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Nitric Oxide Precursors and Dimethylarginines as Risk Markers for Accelerated Measured GFR Decline in the General Population

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Introduction: Nitric oxide (NO) deficiency is associated with endothelial dysfunction, hypertension, atherosclerosis, and chronic kidney disease (CKD). Reduced NO bioavailability is hypothesized to play a vital role in kidney function impairment and CKD. We investigated the association of serum levels of endogenous inhibitors of NO, asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), and precursors of NO, arginine, citrulline, and ornithine, with a decline in glomerular filtration rate (GFR) and new-onset CKD.

Methods: In a prospective cohort study of 1407 healthy, middle-aged participants of Northern European origin in the Renal Iohexol Clearance Survey (RENIS), GFR was measured repeatedly with iohexol clearance during a median follow-up time of 11 years. GFR decline rates were analyzed using a linear mixed model, new-onset CKD (GFR $<$ 60 ml/min per 1.73 m^2) was analyzed with interval-censored Cox regression, and accelerated GFR decline (the 10% with the steepest GFR decline) was analyzed with logistic regression.

Results: Higher SDMA was associated with slower annual GFR decline. Higher levels of citrulline and ornithine were associated with accelerated GFR decline (odds ratio [OR], 1.43; 95% confidence interval [CI] 1.16–1.76 per SD higher citrulline and OR 1.23; 95% CI 1.01 to 1.49 per SD higher ornithine). Higher citrulline was associated with new-onset CKD, with a hazard ratio of 1.33 (95% CI 1.07–1.66) per SD higher citrulline.

Conclusions: Associations between NO precursors and the outcomes suggest that NO metabolism plays a significant role in the pathogenesis of age-related GFR decline and the development of CKD in middle-aged people.

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The prevalence of CKD is increasing, and, at present, CKD affects 8% to 10% of the world's population.[1] CKD is a major contributor to morbidity and mortality, and it is among the few noncommunicable diseases with increasing age-standardized mortality rates worldwide.[1,2] In addition, with an aging population, the incidence of CKD will continue to rise[1] because the loss of GFR with age is an important risk factor for CKD.[3] This age-related loss in GFR varies between persons; some individuals have a moderate age-related GFR loss, whereas others exhibit a steeper GFR decline.[4] A more rapid loss of GFR is associated with an increased risk for end-stage kidney disease and death.[5] However, differences in GFR decline rates are only partly explained by conventional CKD risk factors, such as obesity and hypertension.[6] Therefore, investigations of potential biomarkers that may shed light on underlying mechanisms of age-related GFR decline are essential as an early step toward CKD prevention.

NO is an important vascular regulator of vasodilation and antithrombotic processes.[7] NO synthase converts arginine to NO and citrulline (Figure 1). Ornithine and citrulline are the precursors of arginine through the urea cycle.[8] NO deficiency is considered to

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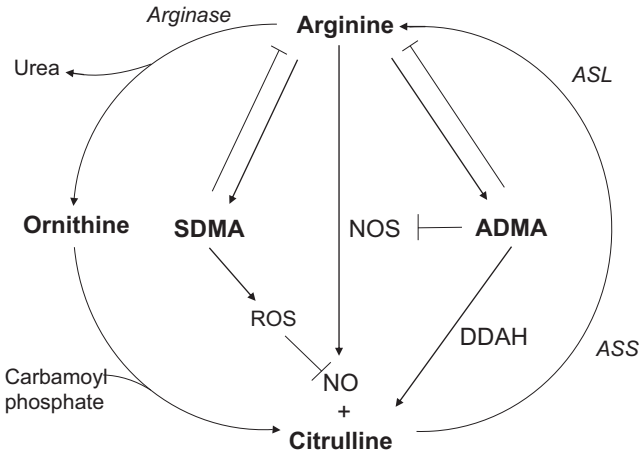

Figure 1. Pathway of NO and arginine metabolism. Arginase converts arginine to ornithine and urea. Arginosuccinate synthase and lyase converts citrulline to arginine, completing the urea cycle. NOS converts arginine to citrulline and NO. ADMA inhibits NOS. SDMA stimulates ROS, which inhibits NO. DDAH converts ADMA to citrulline and dimethylamine. ADMA, asymmetric dimethylarginine; ASL, arginosuccinate lyase; ASS, arginosuccinate synthase; DDAH, dimethylarginine dimethylaminohydrolase; NO, nitric oxide; NOS, nitric oxide synthase; ROS, reactive oxygen species; SDMA, symmetric dimethylarginine.

