

Plasma Arginine Metabolites in Health and Chronic Kidney Disease.

Amy YM Au^{*1,2}, Kevin Mantik^{*3}, Forough Bahadory³, Paul Stathakis³, Hayley Guiney⁴, Jonathan Erlich^{1,2}, Robert Walker⁵, Richie Poulton⁴, and Zoltan H Endre^{1,2}
*co-first authors

1. Department of Nephrology, Prince of Wales Hospital, Sydney, Australia 2. Prince of Wales Clinical School, University of New South Wales, Sydney, Australia 3. Department of Chemical Pathology, New South Wales Health Pathology, Prince of Wales Hospital, Sydney, 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

kevin.mantik@health.nsw.gov.au

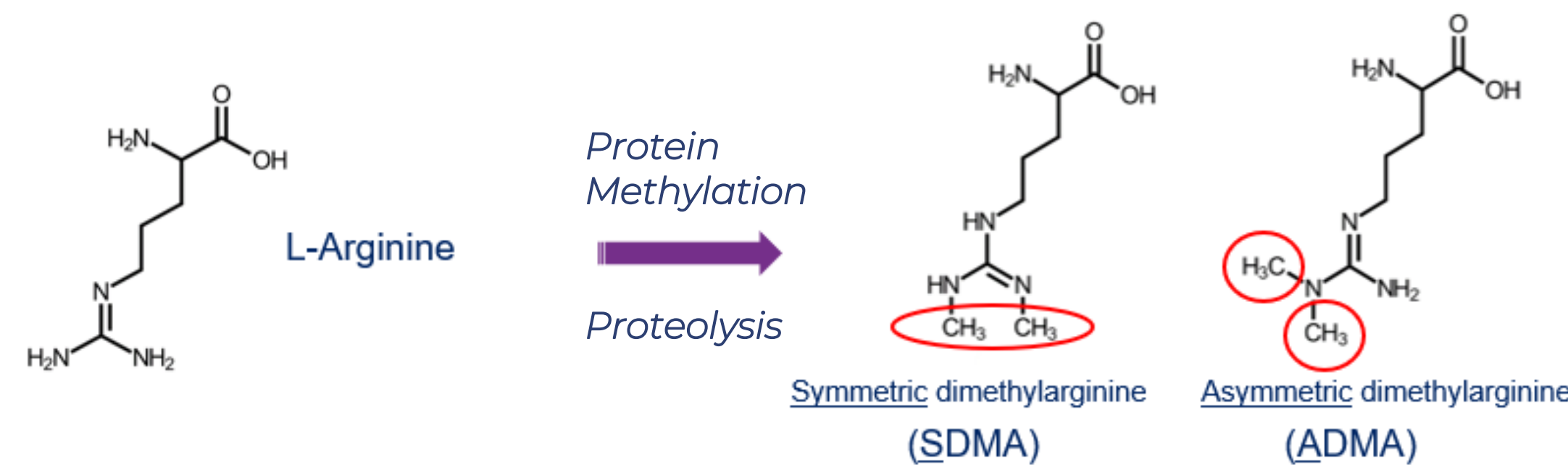


UNSW SYDNEY



Introduction

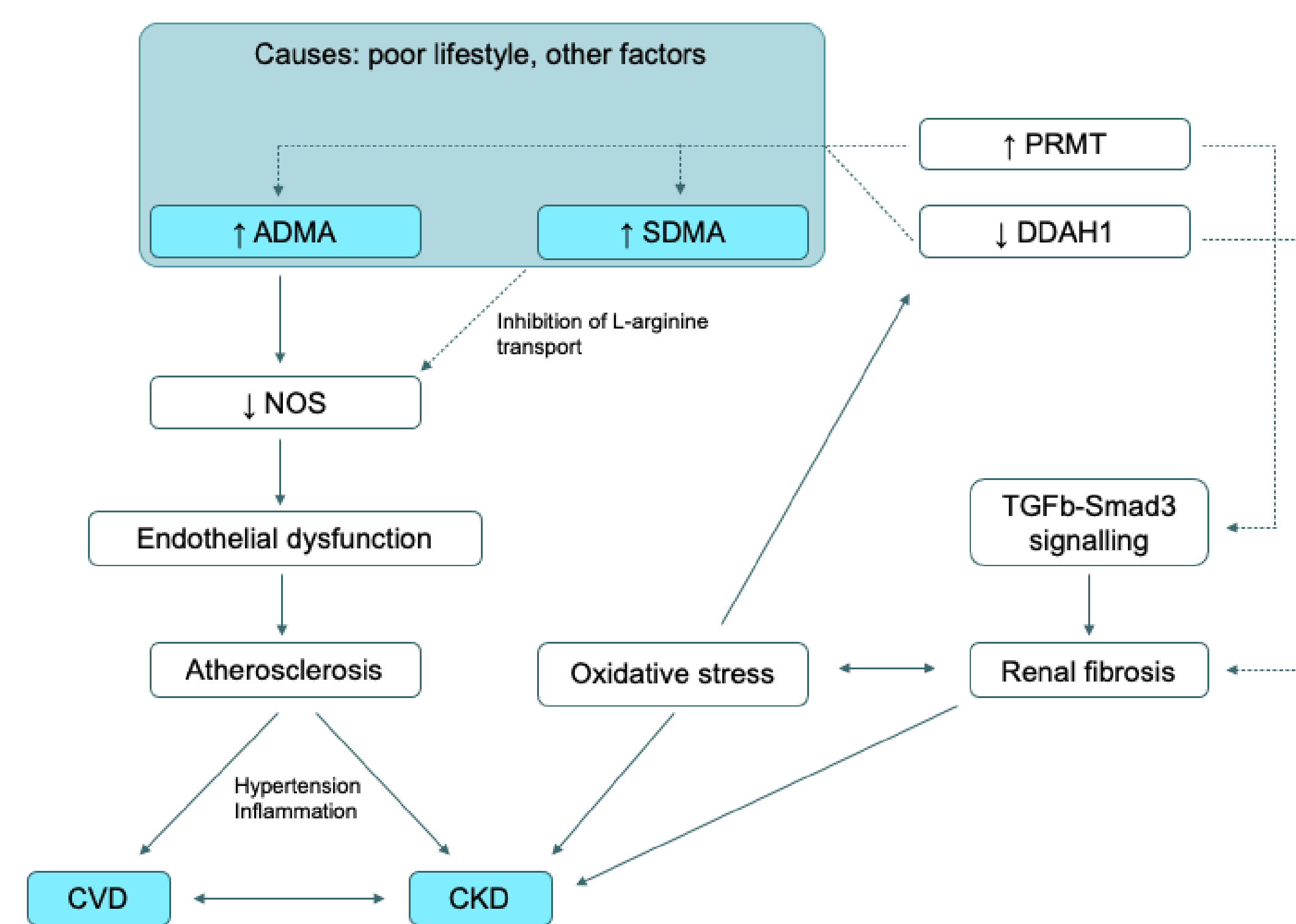
Methylated arginine isomers are produced from normal protein methylation and proteolysis:



- ADMA and SDMA are structural isomers
- When elevated they are both uraemic toxins
- ADMA and SDMA predict chronic kidney disease (CKD) and cardiovascular disease (CVD).

ADMA is a competitive inhibitor of Nitric Oxide Synthase (NOS), therefore decreasing Nitric Oxide (NO), and its beneficial effects.

Normally, NO functions to maintain vascular health (vasodilation, angiogenesis, decreasing inflammation) and kidney function (increased GFR, decreased nephropathy, oxidative stress).



PRMT, protein arginine methyltransferase; DDAH, dimethylarginine dimethylaminohydrolase; TGFb, transforming growth factor beta

Scope

The Nephrology department, Prince of Wales Hospital in Sydney, Australia, in association with the

Dunedin Multidisciplinary Health and Development (DMHD) research unit, New Zealand:

- Developed an interest in methylated arginine's with respect to healthy individuals and CKD patients.

The DHMD research unit manages a longitudinal study, and tracks 1037 individuals born between 1972 – 1973.

