The Association of Longitudinal Trend of Fasting Plasma Glucose With Retinal Microvasculature in People Without Established Diabetes

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Plasma glucose is an important and modifiable factor that relates closely to cardiovascular health. Among people with diabetes, vascular insults from abnormal glucose metabolism have been investigated for decades. Hyperglycemia is believed as of paramount importance in incurring vascular deteriorations,1 and detrimental impacts of longitudinal glucose fluctuation and trend among diabetic people also have been proposed recently and discussed.2–8

Diabetes has been defined traditionally based on fasting plasma glucose and 2-hour value of oral glucose tolerance test (OGTT), and glycated hemoglobin that was proposed recently. Arbitrarily cutoffs often are chosen to classify those with and without diabetes because limited evidences suggest that people with measurements above these cutoffs are more likely to have retinal microvasculopathy.9–10 a specific vascular complication of diabetes. However, this has been challenged by a multiethnic study where it was reported that an apparent cutoff did not exist in terms of the prevalence of retinopathy and its association with plasma glucose level.11 This may suggest that vascular damage from abnormal glucose metabolism, such as greater glycemic level, glucose fluctuation, and rising trend, might already have developed among people without established diabetes. Nevertheless, these conjectures rarely have been validated.

With regard to the ultimate goal of preventing lethal vascular events, detection, and prevention of early vascular insults are of great significance, especially among people without severe cardiovascular diseases. Retinal vessel is a specific part of the vascular system that can be observed noninvasively and quantitatively in vivo,12 and, thus, is a better entity in assessing early changes of the vasculature. Narrowed retinal arterioles and widened venules have been described as surrogates of systemic microvascular change,13,14 and also are considered as predictors for major vascular events.12 Associations of altered retinal vascular caliber with various cardiovascular risk factors, such as hypertension, diabetes, obesity, and dyslipidemia, have been reported by some cross-sectional studies.15–17 Retinal arteriolar and venular calibers increase significantly among people with impaired fasting glucose, and further increase for those with established diabetes,18 and consistently reported positively associated with glucose level in the general population and diabetic people.17,19 However, no information exists regarding long-term glucose level, longitudinal glycemic trend, and fluctuation, and specifically for people without diabetes.

In this report, we assessed the associations of long-term glucose level, longitudinal glycemic trend, and fluctuation with retinal vascular caliber in a cohort of Chinese people without diabetes.
established diabetes at baseline, using 5-year annual data on fasting plasma glucose (FPG).

METHODS

Study Design

The Lingtou Eye Cohort Study is an ongoing prospective study investigating associations of retinal abnormalities with systemic cardiovascular and metabolic conditions. Participants were enrolled through the Guangzhou Government Servant Physical Check-up Center, Lingtou, Guangzhou. This population was chosen because of the availability of annual visit data and the high retention rate in the long-term follow-up visits. The study was approved by the Ethics Committee of the Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Individuals, aged 40 years or older, without history of major cardiovascular events, such as stroke and myocardial infarction, were considered eligible for the study. Participants recruitment was conducted from March to December, 2008. A short set of questions on individual’s age and accompanied disease was carried out to identify eligible participants. Subjects with hypertension, diabetes, or dyslipidemia were sampled at higher rates than the others because these individuals were thought to be at increased risk of developing cardiovascular diseases. Baseline evaluations were performed in 2008 that included physical and ocular examinations as well as questionnaire administered during an in-person interview. Height, weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG, triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), and high density lipoprotein cholesterol (HDL-c) were measured per standardized protocols throughout the study. Lifestyle patterns, such as dietary preference, cigarette smoking, physical activity, and sedentary behavior, were collected and detailed medical history, including medication use and physician diagnoses of cardiovascular and metabolic disorders (confirmed by medical records), were recorded. All devices were calibrated every week before the use on patients. Body mass index (BMI, weight [kg]/height [m]$^2$) and mean arterial pressure (MAP, 1/3 SBP plus 2/3 DBP) were calculated. All the subjects who participated in the baseline survey were invited to take part in the subsequent annual re-examinations during the follow-up period. Procedures of the follow-up examinations were the same as those at baseline.

