Investigation of the association between lens autofluorescence ratio and diabetes: a cross-sectional study

Abstract

Aims/hypothesis Lens autofluorescence ratio (LFR) is a novel approach to detect advanced glycation end products in a time-saving and non-invasive manner. However, its associations with glycemia and diabetes remain unclear. We conducted this study to address this issue in Chinese adults.

Methods We enrolled a total of 4,705 participants aged 20-70 years in China between May 2020 and January 2021 in a cross-sectional study. LFR was determined by biomicroscopy (ClearPath DS-120). Diabetes was ascertained by oral glucose tolerance test, self-reported history, and/or antidiabetic medication use. Correlation and logistic regression analyses were performed.

Results LFR was higher in participants with diabetes than those without (23.27 ± 6.51 vs. 19.45 ± 5.08, $p < 0.001$). LFR correlated with fasting plasma glucose and hemoglobin A1c in the overall and diabetes-stratified populations. The odds of diabetes was increased by 6% per one percent higher of LFR after multivariable-adjustment (odds ratio (OR) 1.06, 95% CI 1.04-1.08, $p < 0.001$). Participants in the highest quartile of LFR had higher odds of diabetes compared with those in the lowest quartile (OR 1.83, 95% CI 1.33-2.52, $p < 0.001$). Mediation analysis showed that, insulin resistance, as assessed by triglyceride-glucose index, may underline the relationship between high LFR and increased odds of diabetes.

Conclusions LFR, a non-invasive indirect measure of advanced glycation end products, appears to be associated with glycemia and the risk of developing diabetes in Chinese adults.

Keywords Advanced glycation end products; lens autofluorescence ratio; diabetes; glycemia

1. Introduction

Advanced glycation end products (AGEs), formed in a multistep process by glycation and oxidation of free amino groups of proteins, lipids and nucleic acids [1], are reported to underpin the development of diabetes and associated complications. AGEs are commonly measured in serum, using the radioreceptor assay, enzyme-linked immunosorbent assay, or high-performance liquid chromatography. Yet procedures from these approaches are both labor-intensive and time-consuming. In this context, skin AGEs, assessed by skin autofluorescence (SAF), have come into use as a non-invasive and convenient measure [2]. However, results from this measurement are often affected by the skin reflection, the application of creams, and the extreme vasodilatation and vasoconstriction at the measurement site [3].

Other than in serum or in the skin, recent studies suggest that AGEs can also be detected in the lens by autofluorescence based on the fluorescent properties of AGEs accumulated in lens. To this end, lens fluorescence ratio (LFR) measured by confocal biomicroscopy was developed as a novel, rapid, and non-invasive technique for the measurement of AGEs. There is accumulating evidence that LFR is augmented in people with diabetes or diabetic peripheral neuropathy [4-7]. For example, in a comparative study of patients with type 2 diabetes (T2D) (n = 82) and healthy subjects (n = 109), LFR was found to be higher in the T2D patients than in the healthy subjects. LFR was also found to be positively related to hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) [7]. However, interpretations of these observations were largely limited by the small sample sizes and the potential for selection bias. It remains unclear as to whether LFR is related to glycemia in the general population and, if so, why.

Insulin resistance (IR) is key to the pathogenesis of diabetes [8]. Several studies have shown that serum AGEs levels are related to IR in non-diabetic subjects [9, 10]. Moreover, inhibition of the formation of AGEs was shown to improve IR in diabetic rodents [11]. These observations suggest a potential role of IR in linking LFR to diabetes. As a result, it would be of interest to examine whether IR could mediate the association of LFR with diabetes in the real-world setting.

Given these, the primary aim of the present study was to investigate the associations of LFR with glycemia and the risk of diabetes in the general population, and the secondary aim was to evaluate the relevance of IR, assessed by triglyceride-glucose (TyG) index [12], to the association of diabetes.

