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Normalized Creatinine-to-Cystatin C Ratio and Risk of Diabetes in Middle-Aged and Older Adults: The China Health and Retirement Longitudinal Study

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Background: Creatinine-to-cystatin C ratio is recently suggested to be a surrogate marker for sarcopenia. However, little is known about its association with diabetes. This study aimed to fill in this gap based on a large-scale prospective cohort.
Methods: A population-based representative sample of 5,055 participants aged ≥45 years from the China Health and Retirement Longitudinal Study was enrolled between 2011 and 2012 and followed at least once during the subsequent surveys at 2013, 2015, or 2018. Creatinine-to-cystatin C ratio was calculated and normalized by body weight. Incident diabetes was ascertained by plasma glucose, glycosylated hemoglobin, self-reported history, or use of anti-diabetic drugs. Logistic regression analysis and mediation analysis were employed.
Results: During follow-up, 634 participants developed diabetes. The risk of diabetes was gradually and significantly decreased with increased normalized creatinine–cystatin C ratio. The multivariable-adjusted odds ratio for diabetes was 0.91 (95% confidence interval, 0.83 to 0.99) per 1 standard deviation higher of normalized creatinine-to-cystatin C ratio, and this relationship remained significant after controlling for muscle strength. The risk reduction in diabetes was significantly larger in participants with normal-weight and high normalized creatinine-to-cystatin C ratio compared with those with overweight/obesity and high normalized creatinine-to-cystatin C ratio ($P_{interaction}$=0.01). Insulin resistance and inflammation appeared to be key mediators accounting for the observed relationship between normalized creatinine-to-cystatin C ratio and risk of diabetes, with their mediating effect being 93.1% and 22.0%, respectively.
Conclusion: High normalized creatinine-to-cystatin C ratio is associated with reduced risk of diabetes in middle-aged and older adults.

Keywords: Creatinine; Cystatin C; Diabetes mellitus; Insulin resistance; Longitudinal studies; Muscle strength

INTRODUCTION

Being extensively presented in nucleated cells, cystatin C is recognized as a novel biomarker that outperforms creatinine in reflecting glomerular filtration [1]. In recent years its ratio from creatinine, namely creatinine-to-cystatin C ratio, has been indicated to reflect health wellbeing in a wide range of clinical contexts [2-5]. For example, creatinine-to-cystatin C ratio has been advocated to be a surrogate marker for sarcopenia [2-6]. Moreover, several prospective cohort studies have

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found that creatinine-to-cystatin C ratio is associated with prognostic outcomes, which include all-cause and cardiovascular mortality [1,7,8], as well as bone fracture [9].

Diabetes has become a worldwide public health burden and its global prevalence is estimated to be projected from 9.3% in 2019 to 10.2% by 2030 and 10.9% by 2045 [10]. Recent evidence points out that low muscle mass assessed by dual X-ray absorptiometry or computed tomography might be implicated in the development of diabetes in middle-aged and older adults [11,12]. However, these gold standard measurement approaches are not feasible for large-scale epidemiological surveys, and the efficacy of the alternative method—bioelectrical impedance analysis in quantifying muscle mass has been questioned [13]. Creatinine-to-cystatin C ratio has recently received substantial interest as a measure for muscle mass, and its accuracy has been well validated against computed tomography in assessing muscle mass among different populations including older adults and cancer patients [2,6,14]. Moreover, the evidence that creatinine-to-cystatin C ratio carries a superior power in detecting myopenia over bioelectrical impedance analysis adds further support to its accuracy [14]. However, to our knowledge, no population-based studies have been conducted to assess whether this ratio is related to the development of diabetes.

To fill in this gap, we investigated the relationship of creatinine-to-cystatin C ratio with risk of diabetes in a cohort of middle-aged and older adults from the China Health and Retirement Longitudinal Study (CHARLS) [15-17]. However, in the present study creatinine-to-cystatin C ratio was normalized by body mass [18], given the evidence that substantial covariance exists between muscle mass or muscle strength and body mass [19,20], and that normalized creatinine-to-cystatin C ratio had a better correlation with insulin sensitivity than the non-normalized ratio [18].

