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Plasma arginine metabolites in health and chronic kidney disease

Amy Y.M. Au[1,2,†], Kevin Mantik[3,†], Forough Bahadory[3], Paul Stathakis[3], Hayley Guiney[4], Jonathan Erlich[1,2], Robert Walker[5], Richie Poulton[4], Andrea Rita Horvath[3] and Zoltan H. Endre[1,2]

[1]Department of Nephrology, Prince of Wales Hospital, Sydney, NSW, Australia
[2]Faculty of Medicine & Health, University of New South Wales, Sydney, NSW, Australia
[3]Department of Chemical Pathology, New South Wales Health Pathology, Prince of Wales Hospital, Sydney, NSW, Australia
[4]Department of Psychology, Dunedin Multidisciplinary Health and Development Research Unit, University of Otago, Dunedin, New Zealand
[5]Department of Medicine, Otago Medical School, University of Otago, Dunedin, New Zealand
Correspondence to: Amy Y.M. Au; E-mail: yokemooi.au@unsw.edu.au
[†]Co-first authors.

ABSTRACT

Background. Elevated plasma asymmetric and symmetric dimethylarginine (ADMA and SDMA) are risk factors for chronic kidney disease (CKD) and cardiovascular disease. Using plasma cystatin C (pCYSC)-based estimated glomerular filtration rate (eGFR) trajectories, we identified a cohort at high risk of poor kidney-related health outcomes amongst members of the Dunedin Multidisciplinary Health and Development Study (DMHDS). We therefore examined associations between methylarginine metabolites and kidney function in this cohort.

Methods. ADMA, SDMA, L-arginine and L-citrulline were measured in plasma samples from 45-year-olds in the DMHDS cohort by liquid chromatography–tandem mass spectrometry.

Results. In a healthy DMHDS subset ($n = 376$), mean concentrations were: ADMA (0.40 ± 0.06 µmol/L), SDMA (0.42 ± 0.06 µmol/L), L-arginine (93.5 ± 23.1 µmol/L) and L-citrulline (24.0 ± 5.4 µmol/L). In the total cohort ($n = 857$), SDMA correlated positively with serum creatinine (Pearson's $r = 0.55$) and pCYSC ($r = 0.55$), and negatively with eGFR ($r = 0.52$). A separate cohort of 38 patients with stage 3–4 CKD (eGFR 15–60 mL/min/1.73 m^2) confirmed significantly higher mean ADMA (0.61 ± 0.11 µmol/L), SDMA (0.65 ± 0.25 µmol/L) and L-citrulline (42.7 ± 11.8 µmol/L) concentrations. DMHDS members classified as high-risk of poor kidney health outcomes had significantly higher mean concentrations of all four metabolites compared with individuals not at risk. ADMA and SDMA individually predicted high-risk of poor kidney health outcomes with areas under the ROC curves (AUCs) of 0.83 and 0.84, and together with an AUC of 0.90.

Conclusions. Plasma methylarginine concentrations facilitate stratification for risk of CKD progression.

