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Plasma creatine and incident type 2 diabetes in a general population-based cohort: The PREVEND study

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Abstract

Background: Type 2 diabetes is associated with both impaired insulin action at target tissues and impaired insulin secretion in pancreatic beta cells. Mitochondrial dysfunction may play a role in both insulin resistance and impaired insulin secretion. Plasma creatine has been proposed as a potential marker for mitochondrial dysfunction. We aimed to investigate the association between plasma creatine and incident type 2 diabetes.

Methods: We measured fasting plasma creatine concentrations by nuclear magnetic resonance spectroscopy in participants of the general population-based PREVEND study. The study outcome was incident type 2 diabetes, defined as a fasting plasma glucose ≥7.0 mmol/L (126 mg/dl); a random sample plasma glucose ≥11.1 mmol/L (200 mg/dl); self-report of a physician diagnosis or the use of glucose-lowering medications based on a central pharmacy registration. Associations of plasma creatine with type 2 diabetes were quantified using Cox proportional hazards models and were adjusted for potential confounders.

Results: We included 4735 participants aged 52 ± 11 years, of whom 49% were male. Mean plasma creatine concentrations were 36.7 ± 17.6 μmol/L, with lower concentrations in males than in females (30.4 ± 15.1 μmol/L vs. 42.7 ± 17.7 μmol/L; p for difference <.001). During 7.3 [6.2–7.7] years of follow-up, 235 (5.4%) participants developed type 2 diabetes. Higher plasma creatine concentrations were associated with an increased risk of incident type 2 diabetes (HR per SD change: 1.27 [95% CI: 1.11–1.44]; p < .001), independent of potential confounders. This association was strongly modified by sex (p interaction <.001). Higher plasma creatine was associated with an increased risk of incident type 2 diabetes in males (HR: 1.40 [1.17–1.67]; p < .001), but not in females (HR: 1.10 [0.90–1.34]; p = .37).

Conclusion: Fasting plasma creatine concentrations are lower in males than in females. Higher plasma creatine is associated with an increased risk of type 2 diabetes in males.

KEYWORDS
creatine, general population, type 2 diabetes

1 | INTRODUCTION

Type 2 diabetes is the most common metabolic disease, and it is one of the leading causes for blindness, cardiovascular disease and end-stage kidney disease.[1,2] Insulin resistance at target tissues and an impaired insulin secretion in pancreatic beta cells are central to the development of type 2 diabetes.[3–5] Increasing evidence suggests that mitochondria play a role in both processes and mitochondrial dysfunction has therefore been implicated to have a key role in the development of type 2 diabetes.[2,6–9] Creatine is a natural nitrogenous organic acid that is integral to cellular energy metabolism. It should be differentiated from creatinine, which is a product arising from non-enzymatic degradation of creatine and creatine phosphate.[10] Circulating creatine, either derived from animal-based protein sources in the diet, from endogenous production or both, is actively transported into tissues by the creatine transporter (*CRT*;*SLC6A8*).[11] Data demonstrating a relationship between a high extracellular creatine concentration and a low intracellular phosphocreatine:creatine ratio,[12] a reflection of a low cellular energetic state, have led to the hypothesis that plasma creatine is a biomarker for mitochondrial dysfunction.[12,13] Indeed, elevated plasma creatine concentrations have been found in a variety of mitochondrial diseases.[12,14–16] However, to date, no studies have explored plasma creatine in relation to incident type 2 diabetes. Therefore, in the current study, we aimed to determine the plasma creatine concentrations in a large population-based cohort in order to cross-sectionally investigate the determinants of plasma creatine concentrations and to prospectively investigate the association of plasma creatine with incident type 2 diabetes. Given the established sex-differences in the rates of type 2 diabetes development[17,18] and the fact that location of the creatine transporter gene resides on the X-chromosome,[19,20] we also aimed to evaluate potential effect-modification by sex and to perform sex-stratified analyses.

2 | METHODS

2.1 | Study design and participants

The current study was conducted within the framework of the Prevention of Renal and Vascular End-stage Disease (PREVEND) study. PREVEND is an observational prospective cohort study which investigates the prevalence and consequences of microalbuminuria in adults of the city of Groningen (The Netherlands). The objectives and design have been described in detail elsewhere.[21] In brief, during 1997 and 1998, all 85,421 inhabitants of the city of Groningen between the ages of 28 and 75 years were invited to participate in the study and were sent a one-page questionnaire regarding demographics, cardiovascular morbidity, use of medication, menstruation, and pregnancy along with a vial to collect a first morning void urine sample. A total of 40,856 (47.8%) responded, in whom the urinary albumin concentration, was determined. Because of the well-established link between cardiovascular or renal disease and microalbuminuria in individuals with insulin-dependent diabetes mellitus, these individuals were excluded from the PREVEND study. Since pregnant females may present with temporary microalbuminuria, pregnant females were also excluded. After further exclusion of individuals unable or unwilling to participate in the study, a total of 6000 individuals with a urinary albumin concentration of 10 mg/L or greater and a randomly chosen control group of 2592 individuals with a urinary albumin concentration of less than 10 mg/L completed the screening protocol and constitutes the PREVEND cohort (n = 8592). A second screening round took place from 2001 to 2003, encompassing 6894 participants and was considered the 'baseline' for the current study. We excluded participants with type 2 diabetes at baseline, participants with no data on plasma creatine concentration and participants with no follow-up data on the incidence of type 2 diabetes, leading to a total of 4375 participants for the current study. Detailed information on the flow of participants through the study is provided in Figure S1. The PREVEND study has been approved by the local medical ethics committee and was undertaken in accordance with the Declaration of Helsinki. All participants provided written informed consent.