METHODS

Study population

CHARLS is an ongoing prospective longitudinal study that enrolls middle-aged and older adults (45 years and above), who were randomly and representatively selected from 150 county-level units from 28 provinces in China. The cohort profile of CHARLS has been described previously [15-17] and the detailed information about the sampling approach and study design was also reported on the website at http://charls.pku.edu.cn/index/en.html. In brief, the baseline survey of CHARLS was conducted from June 2011 to March 2012. Participants with computer-assisted personal interview data at baseline were followed consecutively. To date, three waves of follow-up survey had been conducted at 2013, 2015, and 2018. Information relating to demographics, family structure, health status and functioning, and health outcomes including blood biomarkers measurement, were collected. The study design and protocol of CHARLS were approved by the ethical review committee at Peking University (approval No. IRB 00001052-11014), and informed consent was obtained from all participants.

In the present study, we included participants who had information at baseline (at 2011–2012) and were followed at least once during the subsequent three waves (that is, at 2013, 2015, or 2018). Participants were excluded if they: (1) had no data on cystatin C, creatinine, or glycemic measures such as fasting plasma glucose (FPG) or glycosylated hemoglobin (HbA1c); (2) were diagnosed with diabetes at baseline based on the American Diabetes Association 2010 criteria [21]; (3) had missing information on gender, body weight, body mass index (BMI), systolic blood pressure (SBP), or diastolic blood pressure (DBP); (4) were with outlier data (<1 or >99 percentile) on BMI, SBP, or DBP; (5) were with kidney disease or an estimated glomerular filtration rate (eGFR; calculated using the Chinese-based equation [22]) of less than 30 mL/(min×1.73 m^2); and (6) aged <45 years. Finally, a total of 5,055 middle-aged and older adults were included in this study (Supplementary Fig. 1).

Assessment of demographic and anthropometric information

Information on age, gender, history of smoking (smoker and non-smoker) or drinking (drinker or non-drinker), history of disease (e.g., hypertension, heart disease, kidney disease) was collected using standardized questionnaires. Height (cm) and body weight (kg) were measured. Blood pressure was measured trice in the morning using an automatic monitor (Omron™ HEM-7200 Monitor, Omron [Dalian] Co. LTD., Dalian, China) in a relaxed state. Hypertension was defined as a mean of SBP ≥140 mm Hg, a mean of DBP ≥90 mm Hg, a history of hypertension, or the use of anti-hypertensive agents. History of heart disease or kidney disease was ascertained by questionnaires.

Measurement of blood biomarkers

All participants were asked to fast overnight, and blood samples were taken in the next morning. However, for a small pro-

portion (about 8%) of participants who did not fast, blood samples were also taken, while their plasma glucose data were treated as random plasma glucose. The whole blood and plasma samples were stored at −80°C and were later used to measure FPG, HbA1c, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), uric acid (UA), creatinine, high-sensitivity C-reactive protein (hs-CRP), and cystatin C. Details of the methods for these blood-based bioassays were described in the "2011–2012 National Baseline Blood Data Users' Guide" (http://charls.pku.edu.cn/index/en.html). Specifically, creatinine was measured by rate-blanked and compensated Jaffe creatinine method (within-assay and between-assay coefficient of variation was <1.60% and <2.10%, respectively), and cystatin C was by particle-enhanced turbimetric assay (within-assay and between-assay coefficient of variation was <5.00% and <5.00%, respectively). Moreover, in this study dyslipidemia was defined as TC ≥240 mg/dL, LDL ≥160 mg/dL, HDL <40 mg/dL, TG ≥200 mg/dL, a history of dyslipidemia, and/or the use of lipid-lowering medications.

