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Research Article

# Low urinary citrulline/arginine ratio associated with blood pressure abnormalities and arterial stiffness in childhood chronic kidney disease

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## Abstract

Arginine (ARG) and citrulline (CIT) are essential for nitric oxide (NO) synthesis. Their metabolites are interrelated, and involved in blood pressure (BP) control, chronic kidney disease (CKD), and cardiovascular disease (CVD). Although CVD is the leading cause of mortality in CKD, little is known about subclinical CVD in early-stage childhood CKD. Twenty-four–hour ambulatory BP monitoring and arterial stiffness assessment allows the earlier possible detection of subclinical CVD. We investigated whether urinary CIT and ARG metabolites and their ratios are correlated with BP load and vascular abnormalities in children and adolescents with early-stage CKD. We enrolled 55 pediatric patients with mild-to-moderate CKD. Seventy percent (30/43) had at least one out of BP load abnormality on ambulatory BP monitoring, mainly increased asleep systolic BP (SBP) load (40%), asleep SBP or diastolic BP load > 95th percentile (40%), and nocturnal SBP nondipping (35%). Low urinary CIT level and CIT/ARG ratio were associated with BP load abnormalities in children with early CKD. Urinary CIT/ARG ratio was correlated with arterial stiffness, represented as pulse-wave velocity and augmentation index. SBP and diastolic BP loads were negatively correlated with urinary CIT, ARG, asymmetric dimethylarginine (an endogenous NO synthase inhibitor), and CIT/ARG ratio, while positively associated with dimethylamine/asymmetric dimethylarginine ratio and pulse-wave velocity. Early assessments of BP load abnormalities, urinary biomarkers in the CIT-ARG-NO pathway, and arterial stiffness parameters should increase early preventive care toward decreasing hypertension and CV remodeling in pediatric CKD. J Am Soc Hypertens 2015;■(■):1–9. 

*Keywords:* Arginine; chronic kidney disease; hypertension; nitric oxide.

## Introduction

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality in children and adults with chronic kidney disease (CKD). Twenty-four–hour ambulatory blood pressure monitoring (ABPM) is correlated better with cardiovascular outcomes than office blood pressure (BP) in children.[1] Because children with CKD rarely present with cardiovascular events, the detection of subclinical CVD in childhood requires noninvasive measurement of endothelial function, arterial stiffness, and vascular phenotypes. Although there are several techniques available used to detect subclinical CVD, little data are available from children.[2] These vascular assessments include carotid artery intima-media thickness (cIMT), flow-mediated dilatation (FMD), pulse-wave velocity (PWV), augmentation index (AI), and ABPM-derived arterial stiffness index (AASI).[2,3]

Conflict of interest: The authors declare no conflict of interest.

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Urinary and plasma biomarkers are increasingly being investigated for their utility in predicting CVD in patients with CKD.[4] Nitric oxide (NO) deficiency contributes to hypertension, CVD, and CKD.[5] Arginine (ARG) and citrulline (CIT) are two important amino acids that are essential for NO synthesis. ARG is a substrate for NO synthase to generate NO and CIT. ARG can also be metabolized by arginase to generate ornithine, which can be further converted to CIT by ornithine carbamoyltransferase. The body can use CIT to make ARG via the argininosuccinate pathway.[6] ARG can be methylated by protein ARG methyltransferase to produce asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA).[7] ADMA is an endogenous NO synthase inhibitor. Dimethylarginine dimethylaminohydrolase (DDAH) can metabolize ADMA to dimethylamine (DMA) and CIT. Therefore, the metabolites of ARG and CIT are closely interrelated and maintain NO homeostasis (Figure 1).

The kidney is a major site of net de novo ARG synthesis and ADMA metabolism. In advanced CKD stages, plasma CIT and ADMA levels are increased, renal CIT uptake is diminished, and the amount of CIT converted to ARG in the kidney is reduced.[8,9] Nevertheless, plasma ARG levels demonstrate conflicting results in different models of kidney diseases and CVD, confirming the complexity of ARG metabolism.[10] We and others demonstrated that reduced renal ARG availability and increased ADMA levels precede hypertension in young spontaneously hypertensive rats (SHRs).[11,12] We also found that plasma ARG/ADMA ratio and ADMA/SDMA ratio are better markers than each parameter alone (ie, ARG, ADMA) to predict hypertension in young SHRs.[12] In addition, our recent report showed that high plasma CIT/ARG ratio is related to BP load abnormalities in children with early-stage CKD.[13] Although ARG and CIT supplementation have been used therapeutically in CVD and CKD,[14,15] little effort has been made to better understand their metabolites and combined ratios in the development of hypertension and CVD in children with early-stage CKD. Given the important roles of the kidney in amino acid metabolism, de novo ARG synthesis, and ADMA metabolism, we hypothesized that CKD severity would affect the urinary excretion of these amino acids, and that their levels and/or ratios in the urine could predict hypertension in children with mild-to-moderate CKD. Therefore, the aim of this study was to elucidate whether urinary ADMA, DMA, SDMA, ARG, and CIT concentrations and their combined ratios are correlated with abnormalities of BP load and vascular parameters in children and adolescents with early-stage CKD.