2.2 | Clinical and laboratory measurements

Each screening comprised two visits to an outpatient clinic separated by 3 weeks.[10] Self-administered questionnaires concerning demographics, cardiovascular and renal disease history, smoking habits and medication use were provided by all participants prior to the first visit. Information on medication use was combined with information from IADB.nl, a data base containing information of prescribed medication in public pharmacies in The Netherlands since 1999 (http://www.iadb.nl/). Height and weight were measured with the participants standing without shoes and heavy outer garments. Body mass index (BMI) was calculated by dividing weight in kilograms by height, in metres, squared. Systolic and diastolic blood pressure were measured on the right arm and calculated as the arithmetic mean of the last two measurements of the two visits using an automatic Dinamap XL Model 9300 series device. Baseline EDTA plasma samples were drawn between 8:00 and 10:00 a.m. from all participants, and aliquots of these samples were immediately stockpiled at −80°C until analysis.

Creatine was measured using a Vantera® NMR Clinical Analyzer (LabCorp, Raleigh, NC). We performed creatine measurements in EDTA plasma samples of participants who had been instructed to perform an overnight fast, which minimalizes a potential influence of alimentary creatine ingested with fish or meat on circulating creatine concentrations.[22] Plasma samples were mixed (3:1 v/v) with citrate/phosphate buffer to adjust the pH to 5.3. This was necessary to separate the creatine and creatinine peaks, which overlap at physiological pH, to allow accurate quantification. Proton NMR spectra were acquired as previously described.[23,24] The creatine peak (3.00 ppm) was quantified using a proprietary lineshape deconvolution by a non-negative least squares fitting algorithm which models the peak as Lorentzian and Gaussian lineshapes. The creatine signal amplitudes were converted to concentration units (μmol/L) using an empirical factor determined from standard spiking. Creatine results (n = 44) from the NMR quantification software agrees well with routine enzymatic (creatinase)/spectrophotometry assay (R^2 = .995, slope = 0.99, intercept = 12.2). The coefficient of variation for intra- and inter-assay precision for the NMR assay was 4.0%–4.9%.

Serum creatinine was measured with an enzymatic method on a Roche Modular analyser, using reagents and calibrators from Roche (Roche Diagnostics). Fasting plasma glucose was measured by dry chemistry (Eastman Kodak). Total cholesterol, HDL cholesterol triglycerides, insulin, serum creatinine and serum cystatin C were measured using standard protocols, which have been described previously.[25–28] Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation.[29]

The homeostatic model assessment for insulin resistance (HOMA-IR) and the homeostatic model assessment for beta cell function (HOMA-β) were calculated as follows:

$$\text{HOMA - IR} = (\text{fasting plasma insulin (mU/L)} * \text{fasting plasma glucose (mmol/L)}) \,/\, 22.5.$$

$$\text{HOMA} - \beta\ \% = 20 * \text{fasting plasma insulin (mU / L)} \,/\, (\text{fasting plasma glucose (mmol / L)} - 3.5).$$

The homeostatic model assessments were calculated and analysed only in the subset of participants with a fasting glucose >4.5 mmol/L and a fasting insulin >5 mU/L, since the interpretation of HOMA-IR and HOMA-β is invalid when calculated using fasting glucose <4.5 mmol/L or an insulin <5 mU/L.[30]

2.3 | Outcome

The outcome of the present study was incident type 2 diabetes. Type 2 diabetes was defined as a fasting plasma glucose greater than 7.0 mmol/L, a non-fasting plasma glucose greater than 11.1 mmol/L, self-report of a physician diagnosis or the use of anti-diabetic drugs. Follow-up time for incident type 2 diabetes was estimated using a midpoint imputation method, and censoring was defined as the exact date of loss to follow-up or the end of the follow-up period, whichever came first.

2.4 | Statistical analyses

Statistical analyses were performed with R version 3.6.1 (Vienna, Austria) (http://cran.r-project.org/). Results were expressed as mean ± standard deviation (SD), median [interquartile range] or number (percentage) for normally distributed, skewed and categorical data, respectively. A two-sided p-value <.05 was considered to indicate statistical significance. Baseline characteristics are presented for the whole cohort and according to sex. Differences in baseline characteristics between males and females are tested using independent sample t test, Wilcoxon–Mann Whitney test or chi-square test. Multivariable linear regression was used to assess the association of plasma creatine concentrations with baseline variables. Regression models were adjusted for age and sex. For these analyses, regression coefficients were given as standardized beta values, referring to the number of standard deviations a dependent variable changes per standard deviation increase in the independent variable, thereby allowing for comparison of the strength of the associations of different variables. Potential modification by sex was explored by including product terms to the age and sex adjusted models. A $p_{\text{interaction}}$ <.10 was considered to indicate significant modification. Analyses were performed for the total population and stratified according to sex. The assumption of normally distributed error terms was validated by inspection of Q-Q plots of the residuals.

Cox proportional hazards models were used to investigate the associations of plasma creatine concentrations with incident type 2 diabetes. Hazards ratios were computed per standard deviation increase in plasma creatine. The proportional hazards assumption was verified visually with plots of the scaled Schoenfeld residuals and was not violated in any of the models. Potential modification of the effect of plasma creatine on the risk of type 2 diabetes by sex was explored by including product terms in the model. Analyses were performed for the total population and stratified according to sex. Adjustments were made for a priori selected variables, including age, sex, BMI, eGFR, urinary albumin excretion, systolic blood pressure, NT-ProBNP, total cholesterol, HDL cholesterol, triglycerides, parental history of type 2 diabetes, alcohol intake, smoking status, plasma glucose and plasma insulin. Lastly, in separate models including a subset of the data, adjustments were made for HOMA-IR and HOMA-β. To account for potential bias that could result from the exclusion of participants with missing values,[31] multiple imputation using fully conditional specification

was performed to obtain 5 imputed data sets, in which Rubin's rules were applied to acquire pooled estimates of the regression coefficients and their standard errors across the imputed data sets.[31,32] Apart from the baseline table and unless otherwise stated, analyses were performed using imputed data sets. To visualize the continuous associations of plasma creatine with type 2 diabetes, plasma creatine, as a continuous variable, was plotted against the risk of type 2 diabetes. Sensitivity analyses were conducted to evaluate the robustness of the findings, wherein any potential bias caused by outliers in plasma creatine was accounted for by excluding participants with plasma creatine values in the highest and lowest 2.5 percentiles. Similarly, an additional sensitivity analysis was performed in which participants with plasma creatine values in the highest 5 percentiles were excluded. Given the strong predictive value of glucose, we performed an additional sensitivity analysis after exclusion of participants with an impaired fasting glucose, defined as a fasting glucose between 5.6 and 6.9 mmol/L.[17] Furthermore, we performed sensitivity analyses in which we excluded participants with micro- and macroalbuminuria, that is a urinary albumin excretion >30 mg per 24-h. Lastly, to account for possibility of maternal inheritance of type 2 diabetes, we performed a sensitivity analyses after exclusion of participants whose mother was diagnosed with type 2 diabetes.