Keywords: ADMA, arginine, heart disease, kidney disease, SDMA

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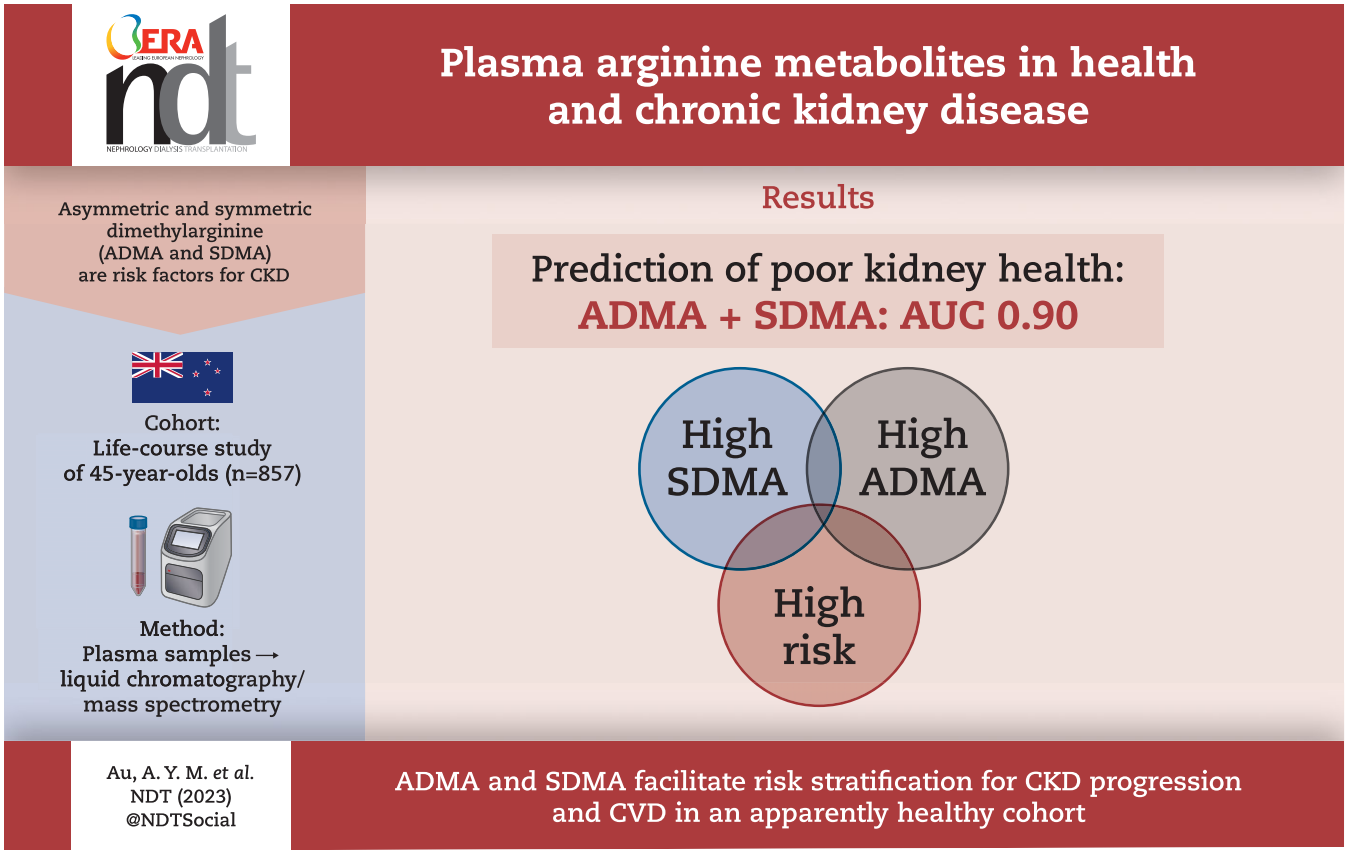
GRAPHICAL ABSTRACT



KEY LEARNING POINTS

What was known:

- Elevated plasma asymmetric and symmetric dimethylarginine (ADMA and SDMA) are risk factors for chronic kidney disease (CKD) and cardiovascular disease (CVD).

This study adds:

- In a whole of life cohort study, ADMA and SDMA predicted individuals at high risk of poor kidney-related outcomes as defined by cystatin C estimated glomerular filtration rate trajectory analysis. Arginine metabolites are complimentary to traditional kidney (and CVD) risk biomarkers.

Potential impact:

- ADMA and SDMA facilitates risk stratification for CKD progression in an apparently healthy cohort.

INTRODUCTION

Chronic kidney disease (CKD) has a global incidence of 9.1%, is the 12th leading cause of death and, with an increase of 41.5% between 1990 and 2017 [1], is predicted to become the fifth highest cause of years of life lost globally by 2040 [2]. Deaths attributable to CKD (1.2 million) or cardiovascular disease (CVD) attributable to reduced kidney function (1.4 million) represented 4.6% of total mortality in 2017 [1]. CVD is the major cause of death in patients with CKD with most patients dying prior to developing end-stage kidney disease, although CVD also remains the most common cause of death in dialysis patients [3]. There is thus considerable interest in identifying and modifying risk factors for CVD associated with reduced kidney function. In parallel, there is growing awareness of the bidirectional interaction between the kidney and heart in acute and chronic disease of either organ. This has led to classification of the cardiorenal syndromes [4] and interest in identifying biomarkers that characterize these interactions [5–7].

Biomarkers predictive of CVD in early CKD and in the general community include eGFR and albuminuria [8]. A number of biomarkers associated with inflammation are associated with CVD in CKD patients on dialysis [9]. Between these extremes of kidney function, many potential biomarkers of CVD have been identified [5–7]. However, when circulating low molecular weight biomarkers are cleared by the kidney through filtration or tubular secretion, the specificity of such biomarkers for CVD becomes uncertain because an elevation may simply reflect an increased half-life of the biomarker resulting from reduced clearance, as kidney function decreases in CKD. Selection of biomarkers that predict increased risk of cardiovascular events in individuals with CKD should utilize biomarkers in the causal pathway for CVD. Corresponding reference intervals are needed for such

biomarkers in healthy members and for variation with kidney function.