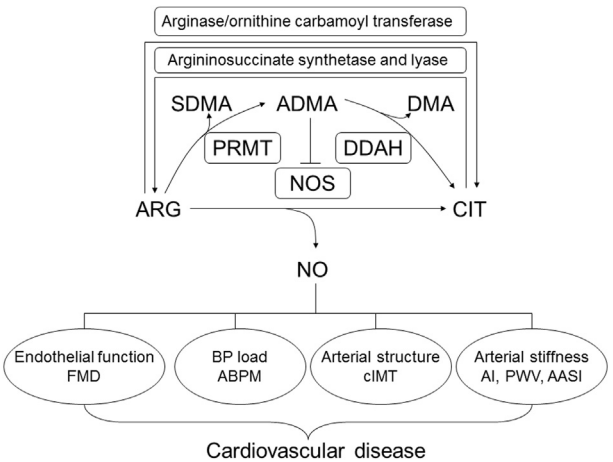


**Figure 1.** Diagram of the synthesis and metabolism of citrulline (CIT), arginine (ARG), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) in the nitric oxide (NO) pathway and their relationships to blood pressure load, endothelial function, arterial structure, and arterial stiffness. AASI, ambulatory blood pressure monitoring–derived arterial stiffness index; ABMP, ambulatory blood pressure monitoring; AI, augmentation index; BP, blood pressure; cIMT, carotid artery intima-media thickness; DDAH, dimethylarginine dimethylaminohydrolase; DMA, dimethylarginine; FMD, flow-mediated dilatation; NOS, nitric oxide synthase; PRMT, protein arginine methyltransferase; PWV, pulse-wave velocity.

## Materials and Methods

### *Study Population*

We enrolled a total of 55 children and adolescents attending the pediatric clinic at Kaohsiung Chang Gung Memorial Hospital. The study was approved by the Chang Gung Memorial Hospital Institutional Review Board (102-4131C), and followed the 1964 Declaration of Helsinki. Informed consent was obtained from all participants before the study. Renal function was determined by estimated glomerular filtration rate (eGFR) using the Schwartz formula on the basis of body height and creatinine (Cr) level.[16] The eGFR categories were defined according to the K/DIGO guidelines.[17] All participants were assigned to eGFR category G1 (eGFR $\geq$90 mL/min/1.73 $m^2$), G2 (eGFR 60–89 mL/min/1.73 $m^2$), or G3a (eGFR 45–59 mL/min/1.73 $m^2$). The exclusion criteria included current pregnancy, history of congenital heart disease, renal transplantation, and inability to complete major data collection procedures. The following assessments were performed in study participants at the same clinic visit: (1) history taking and physical examination; (2) anthropometry, including height and weight; (3) office BP measurement; and (4) laboratory investigations. Height-for-age and weight-for-age z-scores were calculated with the reference population.[18]

### *Biochemistry and High-Performance Liquid Chromatography Analysis*

We educated the parents to prevent their children from eating foods high in ARG (eg, peanuts and gelatin), CIT (eg, watermelon), and DMA (eg, eggs) 1 week before blood and urine sampling. Fasting plasma specimens and spot urine samples were aliquoted and stored at −80°C until analysis. Uric acid, glucose, total cholesterol, low-density lipoprotein cholesterol, triglyceride, sodium, potassium, calcium, phosphate, hemoglobin, and urine total protein/Cr ratio were measured by standard laboratory assays. The levels of ADMA, SDMA, CIT, and ARG in urine were measured using high-pressure liquid chromatography (HP series 1100; Agilent Technologies, Santa Clara, CA, USA) with the o-phthaldialdehyde 3-mercaptopropionic acid derivatization reagent as previously described.[13] The standards contained ADMA, SDMA, CIT, and ARG at concentrations of 0.5–5.0 $\mu$M, 0.5–5.0 $\mu$M, 1–100 $\mu$M, and 1–100 $\mu$M. For DMA, 9-fluorenylmethyl chloroformate was used as the derivatization reagent, as previously described.[19] The standards contained DMA concentrations of 1.625–50 $\mu$M. The recovery rate was approximately 90%–105%. The urinary concentration of each amino acid was corrected for urine Cr concentration, which was represented in $\mu$mol/mmol.