Calculation of normalized creatinine-to-cystatin C ratio and other parameters

In this study normalized creatinine-to-cystatin C ratio was determined as: creatinine (mg/dL)/cystatin C (mg/L)×10/body mass (kg). Insulin resistance was assessed by the metabolic score for insulin resistance (METS-IR), which was calculated as: ln (2×FPG [mg/dL]+TG [mg/dL])/ln (HDL [mg/dL])×BMI (kg/m^2) [23]. BMI was obtained as: body weight (kg)/height (m)^2, and overweight or obesity was defined as BMI ≥24 kg/m^2 [24]. Mean arterial pressure (MAP) was calculated as: (SBP [mm Hg]+2×DBP [mm Hg])/3, and eGFR was as: 2,374.78×([creatinine×88.4]^[−0.54753])×(age^[−0.25011]) for male and 2,374.78×([creatinine×88.4]^[−0.54753])×(age^[−0.25011])×0.8526126 for female based on the Xiangya equation [22].

Assessment of muscle strength

Muscle strength was evaluated by handgrip strength (kg) and chair-rising time (second). For handgrip strength, it was determined using a handgrip dynamometer (YuejianTM WL-1000 dynamometer, Nantong Yuejian Physical Measurement Instrument Co. Ltd., Nantong, China) twice, and the average data for the dominant hand were chosen for the analysis. And for chair-rising time, it was assessed using a stopwatch by guiding participants to stand-up and sit-down on a chair at their fastest pace for five times [15].

Ascertainment of diabetes

Incident diabetes was ascertained by: FPG ≥7.0 mmol/L (126 mg/dL), random plasma glucose ≥11.1 mmol/L (200 mg/dL), HbA1c ≥6.5% (48 mmol/mol), a self-reported history of diabetes, or the use of anti-diabetic drugs [21,25].

Statistical analysis

Continuous variables were presented as mean±standard deviation (SD), and categorical variables were as numbers (%). One-way analysis of variance, unpaired *t*-test, and chi-square test were employed to compare differences when appropriate. Normalized creatinine-to-cystatin C ratio was managed by two approaches: (1) being categorized into three groups (lowest, middle, and highest) defined by tertiles, and (2) being treated as a continuous variable expressed as per 1 SD higher. Odds ratio (OR) and 95% confidence interval (CI) for the association of normalized creatinine-to-cystatin C ratio with risk of diabetes were examined by logistic regression analysis. For this, three different models were introduced: model 1 without adjustment, model 2 adjusted for age and gender, and model 3 additionally adjusted for history of smoking and drinking, disease status (hypertension, dyslipidemia, and heart disease), MAP, HbA1c, TC/HDL cholesterol, TG, LDL, UA, and hs-CRP. For participants with available data on muscle strength, separate analyses based on model 3 were conducted by further controlling for handgrip strength and chair-rising time, respectively.

Restricted cubic spline curve, adjusted for covariables in model 3, was used to depict the association of normalized creatinine-to-cystatin C ratio with risk of diabetes, with the nonlinearity being assessed by Wald test. Subgroup analysis of age (≥60 years vs. <60 years), gender (male vs. female), history of smoking (yes vs. no), or history of drinking (yes vs. no), and sensitivity analysis by excluding participants without fasting samples, were also performed. Mediation analysis based on generalized structural equation model [26,27] was employed to evaluate the role of cardiometabolic factors (such as blood pressure, glycemic markers, lipid profiles, and inflammatory marker) in mediating the risk of diabetes associated with normalized creatinine-to-cystatin C ratio. For this, all the cardiometabolic factors and normalized creatinine-to-cystatin C ratio were expressed in continuous scales, and the risk of diabe-

tes was as a dichotomous variable. The mediation effect was calculated as the indirect effect (mediated by cardiometabolic factors)/total effect×100%.