3 | RESULTS

3.1 | Baseline characteristics

The mean age of the 4375 participants was 52 ± 11 years of whom 2123 (49%) were male. Baseline plasma creatine concentrations were 36.7 ± 17.6 μmol/L. Plasma creatine concentrations were substantially higher in females than males (42.7 ± 17.7 μmol/L vs. 30.4 ± 15.1 μmol/L; $p < .001$). Unlike creatine, plasma creatinine (the product of non-enzymatic conversion of creatine and creatine phosphate) was higher in males than females (79.8 ± 14.0 μmol/L vs. 64.7 ± 10.4 μmol/L; $p < .001$). The distributions of plasma creatine concentrations in males and females are shown in Figure 1. A scatter plot of plasma creatine versus plasma creatinine concentrations in males and females is shown in Figure S2. Among females, plasma creatine was higher in postmenopausal females, as compared to premenopausal females (45.5 ± 18.1 μmol/L vs. 40.0 ± 17.0 μmol/L; $p < .001$). The distributions of plasma creatine concentrations in pre- and postmenopausal females are shown in Figure S3. Unlike plasma creatine, plasma creatinine did not differ between postmenopausal and premenopausal females (64.9 ± 11.4 vs. 64.5 ± 9.5 μmol/L; $p = .36$). Baseline characteristics of the study participants according to sex are shown in Table 1. Males had higher age, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, urinary albumin excretion, alcohol intake, triglycerides, plasma glucose and plasma insulin, compared to females (all $p < .05$). Females had higher NT-ProBNP and HDL cholesterol compared to males (both $p < .05$).

3.2 | Linear regression analyses

Multivariable linear regression analyses are shown in Table 2. Baseline plasma creatine was positively associated with age, BMI, waist circumference, eGFR, urinary albumin excretion, smoking status, total cholesterol, triglycerides, glucose, insulin and HOMA-IR in both males and females (all $p < .05$), all with higher standardized beta values in males than in females. Baseline creatine was positively associated with systolic blood pressure, diastolic blood pressure and alcohol intake in males, but not in females. Plasma creatine was inversely associated with plasma creatinine, NT-ProBNP and HDL cholesterol in both males and females (all $p < .05$). Modification by sex was found for age, BMI, waist circumference, diastolic blood pressure, urinary albumin excretion, smoking status, alcohol intake, HDL cholesterol, triglycerides and glucose (all p interaction <.10).

3.3 | Prospective analyses

Among 4.375 participants at risk, 235 (5.4%) developed incident type 2 diabetes during a follow-up of 7.3 [6.2–7.7] years. Among 2123 males and 2252 females at risk, 144 (6.8%) males and 91 (4.0%) females developed incident type 2 diabetes. Participants who developed incident type 2 diabetes had significantly higher plasma creatine concentrations compared to those who did not develop type 2 diabetes (41.8 ± 18.9 μmol/L vs. 36.5 ± 17.5 μmol/L; $p < .001$). An overview of Cox regression analyses is shown in Table 3. For every standard deviation increase in plasma creatine, the hazard ratio for incident type 2 diabetes was 1.32 (95%CI: 1.18–1.48; $p < .001$). After subsequent adjustment for age, sex and BMI, the hazard ratio changed to 1.28 (95%CI 1.13–1.46; $p < .001$). Further adjustment for other potential confounders, including eGFR, systolic blood pressure, diastolic blood pressure, NT-ProBNP, total cholesterol, HDL cholesterol, triglycerides, parental history of type 2 diabetes, alcohol intake, smoking status, plasma glucose and plasma insulin, did not materially change the association. The association between plasma creatine and incident type 2 diabetes was subject to substantial effect-modification by sex, as evidenced by a significant interaction term ($p < .001$). Sex-stratified analyses of the association between plasma creatine and type 2 diabetes indicate that the association in the whole cohort is primarily driven by an effect in males. In males, plasma creatine was strongly associated with the risk of incident type 2 diabetes. For every standard deviation increase in plasma creatine, the crude hazard ratio for incident type 2 diabetes in males was 1.69 (95%CI: 1.49–1.97; $p < .001$). After subsequent adjustment for age and BMI, the hazard ratio changed to 1.41 (95%CI 1.19–1.67). Further adjustment for the other potential confounders did not materially change the association. In females, plasma creatine was associated with an increased risk of incident type 2 diabetes in the crude model, HR: 1.29 (95%CI: 1.07–1.56; $p = .009$). However, this association lost significance after adjustment for age and BMI (HR: 1.11 (95%CI: 0.91–1.35; $p = .29$)). A graphical representation of the

