used in each case: anterior, lateral, and posterior oblique. The images were focused on the posterior (far) wall, which were recorded from the angle showing the greatest distance between the lumen–intima interface and the media–adventitia interface.26 All scans were recorded on super-VHS videotape for subsequent off-line analysis. All scans were analyzed by the same investigator (P.C.), blinded to subject identity, using an automatic computerized edge-detection and measurement system.20

Endothelial-Dependent and Independent Vascular Dilation Measurement
Endothelial function of the brachial artery was assessed on each case. The procedures and rationale have been documented previously.10–13 Briefly, the diameter of the brachial artery was measured at rest, during reactive hyperemia (to induce endothelium-dependent flow-mediated dilatation [FMD]), again at rest, and after sublingual glyceryl trinitrate (GTN), which is an endothelium-independent dilator. Physiologically, increased blood flow stimulates the release of vasodilators from the endothelium, such as nitric oxide, which in turn causes arterial dilatation (FMD); impaired FMD is observed in the presence of endothelial dysfunction. By contrast, GTN acts directly on the arterial smooth muscle and induces endothelium-independent dilatation. One observer, unaware of the patient’s clinical details, measured the vessel diameter, as described elsewhere.10–13

Statistical Analyses
Data from normally distributed parameters are presented as mean±SD, whereas skewed data were logarithmically transformed and expressed as geometric mean with 95% confidence intervals. Differences in gender and disease frequencies between the normoglycemic and diabetic populations and increasing tertiles of fasting plasma glucose were analyzed using the χ2 test. Differences in the biochemical and anthropometric parameters between the populations and tertiles of fasting plasma glucose were determined using one-way analysis of variance (ANOVA) with least squares difference post hoc testing. Additionally, analysis of covariance was performed to allow adjustment for a number of cardiovascular risk factors that were also associated with increasing glycemia, including age, gender, SBP and DBP, smoking, BMI, WHR, and total cholesterol.

Gender was coded 0 and 1 for male and female, respectively. The variables included in the analyses were linearly related to the dependent variables. For the stepwise multiple regression, age, gender, glucose, smoking, SBP and DBP, WHR, BMI, and LDL and HDL cholesterol and triglycerides were included in the analyses. The appropriateness of the regression model was judged from the Durbin-Watson statistic (testing for serial correlation of adjacent error terms) and partial plots of the residuals. The tolerance and variance inflation factors were taken as measures of collinearity, with low tolerance and high variance inflation factors being signs of collinearity, indicating that a variable should not be included in the model. The Statistics Package for the Social Sciences (SPSS, version 10.0, 2001; SPSS Inc, Chicago, Ill) was used for the aforementioned analyses.