### *Office BP and ABPM*

Triplicate BP measurements were taken at clinic visits after >5 minutes of sitting rest with at least 1 minute between recordings using a standard mercury sphygmomanometer (cuff with bladder size by a 1:2 width-to-length ratio according to arm circumference). The first appearance of sound (phase I Korotkoff) was the systolic BP (SBP). The disappearance of Korotkoff sound (phase V) was used to define diastolic BP (DBP). The mean value was used as the participant's office BP for analysis. The 24-hour ABPM and vascular assessments were performed during the same week. The 24-hour ABPM data were collected for subjects aged 6–18 years using an Oscar II monitoring device (SunTech Medical, Morrisville, NC, USA), handled by an experienced specialist nurse as previously reported.[13] Briefly, the ABPM was set to record the BP and pulse rate at 20-minute intervals over 24 hours. The subjects and their parents were asked to keep a diary of sleeping and waking times, as well as activities that may influence BP measurements, including stressful situations or exercise. Only measurements with a SBP of 50–200 mm Hg, a DBP of 30–100 mm Hg, and a heart rate of 30–200 beats per minute were accepted as valid and included in analysis. If more than 25% of an individual's recordings were outside these valid ranges, then that individual was excluded from further analysis. BP load was defined as the percentage of the area under the BP curve above set limits. The Oscar II monitoring device was checked against a mercury manometer for calibration purposes. An abnormal ABPM profile was determined based on (1) awake, asleep, SBP, or DBP loads exceeding the 95th percentile based on gender and height using ABPM reference data[20]; (2) awake, asleep, SBP or DBP load of 25% or greater; and (3) asleep decrease of BP load by less than 10% compared with average awake BP load.[20] Next, DBP was plotted against SBP using the individual 24-hour ABPM readings to calculate the linear regression slope. The AASI was defined as 1 minus the regression slope.[21]

### *Vascular Assessments*

All vascular assessments were performed during the same day. Carotid ultrasound assessment was performed by two experienced pediatric cardiologists (Shao-Ju Chien and I-Chun Lin) as previously reported.[22] Participants were placed in the supine position for at least 10 minutes in a quiet room before examination. With their neck hyperextended and turned 30°–45° contralateral to the probe, the bilateral mid-common carotid artery was imaged using a 5–12-MHz linear array transducer. Carotid artery IMT was measured during end diastole as determined by the R wave on an electrocardiogram. These images were obtained using a ProSound $\alpha$7 ultrasound coupled to computer-assisted analysis software (e-TRACKING system; Aloka Co, Tokyo, Japan). For reproducibility of cIMT measurements, the intraobserver coefficient of variation used was 4.1%.[22] Triplicate measurements were taken, and the mean of these values was used for analysis. Next, the arterial stiffness parameters, PWV and AI, were determined by echo-tracking methods (e-TRACKING system; Aloka Co., Tokyo, Japan). Endothelial function was analyzed by measuring FMD, defined as the maximal percentage change in right brachial arterial diameter in response to reactive hyperemia induced by inflating a BP cuff to >200 mm Hg around the forearm for 5 minutes. Brachial artery diameter was measured in longitudinal section with a UST-5412 linear probe and a ProSound $\alpha$7 ultrasound system coupled to computer-assisted analysis software. Each study included a resting scan and reactive hyperemia scan (FMD) recorded 30–60 seconds after the cuff pressure was released, which occluded arterial blood flow for 5 minutes.

### *Statistical Analysis*

Data were expressed as medians (interquartile ranges). The Mann-Whitney $U$ test was used to test the differences in variables between children with eGFR category G1 and G2–G3a. The associations between variables were examined using Pearson's correlation coefficient. Logistic or linear regression analysis was performed to further investigate the effects of biomarkers as predictors of parameters of

vascular function and BP load abnormalities in ABPM. A $P$-value <.05 was considered to be statistically significant. All analyses were performed using SPSS version 14.0 (SPSS, Inc, Chicago, IL, USA).