All analyses were performed using Stata version 14.0 (StataCorp LP, College Station, TX, USA), and a $P<0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of enrolled participants

A total of 5,055 participants (mean age 60.0±9.7 years; 46.9% males) were included in this study. The baseline characteristics of enrolled participants according to the tertiles of normalized creatinine-to-cystatin C ratio are summarized in Table 1. The

Table 1. Baseline characteristics of enrolled participants

Variable	Total	Tertiles of normalized creatinine-to-cystatin C ratio			*P* value[a]
		Lowest (0.10±0.01)	Middle (0.13±0.008)	High (0.18±0.03)	
No. of participants	5,055	1,685	1,685	1,685	
Age, yr	60.0±9.7	60.3±10.0	60.0±9.5	59.6±9.7	0.119
Male sex	2,372 (46.9)	696 (41.3)	814 (48.3)	862 (51.2)	<0.001
Smoking[b]	1,987 (39.3)	585 (34.7)	686 (40.7)	716 (42.5)	<0.001
Drinking	1,643 (32.5)	478 (28.4)	572 (33.9)	593 (35.2)	<0.001
Presence of					
Hypertension	1,987 (39.3)	788 (46.8)	689 (40.9)	510 (30.3)	<0.001
Dyslipidemia	1,958 (38.7)	708 (42.0)	626 (37.2)	624 (37.0)	0.003
Heart disease[b]	531 (10.5)	234 (13.9)	160 (9.5)	137 (8.1)	<0.001
BMI, kg/m^2	23.2±3.4	25.2±3.4	23.0±2.8	21.3±2.7	<0.001
SBP, mm Hg	130.1±20.1	133.1±20.3	130.3±20.1	126.8±19.4	<0.001
DBP, mm Hg	75.0±11.1	76.5±11.2	75.2±11.0	73.4±10.8	<0.001
MAP, mm Hg	93.4±13.0	95.3±13.1	93.6±12.9	91.2±12.6	<0.001
FPG, mg/dL[c]	100.5±10.9	100.8±10.5	100.4±10.7	100.2±11.4	0.227
HbA1c, %	5.1±0.4	5.1±0.4	5.1±0.4	5.1±0.4	0.023
HbA1c, mmol/mol	32±4.4	32±4.4	32±4.4	32±4.4	0.023
METS-IR[c]	34.4±7.1	37.8±7.1	33.9±6.1	31.4±6.4	<0.001
TC, mg/dL	192.6±37.5	189.2±37.6	192.3±36.5	196.3±38.3	<0.001
TG, mg/dL	121.7±74.1	121.6±67.4	119.6±69.6	124.1±84.0	0.210
HDL, mg/dL	52.0±14.7	49.6±13.3	52.4±14.6	54.1±15.9	<0.001
LDL, mg/dL	117.1±34.1	117.2±34.7	117.5±33.2	116.7±34.5	0.812
UA, mg/dL	4.4±1.2	4.3±1.2	4.4±1.2	4.5±1.3	<0.001
Cr, mg/dL	0.77±0.18	0.71±0.17	0.77±0.16	0.84±0.18	<0.001
eGFR, mL/min/1.73 m^2	79.5±9.5	82.7±10.5	79.2±8.4	76.4±8.4	<0.001
hs-CRP, mg/L[d]	2.4±6.0	3.0±6.9	2.3±6.3	2.0±4.4	<0.001

Values are presented as mean±standard deviation or number (%).

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; METS-IR, the metabolic score for insulin resistance; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; UA, uric acid; Cr, creatinine; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein.

[a]Data were compared using one-way analysis of variance or chi-square test when appropriate, [b]There were 4 and 35 participants without information on history of smoking and heart disease, respectively, [c]There were 475 participants without fasting blood samples, [d]It was log-transformed before analysis.

proportions of participants with hypertension, dyslipidemia, or heart disease in different tertiles decreased gradually with increases in normalized creatinine-to-cystatin C ratio (all $P \leq 0.003$), while the percentages of smoker or drinker increased. BMI and blood pressure including SBP, DBP, and MAP were significantly lower in groups with higher normalized creatinine-to-cystatin C ratio (all $P<0.001$). For laboratory biomarkers, HbA1c, METS-IR, and hs-CRP were also lower in the higher normalized creatinine-to-cystatin C ratio tertiles, while TC, HDL, UA, and creatinine were progressively greater (all $P<0.001$).