2. Methods

2.1. Study design and participants

This multi-center, cross-sectional study was conducted in 8 provinces in China between May 2020 and January 2021, enrolling a total of 5,201 participants. Participants were excluded from the analysis if they were pregnant, had mental illness or other diseases precluding them to complete the procedures of this survey, had a history of previous removal of the crystalline lens and replacement with an intraocular lens implant, had a fluorescence angiogram within the past 6 months, received photo dynamic therapy within the past year, had ocular surface (dry eye) disease, or were unable to cooperate or understand clinical instructions. We included a total of 5,056 participants aged 20-70 years

that completed study questionnaires, physical and laboratory examination, and LFR measurement. After further exclusion of 324 subjects due to missing data on laboratory indices (including FPG and 2-hour plasma glucose [2h-PG]), and 27 because of outliers on LFR (that is, ≤ 5% or ≥ 50% [http://www.sinocare.com/web/product/detail?id=45]), data from 4,705 participants were included in the final data analysis (Figure 1).

The study protocol was approved by the ethics committee of Zhongda hospital, Southeast University and other participating institutes. Written informed consent was obtained from each participant prior to the start of this study.

2.2. Clinical and biochemical examination

Information on demographics (including age and gender), health behaviors (including history of smoking and drinking) and medical history (including hypertension, dyslipidemia, diabetes, cardiovascular disease, eye disease, and medication use) were collected by well-trained interviewers. Body weight, height, as well as waist circumference (WC) were measured according to the standard protocols. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured three times by automated sphygmometer (YE680E, yuwell, China), with the averages being used. All eligible participants were informed to maintain their usual lifestyle for at least 3 days and instructed to fast at least 10 hours before ingesting a 75 g glucose solution. Venous blood samples were taken before and 2 hours after an oral glucose tolerance test. All blood samples were centrifuged on site within 30 minutes after collection and then transported at 4 °C by air to the central laboratory. An automatic chemistry analyzer (Synchron LX-20, Beckman Coulter Inc., CA, USA) was used to measure FPG, serum triglyceride (TG), total cholesterols (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and serum creatinine. HbA1c was determined using a D-10 Hemoglobin Analyzer (Bio-Rad Inc., Hercules, CA, USA) based on the HPLC method. Body mass index (BMI) was calculated as body weight (kg) divided by squared height (m). The Cockcroft-Gault CCr formula was used for computing estimated glomerular filtration rate (eGFR) [13]. TyG index was calculated as: ln [TG (mg/dL) × FPG (mg/dL)/2] [14].

2.3. Measurement of LFR

LFR, which reflects the ratio of the autofluorescence of the lens to the scattered light in the center of the lens [5], was measured using a lens fluorescence biomicroscope optical system (ClearPath DS-120®, Freedom Meditech, San Diego, California, United States). The lens fluorescence biomicroscope contains a blue (465 nm) LED excitation light source that generates blue excitation light. Upon the elastic and inelastic interactions on the lens proteins, the scattered light from the blue excitation light will be split into the blue-green and green (fluorescence) segments by a rotating filter wheel. Under the control by software, the scattered light, which goes from the posterior lens capsule to the anterior lens capsule, will be automatically detected. The average ratio of the lens autofluorescence to scattered light (that is, LFR) in the central portion of the lens is then calculated.

2.4. Definitions of diabetes

Diabetes was ascertained by either FPG ≥ 7.0 mmol/L, 2h-PG ≥ 11.1 mmol/L, use of antidiabetic drugs, and/or self-reported history, according to the 1999 WHO diagnostic criteria [15].

2.5. Statistical analyses

Continuous variables were presented as means (standard deviations) or medians (interquartile ranges), whereas categorical variables were presented as numbers (percentages). Differences were compared using the Student's t-test or Mann–Whitney test where appropriate. Pearson correlation analysis with or without adjusting for age was conducted to quantify the associations between LFR and variables regarding glycemic control (that is, FPG, 2h-PG and HbA1c).