Published data support elevated plasma asymmetric and symmetric dimethylarginine (ADMA and SDMA) as risk factors in prediction of CKD and CVD [10–12]. Elevated ADMA strongly predicts CKD progression [13–15] and is an independent risk factor for all-cause mortality in CVD [12]. SDMA levels correlated strongly with kidney function in a meta-analysis of 2136 patients [16] and outperformed ADMA as a predictor of atherosclerotic cardiovascular events and CKD progression [17].

The Dunedin Multidisciplinary Health and Development Study (DMHDS) is an ongoing longitudinal investigation of health and behaviour in a representative 1972–73 birth cohort of 1037 individuals [18]. We recently used group-based trajectory modelling of eGFR based on plasma cystatin C (pCYSC) to identify common kidney-function trajectories between ages 32 and 45 years [19] and establish links between trajectories and kidney-related outcomes at age 45 years. Three trajectory groups were identified and were differentiated by age 32 years: normal (58% of participants), low-normal (36%) and high-risk (6%). Modifiable factors such as blood pressure (BP), body mass index (BMI), inflammation, glycated haemoglobin, smoking and socioeconomic status were associated with steeper age-related declines in kidney function, particularly among low-normal and high-risk groups. Those in low-normal and high-risk groups had more adverse kidney-related outcomes at age 45 years [19].

In order to determine whether ADMA and SDMA can predict kidney status in this unique DMHDS cohort, these metabolites were assayed using liquid chromatography–tandem mass spectrometry (LC-MS/MS). After identifying a reference interval in a healthy subset of this cohort, we examined the association between methylarginine metabolites and kidney health trajectories based on pCYSC eGFR.

MATERIALS AND METHODS

Study cohorts

The DMHDS cohort at age 45 years tracks the development of 1037 individuals born in 1972–73 in Dunedin, New Zealand (NZ). This study was approved by the NZ Health and Disability Ethics Committee (17/STH/25/AM07) [20]. The CKD cohort study was from the Kidney Functional Reserve study at Prince of Wales Hospital, Sydney, Australia and approved by the South Eastern Sydney Local Health District Clinical Ethics Committee (HREC/14/POWH/280) [21]. Study cohort characteristics are described in Supplementary data, S1, Guiney *et al.* [19] and Christiadi *et al.* [21].

Sample preparation

Blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes and processed within 1 h by centrifugation at 1000 r.c.f. for 10 min at 4°C. Plasma was immediately stored at –80°C and used from the first thaw. In-house calibrators were prepared by spiking ADMA, SDMA, L-arginine and L-citrulline certified reference material (Toronto Research Chemicals, Canada) into stripped-plasma (Golden West Diagnostics, USA). Next, 50 µL of plasma, calibrator or control was mixed with 50 µL of working internal standard and 300 µL of isopropyl alcohol with 0.2% formic acid. All samples were vortexed for 1 min and then centrifuged at 16100 r.c.f. for 10 min at 4°C. Then, 150 µL of each supernatant was transferred into a 96-well plate for LC-MS/MS analysis. Chemicals used are listed in Supplementary data, Table S1.

LC-MS/MS instrumentation, detection and validation

The LC-MS/MS method was based on published methods [22–24] with the following modifications: Chromatography was performed with a 2 µL injection of supernatant onto a Shimadzu LC30 HPLC (Nexera Series, Shimadzu, Japan) with separation on a ZIC HILIC analytical column (150 mm × 2.1 mm, 3.5 µm particle size, Merck Millipore, USA) coupled with a HILIC guard column (2.1 mm ID, Phenomenex, USA) maintained at 45°C. The total run time was 9 min at a total flow rate of 0.8 mL/min. Separation of isomers was accomplished by holding mobile phase B at 29.1% from 0.6 to 5.5 min (Supplementary data, Table S2a). Mass spectrometry was performed using positive electrospray ionization and multiple reaction monitoring (MRM) on a Sciex 5500 QTRAP (Concord, Canada). Method characteristics are in Supplementary data, Table S2b and Fig. S1. Batch acceptability was based on pooled patient samples and commercial quality control (Recipe Chemicals and Instruments, Germany) at low, middle and high concentrations within the calibration range. All samples were quantified within the respective calibration ranges. Carryover of <6% was observed from a stock solution with concentrations three to four times greater than the top calibrator (data not shown). LC-MS/MS data were acquired with AB Sciex Analyst 1.7.2 acquisition software and analysed with Multiquant 3.0.3. Method design was validated in line with CLSI-C62-A:2014 and FDA 2018 guidance for in-house clinical LC-MS/MS method validation criteria (Supplementary data, Tables S3 and S4, and Figs S2 and S3).