Normalized creatinine-to-cystatin C ratio and risk of diabetes

During the 7.0-year follow-up, 634 (12.5%) participants developed diabetes. Participants with incident diabetes had a worse metabolic profile than those without in general (Supplementary Table 1), and their normalized creatinine-to-cystatin C ratio was lower (0.13±0.04 vs. 0.14±0.04, $P=0.003$). The association between normalized creatinine-to-cystatin C ratio and risk of diabetes is shown in Table 2, which suggested that the ORs for diabetes were gradually lower with larger normalized creatinine-to-cystatin C ratios. In the unadjusted model, the OR for diabetes was 0.72 (95% CI, 0.59 to 0.88) and 0.64 (95% CI, 0.52 to 0.79) for the middle and highest tertiles, respectively, compared with the reference (the lowest tertile). These associations were slightly attenuated but maintained significant after controlling for different covariables (model 2 and model 3, Table 2). The multivariable-adjusted OR for diabetes was 0.91 (95% CI, 0.83 to 0.99) per 1 SD higher of normalized creatinine-to-cystatin C ratio (Table 2). Notably, this OR was still significant even after controlling for grip strength (0.91; 95% CI, 0.84 to 0.99) or chair-rising time (0.91; 95% CI, 0.83 to 0.99).

Cubic spline analysis suggested that there is no clear evidence of a nonlinear association between normalized creatinine-to-cystatin C ratio and risk of diabetes ($P_{nonlinearity}=0.13$). The risk of diabetes decreased progressively with increases in normalized creatinine-to-cystatin C ratio up to 0.15 but plateaued thereafter (Fig. 1).

Subgroup and joint analyses

The ORs for diabetes per 1 SD higher of normalized creatinine-to-cystatin C ratio did not vary substantially by sub-

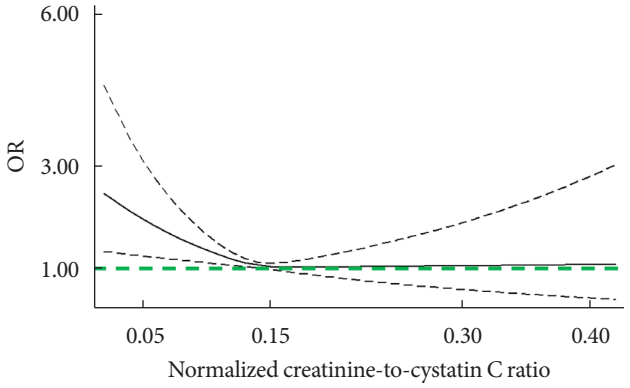

Fig. 1. Cubic spline curve for the relationship between normalized creatinine-to-cystatin C ratio and risk of diabetes. The cubic spline curve analysis was adjusted for age, gender, history of smoking and drinking, presence of hypertension, dyslipidemia, and heart disease, mean arterial pressure, glycosylated hemoglobin, total cholesterol/high-density lipoprotein, triglyceride, low-density lipoprotein, uric acid, and high-sensitivity C-reactive protein. OR, odds ratio.

Table 2. Normalized creatinine-to-cystatin C ratio and risk of diabetes

Variable	Normalized creatinine-to-cystatin C ratio			
	Lowest (tertile 1)	Middle (tertile 2)	Highest (tertile 3)	Per 1 SD higher
Total no.	1,685	1,685	1,685	5,055
No. of cases	261	196	177	634
Model 1[a]	1 (ref)	0.72 (0.59–0.88)	0.64 (0.52–0.79)	0.85 (0.78–0.94)
Model 2[b]	1 (ref)	0.74 (0.60–0.90)	0.66 (0.54–0.81)	0.87 (0.79–0.95)
Model 3[c]	1 (ref)	0.81 (0.65–0.99)	0.76 (0.61–0.94)	0.91 (0.83–0.99)

Values are presented as odds ratio (95% confidence interval).
SD, standard deviation.
[a]Unadjusted, [b]Adjusted for age and gender, [c]Adjusted for age, gender, history of smoking and drinking, presence of hypertension, dyslipidemia, and heart disease, mean arterial pressure, glycosylated hemoglobin, total cholesterol/high-density lipoprotein, triglyceride, low-density lipoprotein, uric acid, and high-sensitivity C-reactive protein.

groups based on gender, age, history of smoking, or history of drinking (all $P_{interaction} \geq 0.34$) (Supplementary Table 2). Moreover, the results remained comparable in analyses that excluded participants without fasting blood samples (OR, 0.89; 95% CI, 0.81 to 0.98).