Logistic regression analyses were performed to examine the association of LFR with diabetes based on 3 models: Model 1, having LFR as the only predictor without adjustment; Model 2, having LFR as the predictor adjusted for age, sex and BMI; and Model 3, having LFR as the predictor, additionally adjusted for WC, HDL-C, TC, TG, SBP, HbA1c and eGFR based on Model 2. In these models, a median value in each quartile of LFR was used to test the linear trends. The primary analysis was based on completed cases in which participants with missing values in covariates were excluded. However, a sensitivity analysis was performed, which enrolled all participants with missing values in covariates imputed using multiple imputation methods. Moreover, we also conducted a sensitivity analysis by including participants with outliers on LFR (that is, ≤ 5% or ≥ 50%) to assess whether this would affect our main outcome. Furthermore, since there is evidence that LFR was higher in cataract groups than age-matched controls and correlated with the severity of nuclear opalescence and color grades [16, 17], we conducted another sensitivity analysis upon the exclusion of participants with known history of cataract. Subgroup analysis on the odds of diabetes in association with LFR was performed based on the following variables: gender (male vs female), age (≥ 45 vs < 45 years old), TC (≥ 5.2 vs < 5.2 mmol/L), BMI (≥ 24 vs < 24 kg/m^2) and eGFR (≥ 90 vs < 90 ml/min/1.73m^2), respectively. Interactions between LFR and gender, age, BMI, TC and eGFR were also explored.

Mediation analysis was performed to assess the total, direct and indirect effects of LFR on diabetes in relation to TyG index. Two-tailed p-value < 0.05 was considered statistically significant. All analyses were performed using the SPSS 25.0, (SPSS Inc., Chicago, IL, USA) and Empower Stats software (X&Y Solutions, Inc., Boston, USA).

3. Results

3.1. Characteristics of study population

The demographic and clinical characteristics of enrolled participants are presented in Table 1. The prevalence of diabetes was 14.2%. Compared with those without diabetes, participants with diabetes were older, and had a higher BMI, WC, blood pressure, FPG, 2h-PG, HbA1c, TC, LDL-C, TG, eGFR and LFR (all $p < 0.001$).

3.2. LFR and glycemia

There were positive relationships between LFR and glycemic indices, including FPG, 2h-PG and HbA1c, in the overall and non-diabetes population. However, the magnitudes of these correlations were weakened after adjusting for sex, age, and BMI, in particular in the overall population (Table 2). In the diabetes group, LFR was related to only FPG and HbA1c, but these relationships were no longer evident after adjusting for sex, age, and BMI (Table 2).

3.3. LFR and diabetes

Table 3 shows the results of the univariate and multivariate associations of LFR with diabetes. The odds of diabetes was increased by 13% (odds ratio (OR) 1.13, 95% CI 1.11-1.15) per one percent higher of LFR in the unadjusted model (Model 1), and 6% (OR 1.06, 95% CI 1.04-1.08) after adjusting for multivariable including age, sex, BMI, WC, HDL-C, TC, TG, SBP, HbA1c and eGFR (Model 3). Categorizing LFR into quartiles, participants in the highest quartile showed higher odds of diabetes compared with those in the lowest quartile after multivariable-adjustment (OR 1.83, 95% CI 1.33-2.52; Model 3). Moreover, sensitivity analyses, which included participants with imputations on missing vales (Table 3) or outliers on LFR (Supplement Table 1), or excluded participants with known history of cataract (Supplement Table 1) showed similar results. Subgroup analysis showed that the association of LFR with diabetes was unaffected by any predefined variables (listed in the Methods) after multivariable-adjustment (all *p* interaction > 0.05) (Figure 2).

3.4. Mediation analysis on the relationship between LFR and diabetes

Figure 3 shows the results of the mediation analysis on the relationship between LFR and diabetes. The association between LFR and diabetes was found to be mediated by TyG index (a surrogate of IR), with a mediation effect of 27.7% (bootstrap 95% CI: 0.022 - 0.031, $p < 0.001$).

4. Discussion

In this multicenter, cross-sectional study of the community-based Chinese population, we observed that (i) LFR was related to glycemic indices (e.g., FPG and HbA1c), (ii) larger LFR was associated with higher risk of diabetes, independent of age, sex, BMI, WC, HDL-C, TC, TG, SBP, HbA1c and eGFR, and (iii) insulin resistance (evaluated by TyG index) might underpin the association between LFR and diabetes. These observations, collectively, might provide evidence supporting that LFR, a non-invasive measure of AGEs, could be used as a marker of hyperglycemia, and potentially an indicator for identifying or predicting diabetes.