At recruitment, a total of 4939 subjects (502 with and 4437 without diabetes) were confirmed eligible and included in the study. The numbers of subjects who participated in the follow-up examination in 2009, 2010, 2011, and 2012 were 4375, 3702, and 4893, respectively.

Definition of Baseline Nondiabetes and Consistent Nondiabetes

Individuals without diabetes at baseline were defined as satisfying all of the following: FPG < 7 mmol/L in 2008, denied use of antidiabetes medication (including insulin) according to the baseline questionnaire, and no documented diagnosed diabetes from previous medical records. People with baseline FPG > 5.6 mmol/L were identified as with prediabetes, while those with baseline normal fasting glucose (NFG) were defined as with FPG less than 5.6 mmol/L.

FPG fluctuation was measured as the standard deviation (SD-FPG) and root mean square error (RMSE-FPG) of visit-to-visit FPG levels. Univariate analyses were performed. The intra- and intergrader intraclass correlation coefficients were 0.83 to 0.93 and 0.81 to 0.93, respectively.

Fasting Plasma Glucose Determinations

Fasting plasma glucose levels were measured annually from 2008 to 2012. Before the FPG measurements, participants were enrolled at the physical examination center, where they stayed for 1 to 2 nights and were instructed to fast from 10 PM the evening before their examination. Venous blood was drawn from the antecubital vein between 7:30 AM and 8:30 AM the next morning. Subjects who arrived at the clinic later than 8:30 AM or had calories intake after 10 PM the previous night were instructed to return on another day. Blood samples were collected in tubes precoated with EDTA and fluoride, and centrifuged within 2 hours. Plasma glucose was assayed with the same device (Boehringer Mannheim, Mannheim, Germany) using a glucose-oxidase method.

Measurement of Retinal Vascular Caliber

In 2012, retinal images were taken using a fundus camera (TRC-NW6S; Topcon, Tokyo, Japan). Standard digital photographs centered on the optic disc were taken for each eye, and images of the right eyes were arbitrarily selected for this analysis. Three experienced graders, masked to the participants’ characteristics, identified each vessel coursing through a specific area (half to one disc diameter from the margin of the optic disc) as an arteriole or venule, and selected a segment (within the area) of each for measurement. The diameters of all the selected segments were measured automatically using the software program IVAN (University of Wisconsin, Madison, WI, USA). Measurements of the arterioles and venules from the right eyes were combined for each individual, and summary estimates of the average retinal vascular caliber were calculated according to the Parr-Hubbard formula. 20,21 and represented as the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE). Reliability was assessed by repeated measurements. The intra- and intergrader intraclass correlation coefficients were 0.83 to 0.93 and 0.81 to 0.93, respectively.

Statistical Analysis

In the present analysis, only the baseline nondiabetic participants who received FPG evaluation in 2008 and had a total of 5 or more FPG measurements were included. Long-term FPG level was assessed as the mean of annual FPG measurements. Intraindividual longitudinal trend of FPG was assessed as the linear regression slope of FPG levels across each time point of FPG examination (recorded as year to date), and FPG fluctuation was measured as the standard deviation (SD-FPG) and root mean square error (RMSE-FPG) of visit-to-visit FPG levels.

The 1-way ANOVA was used for comparison of normally distributed continuous variables and the $\chi^2$ test for comparison of frequencies.