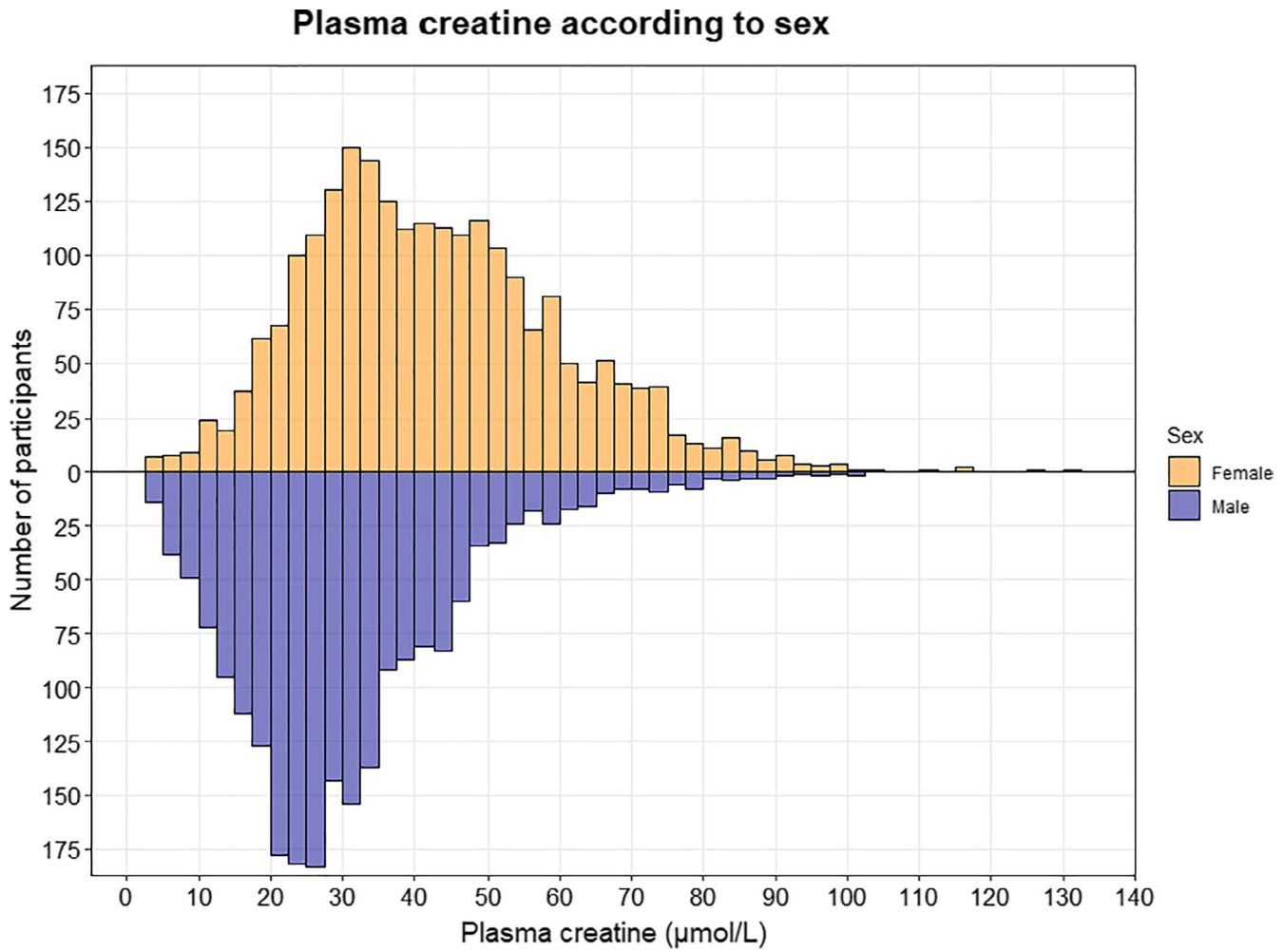

FIGURE 1 Mirror histogram displaying the sex-based differences in plasma creatine. Mean plasma creatine is 42.7 ± 17.7 μmol/L in females and 30.4 ± 15.1 μmol/L in males. *p* for difference <.001

sex-based differences in the association between plasma creatine and the risk of incident type 2 diabetes is shown in Figure 2.

Adjustments for homeostatic model assessment of insulin resistance (HOMA-IR) and beta-cell function (HOMA-β) were performed in a subset of the participants with data available on these parameters. Cox regression models with adjustments for HOMA-IR and HOMA-β are shown separately in Table 4. The associations of plasma creatine with type 2 diabetes did not materially change after adjustment for HOMA-IR and HOMA-β.

3.4 | Sensitivity analyses

Firstly, to determine the influence of potential outliers on the found associations, we performed sensitivity analyses after exclusion of outliers in plasma creatine. Cox regression analyses of plasma creatine with incident type 2 diabetes after exclusion of participants with plasma creatine in the highest or lowest 2.5 percentiles are shown in Table S1. Cox regression analyses of plasma creatine with incident type 2 diabetes after exclusion of participants with plasma creatine in the highest 5 percentiles are shown in Table S2. In both sensitivity analyses, exclusion of outliers in plasma creatine did not materially change the association between plasma creatine and incident type 2 diabetes. Given the strong predictive value of glucose, we also performed a sensitivity analysis after exclusion of participants with impaired fasting glucose, which is shown in Table S3. Excluding participants with impaired fasting glucose did not materially change the associations. Cox regression analyses of plasma creatine with incident type 2 diabetes after exclusion of participants with a urinary albumin excretion >30 mg per 24-h are shown in Table S4. Exclusion of participants with a urinary albumin excretion >30 mg per 24-h did not materially change the associations of plasma creatine with incident type 2 diabetes. We also investigated the possibility of bias introduced by maternal inheritance of type 2 diabetes. Cox regression analyses of plasma creatine with incident type 2 diabetes after exclusion of participants whose mothers were diagnosed with type 2 diabetes are shown in Table S5. Exclusion of participants whose mothers were diagnosed with type 2 diabetes also did again not materially change the associations.