To the best of our knowledge, the current study provided the first evidence on the relationship between LFR and glucose metabolism in the general population, which extended insights made in selective groups of patients with diabetes [6, 7]. In the latter, LFR correlated with HbA1c in patients with uncomplicated type 2 diabetes or those with proliferative diabetic retinopathy. There is also recent evidence suggesting that LFR is related to average blood glucose levels and diabetes duration [5]. These observations suggest that both the duration and the degree of exposure to hyperglycemia are likely to be major determinants of LFR. In keeping with this concept, we found that the relationship between LFR and FPG ($r = 0.30$, $p < 0.001$) or HbA1c ($r = 0.29$, $p < 0.001$) was relatively modest in the general population, but was demonstrably stronger in patients with diabetes. Yet we noted that these correlations were weaker after adjusting for sex, age, and BMI, which, however, should not be surprising given that the latter are recognized risk factors for diabetes [18, 19]. That the relationship between LFR and glycemic indices in the overall and non-diabetic populations remained significant after adjusting for sex, age, and BMI supports the concept that accumulation of AGEs may be responsible for the development

of hyperglycemia [20]. Indeed, elevated skin and lens AGEs often precede with the onset of diabetes [6, 21]. Circulating AGEs were reported to correlate negatively with 2h-PG in healthy individuals (r = - 0.31, $p < 0.05$) [22], but positively with 2h-PG in those at risk of diabetes (r = 0.75, $p < 0.001$) [23]. In contrast, we observed a positive correlation between LFR and 2h-PG in the general population, but not in patients with diabetes. It is unclear whether this disparity reflects the differences in LFR and circulating AGEs. Moreover, serum AGEs are known to be influenced by dietary intake; in patients with type 1 and type 2 diabetes, consumption of a low-AGE diet over two months was associated with a reduction in serum AGEs [24, 25]. To date, there is a lack of research evaluating the impact of dietary intake on LFR.

To follow the investigation of the association of LFR with glycemia, we observed that after adjusting for age, sex, BMI, WC, blood lipids, SBP, HbA1c and eGFR, the risk of diabetes were increased by 6% for every one percent higher of LFR, and that participants in the highest quartile of LFR had higher risk of diabetes than those in the lowest quartile (OR 4.28). Moreover, subgroup analyses revealed that the risk of diabetes with LFR was not affected by TC, age, BMI, eGFR and sex after adjusting for age, sex, BMI, WC, HDL-C, TC, TG, SBP, HbA1c and eGFR, which may support the robustness of our results, at least partly.

Our mediation analysis is indicative of insulin resistance (assessed by TyG index) as a mediator of the relationship between LFR and diabetes. Several lines of evidence support the relevance of AGEs to the development of insulin resistance. In healthy participants, serum levels of AGEs correlate with insulin resistance, independent of age, gender, BMI, WC, smoking, adiponectin or markers of oxidative stress and inflammation[9, 10]. The mechanistic studies in wild-type mice and β-cells revealed that AGEs impaired insulin secretion, signaling, and clearance, via either direct modification of insulin or the receptors for AGEs (RAGE) [26, 27]. In addition, AGEs have the potential to promote inflammation via stimulation of PKCα and upregulation of TNFα [1].

Several limitations should be noted while interpreting our findings. First, despite the efforts to control for different covariables, our results might still be subject to unobserved confounding factors. For example, dietary intake is shown to affect the levels of AGEs [28, 29], while it was not comprehensively assessed, which may weaken the robustness of our findings potentially. Second, LFR was reported to be higher in participants with cataract than controls and might correlate with the severity of nuclear opalescence and color grades [16, 17]. Yet we did not ascertain the presence or severity of cataract by ophthalmologists with reference to the Lens Opacities Classification System but rather based on self-reported history. This may also potentially affect the robustness of our findings, although our sensitivity analysis, which excluded participants with known history of cataract, showed comparable outcomes. Moreover, there is the possibility that corneal opacities or the measuring position (on the eye) might also affect the determination of LFR, while we were not able to evaluate their influences on our present results. Third, we did not assess the impacts of antidiabetic drugs on LFR, although there was only a very small proportion of participants (3.2%) treating with these drugs in our study. Fourth, because we did not measure the circulating levels of C-peptide, insulin, or some specific antibodies such as glutamic acid decarboxylase antibody, the types of diabetes cannot be specified. However,