## Results

We enrolled a total of 55 children and adolescents with eGFR categories G1–G3a, which included 19 cases of renal agenesis or dysgenesis (33.3%), 11 cases of nephrotic syndrome (20%), eight cases of renal cystic disease (14%), five cases of obstructive nephropathy (8.8%), five cases of reflux nephropathy (8.8%), four cases of lupus nephritis (7.3%), three cases of neurogenic bladder (5.5%), one case of IgA nephropathy (1.8%), and one case of genetic disease (1.8%). Children with G1 eGFR were younger, and had higher eGFRs, lower blood urea nitrogen levels, lower Cr levels, and lower uric acid levels compared to those with G2–G3a eGFR (Table 1). However, vascular parameter data were not different between children and adolescents with different categories of eGFR.

Among the 55 patients included, 13 cases (24%) were found to exceed the 95th percentile for gender and height by office BP measurements. A total of 43 patients (78%) aged 6–8 years had undergone complete 24-hour ABPM studies. Among them, 70% (30/43) of children and adolescents with eGFR categories G1–G3a had at least one BP load abnormality in this study (Table 2). ABPM identified 13 (30%), 12 (28%), and 17 patients (40%) with SBP or DBP load >95th percentile at 24 hours, awake, and asleep stages. Other ABPM abnormalities included 15 patients (35%) with increased 24-hour SBP load, six patients (14%) with increased 24-hour DBP load, 13 patients (30%) with increased awake SBP load, five patients (12%) with increased awake DBP load, 17 patients (40%) with increased asleep SBP load, nine patients (21%) with increased awake DBP load, 15 patients (35%) with nocturnal SBP nondipping, and 10 patients (23%) with nocturnal nondipping DBP. Except for 24-hour DBP load ≥25%, there was no difference in BP load abnormalities on ABPM between patients with G2–G3a and G1 eGFR.

As shown in Table 3, the urine biomarkers were corrected for urine Cr level. Urine levels of CIT, ARG, ADMA, SDMA, and DMA, and their combined ratios were not different between children with different stages of CKD. Children with CKD with an abnormal office BP as well as abnormal ABPM profile had lower urinary levels of CIT and CIT/ARG ratios than those with normal BP loads (Table 4).

Table 5 lists the correlations between parameters in the CIT-ARG-NO pathway and vascular parameters. PWV, an arterial stiffness parameter, was negatively correlated with urinary CIT/ARG ratio. We observed that AI was positively correlated with urinary CIT level and CIT/ARG ratio, while negatively with ARG/ADMA ratio. AASI was negatively correlated with urinary ARG and SDMA levels. However, cIMT and FMD were not correlated with alteration of any biomarkers in the CIT-ARG-NO pathway. In addition, awake SBP load was negatively correlated with urinary ARG level, while positively with DMA/ADMA ratio. Awake DBP load was negatively correlated with urinary CIT/ARG ratio, while positively with DMA/ADMA ratio. Asleep SBP load was negatively correlated with urinary CIT and ARG levels. Asleep DBP load was negatively correlated with urinary ARG and DMA levels. Moreover,

**Table 1**
Characteristics of children and adolescents with mild-to-moderate CKD

| GFR Category | G1 | G2–3a |
|---|---|---|
| | N = 33 | N = 22 |
| Gender: M:F | 20:13 | 12:10 |
| Age, years | 8.6 (6.6–14) | 14.9 (11.4–16.8)* |
| Body height z-score | 0 (−0.55–0.75) | −0.35 (−1.2–0)* |
| Body weight z-score | 0.4 (−0.37–1) | −0.42 (−1.28–0.43)* |
| BP > 95th percentile for gender and height by office BP measurements | 7 (21%) | 6 (27%) |
| Blood urea nitrogen, mg/dL | 12 (11–14) | 17 (13–29)* |
| Creatinine, mg/dL | 0.53 (0.45–0.59) | 0.9 (0.74–1.26)* |
| eGFR, $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ | 109 (103–130) | 64 (56–80)* |
| Urine total protein-to-creatinine ratio, mg/g | 83 (61–413) | 95 (48–663) |
| Hemoglobin, g/dL | 13.9 (13–14.9) | 13.9 (12.1–15.1) |
| Total cholesterol, mg/dL | 166 (142–210) | 156 (139–185) |
| LDL, mg/dL | 89 (73–107) | 89 (77–115) |
| Triglyceride, mg/dL | 78 (46–115) | 79 (53–110) |
| Glucose, mg/dL | 89 (83–92) | 87 (83–91) |
| Uric acid, mg/dL | 5 (4.7–6.7) | 7.3 (6.2–8.9)* |
| Sodium, mEq/L | 141 (141–143) | 141 (140–142) |
| Potassium, mEq/L | 4.4 (4.2–4.5) | 4.4 (4.1–4.6) |
| Calcium, mg/dL | 9.5 (9.3–9.9) | 9.5 (9–9.8) |
| Phosphate, mg/dL | 4.7 (4.4–5.2) | 4.2 (3.8–5) |
| cIMT, mm | 0.38 (0.31–0.41) | 0.35 (0.31–0.38) |
| PWV, m/s | 3.9 (3.4–4.38) | 3.75 (3.63–4.05) |
| AI, % | 15.4 (11.3–21.7) | 18.3 (11.1–29.3) |
| AASI | 0.37 (0.26–0.43) | 0.39 (0.27–0.5) |
| FMD, % | 10.9 (5.2–14.8) | 10 (6.1–15) |