To assess the joint effect of BMI and normalized creatinine-to-cystatin C ratio on risk of diabetes, participants were classed as with or without overweight/obesity, or with low (the lowest tertile) or high (the middle and highest tertiles) normalized creatinine-to-cystatin C ratio. Compared with participants with overweight/obesity and low normalized creatinine-to-cystatin C ratio, only those with normal-weight and high normalized creatinine-to-cystatin C ratio showed significantly reduced risk of diabetes (OR, 0.62; 95% CI, 0.49 to 0.78; model 3) (Table 3). Moreover, their magnitude of risk reduction was significantly larger than participants with overweight/obesity and high normalized creatinine-to-cystatin C ratio ($P_{interaction} = 0.01$) but not significantly than participants with normal-weight and low normalized creatinine-to-cystatin C ratio ($P_{interaction} = 0.28$).

Table 3. Joint effect of body mass index and normalized creatinine-to-cystatin C ratio on risk of diabetes

Variable	Overweight/obesity (BMI ≥24 kg/m^2)		Normal-weight (BMI <24 kg/m^2)	
	Low NCCR	High NCCR	Low NCCR	High NCCR
Total no.	1,048	857	637	2,513
No. of cases	188	149	73	224
Model 1[a]	1 (Ref)	0.96 (0.76–1.22)	0.59 (0.44–0.79)	0.45 (0.36–0.55)
Model 2[b]	1 (Ref)	1.01 (0.79–1.28)	0.56 (0.42–0.75)	0.44 (0.36–0.55)
Model 3[c]	1 (Ref)	0.95 (0.74–1.22)	0.77 (0.56–1.05)	0.62 (0.49–0.78)

Values are presented as odds ratio (95% confidence interval). Low NCCR referred to the lowest tertile of NCCR, and high NCCR was defined as the middle and highest tertiles.
BMI, body mass index; NCCR, normalized creatinine-to-cystatin C ratio.
[a]Unadjusted, [b]Adjusted for age and gender, [c]Adjusted for age, gender, history of smoking and drinking, presence of hypertension, dyslipidemia, and heart disease, mean arterial pressure, glycosylated hemoglobin, total cholesterol/high-density lipoprotein, triglyceride, low-density lipoprotein, uric acid, and high-sensitivity C-reactive protein.

Table 4. Association between normalized creatinine-to-cystatin C ratio and cardiometabolic markers

Independent variable	Simple linear regression analysis		Multivariable linear regression analysis[a]	
	Sβ	*P* value	Sβ'	*P* value
SBP	–0.11	<0.001	–0.11	<0.001
DBP	–0.09	<0.001	–0.10	<0.001
FPG	–0.01	0.553	–0.006	0.679
HbA1c	–0.03	0.086	–0.02	0.120
METS-IR	–0.34	<0.001	–0.35	<0.001
TC	0.11	<0.001	0.12	<0.001
TG	0.07	<0.001	0.07	<0.001
HDL	0.10	<0.001	0.10	<0.001
LDL	–0.0001	>0.999	0.009	0.518
UA	0.07	<0.001	0.06	<0.001
eGFR	–0.30	<0.001	–0.41	<0.001
hs-CRP[b]	–0.14	<0.001	–0.15	<0.001

Sβ, standardized regression coefficient; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; METS-IR, the metabolic score for insulin resistance; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; UA, uric acid; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein.
[a]Adjusted for age and gender, [b]It was log-transformed before analysis.