given that type 1 diabetes accounts for only 5-10% of all cases with diabetes and is prevalent in participants aged < 20 years [18], it seems that the vast majority of participants diagnosed with diabetes would be type 2 diabetes in our study. In view of this, our findings might be more applicable to participants with type 2 diabetes. Fifth, there is the controversy on the wavelengths to detect the autofluorescence from AGEs. For example, Karumanchi et al. showed that the excitation wavelength at 435 nm rather than at 340 nm appeared to be more suitable to detect the differences in fluorescence intensity between non-diabetic and diabetic lenses [30], while others suggested that to be at 365 nm [31]. It remains to be explored whether such a discrepancy would affect the association of LFR with diabetes observed in our study that employed the excitation wavelength at 465 nm. Finally, our study was conducted mainly in Chinese population, such that the results may not be generalizable to other populations. Moreover, our study was cross-sectionally designed and the findings are needed to be validated by prospective cohort studies.

5. Conclusions

LFR, which is a non-invasive indirect measure of AGEs, appears to be correlated with glycemia and associated with increased risk of developing diabetes in Chinese adults. These observations may provide the support for the measurement of LFR in the prevention and management of diabetes.

DECLARATIONS

Conflicts of interest

We declare that we have no conflicts of interest.

Founding

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Figures

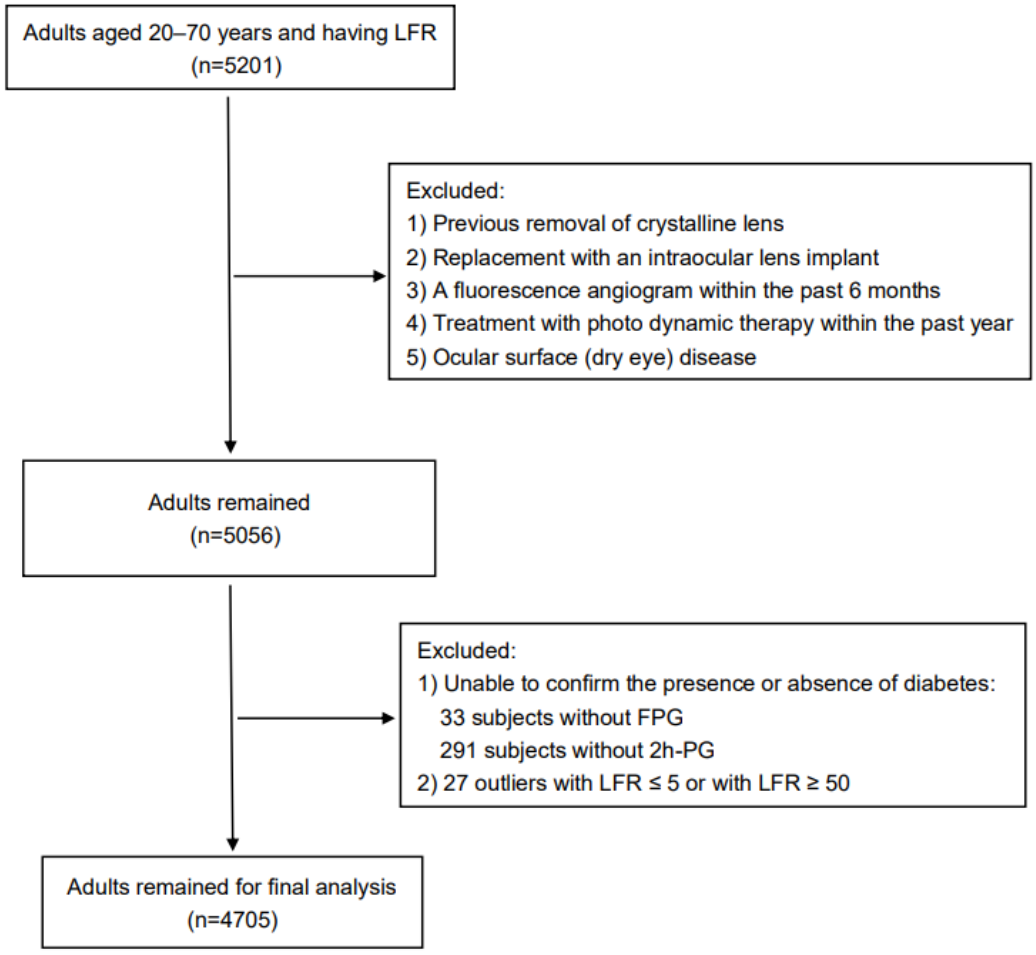

Figure 1. Flow chart of this study.