The associations of retinal vascular caliber with long-term mean FPG level, longitudinal FPG trend, and FPG fluctuation were examined using univariate and multivariate linear regression analyses. In univariate analyses, variables with a $P$ value of $< 0.1$ in association with retinal vascular caliber were considered potential risk factors and, thus, were included in multivariable analysis. The adjustment on the fellow calibers on the same eye (such as CRVE adjusting for CRAE and CRAE adjusting for CRVE) were used as a proxy for immeasurable variables that may be potential confounding factors, such as blood volume, genetic factors, body size, and magnification.
Glycemic Trend and Retinal Vasculature

RESULTS

Among the 4437 nondiabetic participants at baseline, a total of 3645 (82.2%) were available for present analysis; 792 (17.8%) individuals were excluded because of their missing data on baseline FPG (67.1.5%), less than 3 FPG measurements during 5 years (225, 5.0%), unavailable or ungradable retinal photographs (154, 3.5%), or missing data on other portions of physical examination (346, 7.8%). Comparisons of baseline characteristics of participants included and excluded from the study are demonstrated in Table 1. In general, those included in the study were more likely to be older, male, had lower TC, and greater LDL-C level. Baseline BMI, BP, FPG, TG, and HDL-C did not differ between those included and excluded. Average follow-up time of those included was 4.00 ± 0.20 years.

Of the 3645 participants included, from 2008 to 2012, 2486 (68.2%) participated in all 5 annual FPG measurements, 547 (15.0%) participated in 4 measurements, and 612 (16.8%) participated in 3 measurements. By the end of the fifth visit in 2012, a total of 284 (7.8% of 3645) baseline nondiabetic participants had diabetes mellitus, and the remaining 3361 were consistently nondiabetic.

Characteristics of 5-year glucose profile are shown in Table 2. Among nondiabetic participants, 5-year mean FPG level was approximately 5.3 mmol/L. Approximately two-thirds of nondiabetic participants had an annual rising trend of FPG (FPG trend > 0 mmol/L/year) and the median of this rising trend was approximately 0.1 mmol/L/year. The FPG fluctuation in studied nondiabetic population ranged from 0.3 to 0.4 mmol/L.

Tables 3 and 4 summarize the associations of retinal vascular caliber with 5-year mean FPG level, longitudinal FPG trend, and FPG fluctuation (all FPG values were natural logarithm transformed to meet the hypothesis of linear regression). In models using univariate linear regression analyses, FPG trend was not associated with retinal vascular calibers among baseline nondiabetic participants. After adjusted for age, sex, BMI, MAP, serum lipid levels, baseline FPG, and fellow retinal vascular calibers (Model 1), rising FPG trend was found associated with narrower retinal arterial (β = −20.1, P = 0.022) and wider venule (β = 34.3, P = 0.001). The associations became stronger (β = −27.8, P = 0.008 for CRAE, and β = 36.4, P = 0.004) after potential lifestyle confounders, such as antihypertensive medication use, dietary factors, cigarette smoking, physical activity, and sedentary behavior, were further adjusted for (Model 2). The associations were equivalent to a decrease of 2.65 μm (12.2% of the SD) in CRAE and an increase of 3.47 μm (13.0% of the SD) in CRVE for every 10% annual increase in FPG level. These remained statistically significant when those who had diabetes during the follow-up period were excluded from analysis (for CRAE: β = −36.3, P = 0.003; for CRVE: β = 46.8, P = 0.001). Estimated changes in CRAE and CRVE with the percentage of annual change in FPG are demonstrated in the Figure. Mean FPG level and FPG fluctuation were not associated with retinal vascular caliber among people without diabetes in multivariate analyses (all P > 0.05).

The significant associations of rising FPG trend with retinal vascular calibers were mainly driven by the effects among people with NFG at baseline (n = 2657; β = −40.3, P = 0.002 for CRAE; and β = 52.4, P = 0.001 for CRVE in Model 2), while the associations among those with baseline prediabetes were not statistically significant (n = 1008; β = −1.45, P = 0.941 for CRAE; and β = 7.39, P = 0.737 for CRVE in Model 2).