contribute to endothelial dysfunction, atherosclerosis, as well as pulmonary and systemic hypertension.[9,10] ADMA has a direct inhibiting effect on NO,[10] and SDMA has an indirect inhibiting effect on NO.[11] Both have been identified as independent risk factors for cardiovascular disease (CVD) and death in patients with CKD and the general population.[12–15] Because CKD and CVD share several risk factors, increased levels of ADMA and SDMA could potentially also increase the risk of GFR loss and incident CKD. Although cross-sectional studies have found impaired NO metabolism in persons with decreased GFR,[15,16] it is unknown whether markers of impaired NO metabolism, such as deviations of ADMA, SDMA, arginine, citrulline, and ornithine, are associated with increased risk of accelerated age-related GFR decline and incident CKD.

To the best of our knowledge, no longitudinal studies have investigated the association between ADMA and SDMA and the decline in GFR or new-onset CKD in a general population.[15,17,18] A few have investigated the association between arginine, citrulline, and ornithine and GFR decline. In a longitudinal study, citrulline has been associated with new-onset CKD.[19,20] In addition, a cross-sectional study showed increased levels of arginine and citrulline to be associated with reduced estimated GFR (eGFR) and CKD prevalence. These studies were based on eGFR from serum creatinine, a method that lacks precision in the normal range of GFR and is biased by non–GFR-related factors.[21,22]

The present study aimed to investigate the serum levels of ADMA, SDMA, arginine, citrulline, and ornithine as risk markers for GFR decline using iohexol clearance in a population-based cohort study. We hypothesized that higher levels of dimethylarginines and/or deviations in arginine, citrulline, and ornithine levels were associated with accelerated GFR decline and incident CKD.

METHODS

Study Population

The Tromsø Study is a population-based prospective study with repeated health surveys of representative samples of the inhabitants of the municipality of Tromsø in Northern Norway.[23] The RENIS is a substudy of the Tromsø Study. The baseline investigation of RENIS (RENIS-T6), performed between 2007 and 2009, included 1627 individuals aged 50 to 62 years as described in detail elsewhere.[24] Participants with self-reported kidney disease, CVD, or diabetes were excluded. Of the 1627 participants at baseline, 1324 (81%) were examined in the RENIS follow-up study (RENIS-FU, between 2013 and 2015),[24,25] and 1174 (72%) were examined in RENIS-3 (2018–2020)[26] (Figure 2a). The median follow-up time was 10.86 years (range: 4.39–12.82).

In this study, participants without baseline dimethylarginines and NO precursor measurements were excluded ($n = 3$). In addition, participants had to be present at baseline and have at least 1 follow-up to be included, resulting in a study population of 1407 participants for the analyses (Figure 2b).

The Regional Ethics Committee of Northern Norway approved the study, and the study adhered to the Declaration of Helsinki. All participants provided written informed consent.

Baseline Data and Measurements

Measurements were performed in the morning after overnight fasting at the Clinical Research Unit at the University Hospital of Northern Norway. All participants answered a questionnaire about smoking status and current medications. Body height and weight were measured, and body mass index was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured as automated office blood pressure as described previously.[27] Three first-void morning urine samples were collected on separate days before the GFR measurement, the urinary albumin-to-creatinine ratio (ACR) was calculated for each urine specimen, and the mean ACR value was used in the analyses.[28] Fasting plasma samples for glucose and C-reactive protein were drawn for biochemical analyses from a Teflon catheter placed in