LFR, lens fluorescence ratio; FPG, fasting plasma glucose; 2h-PG, 2-hour plasma glucose.

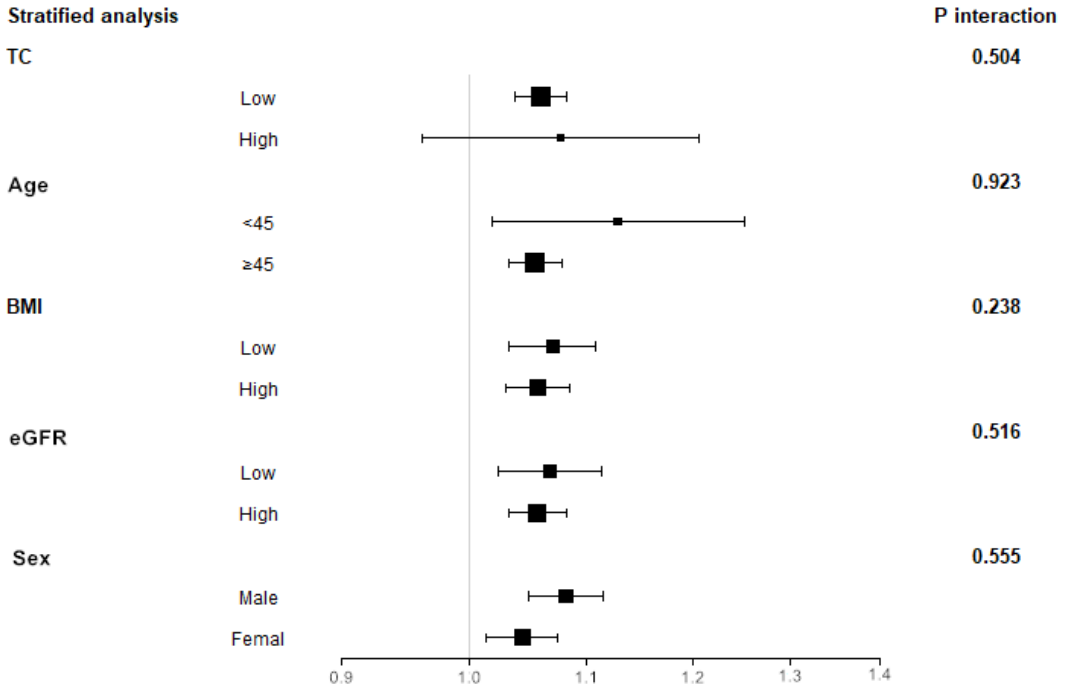

Figure 2. Subgroup analyses of the association between LFR and odds of diabetes.[a]

LFR, lens fluorescence ratio; BMI, body mass index; WC, waist circumference; HDL-C, high-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride; SBP, systolic blood pressure; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate; OR, odds ratio; CI, confidence interval.

[a] Adjusted for age, sex, BMI, WC, HDL-C, TC, TG, SBP, HbA1c and eGFR.

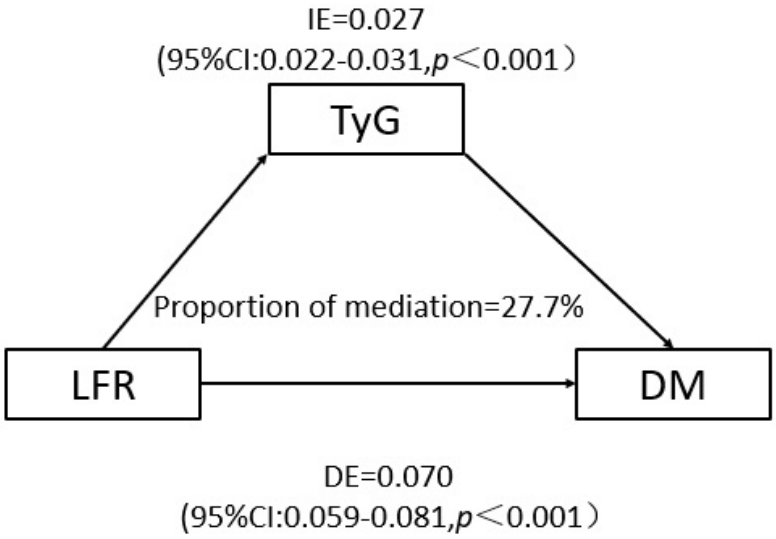

Figure 3. Mediation analysis on the relationship between LFR and diabetes.
TyG, triglyceride-and-glucose; LFR, lens fluorescence ratio; DM, diabetes; IE, indirect effect; DE, direct effect