DISCUSSION

We demonstrate, for the first time to our knowledge, that an annual rising trend in FPG is associated with narrower retinal arterioles and wider venules even among the people
TABLE 3. Association of 5-Year Mean FPG, Longitudinal FPG Trend and Fluctuation With Retinal Arteriolar Caliber in People Without Established Diabetes

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1*</th>
<th>Model 2†</th>
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<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>P</td>
<td>β (95% CI)</td>
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<tr>
<td>Non-DM at baseline, n = 3645</td>
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<tr>
<td>Mean FPG, mmol/L</td>
<td>−11.7 (−18.7, −4.62)</td>
<td>0.001</td>
<td>0.17 (−5.64, 5.97)</td>
</tr>
<tr>
<td>FPG trend, mmol/L/y</td>
<td>−19.1 (−40.1, 1.91)</td>
<td>0.075</td>
<td>−20.1 (−37.3, −2.96)</td>
</tr>
<tr>
<td>FPG fluctuation</td>
<td>0.48 (−17.5, 18.4)</td>
<td>0.958</td>
<td>−6.34 (−20.4, 7.76)</td>
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<tr>
<td></td>
<td>0.49 (−16.7, 17.7)</td>
<td>0.955</td>
<td>−5.86 (−19.4, 7.69)</td>
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<td>Consistent non-DM by the fifth visit, n = 3361</td>
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<tr>
<td>Mean FPG (mmol/L)</td>
<td>−11.5 (−20.9, −1.76)</td>
<td>0.020</td>
<td>1.00 (−6.88, 8.88)</td>
</tr>
<tr>
<td>FPG trend, mmol/L/y</td>
<td>−18.8 (−43.9, 6.35)</td>
<td>0.143</td>
<td>−24.2 (−45.8, −2.56)</td>
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<td>FPG fluctuation</td>
<td>3.31 (−21.9, 28.5)</td>
<td>0.797</td>
<td>−10.9 (−30.8, 9.00)</td>
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<td>−1.96 (−25.6, 19.7)</td>
<td>0.859</td>
<td>−11.3 (−28.3, 5.64)</td>
</tr>
</tbody>
</table>

All FPG values are natural logarithm transformed to meet the hypothesis of linear regression. CI, confidence interval.

* Adjusted for age, sex, BMI, MAP, serum lipid levels (TG, LDL-c, and HDL-c), and fellow retinal vascular caliber at the 5th visit for mean FPG, and baseline FPG level in addition for FPG trend and FPG fluctuation.

† Further adjusted for antihypertensive medications, dietary factors, physical activity, sedentary behavior, and cigarette smoking on the base of Model 1.

TABLE 4. Association of 5-Year Mean FPG, Longitudinal FPG Trend and Fluctuation With Retinal Venular Caliber in People Without Established Diabetes

<table>
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<tr>
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<th>Unadjusted</th>
<th>Model 1*</th>
<th>Model 2†</th>
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<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>P</td>
<td>β (95% CI)</td>
</tr>
<tr>
<td>Non-DM at baseline, n = 3645</td>
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<td></td>
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<tr>
<td>Mean FPG, mmol/L</td>
<td>−1.31 (−9.93, 7.32)</td>
<td>0.766</td>
<td>4.81 (−2.28, 11.9)</td>
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<td>FPG trend, mmol/L/y</td>
<td>19.4 (−6.55, 45.1)</td>
<td>0.140</td>
<td>54.5 (15.4, 55.5)</td>
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<td>FPG fluctuation</td>
<td>24.5 (2.56, 46.5)</td>
<td>0.029</td>
<td>16.7 (−0.53, 33.9)</td>
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<td>19.4 (−1.74, 40.4)</td>
<td>0.072</td>
<td>11.3 (−5.25, 27.9)</td>
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<tr>
<td>Consistent non-DM by the fifth visit, n = 3361</td>
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<tr>
<td>Mean FPG, mmol/L</td>
<td>1.45 (−10.3, 13.2)</td>
<td>0.809</td>
<td>6.78 (−2.87, 16.4)</td>
</tr>
<tr>
<td>FPG trend, mmol/L/y</td>
<td>11.0 (−20.0, 41.9)</td>
<td>0.487</td>
<td>36.3 (9.84, 62.7)</td>
</tr>
<tr>
<td>FPG fluctuation</td>
<td>20.2 (−10.8, 51.2)</td>
<td>0.202</td>
<td>11.2 (−13.1, 35.6)</td>
</tr>
<tr>
<td></td>
<td>15.4 (−11.3, 42.0)</td>
<td>0.259</td>
<td>8.35 (−12.5, 29.2)</td>
</tr>
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</table>

All FPG values are natural logarithm transformed to meet the hypothesis of linear regression.