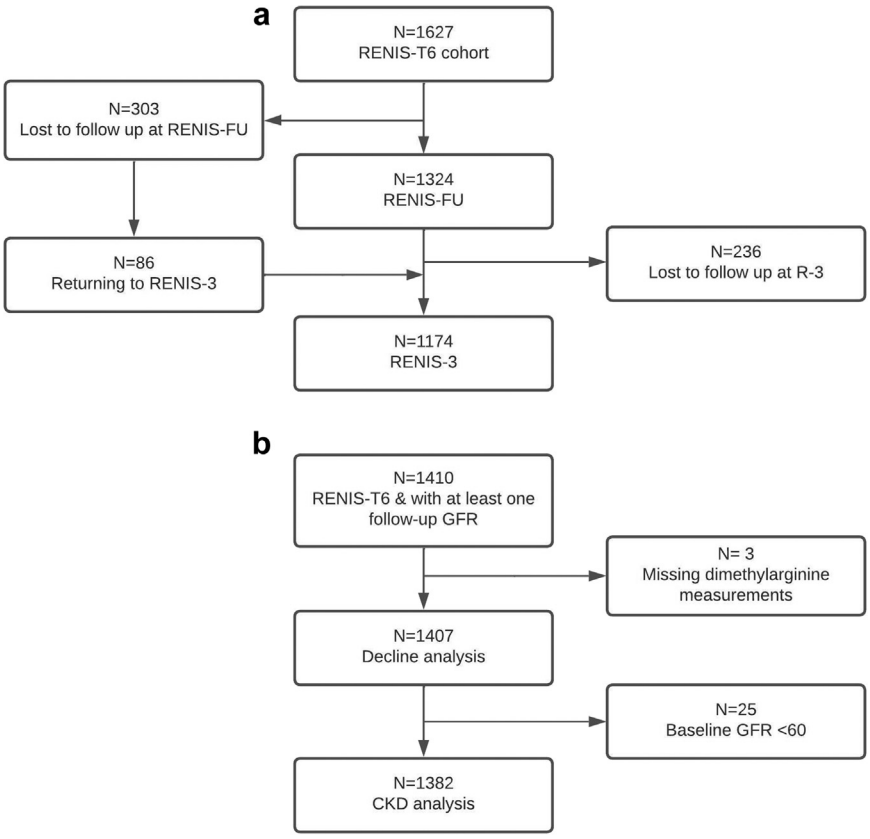

Figure 2. (a) Inclusion of participants in the Renal Iohexol Clearance Survey from Tromsø 6 (RENIS-T6), the first follow-up (RENIS-FU), and third Renal Iohexol Clearance Survey (RENIS-3). (b) Number of baseline participants with at least 1 follow-up GFR measurement and the corresponding number of participants in the different analyses. CKD, chronic kidney disease; GFR, glomerular filtration rate; RENIS-FU, Renal Iohexol Clearance Survey Follow-Up.

an antecubital vein. Serum samples used to measure ADMA, SDMA, arginine, citrulline, and ornithine levels were stored at −80 °C and thawed the day of the analysis.

Iohexol Clearance

GFR was measured at RENIS-T6, RENIS-FU, and RENIS-3 using single-sample plasma iohexol clearance, previously described in detail.[25] Single-sample iohexol clearance has been validated against gold-standard methods.[29] A Teflon venous catheter was used to inject 5 ml of iohexol (Omnipaque, 300 mgI/ml, Amersham Health, London, UK). The optimal time from injection to the sampling of blood for iohexol measurements was calculated using Jacobssons's method.[30] The serum iohexol concentrations were measured by using high-performance liquid chromatography at baseline and RENIS-FU and liquid chromotography–mass spectrometry at RENIS-3.[31] The iohexol measurements in RENIS-T6 and RENIS-3 were calibrated to the RENIS-FU measurements by reanalysis of frozen samples (Supplementary Methods). GFR was calculated using the equations of Jacobsson[30] and standardized to a body surface area of 1.73 m^2.[32] The intra-assay coefficient of variation during the study period was 3.0%, 3.1%, and 2.8% for RENIS-T6, RENIS-FU, and RENIS-R3, respectively.[33,34] The intraindividual variation in the GFR measurements for a random sample of 88 participants who received repeated follow-up measurements within 8 weeks was 4.2%.[35]

Dimethylarginines and NO Precursors

The measurement of ADMA, SDMA, arginine, citrulline, and ornithine in the RENIS cohort has been described in detail previously.[21] Serum levels of dimethylarginines and NO recursors were analyzed by a liquid chromatography–mass spectrometry system consisting of a Waters Acquity UPLC *I*-Class flow through needle system with an autosampler and a binary solvent delivery system (Waters, Milford, MA) interfaced with a Waters Xevo TQ-X benchtop tandem quadrupole mass spectrometer (Waters, Manchester, UK). ADMA, SDMA, arginine, citrulline, and ornithine were obtained from Sigma-Aldrich (St. Louis, MO), and labeled versions were purchased from Toronto Research Chemicals (Ontario, Canada). The precision of the interday coefficient of variation was measured at <8% on 3 different days for ADMA, SDMA, arginine, citrulline, and ornithine.