Results
No significant differences were identified between the subjects from Hong Kong and Macau and we therefore combined the data sets for the subsequent analyses. A total of 242 apparently healthy subjects were initially recruited for the study. Despite the subjects being recruited as apparently healthy, a number of conditions were identified, including 5.0% with hypertension, 38.3% with dyslipidemia, 19.0% with hyperglycemia (impaired fasting glucose 69.6%, diabetes 30.4%), and 41.7% with obesity. The 14 subjects subsequently identified as having diabetes were excluded from the analyses. Exclusion of the diabetic subjects generally did not affect the findings of the study, although it slightly weakened the strength of some of the associations between glycemia and vascular function. The 228 non-diabetic healthy subjects were subgrouped according to indices of glycemia, using tertiles of increasing fasting glucose levels (Table). The upper tertile with glucose levels ranging from 5.7 to 6.9 mmol/L is considered “high normal” for the purposes of the study. Carotid IMT significantly increased but endothelium-independent dilatation decreased across each of the tertiles of increasing glycemia, (Table), whereas the endothelium-dependent dilatation in upper glucose tertile was significantly lower than the other 2 groups, as was the ratio between FMD and GTN. Increasing glycemia was associated with an increasingly adverse metabolic profiles, including elevated blood pressure, dyslipidemia, and obesity. Only 11.2% of the subjects smoked, and smoking was not significantly associated with increasing levels of glycemia. However, as described, smoking was an independent predictor of IMT, but not FMD. Because a number of cardiovascular risk factors increase with increasing glycemia, we also performed ANOVA, which allowed adjustment for age, gender, SBP and DBP, smoking, BMI, WHR, and total cholesterol. After
Demographic Characteristics Associated With Increasing Tertiles of Fasting Plasma Glucose in the 228 Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tertiles of Fasting Plasma Glucose</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n=70)</td>
<td>2 (n=84)</td>
</tr>
<tr>
<td>Glucose (mmol/L) (range)</td>
<td>4.9±0.3</td>
<td>5.4±0.1*</td>
</tr>
<tr>
<td>Age (y)</td>
<td>41.4±12.3</td>
<td>43.7±12.6</td>
</tr>
<tr>
<td>Gender (% males)</td>
<td>47.1</td>
<td>56.0</td>
</tr>
<tr>
<td>Intima-media thickness (mm)</td>
<td>0.53±0.09</td>
<td>0.57±0.09*</td>
</tr>
<tr>
<td>Flow-mediated dilatation (FMD, %)</td>
<td>9.5±2.26</td>
<td>9.20±2.25</td>
</tr>
<tr>
<td>Glyceryl trinitrate-mediated dilatation (%)</td>
<td>19.3±3.2</td>
<td>18.8±3.4*</td>
</tr>
<tr>
<td>FMD:GTN ratio</td>
<td>0.50±0.13</td>
<td>0.50±0.12</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.6±2.9</td>
<td>22.8±3.4*</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.82±0.06</td>
<td>0.85±0.07*</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>113±13</td>
<td>116±13</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>73±9</td>
<td>74±9</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.2±0.9</td>
<td>5.5±1.1</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.33±0.80</td>
<td>3.62±0.99</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.41±0.40</td>
<td>1.37±0.33</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.86 (0.77–0.96)</td>
<td>1.03 (0.94–1.12)*</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>9.4</td>
<td>14.1</td>
</tr>
</tbody>
</table>

Mean±SD or geometric mean (95% CI), GTN glyceryl trinitrate-mediated dilatation, P values are determined by analysis of variance (ANOVA) and describe significance between the 3 groups.

*P<0.05 vs. tertile 1.
†P<0.05 vs. tertile 2.

For the carotid IMT, age (β=0.36, P<0.001), glucose (β=0.19, P=0.004), and smoking (β=0.14, P=0.024) accounted for 25% of the variance (IMT=β[0.003·age] + [0.5·glucose] + [0.05·smoking] + 0.02; R=0.25, F=22.6, P<0.001).

Discussion

In this study of apparently healthy adults, we have found a clear relationship between increasing levels of glycemia, particularly in those subjects with “high normal” glycemia (ie, in the upper glucose tertile of the non-diabetic subjects), and both carotid IMT and vascular reactivity of the brachial artery. The relationship with carotid IMT was linear and increased significantly with increasing tertiles of glucose, whereas there was a similar but inverse relationship with endothelium-dependent and independent vasodilation. These findings were consistent after adjustment for a number of the components of the metabolic syndrome that were also closely associated with the increasing glycemia. The evidence of this relationship across the whole normal range of glucose levels, but most evident in the “high normal” glycemic range as currently defined, emphasizes that the glycemia associated with diabetes is an extreme of a continuum, and that optimal glucose levels for vascular health remain to be determined but are likely at the lower ends of the glycemic spectrum. In part, these detrimental changes in vascular function may parallel the increases in other associated cardiovascular risk factors, such as aging, hypertension, smoking, dyslipidemia, triglyceride levels, or obesity, although the relationship with glyce-
nia remained after statistically adjusting for these metabolic syndrome components using ANOVA and stepwise linear regression analyses. Because increasing glycemia was associated with reductions in FMD and GTN, it is possible that the changes in FMD result from those with GTN. However, the significant decrease in the ratio in upper glucose tertile would suggest a disproportionate affect on the FMD. In agreement, glucose level per se was confirmed as an independent predictor of IMT and FMD, but not GTN, in this group of subjects.