TABLE 1 Baseline characteristics

Variables	Total cohort (*n* = 4375)	Males (*n* = 2123)	Females (*n* = 2252)	*p*-value
Plasma creatine, µmol/L	36.7 ± 17.6	30.4 ± 15.1	42.7 ± 17.7	<.001
Age, years	52 ± 11	53 ± 12	52 ± 11	<.001
BMI, kg/m^2	26.4 ± 4.2	26.6 ± 3.7	26.2 ± 4.7	.007
Waist circumference, cm	91.1 ± 12.4	96.2 ± 10.7	86.3 ± 12.0	<.001
Systolic blood pressure, mmHg	125 ± 18	129 ± 17	121 ± 18	<.001
Diastolic blood pressure, mmHg	73 ± 9	76 ± 9	70 ± 8	<.001
NT-ProBNP, ng/L	40 [20–75]	27 [13–54]	52 [31–87]	<.001
Plasma creatinine, µmol/L	72.0 ± 14.4	79.8 ± 14.0	64.7 ± 10.4	<.001
eGFR, ml/min per 1.73 m^2	94 ± 16	94 ± 17	94 ± 16	.50
Urinary albumin excretion, mg/24-h	8.5 [6.0–14.6]	9.4 [6.6–17.9]	7.7 [5.7–12.2]	<.001
Parental history of T2D, *n* (%)	625 (15)	279 (13)	346 (16)	.07
Smoking status, current *n* (%)	1201 (28)	574 (27)	627 (28)	.63
Alcohol intake, *n* yes (%)	3316 (77)	1755 (84)	1561 (70)	<.001
Antihypertensive drugs, *n* (%)	727 (17)	376 (18)	351 (16)	.06
Lipid lower drugs, *n* (%)	298 (8)	163 (9)	135 (7)	.005
Total cholesterol, mmol/L	5.4 ± 1.0	5.4 ± 1.0	5.4 ± 1.1	.82
HDL cholesterol, mmol/L	1.3 ± 0.3	1.1 ± 0.2	1.4 ± 0.3	<.001
Triglycerides, mmol/L	1.1 [0.8–1.6]	1.2 [0.9–1.8]	1.0 [0.7 -0 1.4]	<.001
Glucose, mmol/L	4.8 ± 0.6	4.9 ± 0.6	4.8 ± 0.6	<.001
Insulin, mU/L	7.9 [5.7–11.7]	8.4 [6.0–12.2]	7.6 [5.5–10.9]	<.001
HOMA-IR[a], (mU mmol/L^2)/22.5	2.1 [1.6–3.1]	2.2 [1.6–3.2]	2.0 [1.5–3.0]	<.001
HOMA-Beta[a], %	125 [94–176]	124 [92–177]	126 [95 -176]	.58

[a]Data on HOMA-IR and HOMA-Beta are only reported in a subset of the participants with a fasting glucose >4.5 mmol/L and a fasting insulin >5 mU/L (*n* = 2690).

Lastly, to investigate whether a similar association of plasma creatinine (the product of non-enzymatic conversion of creatine and creatine phosphate) with incident type 2 diabetes could be present, we performed a Cox regression analysis for the association of plasma creatinine concentrations with incident type 2 diabetes in the overall population and in males and females separately. In these crude analyses, we found no significant association between plasma creatinine and incident type 2 diabetes in the overall population (HR: 1.04 (95%CI: 0.94–1.14; $p = .46$). The same was true in separate analyses in males (HR: 0.89 (95%CI: 0.89–1.11; $p = .30$)) and females (HR: 0.91 (95%CI: 0.63–1.30; $p = .60$)).

4 | DISCUSSION

In this population-based cohort, we validated that plasma creatine concentrations are lower in males than in females. In time-to-event analyses, higher plasma creatine was independently associated with an increased risk of incident type 2 diabetes, and we demonstrate that this association between plasma creatine concentration and the risk of incident type 2 diabetes is heavily modified by sex, with higher plasma creatine being independently associated with an increased risk of incident type 2 diabetes in males, but not in females.

Creatine plays a crucial bioenergetic role in adenosine triphosphate turnover and is especially important in tissues with high and fluctuating energetic demands, that is skeletal muscles, brain and heart.[33,34] In omnivorous humans, creatine is synthesized endogenously, but also ingested through the diet.[35] The first step of the biochemical synthesis of creatine is a facilitated by the enzyme arginine:glycine amidinotransferase (AGAT), which converts arginine and glycine into guanidinoacetate, and it is at this step that regulation of endogenous creatine synthesis occurs.[36–40] In humans, highest AGAT activities are present in kidney and pancreas, while other tissues, such as brain and testes, express lower activities of AGAT.[41,42] The high expression of AGAT in the kidneys likely explains the strongly positive association we found between plasma creatine concentration and kidney function. Generally, due to a reduction in clearance, plasma concentrations of small water-soluble molecules increase with decreasing kidney function. However, we found that lower kidney function was associated with lower plasma creatine, confirming that enzymatic kidney function is likely for a large part responsible for endogenous biosynthesis of creatine in humans.[35,43] The second step of endogenous

TABLE 2 Multivariable linear regression analyses of plasma creatine with selected variables