AASI, ambulatory blood pressure monitoring–derived arterial stiffness index; AI, augmentation index; BP, blood pressure; cIMT, carotid artery intima-media thickness; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; F, female; FMD, flow-mediated dilatation; LDL, low-density lipoprotein; M, male; PWV, pulse-wave velocity.

Data are presented as median (interquartile range).

* $P < .05$.

**Table 2**
Ambulatory blood pressure parameters of children and adolescents with mild-to-moderate CKD

| GFR Category | G1 | G2–3a |
|---|---|---|
| | N = 25 | N = 18 |
| 24-h SBP load, % | 7 (2.5–36.5) | 14 (4.8–48.3) |
| 24-h DBP load, % | 4 (2–11.5) | 6.5 (0–29.3) |
| Awake SBP load, % | 5 (2–28) | 11.5 (4–41.3) |
| Awake DBP load, % | 2 (0–10) | 4 (0–21) |
| Asleep SBP load, % | 12 (0–57) | 16.5 (3.8–65.5) |
| Asleep DBP load, % | 6 (0–16.5) | 0 (0–37.3) |
| Average 24-h SBP or DBP load >95th percentile | 7 (28%) | 6 (33%) |
| Average awake SBP or DBP load >95th percentile | 6 (24%) | 6 (33%) |
| Average asleep SBP or DBP load >95th percentile | 9 (36%) | 8 (44%) |
| 24-h SBP load ≥ 25% | 7 (28%) | 8 (44%) |
| 24-h DBP load ≥ 25% | 1 (4%) | 5 (28%)* |
| Awake SBP load ≥ 25% | 6 (24%) | 7 (39%) |
| Awake DBP load ≥ 25% | 2 (8%) | 3 (17%) |
| Asleep SBP load ≥ 25% | 9 (36%) | 8 (44%) |
| Asleep DBP load ≥ 25% | 3 (12%) | 6 (33%) |
| Nocturnal SBP nondipping | 8 (32%) | 7 (39%) |
| Nocturnal DBP nondipping | 5 (20%) | 5 (28%) |

CKD, chronic kidney disease; DBP, diastolic blood pressure; GFR, glomerular filtration rate; SBP, systolic blood pressure.

Data are presented as median (interquartile range) or n (%).

*$P < .05$.

**Table 3**
Urinary levels of biomarkers and ratios in children with mild-to-moderate CKD

| GFR Category | G1 | G2–3a |
|---|---|---|
| | N = 33 | N = 22 |
| Urine level, μmol/mmol Cr | | |
| CIT/Cr | 0.91 (0.46–1.48) | 0.74 (0.44–1.37) |
| ARG/Cr | 24 (18.7–39.3) | 17.7 (14.6–27.2) |
| ADMA/Cr | 16.9 (11.1–32.1) | 16.5 (11.1–26.1) |
| SDMA/Cr | 7.8 (4.36–10.4) | 4.65 (3.48–6.53) |
| DMA/Cr | 3.28 (2.18–4.15) | 2.47 (1.68–3.6) |
| Urine ratio | | |
| ARG/ADMA | 1.18 (0.97–1.64) | 1.18 (0.94–1.49) |
| ADMA/SDMA | 3.17 (2.17–4.73) | 3.28 (2.52–5.2) |
| CIT/ARG | 0.03 (0.02–0.05) | 0.04 (0.03–0.05) |
| DMA/ADMA | 0.15 (0.11–0.23) | 0.14 (0.12–0.23) |

ADMA, asymmetric dimethylarginine; ARG, arginine; CIT, citrulline; CKD, chronic kidney disease; Cr, creatinine; DMA, dimethylarginine; GFR, glomerular filtration rate; SDMA, symmetric dimethylarginine.

Data are presented as median (interquartile range).

there was a positive correlation between PWV and awake SBP load ($P = .045$). AASI was positively correlated with awake SBP load ($P = .001$), awake DBP load ($P = .011$), asleep SBP load ($P = .01$), and asleep DBP load ($P = .034$).