In previous work, hyperglycemia after an oral glucose tolerance test has been reported to acutely reduce FMD in normoglycemic individuals.37 Acute hyperglycemia has been reported to rapidly induce oxidative stress, inflammatory markers, and hemodynamic effects, such as increased blood pressure, all of which may be important initiators of vascular disease.28–30 Similarly, the parallel increases in glycemia and insulin resistance, for which obesity is a major predisposing risk factor, are associated with abnormal vascular function.22,31,32

Even modest levels of glucose can lead to the accumulative formation of advanced glycation end products, which alter the structure and function of macromolecules.32 These end products are responsible for a range of effects, including quenching nitric oxide, and for contributing to oxidative stress and inflammatory processes.32,33 Glycation end products, which undergo receptor-mediated endocytosis into vascular endothelial and smooth muscle cells, have been associated with atherogenesis, even in normoglycemic subjects.33 The mechanism whereby increasing glucose might act as an independent predictor of both endothelium-dependent and endothelium-independent vasodilatation may be mediated through the formation of these products, which may then disrupt homeostasis of endothelial and smooth muscle cells.

Although our data suggest an independent relationship between glycemia, particularly in the “high normal” range, and abnormal vascular function and structure, this effect may be magnified by the co-existence of a number of other known cardiovascular risk factors that also increased in parallel with glucose levels, including aging, obesity, and dyslipidemia. Aging is closely associated with obesity, particularly when centrally deposited, as determined by the WHR in this study, which has been reported to be a major predisposing factor to the development of hyperglycemia, as well as many other cardiovascular risk factors, such as inflammatory markers, hypertension, elevated triglycerides, and reduced HDL cholesterol levels (metabolic syndrome).34,35 The close association between aging, obesity, and this constellation of risk factors of metabolic syndrome clearly contributes to the detrimental effects of glycemia on endothelial function. It is quite plausible that healthy subjects with upper tertile blood glucose had metabolic syndrome and were insulin-resistant. There were relatively small numbers of smokers in this study. The relationship between smoking and FMD, and indeed IMT, is well known from previous publications.13,20,36

Regression equations are designed to explain the largest proportion of the variance in the model rather than to identify biologically relevant parameters involved in disease pathogenesis. As such, WHR, male gender, or age may act as composite markers of a number of cardiovascular risk factors and therefore appear as strong independent predictors of endothelial function in these analyses. Similarly, exclusion of a parameter does not preclude it from directly contributing to the pathogenesis of the disorder; merely, the variance attributed to the parameter is accounted for by the other variables. In our cross-sectional analyses, SBP and DBP closely correlated with glycemia and endothelial function, yet they were not independent predictors in regression analyses, probably confounded by other parameters such as increasing age.

LDL cholesterol is clearly associated with the development of cardiovascular disease.37 In this study, there was a significant relationship between increasing total and LDL cholesterol profiles and glycemia, although they were not independent predictors of vascular reactivity. LDL cholesterol levels have been reported to contribute to impaired endothelial function and increased IMT in patients with type 2 diabetes.38 Modification of LDL cholesterol, for instance by glycation as in diabetic subjects, oxidation, or incorporation into immune complexes appears to be a major cause of endothelial and vascular smooth muscle dysfunction.22,30

We have previously established that the effects of diabetes are probably similar in Chinese and white subjects21 but that other risk factors may have differential impact on different racial groups.12,13,20 Therefore, caution must be applied when extrapolating these results to white populations. We have also previously shown that increasing Westernization is associated with greater susceptibility to the pro-atherogenic effects of traditional cardiovascular risk factors.20 Furthermore, given the close relationship between diabetes, which represents the extreme of the range of glycemia, and vascular disease, it is not surprising that lower glucose levels also contribute to deteriorating vascular function.

In summary, increasing levels of glycemia, particularly in the “high normal” range, and the coexistence of other cardiovascular risk factors in this group of apparently healthy southern Chinese subjects were adversely associated with vascular reactivity and arterial wall thickening. The observation of these potentially deleterious effects within what is currently defined as “normal” suggests that non-diabetic subjects with “high normal” fasting blood glucose levels may also be at increased cardiovascular risk.

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