	Total cohort (*n* = 4375)			Males (*n* = 2123)		Females (*n* = 2252)	
Dependent variables	**Std. β (95% CI)**	***p*-value**	$p_{Interaction}$	**Std. β (95% CI)**	***p*-value**	**Std. β (95% CI)**	***p*-value**
Age, years	0.17 (0.13; 0.20)	<.001	.04	0.20 (0.15; 0.26)	<.001	0.14 (0.10; 0.18)	<.001
BMI, kg/m^2	0.19 (0.16; 0.22)	<.001	.08	0.24 (0.20; 0.28)	<.001	0.16 (0.11; 0.20)	<.001
Waist circumference, cm	0.17 (0.14; 0.20)	<.001	<.001	0.24 (0.20; 0.28)	<.001	0.13 (0.09; 0.16)	<.001
Systolic blood pressure, mmHg	0.05 (0.02; 0.07)	.001	.09	0.09 (0.05; 0.13)	<.001	0.01 (−0.02; 0.05)	.46
Diastolic blood pressure, mmHg	0.09 (0.06; 0.12)	<.001	.001	0.14 (0.10; 0.19)	<.001	0.05 (0.01; 0.09)	.01
NT-ProBNP, ng/L[a]	−0.11 (−0.13; −0.08)	<.001	.16	−0.11 (−0.16; −0.07)	<.001	−0.10 (−0.13; −0.06)	<.001
Plasma creatinine, μmol/L[a]	−0.18 (−0.20; −0.15)	<.001	.62	−0.17 (−0.21; −0.13)	<.001	−0.18 (−0.22; −0.15)	<.001
eGFR, ml/min per 1.73 m^2	0.15 (0.13; 0.18)	<.001	.76	0.16 (0.12; 0.20)	<.001	0.15 (0.12; 0.18)	<.001
Urinary albumin excretion, mg/24-h	0.08 (0.05; 0.11)	<.001	<.001	0.19 (0.14; 0.24)	<.001	0.05 (0.01; 0.08)	.009
Parental history of T2D, *n* (%)	0.02 (0.01;0.03)	.001	.51	0.01 (−0.01; 0.03)	.10	0.02 (0.01; 0.04)	.006
Smoking status, current *n* (%)	0.07 (0.05; 0.09)	<.001	<.001	0.07 (0.05; 0.09)	<.001	0.03 (0.02; 0.05)	<.001
Alcohol intake, *n* yes (%)	0.01 (−0.01; 0.02)	.75	.01	0.02 (0.01; 0.04)	.02	−0.01 (−0.04; 0.01)	.23
Total cholesterol, mmol/L	0.10 (0.07; 0.14)	<.001	.49	0.13 (0.08; 0.18)	<.001	0.09 (0.05; 0.13)	<.001
HDL cholesterol, mmol/L	−0.10 (−0.13; −0.08)	<.001	.01	−0.15 (−0.19; −0.11)	<.001	−0.07 (−0.11; −0.03)	<.001
Triglycerides, mmol/L[a]	0.14 (0.11; 0.17)	<.001	.03	0.20 (0.15; 0.26)	<.001	0.09 (0.06; 0.13)	<.001
Glucose, mmol/L[a]	0.10 (0.07; 0.13)	<.001	.05	0.15 (0.10; 0.20)	<.001	0.07 (0.03; 0.11)	<.001
Insulin, mU/L	0.15 (0.12; 0.18)	<.001	.76	0.20 (0.15; 0.25)	<.001	0.11 (0.07; 0.15)	<.001
HOMA-IR[b], (mU mmol/L^2)/22.5	0.12 (0.08; 0.16)	<.001	.43	0.14 (0.08; 0.20)	<.001	0.10 (0.05; 0.15)	<.001
HOMA-Beta[b], %	0.06 (0.02; 0.10)	.006	.69	0.04 (−0.02; 0.11)	.17	0.05 (0.01; 0.08)	.01

All models are adjusted for age and sex. Unless otherwise stated, analyses are performed on imputed data sets.

[a]Log_2 transformed for analyses.

[b]Data on HOMA-IR and HOMA-Beta are only reported in a subset of the participants with a fasting glucose >4.5 mmol/L and a fasting insulin >5 mU/L (*n* = 2690).

creatine synthesis is facilitated by the liver enzyme guanidinoacetate N-methyltransferase (GAMT), converting guanidinoacetate to creatine by performing a methylation step, which interestingly, uses up to 40% of all endogenously generated S-adenosylmethionine.[44,45] The importance of creatine and endogenous creatine synthesis becomes apparent from the rare inherited AGAT and GAMT deficiency syndromes, leading to severe mental retardation, autism, movement disorders and epilepsy.[46] After being released into the circulation, creatine is transported into tissues by the creatine transporter 1 (CrT1), encoded by the SLC6A8 gene, located on the X-chromosome.[47] Through the years, several sex-based differences in creatine homeostasis have been identified. Rates of endogenous creatine biosynthesis in females have been found to be 70%–80% lower than in males,[41] which is also reflected by lower serum guanidinoacetate concentrations in females than in males.[48] Usually, dietary creatine intake is also lower in females than in males.[41] Despite the lower production and intake in females, we found significantly higher plasma concentrations of creatine in females, compared to males. In 1989, Delanghe et al. investigated potential sex-based differences in serum creatine in a small population of 60 healthy adults and also found significantly higher creatine concentrations in females compared to males.[49] In their study, serum creatine was on average 9.4 μmol/L higher in females than in males.[49] In our present study, we found a comparable difference of 12.3 μmol/L. Interestingly, the trend of higher creatine concentrations in females was also found in biopsies of the vastus lateralis muscle, where females had 10% higher intracellular creatine concentrations compared to males.[50] This

TABLE 3 Prospective associations of plasma creatine with risk of type 2 diabetes

	Total population		Males		Females	
	Per 1-SD		Per 1-SD		Per 1-SD	
	HR [95% CI]	*p*-value	HR [95% CI]	*p*-value	HR [95% CI]	*p*-value
Model 1	1.32 [1.18–1.48]	<.001	1.69 [1.49–1.97]	<.001	1.29 [1.07–1.56]	.009
Model 2	1.44 [1.27–1.62]	<.001	1.62 [1.39–1.88]	<.001	1.22 [1.00–1.49]	.05
Model 3	1.28 [1.13–1.46]	<.001	1.41 [1.19–1.67]	<.001	1.11 [0.91–1.35]	.29
Model 4	1.26 [1.11–1.44]	<.001	1.37 [1.14–1.64]	<.001	1.12 [0.91–1.37]	.29
Model 5	1.28 [1.12–1.46]	<.001	1.37 [1.14–1.64]	<.001	1.14 [0.93–1.41]	.21
Model 6	1.20 [1.05–1.36]	.008	1.31 [1.09–1.58]	.004	1.07 [0.87–1.32]	.50
Model 7	1.19 [1.04–1.36]	.01	1.35 [1.12–1.63]	.002	1.10 [0.89–1.34]	.38
Model 8	1.23 [1.08–1.40]	.002	1.39 [1.16–1.67]	<.001	1.07 [0.88–1.32]	.49
Participants	*4375*		*2123*		*2252*	
Events	*235*		*144*		*91*	

Model 1: crude. Model 2: adjusted for age and sex. Model 3; as model 2, additionally adjusted for BMI. Model 4, as model 3, additionally adjusted for eGFR and urinary albumin excretion. Model 5, as model 4, additionally adjusted for systolic blood pressure and NT-ProBNP. Model 6, as model 4, additionally adjusted for total cholesterol, HDL cholesterol and triglycerides. Model 7, as model 4, additionally adjusted for parental history of type 2 diabetes, alcohol intake and smoking status. Model 8, as model 4, additionally adjusted for plasma glucose and plasma insulin. The italic values represent the number of participants and events in the analyses.

difference could not be attributed to differences in the proportion of fast and slow twitch fibres.