Statistical Analysis

Continuous variables are given as the means ± SD for normally distributed variables or the median and interquartile range for skewed variables. The baseline data were presented across quartiles of the measured GFR (mGFR).

The association between the dimethylarginines and NO precursors and the annual GFR change rate was analyzed using linear mixed models with random intercept and slope and an unstructured covariance matrix.[5] In these analyses, a negative GFR change rate corresponds to a mean annual reduction in GFR. ADMA, SDMA, arginine, citrulline, and ornithine were studied as independent variables in separate models adjusted for known CKD risk factors (age, sex, body mass index, systolic blood pressure, use of blood pressure–lowering drugs [use of angiotensin-converting enzyme inhibitors, angiotensin receptor II blockers, diuretics, calcium blockers, beta-blockers, or other antihypertensive medications (yes/no)], fasting glucose, smoking status, C-reactive protein and ACR).

There were 3 models for multiple linear mixed models. Model 1 was unadjusted. Model 2 was adjusted for baseline age, sex, and body mass index. Model 3 was adjusted as model 2 with the addition of adjustments for baseline office blood systolic pressure, the use of blood pressure–lowering drugs, fasting glucose, current smoking, ACR, and C-reactive protein as a marker of inflammation because of SDMA's effect on reactive oxygen species. Whether there was an interaction between the GFR change rate and sex was assessed by including a 3-way cross product between sex, the time variable, and the dimethylarginines or NO precursors.

The annual GFR change rate (in ml/min per 1.73 m^2 per year) for each participant was calculated using a linear mixed model adjusted for age, sex, and known CKD risk factors, body mass index, systolic blood pressure, and blood pressure–lowering drugs at baseline using a within-person centered time variable.[36] The centered time variable was used to minimize confounding due to baseline associations between dimethylarginines or NO precursors, CKD risk factors, and GFR.[5,36] The associations between accelerated decline and ADMA, SDMA, arginine, citrulline, and ornithine were studied in separate multiple logistic regression models. Accelerated GFR decline was defined as a dichotomous variable (yes/no), indicating the 10% of participants with the steepest annual GFR decline rate calculated using the mixed model described above.[5,36,37]

Incident CKD was defined as new-onset GFR $<$ 60 ml/min per 1.73 m^2.[38] The exact time when the outcome occurred was not observed but was only known to occur between 2 observations. Therefore, associations between baseline levels of dimethylarginines and NO precursors and incident CKD were examined with interval-censored Cox regression. The time interval of the event was defined as the last observation with GFR $\geq$ 60 ml/min per 1.73 m^2 and the first observation with GFR $<$ 60 ml/min per 1.73 m^2. Participants with GFR $<$ 60 ml/min per 1.73 m^2 at baseline ($n = 25$) were excluded from the interval-censored Cox analyses of incident CKD. Because only 50 of the remaining 1594 persons (3%) died before we started the last wave of iohexol clearance measurements, we did not perform an analysis with death as a competing risk

In the multiple logistic models and interval-censored Cox regression, the same 3 models were used but adjusted for baseline mGFR in models 2 and 3. Interval-censored Cox regression was not adjusted for ACR and C-reactive protein in model 3 because of few events. Interactions between accelerated GFR decline or new-onset CKD and sex were assessed by including a 2-way cross product between sex and dimethylarginine or NO precursor in question.

Interval-censored Cox regression analyses for new-onset CKD with/without albuminuria (mGFR $<$ 60 ml/min per 1.73 m^2 and/or new-onset ACR $>$ 3 mg/mmol) were performed and shown in Supplementary Table S2. As a sensitivity analysis, rapid GFR decline, defined as an annual loss $>$ 3 ml/min per 1.73 m^2 per year calculated using the slope described previously in this article, was analyzed as multiple logistic regression models. For comparison, in additional analyses, we used eGFR instead of mGFR as the dependent variable for each outcome in the supplement.

A P value below 0.05 was considered statistically significant. Statistical analyses were performed with STATA/MP software, version 17.0 (StataCorp LP, College Station, TX).

RESULTS

The baseline characteristics of the study population ($N = 1407$) are presented across quartiles of the mGFR in Table 1.