Tables

Table 1 Characteristics of the study population.

Characteristics	Overall (n = 4705)	Non-diabetes (n =4036)	Diabetes (n = 669)	*p* value
Age (years)	55.94±9.25	55.43±9.42	59.03±7.40	< 0.001
Sex (male/female)	1626/3079	1341/2695	285/384	< 0.001
BMI (kg/m^2)	25.32±3.82	25.11±3.79	26.54±3.79	< 0.001
WC (cm)	84.87±10.53	84.14±0.43	89.29±10.03	< 0.001
SBP (mmHg)	134.54±20.08	133.27±19.65	142.18±20.91	< 0.001
DBP (mmHg)	83.87±12.39	83.37±12.37	86.85±12.11	< 0.001
FPG (mmol/L)	5.63±1.43	5.23±0.49	8.11±2.40	< 0.001
2h-PG (mmol/L)	7.24±2.30	6.83±1.66	12.77±2.59	< 0.001
HbA1c (mmol/mol)	39.83±10.89	37.21±6.19	55.65±17.61	< 0.001
HbA1c (%)	5.79±1.00	5.55±0.57	7.24±1.61	< 0.001
TC (mmol/L)	4.71±0.95	4.67±0.92	4.96±1.06	< 0.001
HDL-C (mmol/L)	1.43±0.29	1.43±0.28	1.43±0.31	0.514
LDL-C (mmol/L)	2.62±0.69	2.60±0.67	2.75±0.78	< 0.001
TG (mmol/L)	1.63±1.51	1.53±1.32	2.24±2.29	< 0.001
eGFR (ml/min/1.73m^2)	111.46±31.71	110.38±30.66	117.96±36.77	< 0.001
LFR (%)	19.99±5.47	19.45±5.08	23.27±6.51	< 0.001

Data are presented as means ± standard deviations, or numbers.

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; 2h-PG, 2-hour plasma glucose; HbA1c, hemoglobin A1c; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglyceride; eGFR, estimated glomerular filtration rate; LFR, lens fluorescence ratio

Table 2. Correlations between lens fluorescence ratio and glycemia.

	LFR		
	Overall	Non-diabetes	Diabetes
Simple correlations			
FPG	0.30 (< 0.001)	0.10 (< 0.001)	0.36 (< 0.001)
2h-PG	0.12 (< 0.001)	0.13 (< 0.001)	-0.03 (0.606)
HbA1c	0.29 (< 0.001)	0.10 (< 0.001)	0.36 (< 0.001)
Age, sex and body mass index-adjusted partial correlations			
FPG	0.11 (< 0.001)	0.11 (< 0.001)	0.02 (0.753)
2h-PG	0.11 (< 0.001)	0.11 (< 0.001)	-0.03 (0.565)
HbA1c	0.11 (< 0.001)	0.10 (< 0.001)	0.08 (0.192)

Data are presented as r (*p*).

LFR, lens fluorescence ratio; FPG, fasting plasma glucose; 2h-PG, 2-hour plasma glucose; HbA1c, hemoglobin A1c

Table 3. Univariate and multivariate logistic regression analyses.