In our study, within the females, we found higher plasma creatine concentrations in females that were postmenopausal, as compared to premenopausal females. A potential explanation for the higher postmenopausal plasma creatine concentrations might be the fact that estrogens are able to activate AMP-activated protein kinase (AMPK), which down-regulates the creatine transporter in the proximal tubulus, leading to enhanced creatine excretion.[51–53]

Sex is known to influence not only creatine homeostasis but also the incidence of type 2 diabetes. Studies have demonstrated that females are more prone to youth-onset type 2 diabetes, whereas males are more prone to midlife type 2 diabetes.[18,54] In the current study, where the mean age was 52 years, and we also found a higher incidence of type 2 diabetes in males. Sex-based differences in disease incidences, such as these, have led to editorial statements calling for clinical trials and large observational studies to report on sex-stratified results.[55,56] In the current study, we demonstrate that there are sex-based differences in both plasma creatine, type 2 diabetes and the interrelation between the plasma creatine and type 2 diabetes. Plasma creatine was more strongly associated with traditional risk factors for type 2 diabetes (among others weight, waist circumference, blood pressure, smoking status and parental history of diabetes) in males than in females. Additionally, plasma creatine itself was strongly associated with an increased risk of incident type 2 diabetes in males, but not in females. In a recent metabolomic study of human erythrocytes, it was shown that the creatine content of erythrocytes in patients with type 2 diabetes was also higher than that of healthy controls.[57] Unfortunately, no sex-stratified analyses were performed in this study. Although we are unable to explore the underlying mechanisms of the association of plasma creatine with incident type 2 diabetes, it may be speculated that it is related to mitochondrial dysfunction, a pivotal mechanism involved in the pathophysiology underlying type 2 diabetes,[2,6–9] for which plasma creatine has been hypothesized as a potential biomarker.[12,13] Interestingly, studies have demonstrated that estrogens have protective effects on mitochondrial functions.[58] A possible explanation for the found sex-based difference is that males are more prone to mitochondrial dysfunction leading to type 2 diabetes, for which high creatine may be an early biomarker. Additionally, it is possible that estrogens have a protective effect against the development of insulin resistance and hyperinsulinemia and that in participants prone to developing type 2 diabetes, higher insulin levels inhibit AMPK, which in turn stimulates creatine transport in the proximal tubulus, leading to higher plasma creatine concentrations.[59,60] In the current study, compared to females, males had more traditional risk factors for diabetes, including higher waist circumference, higher blood pressure and higher triglyceride levels, and males also had the highest incident rate for type 2 diabetes. Nonetheless, males had the lowest creatine concentrations. This paradox may partially be explained by the results of the linear regression analyses, where it is shown that plasma creatine is more strongly associated with traditional risk factors in males than in females. For example, the standardized beta of the association between plasma creatine and triglycerides is nearly twice as high in males as in females. In addition, the association between plasma creatine and systolic blood pressure is significant in males, but not in females. One possibility is that homeostasis of circulating creatine concentrations is different among males and females and that it is more affected by metabolic disturbances in males.