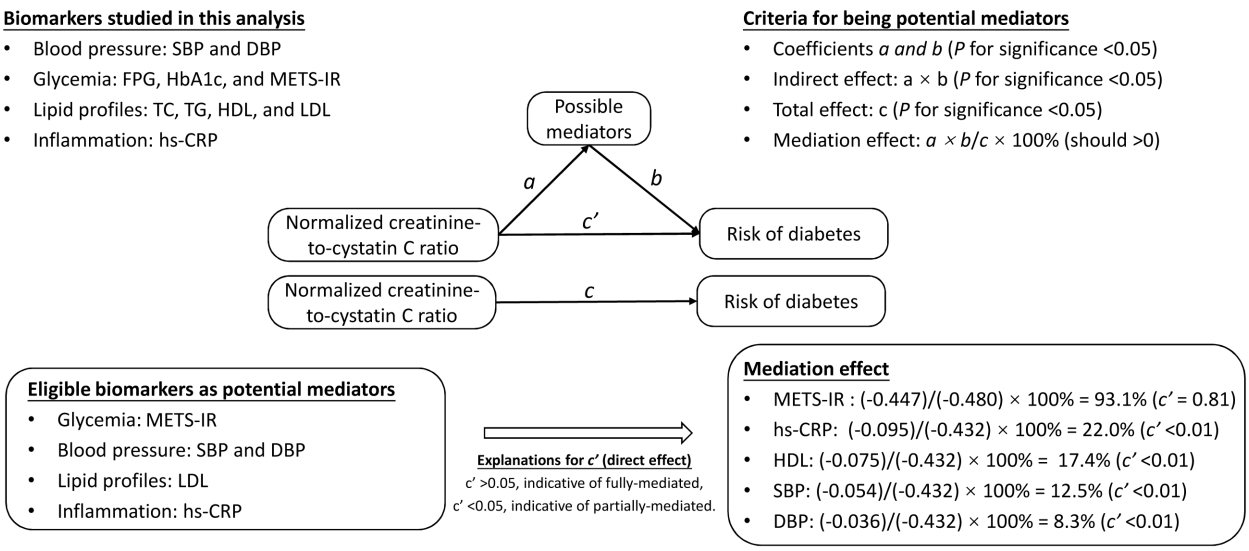

Fig. 2. Mediation analysis for the relationship between normalized creatinine-to-cystatin C ratio and risk of diabetes. Mediation analysis (which included the tests for coefficients of a, b, and c) was conducted based on the generalized structural equation model using "GSEM" command in Stata. SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; METS-IR, the metabolic score for insulin resistance; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate.

Mediation analysis

Both simple and multivariable linear regression analyses suggested that normalized creatinine-to-cystatin C ratio was negatively and significantly correlated with blood pressure, METS-IR, eGFR, and hs-CRP, positively and significantly with TC, TG, HDL, and UA (all $P<0.001$) (Table 4), but non-significantly with FPG or HbA1c. Mediation analysis showed further that METS-IR may fully mediate the relationship between normalized creatinine-to-cystatin C ratio and risk of diabetes, with its mediating effect being 93.1% (Fig. 2). Moreover, hs-CRP, HDL, SBP, and DBP may also mediate this relationship, with their mediating effect at 22.0%, 17.4%, 12.5%, and 8.3%, respectively (Fig. 2). However, FPG, HbA1c, TC, TG, and LDL did not have any significant moderating effects.

DISCUSSION

Summary of main findings

Our study, which is based on a large-scale population-based prospective cohort, showed for the first time in middle-aged and older adults that: (1) higher normalized creatinine-to-cystatin C ratio was associated with lower risk of diabetes independent of traditional cardiometabolic factors and muscle strength; (2) reducing body weight could lower the risk of diabetes in participants with a high normalized creatinine-to-cystatin C ratio; and (3) the association of normalized creatinine-to-cystatin C ratio with risk of diabetes appeared to be mediated primarily by insulin resistance (assessed by METS-IR) and inflammation (evaluated by hs-CRP).