* Adjusted for age, sex, BMI, MAP, serum lipid levels (TG, LDL-c, and HDL-c), and fellow retinal vascular caliber at the 5th visit for mean FPG, and baseline FPG level in addition for FPG trend and FPG fluctuation.

† Further adjusted for antihypertensive medications, dietary factors, physical activity, sedentary behavior, and cigarette smoking on the base of Model 1.

without established diabetes, while long-term mean FPG level and FPG fluctuation are not associated with retinal vascular caliber. This novel finding indicates potential vascular risks from rising glucose trend, before the diagnosis of diabetes.

Unlike macro-vasculopathies, diabetic retinal lesions are considered as vascular insults caused specifically by abnormalities of glucose metabolism. Considerable prevalence (varying at approximately 10%) of typical retinal microvascular signs reported among nondiabetic people may suggest other cardiovascular risk factors, such as hypertension, may have a role as suggested by some studies; however, this also may be attributable to the long-term glycemic fluctuation and rising trend as suggested by the current study. In diabetic populations, elevation and fluctuation of glucose are considered to be potentially harmful for the vasculature. In nondiabetic populations, however, vascular impacts of their glucose metabolism seldom have been investigated.

General narrowing in retinal arterioles and widening in venules are not as specific as the typical lesions of diabetic retinopathy, but are considered as early signs of vascular change. Altered retinal vascular calibers are associated with retinal vascular disorders and even with systemic cardiometabolic conditions. Narrowed retinal arterioles and widened venules suggest early metabolic changes associated with systemic vascular health, such as hypoxia, inflammation, and endothelial dysfunction. Retinal vascular changes have been considered as predictors for macrovascular disorders, as well as microvasculopathies. Widened retinal venules are potential indicators of incidence and progression of diabetic retinopathy, and incident renal microvasculopathy. Narrower retinal arterioles and wider venules are proved further to be associated with mortality and morbidity of coronary heart disease, and wider retinal venules are also proved associated with incident stroke.
Several previous studies have investigated associations of retinal vascular caliber with serum glucose level and diabetes. Elevated serum glucose has been proved associated with larger retinal arterioles and venules. Increased retinal arterioles and venules also have been observed among people with impaired fasting glucose or diabetes. Besides elevated serum glucose, glycemic fluctuation and rising trend are two potential patterns of abnormal glucose metabolism that may be relevant to the insults of microvasculature among nondiabetic people. However, associations of these glycemic alterations with retinal vascular caliber have seldom been examined in such a population.

To our knowledge, our study is the first to investigate associations of retinal vascular caliber with long-term glucose level, longitudinal glycemic trend, and fluctuation among people without diabetes. Results from our study suggested that annual rising fasting glucose, even when it is in normal range, is common in people without established diabetes and is associated with altered vasculature in the retina and probably also is associated with changes of systemic vasculature. One may note that the effects of rising glucose trend on retinal vascular caliber are modest: 10% annual rising of FPG would result in approximately 2.65 μm (equivalent to 12.2% of the SD) narrowing of CRAE and 3.47 μm (equivalent to 13.0% of the SD) widening of CRVE in 5 years. Although this level of effects was comparable to other identified risk factors, such as age, hypertension, and obesity, further studies that look into how these modest changes may lead to pathologic changes on the vessels would be very important to explore.

Being relatively simple and inexpensive, fasting plasma glucose measurement is widely used in clinical practice, either as a routine in medical management for patients or in regular checkup for healthy people. It can identify individuals with
Glycemic Trend and Retinal Vasculature

References