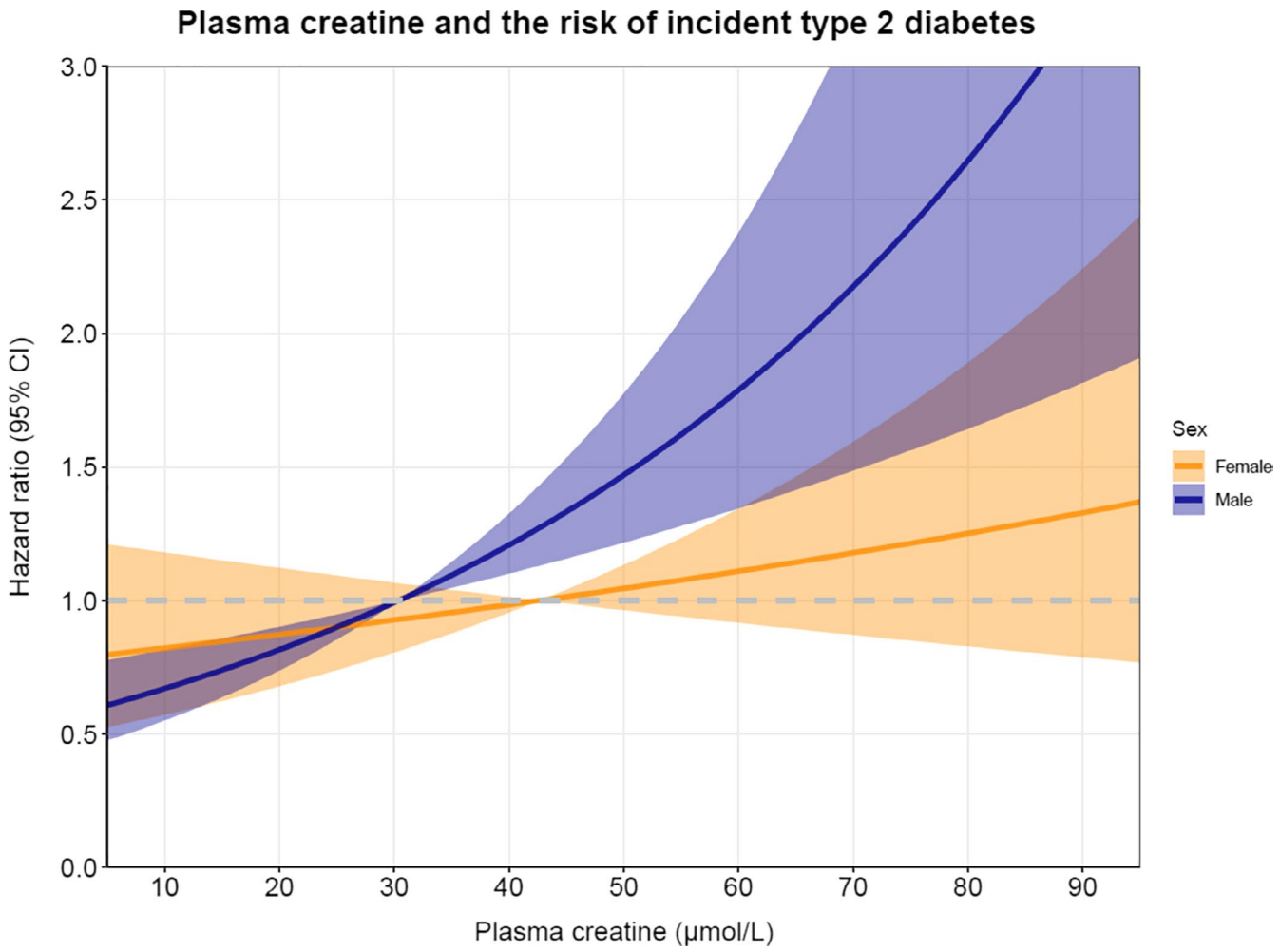

FIGURE 2 Graphical representation of the association of plasma creatine and type 2 diabetes in males and females. The lines show the adjusted hazard ratio (HR) and the shaded area corresponds to the 95% pointwise confidence interval (CI). The analyses were stratified for sex and adjusted for age and BMI. *p*-effect is .29 and <.001 in females and males, respectively

TABLE 4 Prospective associations of plasma creatine with risk of type 2 diabetes with adjustments for HOMA-IR and HOMA-β

	Total population		Males		Females	
	Per 1-SD		Per 1-SD		Per 1-SD	
	HR [95% CI]	*p*-value	HR [95% CI]	*p*-value	HR [95% CI]	*p*-value
Model 1	1.18 [1.02–1.36]	.03	1.37 [1.12–1.67]	.002	1.00 [0.80–1.24]	.97
Model 2	1.17 [1.01–1.36]	.03	1.37 [1.13–1.67]	.002	0.97 [0.78–1.22]	.82
Model 3	1.19 [1.03–1.37]	.02	1.37 [1.13–1.67]	.002	1.01 [0.81–1.26]	.95
Model 4	1.20 [1.04–1.39]	.01	1.40 [1.15–1.70]	.001	1.01 [0.81–1.26]	.92
Participants	*2690*		*1436*		*1254*	
Events	*207*		*126*		*81*	

Model 1: Adjusted for age, sex, BMI, eGFR and urinary albumin excretion. Model 2, as model 1, additionally adjusted for HOMA-IR. Model 3, as model 1, additionally adjusted for HOMA-β. Model 4, as model 1, additionally adjusted for HOMA-IR and HOMA-β. The italic values represent the number of participants and events in the analyses.

Regardless of the underlying mechanisms, the findings of the current study further underscore the need for sex-stratified analyses in studies regarding creatine homeostasis or type 2 diabetes.

Worth noting is that this study only investigated the association of plasma creatine in participants not taking creatine supplements and that these findings do not implicate an adverse effect of

creatine supplementation. In contrast, a small-scale clinical trial has demonstrated that creatine supplementation in combination with an exercise programme is able to improve glycemic control in type 2 diabetes.[61]

Noteworthy strengths of this study were the size of the study, the long-term follow-up, and the extensive data collection, allowing for the adjustment for a wide variety of potential confounders. However, there are several limitations that should be addressed, including the fact that the evaluation of plasma creatine in the current study was based on a single measurement, rather than on repeated measurements over time. Circulating creatine concentrations are known to follow diurnal variations, which have been shown to be mainly attributable to post-prandial effects of ingestion of alimentary creatine with meals containing meat or fish, leading to temporary increases in circulating creatine concentrations which are in proportion to the amount of creatine ingested with food.[22] The plasma samples in the current study were obtained between 8:00 and 10:00 a.m. after an overnight fast prior to blood collection, which minimalizes a potential influence of alimentary creatine ingested with fish or meat on circulating creatine concentrations.[22] According to data of Pasternack et al., circulating creatine concentrations remain stable over the day if subjects remain in the fasting state, with an intra-individual variation over the day of ~10%.[22] However, several other limitations of this study need to be addressed. Firstly, due to the observational design of this study, we were unable to investigate whether the relationship between plasma creatine and incident type 2 diabetes is causal or associative. Similarly, the observational design of this study does not allow to elucidate the biological mechanisms underlying the association of plasma creatine and incident type 2 diabetes.

In conclusion, we demonstrated that plasma creatine concentrations are lower in males than in females. In time-to-event analyses, higher plasma creatine is associated with an increased risk of incident type 2 diabetes, independent of potential confounders. This association was strongly modified by sex. Higher plasma creatine was associated with an increased risk of incident type 2 diabetes in males, but not in females. Future studies are warranted to define in more detail the underlying mechanisms for these sex-based differences of the association between plasma creatine and type 2 diabetes.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

All authors have substantially contributed to the manuscript design and/or revision and have approved this final version of the work. The authors have agreed to take accountability for all aspects of this study. The authors' responsibilities were as follows: AP conducted the literature search, analysed the data and created the figures. AP and SJLB drafted the initial manuscript. AP, DG, JCS, JLFG, JCS, RMD, IPK, RAB, EG, MAC, TW, RPFD, CFMF and SJLB revised and edited the manuscript. MAC and EG acquired the creatine data. All authors have read and approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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