To control for the effects of other factors (eg, age, sex, eGFR) on BP load abnormalities, multivariate logistic regression models were analyzed (Table 6). Our results showed that low urinary ARG/ADMA ratio and high DMA/ADMA ratio were associated with abnormal office BP measurements. Furthermore, low CIT level, low CIT/ARG ratio, and low ARG level were significantly associated with ABPM abnormalities, awake and asleep SBP and DBP loads ≥25%, and nocturnal SBP nondipping.

## Discussion

This study provides insight into the different prognostic abilities of urinary CIT, ARG, their metabolites, and combined ratios for subclinical CVD in children and adolescents with mild-to-moderate CKD. The key findings are as follows: (1) 70% of children and adolescents with mild-to-moderate CKD had BP load abnormalities on ABPM; (2) low urinary CIT level and CIT/ARG ratio were associated with BP load abnormalities in children with mild-to-moderate CKD; (3) urinary ARG level was negatively correlated with asleep SBP and DBP loads; (4) urinary CIT/ARG ratio was correlated with arterial stiffness, represented as AI and PWV; and (5) urinary DMA/ADMA ratio was positively correlated with AASI, awake SBP load, and awake DBP load.

In the present study, 70% of children with eGFR categories G1–G3a had abnormal ABPM profiles in spite of their eGFR categories. Our data showed that increased BP load in children with mild-to-moderate CKD is frequently masked by office BP measurements and is not associated with eGFR staging, consistent with previous reports.[20,23]

In contrast to our previous study, which showed that high CIT/ARG ratio in plasma correlated with abnormal ABPM profile, this present study highlighted that BP load abnormalities are associated with low urinary CIT level and CIT/ARG ratio. CIT uptake and CIT/ARG conversion in the kidney was reduced, whereas urinary CIT excretion was increased in advanced CKD.[8,9] Thus, our findings suggest that in mild-to-moderate CKD, the kidney experiences a compensatory increase in CIT uptake and CIT/ARG conversion to increase renal ARG bioavailability in response to the elevation of BP load. CIT has been shown to be superior to ARG at augmenting NO as it can bypass hepatic metabolism, is well-tolerated, and is not metabolized by arginase.[15] Emerging evidence supports the protective effects of CIT supplementation against hypertension,[15,24,25] and early intervention targeting the CIT-ARG-NO pathway

**Table 4**
Urine levels of biomarkers and ratios in children with mild-to-moderate CKD stratified by office BP loads and ABPM profile

| | Office BP Loads | | ABPM Profile | |
|---|---|---|---|---|
| | Systolic and Diastolic BP Loads Less Than 95th Percentile for Gender and Height by Office BP Measurements | Systolic or Diastolic BP Loads Exceed the 95th Percentile for Gender and Height by Office BP Measurements | Both Awake and Asleep Systolic and Diastolic BP Loads < 25%, and Asleep SBP and DBP Dipping >10% | Awake or Asleep Systolic (or Diastolic) BP Loads ≥ 25% or Nocturnal SBP (or DBP) Nondipping |
| | N = 33 | N = 10 | N = 13 | N = 30 |
| GFR category, n (%) | | | | |
| G1 | 19 (76%) | 6 (24%) | 7 (28%) | 18 (72%) |
| G2–3a | 14 (78%) | 4 (22%) | 6 (33%) | 12 (67%) |
| Urine level, μmol/mmol Cr | | | | |
| CIT/Cr | 0.83 (0.52–1.47) | 0.44 (0.36–0.65)* | 1.18 (0.62–1.9) | 0.58 (0.42–0.95)* |
| ARG/Cr | 20 (15.2–31.2) | 21.7 (15.1–25.6) | 26.1 (16.4–34.2) | 20.4 (13.1–26.7) |
| ADMA/Cr | 16.6 (11.4–25.5) | 13.1 (6–19.7) | 15.7 (11.3–24.9) | 16.1 (9.3–25.5) |
| SDMA/Cr | 5.16 (3.3–8.51) | 6.3 (3.3–9.32) | 5.14 (3.45–9) | 5.5 (3.3–8.51) |
| DMA/Cr | 2.63 (1.78–3.81) | 2.19 (1.97–3.59) | 2.31 (1.68–3.64) | 2.32 (1.84–3.73) |
| Urine ratio | | | | |
| ARG/ADMA | 1.18 (0.99–1.42) | 1.54 (1.14–2.31) | 1.22 (1–1.58) | 1.2 (1.09–1.67) |
| ADMA/SDMA | 3.17 (2.54–4.66) | 2.43 (1.25–4.09) | 3.39 (2.58–4.8) | 2.91 (2.16–4.54) |
| CIT/ARG | 0.04 (0.03–0.05) | 0.02 (0.02–0.04)* | 0.05 (0.04–0.08) | 0.03 (0.02–0.04)* |
| DMA/ADMA | 0.14 (0.12–0.2) | 0.21 (0.13–0.43) | 0.13 (0.11–0.16) | 0.17 (0.12–0.25) |