Sample calculation and statistics

Each metabolite distribution was examined and transformed for normalization. Differences between groups of normally distributed data were tested using Tukey's HSD. Pearson's correlation was used and $|r| > 0.30$ and $P < .05$ were considered significant. Analyses were performed using JMP Version 16 (SAS Institute, USA).

RESULTS

Healthy DMHDS subset

A complete data set was available for 857 DMHDS members. A total of 376 study members were selected as a healthy subset. The healthy DMHDS subset: did not have diabetes, CKD or known heart disease; were not taking medications for BP or cholesterol; were non-smokers; did not have albuminuria [i.e. urine albumin-creatinine ratio (UACR) >3 g/mol] or eGFR <60 mL/min/1.73 m^2; did not have systolic BP >140 mmHg or diastolic BP >90 mmHg; and did not have high-sensitivity C-reactive protein (hsCRP) >3 mg/L (Supplementary data, Fig. S4 and Table S5). Healthy subset characteristics are summarized in Table 1, including mean sCr and pCYSC concentrations, and eGFR based on sCr (eGFRcr), pCYSC (eGFRcy) or both (eGFRcrcy). In this healthy cohort, females had lower mean concentrations than males of ADMA, SDMA, L-arginine and L-citrulline.

DMHDS cohort

The characteristics for the DMHDS cohort ($n = 857$) are shown in Table 2. As in the healthy DMHDS subset, females in the DMHDS cohort had lower SDMA, L-arginine and L-citrulline concentrations, but not ADMA, compared with males. There was no difference in mean ADMA, SDMA, L-arginine or L-citrulline

Table 1: Characteristics of the healthy DMHDS subset.

	Healthy DMHDS subset		Female		Male	
***n* (% total)**	**376**		**191 (50.8)**		**185**	
Age, years	**45**		**45**		**45**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
BMI, kg/m^2	26.8	4.3	26.3	4.8	27.3	3.6
Systolic BP, mmHg	115.8	9.9	112.7	9.6	119.0	9.2
Diastolic BP, mmHg	76.7	7.8	73.8	7.8	79.6	6.7
sCr, µmol/L	77.1	12.6	68.7	9.11	85.8	9.4
eGFRcr, mL/min/1.73 m^2	93.3	11.1	92.8	12.0	93.8	10.1
pCYSC, mg/L	0.64	0.09	0.60	0.07	0.68	0.09
eGFRcy, mL/min/1.73 m^2	120.1	7.8	119.8	6.9	120.4	8.6
eGFRcrcy, mL/min/1.73 m^2	109.2	9.8	109.0	6.9	109.4	9.4
UACR, g/mol	1.11	0.31	1.15	0.37	1.06	0.23
ADMA, µmol/L	0.40	0.06	0.39	0.07	0.41[a]	0.06
SDMA, µmol/L	0.42	0.06	0.40	0.06	0.44[b]	0.05
Arginine, µmol/L	93.5	23.1	87.7	24.0	99.6[b]	20.5
Citrulline, µmol/L	24.0	5.42	23.0	5.40	25.1[b]	5.2

[a]$P < .01$ and [b]$P < .001$ Tukey–Kramer HSD between genders.
SD, standard deviation.

Table 2: Characteristics: of the DMHDS cohort.

	DMHDS cohort		Female		Male	
***n* (% total)**	**857**		**425 (49.6)**		**432**	
Age, years	**45**		**45**		**45**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
BMI, kg/m^2	28.5	5.8	28.5	6.7	28.6	4.7
Systolic BP, mmHg	121.4	14.8	117.0	14.0	125.7	14.2
Diastolic BP, mmHg	80.3	10.3	76.0	9.1	84.6	9.6
sCr, µmol/L	77.0	12.5	68.9	8.8	85.0	10.4
eGFRcr, mL/min/1.73 m^2	93.5	11.3	92.5	11.7	94.6	10.8
pCYSC, mg/L	0.66	0.10	0.62	0.08	0.70	0.10
eGFRcy, mL/min/1.73 m^2	118.3	9.0	117.8	7.7	118.8	10.1
eGFRcrcy, mL/min/1.73 m^2	108.2	10.2	107.5	10.2	108.8	10.3
UACR, g/mol	1.76	4.43	1.75	3.30	1.77	5.32
ADMA, µmol/L	0.41	0.06	0.41	0.07	0.41	0.06
SDMA, µmol/L	0.42	0.06	0.40	0.06	0.44[a]	0.06
Arginine, µmol/L	94.4	22.8	89.5	23.8	99.1[a]	20.7
Citrulline, µmol/L	24.4	5.8	23.1	5.9	25.7[a]	5.5