GFR was negatively correlated with ADMA ($r = -0.18$), SDMA ($r = -0.43$), and citrulline ($r = -0.20$) with a P value of <0.001 (Supplementary Table S1). ACR did not correlate with any of the dimethylarginines or the NO precursors at baseline (Supplementary Table S1). The pairwise correlations between the dimethylarginines and the NO precursors were in the range of 0.15 to 0.47.

In the multivariable-adjusted linear mixed model analyses, ornithine was associated with a steeper

Table 1. Baseline characteristics of the Renal Iohexol Clearance Survey participants according to mGFR quartiles ($N = 1407$)

	Quartile of glomerular filtration rate, range (ml/min per 1.73 m^2)			
	Quartile 1 ($n = 352$)	Quartile 2 ($n = 352$)	Quartile 3 ($n = 352$)	Quartile 4 ($n = 351$)
Characteristic	**≤85.3**	**85.4–93.9**	**94–103.5**	**>103.6**
Sex, men *n* (%)	111 (32)	161 (46)	188 (53)	236 (67)
Age, yr	59.1 (3.5)	58.2 (3.8)	57.5 (3.9)	57.1 (4.0)
BMI, kg/m^2	27.3 (4.2)	27.0 (3.9)	27.2 (3.7)	27.4 (3.7)
Systolic BP, mm Hg	129.4 (17.9)	129.5 (17.8)	129.1 (17.5)	129.0 (16.3)
Diastolic BP, mm Hg	83.17 (10.1)	83.89 (10.0)	83.08 (9.9)	83.40 (8.9)
Antihypertensive medication, *n* (%)	72 (20)	57 (16)	62 (18)	58 (17)
Fasting glucose, mmol/l	5.2 (0.5)	5.3 (0.5)	5.4 (0.5)	5.5 (0.6)
Current smoking, *n* (%)	55 (16)	59 (17)	62 (18)	91 (26)
CRP, mg/l (IQR)	1.2 (0.7–2.3)	1.1 (0.6–2.1)	1.2 (0.7–2.2)	1.2 (0.6–2.1)
ACR, mg/mmol (IQR)	0.2 (0.1–0.5)	0.2 (0.1–0.5)	0.2 (0.1–0.5)	0.3 (0.1–0.5)
mGFR ml/min per 1.73 m^2	76.1 (9.1)	89.6 (2.5)	98.6 (2.7)	111.7 (6.9)
ADMA, µmol/l	0.442 (0.05)	0.435 (0.05)	0.423 (0.05)	0.417 (0.05)
SDMA, µmol/l	0.67 (0.1)	0.64 (0.1)	0.61 (0.1)	0.57 (0.1)
Arginine, µmol/l	92.3 (16.3)	93.4 (16.5)	93.3 (16.6)	95.4 (17.5)
Citrulline, µmol/l	23.0 (6.4)	22.0 (6.1)	20.6 (6.0)	19.7 (5.6)
Ornithine, µmol/l	63.5 (14.5)	63.0 (14.3)	62.0 (14.2)	62.3 (14.6)

ACR, albumin-to-creatinine ratio; ADMA, asymmetric dimethylarginine; Arg, arginine; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; DMA, dimethylarginine; GABR, global arginine bioavailability ratio, GFR, glomerular filtration rate; IQR, interquartile range, mGFR, measured glomerular filtration rate; SDMA, symmetric dimethylarginine.
Data are shown as mean (SD), median (interquartile range), or *n* (%).

annual GFR change rate (per SD increment; −0.07 ml/min per 1.73 m^2 per year, 95% CI, −0.13 to −0.001; $P < 0.05$) (model 3, Table 2). Participants with a higher baseline level of SDMA, however, had a slower annual GFR decline rate (0.07 ml/min per 1.73 m^2 per year; 95% CI, 0.002–0.13; $P = 0.04$). The other biomarkers were not statistically significantly associated with the annual GFR change rate. There was no interaction between sex and the levels of dimethylarginines or the NO precursors (data not shown).

The cutoff for the 10% with steepest mGFR decline was set at >1.62 ml/min per 1.73 m^2 per year.

Higher baseline levels of citrulline and ornithine were associated with higher odds of accelerated GFR decline (Table 3). The odds of an accelerated GFR decline increased by 43% (OR, 1.43; 95% CI, 1.16–1.76) per SD increment citrulline level and 23% (OR, 1.23; 95% CI, 1.01–1.49) per SD higher ornithine level in the multivariable-adjusted logistic regression analyses (model 3, Table 3).