Aims

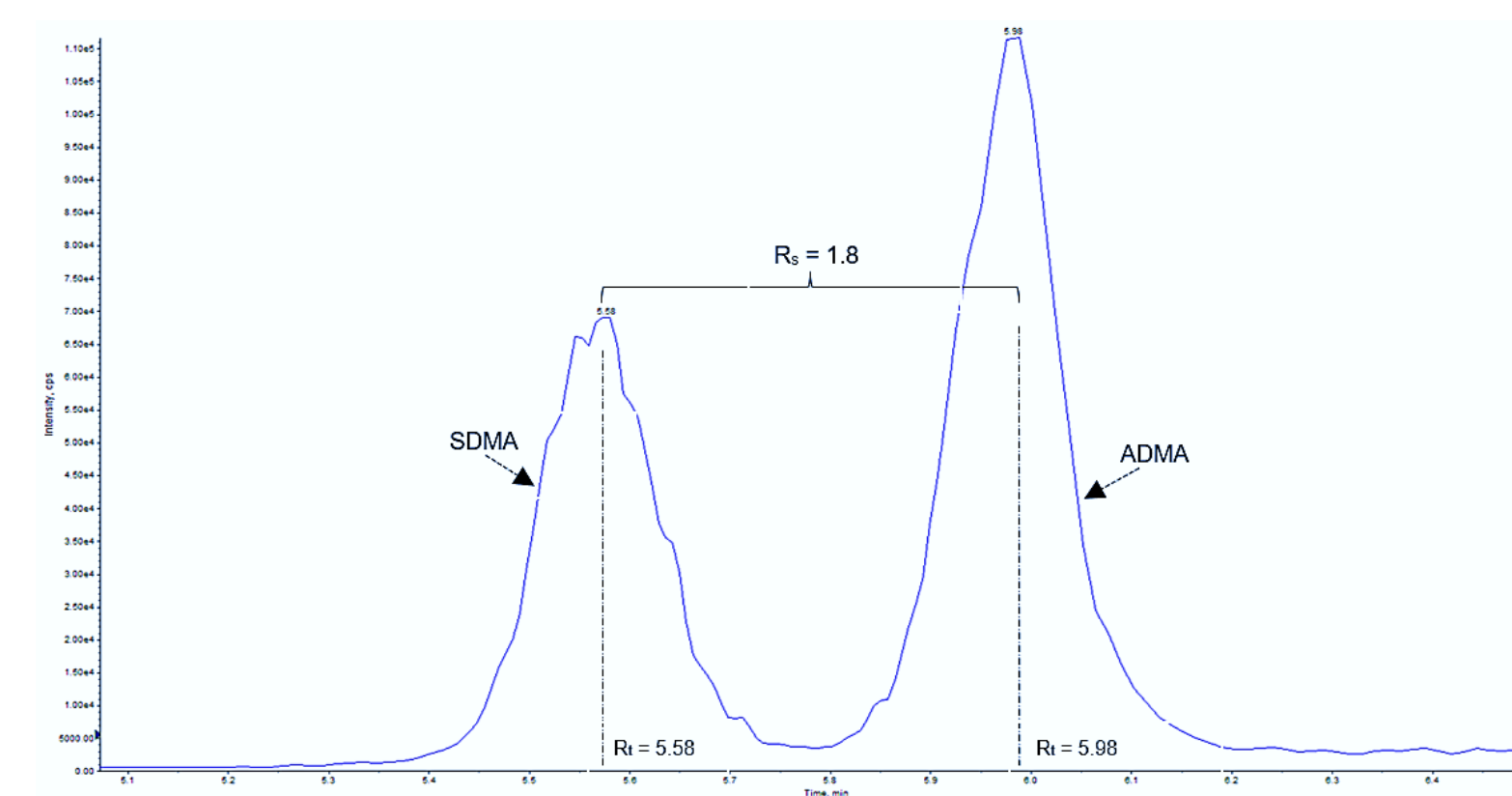
- Develop and validate an LC-MS/MS method to measure methylated arginine's and NO-related amino acid metabolites
- Determine values from healthy subjects within the DHMD study
- Compare results with Australian CKD level 3 and level 4 patients

Aim 1:

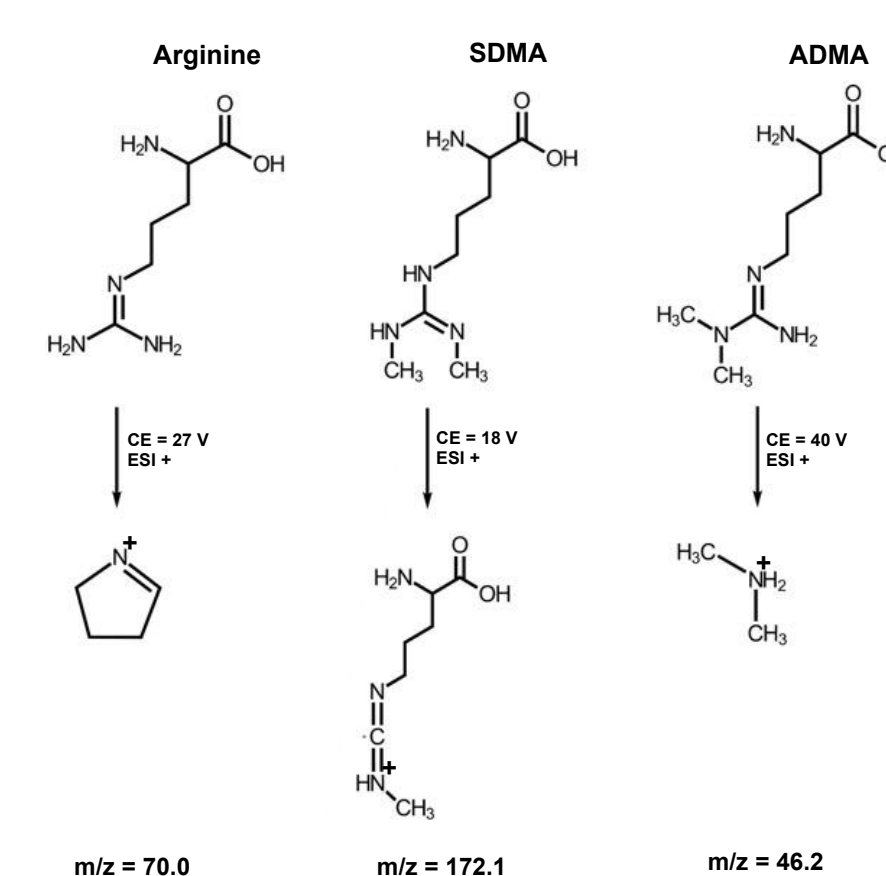
To develop and validate an LC-MSMS Method

- 50 μ L of sample \rightarrow added to 50 μ L of labelled internal standard \rightarrow added to 300 μ L of isopropyl alcohol / 0.2% formic acid \rightarrow Centrifuge high speed \rightarrow Supernatant.
- 2 μ L injection Shimadzu LC30 HPLC with isocratic separation on a ZIC HILIC analytical column (150mm x 2.1mm, 3.5 μ m, Merck) at 45°C, flow rate 0.8mL/min, total 9mins.
- ESI+ / MRM on a Sciex 5500 QTRAP
- QC: Pooled patient plasma and commercial (Recipe Chem + Instr, GmbH)

SDMA and ADMA separated by "Zwitterion" Chromatography: weak electrostatic and hydrophilic partitioning:



Multiple reaction monitoring (MRM) settings for ESI+ LC-MS/MS detection.



Compound	Q1 [M+H] ⁺	Q3 (Quantifier/Qualifier)	Collision Energy (V)	Declustering Potential (V)	Collision Cell Entrance / Exit Potential (V)	Retention time (min)	
Arg	175.2	70.0	116.2	27	39	6.93	
Arg-PCB, HMA	185.2	75.0	30	-	70	6.93	
Cit	176.2	70.1	113.0	29	21	30 / 45	4.40
Cit-D6	182.2	76.1	-	29	-	30	4.40
SDMA	203.2	172.1	57.0	18	70	60	5.60
SDMA-D6	209.2	178.0	-	18	-	60	5.60
ADMA	203.2	46.2	158.0	40	20	65 / 70	6.00
ADMA-D6	209.2	52.0	-	41	-	61	6.00

Method Validation

For research-only, carried out to the principles of CLSI C-62A and FDA 2018 validation guidelines including:

- LINEAR RANGE
- LLOQ (%CV < 20, and S/N > 10)
- IMPRECISION
- SPECIFICITY
- ANALYTE RECOVERY
- MATRIX EFFECTS
- STABILITY
- METHOD COMPARISON