[a]$P < .001$ Tukey–Kramer HSD between genders.
SD, standard deviation.

concentrations when the DMHDS cohort was stratified into high and low BP groups (BP <140/90 and BP >140/90, Supplementary data, Table S6). Mean sCr and pCYSC concentrations were slightly lower in the high BP groups compared with the low BP group; however, no difference in eGFR was observed. The results were similar when study members taking BP medication were excluded (data not shown). When BP groups were divided by gender, mean SDMA, L-arginine and L-citrulline concentrations were higher in males with BP <140/90 compared with females with BP <140/90; however, there was no difference in mean L-arginine and L-citrulline concentrations between males or females with BP >140/90 (Supplementary data, Table S7). There was no difference in mean ADMA concentrations between genders with high or low BP. When individuals taking BP medication were excluded, there was no significant change (data not shown).

SDMA and kidney function: DMHDS cohort

In the DMHDS cohort, SDMA correlated with sCr ($r = 0.55$), pCYSC ($r = 0.55$) and eGFRcrcy, $r = -0.52$ (Fig. 1). ADMA correlated with pCYSC ($r = 0.36$), but there was no correlation between ADMA and sCr or between L-arginine or L-citrulline and kidney function (eGFRcrcy, sCr or pCYSC) (Supplementary data, Table S8). No evidence of a correlation between BP and the four metabolites (ADMA, SDMA, L-arginine or L-citrulline) was found in the DMHDS cohort (data not shown).

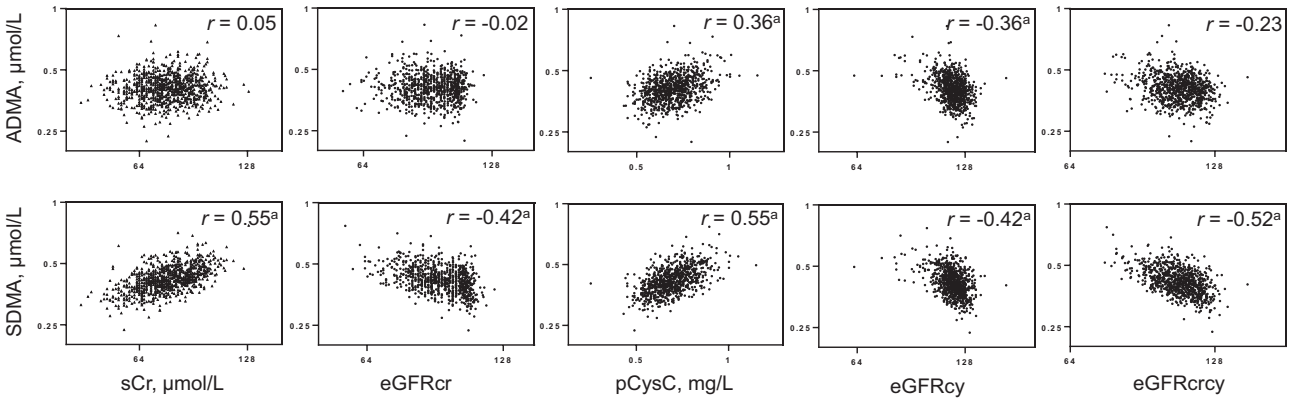


Figure 1: Correlations of sCr, eGFRcr, pCYSC, eGFRcy and eGFRcrcy with ADMA and SDMA from the DMHDS cohort. [a]Pearson's correlation, $|r| > 0.30$ and $P < .05$ were considered significant.

Table 3: Characteristics of CKD patients.