Variable	Model 1		Model 2		Model 3	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Main analysis						
Per one percent higher of LFR	1.13 (1.11,1.15)	< 0.001	1.14 (1.12,1.16)	< 0.001	1.06 (1.04,1.08)	< 0.001
LFR (%)						
Q1 (<16.06)	1 (Ref)		1 (Ref)		1 (Ref)	
Q2 (16.06-19.30)	1.38 (1.04,1.85)	0.027	1.47 (1.09,1.97)	0.011	1.18 (0.84,1.66)	0.350
Q3 (19.30-23.26)	1.97 (1.50,2.59)	< 0.001	2.14 (1.62,2.83)	< 0.001	1.13 (0.81,1.59)	0.470
Q4 (>23.26)	4.28 (3.32,5.52)	< 0.001	4.85 (3.72,6.32)	< 0.001	1.83 (1.33,2.52)	< 0.001
p for trend	< 0.001		< 0.001		< 0.001	
Sensitivity analysis (Missing values in covariates were imputed with multiple imputation methods)						
Per one percent higher of LFR	1.13 (1.11,1.15)	< 0.001	1.14 (1.12,1.16)	< 0.001	1.06 (1.04,1.08)	< 0.001
LFR (%)						
Q1 (<16.06)	1 (Ref)		1 (Ref)		1 (Ref)	
Q2 (16.06-19.30)	1.38 (1.04,1.85)	0.027	1.47 (1.09,1.97)	0.011	1.18 (0.84,1.67)	0.337
Q3(19.30-23.26)	1.97 (1.50,2.59)	< 0.001	2.14 (1.62,2.83)	< 0.001	1.15 (0.82,1.62)	0.421
Q4 (>23.26)	4.28 (3.32,5.52)	< 0.001	4.85 (3.72,6.32)	< 0.001	1.86 (1.35,2.58)	< 0.001
p for trend	< 0.001		< 0.001		< 0.001	

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; HbA1c, hemoglobin A1c; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; eGFR, estimated glomerular filtration rate; LFR, lens fluorescence ratio; CI: confidence interval; OR: odds ratio

Model 1 had LFR as the only predictor.

Model 2 had LFR as a predictor adjusted for age, sex and BMI.

Model 3 had LFR as a predictor adjusted for age, sex, BMI, WC, HDL-C, TC, TG, SBP, HbA1c and eGFR.

Supplement Table 1. Sensitivity analyses on the association of LFR with odds of diabetes.

Variable	Model 1		Model 2		Model 3	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Analysis upon the inclusion of participants with outliers on LFR (<5% or ≥50%)						
Per one percent higher of LFR	1.12(1.11,1.14)	< 0.001	1.14(1.12,1.15)	< 0.001	1.06(1.04,1.08)	< 0.001
LFR (%)						
Q1 (<16.06)	1 (Ref)		1 (Ref)		1 (Ref)	
Q2 (16.06-19.30)	1.32(1.00,1.76)	0.052	1.39(1.04,1.86)	0.026	1.10(0.79,1.54)	0.570
Q3(19.30-23.26)	1.92(1.47,2.51)	< 0.001	2.07(1.58,2.73)	< 0.001	1.09(0.78,1.53)	0.605
Q4 (>23.26)	4.12(3.21,5.28)	< 0.001	4.68(3.61,6.06)	< 0.001	1.82(1.32,2.51)	< 0.001
p for trend	< 0.001		< 0.001		< 0.001	
Analysis upon the exclusion of participants with history of cataract						
Per one percent higher of LFR	1.12(1.11,1.13)	< 0.001	1.13(1.12,1.14)	< 0.001	1.06(1.05,1.07)	< 0.001
LFR (%)						
Q1 (<16.06)	1 (Ref)		1 (Ref)		1 (Ref)	
Q2 (16.06-19.30)	1.09(0.94,1.25)	0.251	1.11(0.96,1.13)	0.161	0.99(0.84,1.17)	0.922
Q3(19.30-23.26)	1.70(1.49,1.94)	< 0.001	1.77(1.55,2.03)	< 0.001	1.11(0.94,1.30)	0.222
Q4 (>23.26)	3.36(2.96,3.82)	< 0.001	3.67(3.22,4.19)	< 0.001	1.75(1.49,2.05)	< 0.001
p for trend	< 0.001		< 0.001		< 0.001	

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; HbA1c, hemoglobin A1c; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; eGFR, estimated glomerular filtration rate; LFR, lens fluorescence ratio; CI: confidence interval; OR: odds ratio

Model 1 had LFR as the only predictor.

Model 2 had LFR as a predictor adjusted for age, sex and BMI.

Model 3 had LFR as a predictor adjusted for age, sex, BMI, WC, HDL-C, TC, TG, SBP, HbA1c and eGFR.