Interpretations and implications

Our study showed that high normalized creatinine-to-cystatin C ratio reduced risk of diabetes in middle-aged and older adults. One might assume that this relationship is attributable to variations in muscle mass and/or muscle strength [5,6,18, 28], both of which are identified determinants for risk of diabetes [20,29,30]. However, in the present study, the association between high normalized creatinine-to-cystatin C ratio and low risk of diabetes was independent of muscle strength. Moreover, the correlation between creatinine-to-cystatin C ratio and muscle mass or muscle strength was reported to be weak to moderate (the correlation coefficient ranged from 0.27 to 0.57) [3,18,28,31]. It is therefore plausible that there exist factors complementing muscle mass or muscle strength. Indeed, we observed that normalized creatinine-to-cystatin C ratio was also inversely associated with blood pressure, insulin

resistance (assessed by METS-IR), and inflammation (presented by hs-CRP), all of which were closely related to risk of diabetes [32-34]. Moreover, normalized creatinine-to-cystatin C ratio was also found to be significantly and negatively correlated with BMI (data not shown), suggesting that this ratio may additionally help to reflect the extent of fat mass accumulation, at least partly. Notably, our mediation analysis revealed that the amelioration of insulin resistance or inflammation, as a result of high normalized creatinine-to-cystatin C ratio, might be the key drivers in reducing risk of diabetes, with their mediating effect at around 93% and 22%, respectively.

Although our restricted cubic spline analysis showed that there was no significant evidence of a nonlinear association between normalized creatinine-to-cystatin C ratio and risk of diabetes, improvements in normalized creatinine-to-cystatin C ratio beyond 0.15 do not appear to yield additional benefit in reducing risk of diabetes, indicative of a "threshold effect." Moreover, our study found that participants with normal-weight and high normalized creatinine-to-cystatin C ratio showed significantly larger risk reduction in diabetes than those with overweight/obesity and high normalized creatinine-to-cystatin C ratio. This indicates that reducing body weight could help to lower the risk of diabetes, even in participants who had a high normalized creatinine-to-cystatin C ratio. However, neither sex nor age affected the association of normalized creatinine-to-cystatin C ratio with risk of diabetes in our study.

Strengths and limitations

The strengths of this study include a nationally representative sample of middle-aged and older Chinese adults, a relatively long follow-up period, a standardized and validated measurement of creatinine and cystatin C, as well as the use of subgroup analysis, cubic spline analysis, and mediation analysis, which facilitates an enriched interpretation of the association of normalized creatinine-to-cystatin C ratio with risk of diabetes. However, our study has several limitations. First, despite of the effort to adjust for a series of covariables, residual confounding could not be completely ruled out. For example, cardiorespiratory fitness may affect the onset of diabetes [35], while the lack of this information precluded an opportunity to undertake this analysis. Second, creatinine and cystatin C were measured only once, while this may introduce the risk of measurement error or overestimation. However, the protocols for their measurements were well-validated (http://charls.pku.edu.cn/index/en.html). Third, oral glucose tolerance test was not conducted to ascertain incident diabetes, which may underestimate the incidence of diabetes. Finally, our study focused mainly on Chinese Han adults, and it remains unclear whether our conclusions could be generalized for other ethnic populations.

In conclusion, high normalized creatinine-to-cystatin C ratio is associated with reduced risk of diabetes in middle-aged and older adults, and this association might be primarily attributable to the amelioration of insulin resistance and inflammation. Interventions designed to increase normalized creatinine-to-cystatin C ratio is therefore worth being recommended to lower risk of diabetes.

SUPPLEMENTARY MATERIALS

Supplementary materials related to this article can be found online at https://doi.org/10.4093/dmj.2021.0074.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: S.Q., X.C., Z.S., T.W.
Acquisition, analysis, or interpretation of data: S.Q., X.C., B.X., Y.Y., Z.S.
Drafting the work or revising: S.Q., B.X., Y.Y., T.W.
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