Of the 1382 participants without CKD at baseline, 94 (7%) developed CKD during follow-up. The interval-censored Cox regression analyses showed an association between the development of CKD and higher baseline levels of citrulline (hazard ratio, 1.33; 95% CI, 1.07–1.66 per SD higher citrulline) in the multivariable-adjusted model (model 3, Table 4).

In sensitivity analyses, 48 (3.6%) participants had a rapid GFR decline >3 ml/min per 1.73 m^2 (Supplementary Table S3). Analyses with eGFR instead of mGFR are presented in Supplementary Tables S4, S5, and S6. SDMA and citrulline show associations with

Table 2. Linear mixed model regression analyses of the associations between dimethylarginines, nitric oxide precursors, and their ratios with measured glomerular filtration rate change rates ($N = 1407$)

	Model 1		Model 2		Model 3	
Independent variable	**ml/min per 1.73 m^2 per yr per SD[a] (95% CI)**	***P* value**	**ml/min per 1.73 m^2 per yr per SD[a] (95% CI)**	***P* value**	**ml/min per 1.73 m^2 per yr per SD[a] (95% CI)**	***P* value**
ADMA	−0.00 (−0.07 to 0.06)	0.99	−0.00 (−0.07 to 0.06)	0.97	0.00 (−0.06 to 0.07)	0.93
SDMA	0.07 (0.00–0.13)	0.05	0.06 (−0.00 to 0.13)	0.05	0.07 (0.00–0.13)	0.04
Arginine	−0.05 (−0.11 to 0.02)	0.14	−0.05 (−0.12 to 0.02)	0.13	−0.05 (−0.12 to 0.01)	0.13
Citrulline	−0.05 (−0.11 to 0.02)	0.17	−0.05 (−0.11 to 0.02)	0.15	−0.04 (−0.10 to 0.03)	0.26
Ornithine	−0.07 (−0.14 to −0.01)	0.03	−0.07 (−0.14 to −0.01)	0.03	−0.07 (−0.13 to −0.00)	0.04

ADMA, asymmetric dimethylarginine; Arg, arginine; CI, confidence interval; OR, odds ratio; SDMA, symmetric dimethylarginine.
[a]A negative coefficient indicates a steeper decline.Model 1: crude.
Model 2: adjusted for age, sex, and BMI.
Model 3: model 2 and adjusted for systolic blood pressure, use of angiotensin-converting enzyme inhibitors, angiotensin receptor II blockers, diuretics, calcium blockers, beta-blockers, or other antihypertensive medications (yes/no), fasting glucose, smoking status (yes/no), C-reactive protein, and albumin-to-creatinine ratio.
Each row represents a separate regression model.

Table 3. Associations of biomarkers with accelerated GFR decline defined as the 10% with the steepest GFR decline rate

	Model 1		Model 2		Model 3	
Independent variable	OR per SD (95% CI)	*P* value	OR per SD (95% CI)	*P* value	OR per SD (95% CI)	*P* value
ADMA	1.16 (0.97–1.37)	0.1	1.13 (0.94–1.35)	0.2	1.11 (0.91–1.36)	0.30
SDMA	1.06 (0.89–1.27)	0.50	1.08 (0.88–1.33)	0.47	1.14 (0.91–1.41)	0.26
Arginine	1.28 (1.08–1.52)	0.005	1.19 (0.99–1.42)	0.06	1.07 (0.88–1.30)	0.48
Citrulline	1.39 (1.17–1.64)	<0.001	1.52 (1.26–1.84)	<0.001	1.43 (1.16–1.76)	0.001
Ornithine	1.39 (1.18–1.63)	<0.001	1.36 (1.15–1.62)	<0.001	1.23 (1.01–1.49)	0.04

ADMA, asymmetric dimethylarginine; CI, confidence interval; GFR, glomerular filtration rate; OR, odds ratio; SDMA, symmetric dimethylarginine.
Model 1: crude.
Model 2: adjusted for age, sex, BMI, and baseline GFR.
Model 3: model 2 and adjusted for systolic blood pressure, use of angiotensin-converting enzyme inhibitors, angiotensin receptor II blockers, diuretics, calcium blockers, beta-blockers, or other antihypertensive medications (yes/no), fasting glucose, smoking status (yes/no), C-reactive protein, and albumin-to-creatinine ratio.
Each row represents a separate regression model.