	CKD cohort 38	
N	**Mean**	**SD**
Age, years	66.5	12.3
Systolic BP, mmHg[b]	141.2	15.9
Diastolic BP, mmHg[b]	76.0	8.9
sCr, µmol/L	173.2	62.4
eGFRcr, mL/min/1.73 m^2	39.1	15.2
pCYSC, mg/L	1.98	0.68
eGFRcy, mL/min/1.73 m^2	40.1	19.2
eGFRcrcy, mL/min/1.73 m^2	37.1	15.4
ADMA, µmol/L	0.61[a]	0.11
SDMA, µmol/L	0.65[a]	0.25
Arginine, µmol/L	100.1	29.7
Citrulline, µmol/L	42.7[a]	11.8

[a]$P < .001$ Tukey–Kramer HSD compared with the healthy DMHDS subset.
[b]One patient systolic and diastolic blood pressure was not measured ($n = 37$).
SD, standard deviation.

Albuminuria (UACR >3 g/mol) was not associated with differences in ADMA, SDMA, L-arginine or L-citrulline concentrations (Supplementary data, Table S9). When stratified by function, individuals with eGFRcr <90 mL/min/1.73 m^2 and albuminuria had higher mean ADMA (0.44 ± 0.08 vs 0.41 ± 0.07 µmol/L) and L-citrulline (28.5 ± 7.4 vs 25.3 ± 6.0 µmol/L) compared with those with eGFRcr <90 mL/min/1.73 m^2 and without albuminuria (Supplementary data, Table S10). SDMA also showed a similar trend (0.47 ± 0.09 vs 0.44 ± 0.06 µmol/L).

SDMA and kidney function: CKD patients

Mean ADMA, SDMA and L-citrulline concentrations in CKD patients were significantly higher than in the healthy DMHDS subset: ADMA (0.61 ± 0.11 vs 0.40 ± 0.06 µmol/L), SDMA (0.65 ± 0.25 vs 0.42 ± 0.06 µmol/L) and L-citrulline (42.7 ± 11.8 vs 24.0 ± 5.4 µmol/L) (Table 3). Mean L-arginine concentration was slightly higher in CKD compared with the healthy subset (100.1 ± 11.8 vs 93.5 ± 23.1 µmol/L). As observed in the total DMHDS cohort, there was a strong correlation between kidney function and SDMA in the CKD cohort (eGFRcrcy, $r = –0.60$; Supplementary data, Table S11). There was also a correlation between kidney function and L-citrulline (eGFRcrcy, $r = –0.54$). There was no significant correlation between kidney function and ADMA in the CKD cohort, but when all cohorts were combined (DMHDS and CKD cohorts, $n = 895$) there was a correlation between kidney function and ADMA (eGFRcrcy, $r = –0.53$; Supplementary data, Fig. S5).

SDMA and ADMA: kidney function trajectory

From the DMHDS cohort, 803 individuals were categorized by Guiney *et al.* [19] into three groups based on risk of declining kidney function using pCYSC eGFR trajectory analysis: (i) high-risk (5%), (ii) low-normal risk (36%) and (iii) normal risk (59%). The trajectory analysis was based on data from three separate timepoints (cohort at age 32, 38 and 45 years) as described in Supplementary data, S2. Individuals in the high-risk group had low eGFRcy levels from age 32 to 45 years, the normal-risk trajectory group had normal eGFRcy levels from age 32 to 45 years, while the low-normal risk group started with normal eGFRcy levels at age 32 years and decrease below normal at age 45 years. These three trajectory groups were used as surrogates for kidney-related outcomes. The mean ADMA, SDMA, L-arginine and L-citrulline concentrations were higher in the high-risk trajectory group compared with the low-normal and normal-risk trajectory groups (ADMA: 0.48 vs 0.43 and 0.40 µmol/L; SDMA: 0.49 vs 0.44 and 0.40 µmol/L; L-citrulline: 28.6 vs 25.4 and 23.5 µmol/L; L-arginine: 103.1 vs 95.1 and 93.5 µmol/L, respectively) (Table 4). In addition, pCYSC and sCr concentrations at age 38 years correlated with SDMA ($r = 0.62$ and $r = 0.63$, respectively) and L-citrulline concentrations at age 45 years ($r = 0.34$ and $r = 0.36$, respectively) (Supplementary data, Table S12). At age 32 years, sCr and pCYSC concentrations also correlated with SDMA at age 45 years ($r = 0.46$ and $r = 0.40$, respectively; Supplementary data, Table S13).