Analyte	Sensitivity (CV% = 10, 20, 30, 40, 50%) (n = 10)	Calibrator Accuracy (n = 9)					Imprecision				
		LLOQ (μmol/L)	Level (μmol/L)	Ave Bias (%)	Slope	R ²	QC (μmol/L)	Accuracy (%)	Within-run Repeatability (%CV)	Between-Run Precision (%CV)	Total %CV
ADMA	0.018	0.1 - 4.5	-0.10	0.964	12.5%	0.9996	0.3 - 3.7	98 - 103	2.1 - 6.3%	3.1 - 6.6%	9.1%
SDMA	0.018	0.1 - 5.9	0.04	0.328	7.3%	0.9996	0.4 - 4.7	93 - 100	2.6 - 3.3%	2.8 - 4.7%	5.9%
Citrulline	0.293	1.4 - 71	-0.20	0.132	7.6%	0.9995	4.4 - 56.6	97 - 102	1.6 - 3.0%	2.5 - 3.3%	4.4%
Arginine	n.d.	6.0 - 145	-0.50	0.016	10.3%	0.9997	10.4 - 95.5	96 - 101	3.2 - 4.7%	3.5 - 6.9%	8.2%

Aim 2:

Determine values from healthy subjects within the total DHMD study

A healthy cohort was derived from the total DHMD cohort by excluding:

- Diabetes, self-reporting CKD, Heart problems
- Abnormal tests, e.g., blood pressure levels, urine Albumin / Creatinine ratios, eGFR and hsCRP

Results & Discussion

Total DMHD study cohort and Reference intervals for comparison. Note: the age of all our subjects was 45.

Total DMHD cohort			Reference Intervals				
			Bonnitcha, et al		Mayo Clinic Labs		
n (total)	857		n (total)	240			
Age (years)	45		Age (years)	18 - 65	> 18 yrs		
	Mean	SD		Mean	SD	Mean	SD
sCr	77.01	12.55	ADMA	0.49	0.08	0.50	0.09
eGFRcr	93.54	11.30	SDMA	0.40	0.07		
ADMA	0.41	0.06	Arg			76.00	22.00
SDMA	0.42	0.06	Cit			31.50	7.25
Arg	94.35	22.79					
Cit	24.41	5.84					

Bonnitcha et al, Pathology 54:591
<https://www.mayocliniclabs.com/test-catalog/overview/607697#Overview>

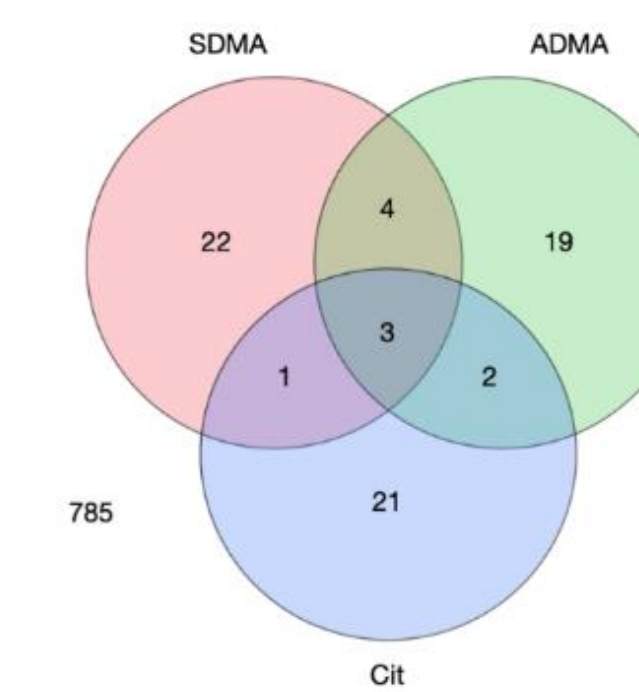


Figure details metabolites above the 97.5th percentile healthy cohort cut-off concentrations (see below) in all DMHD members (n = 857)

Aim 3:

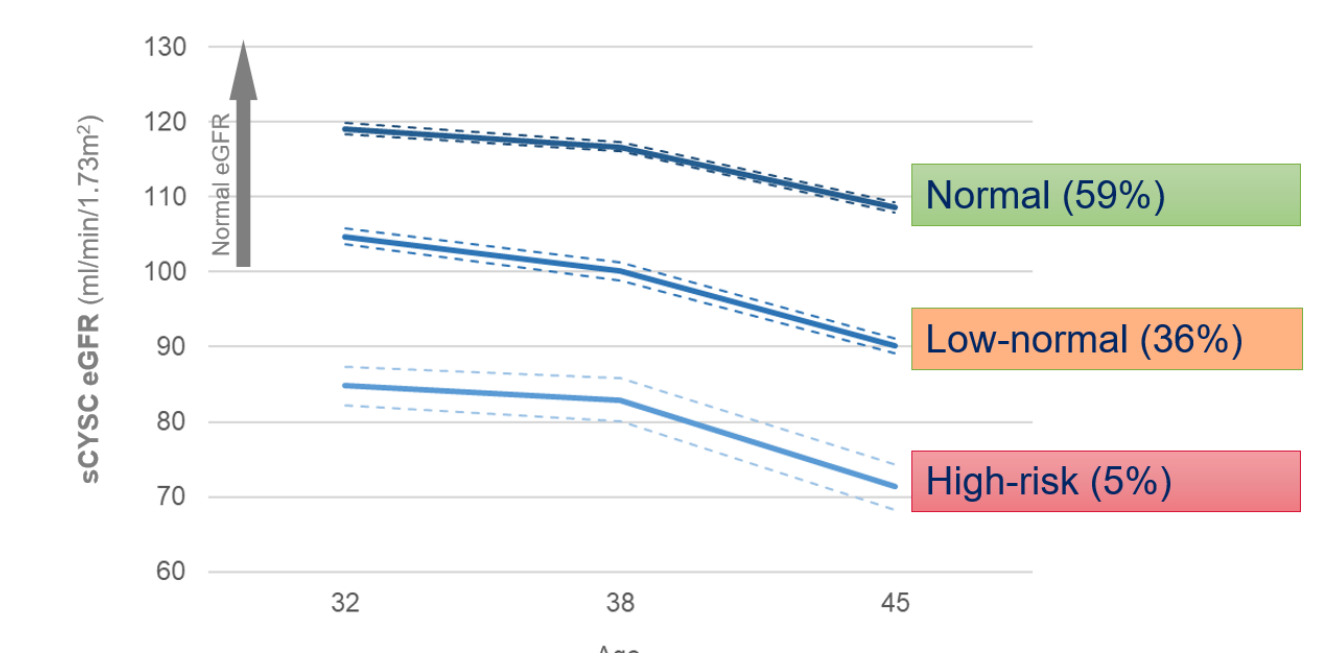
Compare Healthy DMHD with Australian CKD level 3 and 4 patients:

In CKD we see higher ADMA, SDMA, and Citrulline; while Arg is not significantly elevated.

CKD cohort			Healthy cohort		
n (total)	38		n (total)	376	
Age (yrs)	66.53	12.33	Age (years)	45	
sCr	173.18	62.38	sCr	77.11	12.58
eGFRcr	39.71	18.28	eGFRcr	93.30	11.10
ADMA	0.61**	0.11	ADMA	0.40	0.06
SDMA	0.65**	0.25	SDMA	0.42	0.06
Arg	100.08	29.69	Arg	93.54	23.09
Cit	42.69**	11.82	Cit	24.02	5.42

What about RISK of kidney related outcomes?

- Our NZ collaborators developed trajectory group modelling based on observed patterns (early-life: SES, modifiable: BP, BMI, smoking, HbA1c) from the DHMDs and applied logistic regression analyses.
- 95% CI trajectories of Normal, Low-normal, and High risk groups for KIDNEY RELATED OUTCOMES



Guiney, Hayley et al., Kidney-Function Trajectories From Young Adulthood to Midlife: Identifying Risk Strata and Opportunities for Intervention, Kidney International Reports (2023) 8, 51-63

Applying trajectory group modelling:

High-risk DMHDs individuals had significantly higher mean concentrations of all 4 metabolites within the total cohort

	High-risk		Low-normal		Normal	
	N (% total)		Mean	SD	Mean	SD
ADMA	41 (5%)	0.48**	0.43	0.06	0.40	0.05
SDMA		0.49**	0.44	0.06	0.40	0.06
Arg		103.1*	95.1	22.4	93.5	22.9
Cit		28.6**	25.4	6.08	23.5	5.29

Conclusion

- ADMA and SDMA disrupt NO metabolism, and predict the progression of CKD.
- Known CKD patients, and High-Risk individuals have higher levels of arginine metabolites than healthy study participants
- The LCMSMS method was applied to study members at age 45
- The next stage of the study will be follow-up of the participants at age 52 in 2024.

Acknowledgements

This study is a collaboration between: New South Wales health pathology clinical mass spectrometry, Prince of Wales hospital nephrology team, researchers at UNSW, the University of Otago in New Zealand, and the Dunedin study, which is a multidisciplinary health and development research unit in New Zealand.