the 10% with accelerated eGFR decline, OR 1.41 (CI, 1.16–1.73) and 1.24 (CI, 1.03–1.49), respectively. With eGFR, 58 (4.3%) participants fulfilled the criteria for CKD. In the analysis with Cox interval-censored regression, citrulline (HR, 1.34; 95% CI, 1.03–1.73) was associated with new-onset CKD. There were no statistically significant interactions between sex and the levels of dimethylarginines or NO precursors for accelerated GFR decline or incident CKD (data not shown).

DISCUSSION

Although NO deficiency has been proposed as a common pathway for progressive kidney disease, its role in early CKD development has been unclear. In the present prospective, population-based cohort study, there were no associations between higher levels of ADMA or lower arginine with accelerated GFR decline or new-onset CKD. However, participants with higher baseline levels of serum citrulline had a higher risk of accelerated GFR decline and new-onset CKD, whereas higher baseline levels of serum ornithine were associated with a higher risk of accelerated GFR decline.

To our knowledge, this is the first study assessing the association between ADMA and SDMA with the decline of GFR or new-onset CKD in a general population.[15,17,18] Consistent with previous cross-sectional studies, we found a correlation between higher SDMA and reduced GFR.[39,40] This is not surprising because 90% of SDMA is eliminated by glomerular filtration in the kidneys.[17] In contrast, ADMA was poorly associated with mGFR. This may partially be due to ADMA clearance being more dependent on the metabolism of dimethylarginine dimethylaminohydrolase than the GFR.[12,16,17]

NO deficiency has been of interest when investigating the underlying mechanism behind endothelial dysfunction in CKD.[41] Animal studies performed *in vitro* and *in vivo* have investigated the association between dimethylarginines and NO precursors.[7,9,11,16,41] Higher levels of ADMA and lower levels of arginine have been proposed as the underlying mechanisms leading to GFR decline and CKD progression.[7] SDMA limits the arginine supply to NO metabolism[11] and has also been suggested as a marker of kidney function.[42] Therefore, our findings that higher SDMA was associated with slower annual GFR decline may be contraintuitive relative to previous studies. However, the effect on the GFR change rate was small, may not be clinically relevant, and should be interpreted with caution. Most previous studies investigating ADMA and SDMA as biomarkers for diseases have assessed the risk of CVD and mortality[12–15,17,43–45] and not kidney outcomes. A small number of studies have observed an association between

Table 4. Cox interval–censored regression analyses for new-onset chronic kidney disease with measured glomerular filtration rate < 60 ml/min per 1.73 m^2 ($n = 94$)

	Model 1		Model 2		Model 3	
Independent variable	HR per SD (95% CI)	*P* value	HR per SD (95% CI)	*P* value	HR per SD (95% CI)	*P* value
ADMA	1.29 (1.06–1.57)	0.01	1.03 (0.85–1.26)	0.75	1.03 (0.84–1.28)	0.76
SDMA	1.84 (1.52–2.22)	<0.001	1.25 (1.00–1.56)	0.05	1.23 (0.98–1.54)	0.07
Arginine	1.05 (0.85–1.29)	0.68	1.06 (0.87–1.30)	0.56	1.07 (0.87–1.31)	0.51
Citrulline	1.59 (1.31–1.93)	<0.001	1.30 (1.06–1.58)	0.01	1.33 (1.07–1.66)	0.01
Ornithine	1.17 (0.95–1.43)	0.13	1.19 (0.97–1.47)	0.10	1.17 (0.93–1.47)	0.19

ADMA, asymmetric dimethylarginine; CI, confidence interval; HR, hazard ratio; SDMA, symmetric dimethylarginine.
Model 1: crude.
Model 2: adjusted for age, sex, BMI, and baseline GFR.
Model 3: model 2 and adjusted for systolic blood pressure, use of angiotensin-converting enzyme inhibitors, angiotensin receptor II blockers, diuretics, calcium blockers, beta-blockers, or other antihypertensive medications (yes/no), fasting glucose, and smoking status (yes/no).
Each row represents a separate regression model.