ABMP, ambulatory blood pressure monitoring; ADMA, asymmetric dimethylarginine; ARG, arginine; BP, blood pressure; CIT, citrulline; CKD, chronic kidney disease; Cr, creatinine; DBP, diastolic blood pressure; DMA, dimethylarginine; SBP, systolic blood pressure; SDMA, symmetric dimethylarginine.

Data are presented as median (interquartile range) or n (%).

* $P < .05$.

might therefore be a promising strategy to treat prehypertension in children with mild-to-moderate CKD.

In addition to CIT/ARG ratio, we found that ARG level was negatively associated with awake SBP load and asleep SBP and DBP loads. Given that ARG is a substrate for NO production, our data were consistent with the ample available evidence showing that NO deficiency is involved in the development of hypertension.[1,2] On the other hand, ADMA can compete with ARG to generate NO. Therefore, the ARG/ADMA ratio has been considered to represent NO bioavailability.[26] Our present study showed that urinary ARG/ADMA ratio was not correlated with BP load. Because current methodologies used to measure NO production in humans remain inconclusive,[27] there is a need to elucidate which marker (eg, ARG, ARG/ADMA ratio) can truly reflect bioactive NO in vivo in future studies.

Next, we found that high DMA/ADMA ratios were correlated with high AASI, awake SBP load, and awake DBP load. ADMA is mainly metabolized by DDAH into DMA and CIT in the kidney. Thus, urinary DMA/ADMA ratio has been proposed to estimate whole body DDAH activity.[28] A previous study indicated that higher urinary DMA/ADMA ratio was associated with poor cardiovascular outcomes in patients with coronary artery disease.[29] Our data demonstrated that a high urinary DMA/ADMA ratio was associated with high awake SBP and DBP loads in children with mild-to-moderate CKD. High DMA/ADMA ratio reflects high DDAH activity. Increased DDAH activity can reduce ADMA and prevent hypertension in young SHRs.[30] Therefore, our data suggests that elevated DDAH activity to increase ADMA degradation might be a compensatory mechanism to maintain a steady ADMA concentration in response to early-stage CKD.

Furthermore, we examined endothelial function and arterial stiffness parameters. Decreases in FMD and increases in cIMT have been observed in different chronic childhood diseases,[6] including CKD.[31,32] In the present study, we did not observe abnormal FMD and cIMT in mild-to-moderate CKD and their associations with other urinary markers. It is possible that the small number of patients included (n = 55) may have not provided sufficient power to determine small differences in the vascular measurements compared with the normative data for this pediatric population. However, we found that urinary CIT/ARG ratio was correlated with AI and PWV, and that AASI was correlated with urinary ARG and SDMA levels. Given that measurement of arterial stiffness enables evaluation of arterial dysfunction, which may precede structural vascular remodeling evaluated by cIMT,[33] our data suggest that assessment of arterial stiffness might be more reliable than endothelial function and

**Table 5**
Correlation between urine levels of biomarkers and ratios and vascular assessments in children with mild-to-moderate CKD

| Urinary Biomarkers | PWV | | AI | | AASI | | Awake SBP Load | | Awake DBP Load | | Asleep SBP Load | | Asleep DBP Load | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | r-Value | *P*-Value | r-Value | *P*-Value | r-Value | *P*-Value | r-Value | *P*-Value | r-Value | *P*-Value | r-Value | *P*-Value | r-Value | *P*-Value |
| CIT | −0.11 | .436 | 0.27 | .047* | −0.14 | .357 | −0.26 | .09 | −0.21 | .174 | −0.31 | .045* | −0.3 | .053 |
| ARG | 0.04 | .764 | 0.09 | .528 | −0.32 | .034* | −0.32 | .04* | −0.23 | .147 | −0.32 | .037* | −0.37 | .014* |
| ADMA | −0.03 | .849 | 0.19 | .166 | −0.22 | .156 | −0.22 | .159 | −0.18 | .246 | −0.26 | .098 | −0.25 | .11 |
| SDMA | 0.25 | .070 | 0.14 | .331 | −0.33 | .032* | −0.22 | .154 | −0.22 | .153 | −0.25 | .112 | −0.29 | .055 |
| DMA | 0.07 | .629 | 0.24 | .085 | −0.11 | .494 | −0.19 | .219 | −0.1 | .508 | −0.2 | .19 | −0.3 | .05* |
| ARG/ADMA | 0.25 | .067 | −0.28 | .041* | 0.08 | .629 | 0.12 | .452 | −0.1 | .947 | 0.08 | .633 | −0.02 | .88 |
| ADMA/SDMA | −0.25 | .065 | 0.23 | .102 | 0.01 | .965 | −0.1 | .542 | −0.02 | .893 | −0.02 | .893 | −0.01 | .963 |
| CIT/ARG | −0.38 | .005† | 0.3 | .031* | −0.02 | .879 | −0.22 | .161 | −0.34 | .028* | −0.26 | .098 | −0.29 | .056 |
| DMA/ADMA | 0.22 | .113 | −0.21 | .127 | 0.35 | .022* | 0.41 | .007† | 0.46 | .002† | 0.3 | .053 | 0.28 | .065 |