ADMA and SDMA can predict risk of poor kidney-related trajectory outcomes

ADMA and SDMA each predicted DMHDS members at high risk of a poor kidney-related trajectory with area under the ROC curves (AUCs) of 0.83 [95% confidence interval (CI) 0.76–0.89] and 0.84 (95% CI 0.76–0.90), respectively, from individuals with normal risk. Using both metabolites, ADMA plus SDMA, was the most parsimonious model, predicting DMHDS members at high risk of a poor kidney-related trajectory with an AUC 0.90 (95% CI 0.84–0.93) versus DMHDS members with normal risk (Supplementary data, Fig. S6, Tables S14 and S15). Model

Table 4: Association of plasma metabolites and risk of poor kidney-related outcome trajectory groups in the DMHDS cohort ($n = 803$).

n (% total)	High-risk 41 (5)		Low-normal 290 (36)		Normal 472 (59)		
	Mean	SD	Mean	SD	Mean	SD	*P*-value[a]
ADMA, µmol/L	0.48	0.07	0.43	0.06	0.40	0.05	<.001
SDMA, µmol/L	0.49	0.08	0.44	0.06	0.40	0.06	<.001
Arginine, µmol/L	103.1	21.7	95.1	22.4	93.5	22.9	.030
Citrulline, µmol/L	28.6	7.5	25.4	6.1	23.5	5.3	<.001

[a]*P*-value across the three risk groups

Figure 2: DMHDS members at risk of CKD[a].
[a]DMHDS members with metabolites above the respective 97.5th percentile cut-off concentrations for SDMA, ADMA and L-citrulline.

comparisons with L-citrulline and L-arginine are shown in Supplementary data, Table S15.

Using ADMA, SDMA and L-citrulline cut-off concentrations to identify DMHDS members who may be at risk of CKD

As the CKD cohort had higher concentrations of ADMA, SDMA and L-citrulline, we used metabolite concentrations above the 97.5th percentile from the healthy subset as a cut-off to identify DMHDS members at risk of CKD (Supplementary data, Table S16). Approximately 3% of the DMHDS cohort had at least one metabolite above the 97.5th percentile cut-off concentration. Three DMHDS members had ADMA, SDMA and L-citrulline above the cut-off concentrations, while 72 study members had at least one of the three metabolites above the 97.5th % cut-off, while the remaining 785 study member concentrations were all below 97.5th percentile cut-off (Fig. 2). Ten DMHDS members had at least two metabolites above the 97.5th percentile cut-off concentrations: of these nine had eGFRcr <90 mL/min/1.73 m^2 and six were pre-diabetic, with half (three) having albuminuria (Supplementary data, Table S17).

For those identified at risk of CKD (i.e. above the 97.5th percentile cut-off for either ADMA, SDMA or L-citrulline from Supplementary data, Table S17), over 17% were in the high-risk trajectory group (Supplementary data Table S18; $P < .001$). Nine out of 10 DMHDS members with two or more metabolites above the 97.5th percentile cut-offs were classified in the high-risk or low-normal groups, while one was classified in the normal-risk trajectory group (Supplementary data, Fig. S7). This is consistent with the observation that not all patients with established CKD stage 3 or 4 had elevated metabolites above the 97.5th percentile cut-off: only 65% were above the 97.5th percentile cut-off for either ADMA, SDMA or L-citrulline, while 5 (15.8%) CKD patients had ADMA, SDMA and L-citrulline concentrations below the 97.5th percentile cut-off (Supplementary data, Table S19). Nevertheless, each CKD patient had at least one metabolite above the healthy 90th percentile concentration (data not shown).

DISCUSSION

This study established concentration ranges of arginine metabolites (ADMA, SDMA, L-citrulline and L-arginine) in a healthy group of 45-year-olds by exclusion of individuals with diabetes, CKD, known heart disease, albuminuria, high BP, low eGFRcr (<60 mL/min/1.73 m^2), high hsCRP, smokers, or taking medications for BP or cholesterol. There were clear inverse relationships between kidney function and ADMA and SDMA in the DMHDS cohort. These inverse relationships were confirmed by comparison with an unrelated cohort of patients with stages 3–4 CKD. L-citrulline concentrations were higher in CKD patients compared with the healthy DMHDS subset. While most DMHDS cohort members had little evidence of overt cardiovascular or kidney disease, high ADMA and SDMA concentrations suggested risk of CKD or CVD or both. This increased risk was also suggested by the strong association between elevated ADMA and SDMA and the high-risk pCYSC eGFR trajectory group previously identified [19]. ADMA and SDMA individually predicted the high-risk trajectory group with AUCs of 0.83 and 0.84, respectively, and together with an AUC of 0.90.