increased serum concentrations of ADMA and SDMA and the progression of CKD.[12,13,17,41,43] In comparison to our study, however, these studies included participants with preexisting CKD. In addition, they assessed kidney function with eGFR, which may be influenced by non–GFR-related factors[46] such as CVD risk factors, inflammation, and levels of dimethylarginines,[21,22,47] possibly leading to spurious associations with GFR change rates during follow-up. Indeed, SDMA was associated with accelerated GFR decline using eGFR (Supplementary Table S4), but not with mGFR, indicating confounding due to non–GFR-related factors. Another explanation for the unexpected association of SDMA with a slower mean mGFR decline rate could be an association of SDMA (an inflammatory marker) with hyperfiltration, leading to a phase of increasing GFR in participants with, for example, prediabetes or metabolic syndrome. A phase of hyperfiltration may persist for several years in relatively healthy persons, for example, with those with prediabetes.[33] In line with this hypothesis, SDMA was borderline associated with an increased risk of incident CKD at the end of follow-up.

We found associations between serum citrulline and accelerated GFR decline and new-onset CKD, as well as an association between serum ornithine and accelerated GFR decline. Ornithine competes for the same transport system as arginine.[48] An increase in ornithine may result in a reduction in intracellular arginine availability. Only a few longitudinal studies investigated the association of citrulline with new-onset CKD in the general population.[19,20] Consistent with our observations, the Framingham Heart Study observed a 50% increased risk of CKD development per SD increase in citrulline levels.[19] Another cohort study found a strong association between high levels of citrulline and new-onset CKD.[20] Citrulline is produced from ornithine and further to arginine in the urea cycle,[8] and all are necessary precursors in the biological pathway of NO (Figure 1). An early elevated level of citrulline for persons developing CKD may be explained by reduced citrulline to arginine conversion[49] and/or overproduction of citrulline trying to compensate for the NO shortage.[50] Although no association between arginine and new-onset CKD or accelerated GFR decline was found in our study, disorders of arginine metabolism may be associated with kidney function decline. The citrulline and ornithine association suggests a disorder in endothelial function not captured by measuring serum arginine levels.

The main strength of the present study is the prospective design and the repeated measurements by iohexol clearance in a cohort recruited from the general population, an accurate method to establish GFR without the influence of non–GFR-related factors.[21,22] Previous studies have used eGFR with creatinine or cystatin C to examine the association. Another strength is the relatively long observation period from 2007 to 2020, where only a few participants (27.8%) were lost to follow-up.

Our study has limitations. The enrollment of only middle-aged participants of Northern European origin limits the generalizability of the study. In addition, individuals participating in cohorts over time tend to be healthier,[51] and the study had few (94 [7%]) CKD events. The concentration of the dimethylarginines and NO precursors was analyzed at one time point only. The concentration of the dimethylarginines and NO precursors may vary over the years.

In conclusion, higher levels of citrulline and ornithine, but not ADMA, SDMA, and arginine, were associated with accelerated GFR decline in a general population without diabetes, CKD, or CVD. This suggests that NO metabolism plays a role in age-related GFR decline and early CKD. Our findings warrant further investigation into the pathogenesis of NO metabolism and the possible therapeutic targets and preventive measures.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods. Calibration of the HPLC and LC/MS analyses of serum iohexol.

Table S1. Spearman correlation coefficients between dimethylarginines and the nitric oxide precursors and the baseline characteristics.

Table S2. Interval-censored Cox regression analyses for chronic kidney disease (defined as new-onset measured glomerular filtration rate < 60 ml/min per 1.73 m^2 and/or new-onset albumin-to-creatinine ratio > 3 mg/mmol).

Table S3. Associations of biomarkers with rapid GFR decline defined as an annual loss > 3 ml/min per 1.73 m^2 ($n = 48$).

Table S4. Associations of biomarkers with accelerated GFR decline defined as the 10% with the steepest estimated

GFR decline rate (cutoff 10-percentile −1.48 ml/min per 1.73 m^2 annually).

Table S5. Cox interval–censored regression analyses for new-onset chronic kidney disease with estimated glomerular filtration rate < 60 ml/min per 1.73 m^2 ($n = 58$).

Table S6. Linear mixed model regression analyses of the associations between dimethylarginines, nitric oxide precursors, and their ratios with estimated glomerular filtration rate change rates ($N = 1407$).

Strobe Checklist.

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