AASI, ambulatory blood pressure monitoring–derived arterial stiffness index; ADMA, asymmetric dimethylarginine; AI, augmentation index; ARG, arginine; CIT, citrulline; DBP, diastolic blood pressure; DMA, dimethylamine; PWV, pulse-wave velocity; SBP, systolic blood pressure; SDMA, symmetric dimethylarginine.

* $P < .05$.

† $P < .01$.

vascular structure for refining cardiovascular risk in children with early-stage CKD. High PWV and CKD have been reported as predictors of cardiovascular events in adult hypertensive patients.[34] This concept is supported by our data, which show a positive correlation between PWV and awake SBP load in children with mild-to-moderate CKD. Nevertheless, we found no associations between arterial stiffness parameters and eGFR categories. Our data support a previous report indicating that BP load, not renal function, is the major determinant of arterial stiffness in adult CKD.[35] Given that validation studies of arterial stiffness have not been reproduced in children,[6] further studies are warranted to elucidate the prognostic abilities of PWV, AI, and AASI for cardiovascular outcomes in larger cohorts of children with CKD.

This study has some limitations that should be considered. First, the low number of patients included might not have been sufficient to reveal the true relationships present. Second, we used ABPM reference values from studies performed in Germany.[20] Ethnic differences should therefore be considered, and our results may be not be applicable to other populations. Third, so far there are no reference values of cIMT, PWV, AI, AASI, and FMD to define a cut-off point between healthy subjects and children with CKD. Further studies using larger populations may be warranted in the future.

## Conclusions

BP load abnormalities are extremely prevalent in children and adolescents with mild-to-moderate CKD, which are associated with low urinary level of CIT and CIT/ARG ratio. Urinary CIT/ARG ratio was correlated with the arterial stiffness parameters AI and PWV. In conclusion,

**Table 6**
Logistic regression on urine levels of biomarkers and ratios in children with mild-to-moderate CKD

| Dependent Variables | Explanatory Variable | Adjusted OR (95% CI) | *P*-Value | Model *P*-Value |
|---|---|---|---|---|
| CIT | Awake and asleep systolic and diastolic BP loads ≥ 25% or nocturnal SBP (or DBP) nondipping | 0.023 (0.001–0.662) | .028 | <.001 |
| ARG | Nocturnal SBP nondipping | 0.909 (0.835–0.99) | .029 | .007 |
| ARG/ADMA | BPs exceed the 95th percentile for gender and height by office BP measurements | 0.041 (0.002–0.717) | .029 | .002 |
| CIT/ARG | Awake and asleep systolic and diastolic BP loads ≥ 25% | 0 (0–0.226) | .042 | .002 |
| DMA/ADMA | BPs exceed the 95th percentile for gender and height by office BP measurements | 2.61 + 019 (21.974–3.07E+034) | .012 | .002 |

ADMA, asymmetric dimethylarginine; ARG, arginine; BP, blood pressure; CI, confidence interval; CIT, citrulline; CKD, chronic kidney disease; DBP, diastolic blood pressure; DMA, dimethylarginine; eGFR, estimated glomerular filtration rate; OR, odds ratio; SBP, systolic blood pressure.

Each OR of the variable was adjusted by the other factors in the logistic regression model. All models included age, sex, eGFR, proteinuria, and plasma uric acid as covariates.

early detection of BP load abnormalities, urinary biomarkers in the CIT-ARG-NO pathway, and arterial stiffness parameters will improve early preventive care with respect to decreasing hypertension and CV remodeling in pediatric CKD patients.

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