These results are consistent with previous studies demonstrating associations between kidney function and ADMA or SDMA in CKD [16, 17, 25] and comparable to a study in a 'non-diseased' population of 120 males and 120 females [24] that found modest correlations with eGFRcr. The DMHDS cohort encompassed an eGFRcr range of 57–122 mL/min/1.73 m^2. When combined with the CKD cohort, this extended the eGFRcr range to 12–122 mL/min/1.73 m^2 and revealed stronger correlations between kidney function and ADMA or SDMA as well as an inverse correlation between L-citrulline and eGFR.

In the CKD cohort, mean ADMA and SDMA concentrations were significantly higher than in the healthy DMHDS subset, consistent with previous results [26–28]. Elevated ADMA precedes decreased eGFR in CKD [28] and suggests that increased ADMA induces aberrant vascular remodelling, perhaps via decreased

nitric oxide (NO) production secondarily reducing kidney function (Supplementary data, Fig. S8a). An increase in protein arginine methyltransferase (PRMT) enzyme activity and decrease in dimethylarginine dimethylaminohydrolase 1 (DDAH1) activity may contribute to increased ADMA. In addition to vascular remodelling, ADMA inhibition of NO synthase (NOS) 'uncouples' the normal arginine substrate, shifting from NO to superoxide (O_2^-) production. Superoxide then scavenges available NO to form other reactive oxygen species (ROS), which activates PRMT-1 and inhibits DDAH, causing further elevations in ADMA and initiating an abnormal cycle (Supplementary data, Fig. S8b) [29–31].

SDMA is almost entirely excreted in the urine, and plasma levels correlate strongly with eGFR. SDMA is therefore sensitive to early GFR loss but unaffected by muscle mass, diet, age and gender. Nevertheless, a link between SDMA and kidney disease is not well described. SDMA is a weak or indirect inhibitor of NOS, but its inhibitory activity on the uptake of L-arginine through the y+ transporter may contribute to dysregulation of NO production and thus endothelial dysfunction. SDMA may also upregulate ROS production in monocytes by acting on Ca^{2+} channels [32].

ADMA concentration has been reported to be higher in patients with high BP [12, 33, 34]. Notsu *et al.* [35] found that the arginine/ADMA ratio was a sensitive risk marker for atherosclerosis and associated with carotid intima-media thickness independent of age, sex, BMI and the presence of hypertension. In the DMHDS cohort, there was no direct correlation between ADMA and BP and no significant difference in the arginine/ADMA ratio between high and low hypertension groups (data not shown). Absent evidence of association may reflect the limited number of DMHDS members with high BP.

Maintenance of stable L-arginine concentrations in CKD is hypothesized to result from *de novo* arginine synthesis following adaptive increased citrulline synthesis leading to high citrulline plasma concentrations [36–38]. Consistent with this, significantly higher mean L-citrulline concentrations were observed in CKD compared with the healthy DMHDS subset, but only slightly higher mean L-arginine concentrations. DMHDS members with reduced eGFRcr (<90 mL/min/1.73 m^2) and albuminuria had higher mean ADMA, SDMA and L-citrulline concentrations. However, mean L-arginine compared similarly with DMHDS members having eGFRcr <90 mL/min/1.73 m^2 and no albuminuria.

Mean concentrations for all four metabolites were higher in the high-risk trajectory group for poor kidney-related outcomes compared with the normal-risk group. As this high-risk group is associated with BP, BMI, inflammation, glycated haemoglobin, smoking and socioeconomic status [19], this suggests that arginine metabolites associate with the same parameters. ADMA and SDMA predicted the high-risk trajectory group with AUC 0.90 and may thus independently help screening for progression of CKD and for increased risk of CVD.

This is one of the largest studies of plasma methylarginine metabolites and, like a recent larger study [39], confirms the predictive value of both dimethylarginines and NO precursors as risk factors for accelerated decline in kidney function. Study limitations include that the DMHDS cohort comprised only 45-year-olds of predominantly white European descent. Further analysis of previous DHMDS cohort samples (at age 32 and 38 years) could provide the trajectories of the methylarginine metabolites. The association between increased ADMA and SDMA and the high-risk kidney function trajectory requires follow-up of DMHDS members to determine whether ADMA or SDMA correctly predicts CKD progression and cardiovascular disease in this cohort.

SUPPLEMENTARY DATA

Supplementary data are available at *ndt* online.

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AUTHORS' CONTRIBUTIONS

A.Y.MA., R.W., R.P., A.R.H. and Z.H.E. designed the study. K.M., F.B. and P.S. performed the assays. A.Y.MA., K.M., F.B., H.G., J.E. and Z.H.E. analysed the data. A.Y.MA., K.M. and Z.H.E. drafted the manuscript. All authors revised and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

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