Cardiovascular disease (CVD) is a major cause of morbidity and mortality in children and adults with chronic kidney disease (CKD). Unlike adults, young CKD patients rarely present with CV events. Thus assessment of CVD in childhood mainly relies on the characteristics of vascular phenotype. Less attention has been paid to evaluating subclinical CVD in the early stage of CKD in the pediatric population. It is noteworthy that CKD children with subclinical CVD are at risk of subsequent development of CVD in adulthood. Thus, identification of surrogate markers to detect subclinical CVD in early CKD children may aid in reducing the global burden of CKD.

Deterioration in endothelial function and arterial stiffness are early events in the development of CVD. Nitric oxide (NO), a vasodilator, regulates endothelial function and blood pressure (BP). Our previous review indicated emerging evidence supporting that a deficiency of NO links endothelial dysfunction to CVD in pediatric CKD.

Asymmetric dimethylarginine (ADMA) is an analog of L-arginine (ARG) that inhibits nitric oxide (NO) production; thus the ARG-to-ADMA ratio (AAR) is an index of NO. Homocysteine (HCY) is a risk factor for CVD and it can be metabolized to L-cysteine (CYS). Given that HCY and ADMA/NO are closely linked and related to hypertension, we therefore investigated whether ARG and HCY metabolites, arterial stiffness parameters, ABPM profile, and left ventricular hypertrophy (LVH) are interrelated in children and adolescents with early CKD.

Background: Less attention has been paid to evaluating subclinical cardiovascular disease (CVD) in the early stage of pediatric chronic kidney disease (CKD). Ambulatory blood pressure monitoring (ABPM) and arterial stiffness are the earliest detectable assessments of subclinical CVD. Asymmetric dimethylarginine (ADMA) is an analog of L-arginine (ARG) that inhibits nitric oxide (NO) production; thus the ARG-to-ADMA ratio (AAR) is an index of NO. Homocysteine (HCY) is a risk factor for CVD and it can be metabolized to L-cysteine (CYS). Given that HCY and ADMA/NO are closely linked and related to hypertension, we therefore investigated whether ARG and HCY metabolites, arterial stiffness parameters, ABPM profile, and left ventricular hypertrophy (LVH) are interrelated in children and adolescents with early CKD.

Methods and Results: This cross-sectional study included 57 pediatric patients with CKD stages 1–3. Two-thirds of the children with CKD stages 1–3 exhibited BP abnormalities accessed by ABPM. Children with CKD stages 2–3 had higher HCY, but lower CYS levels. The plasma HCY level was increased in children with LVH and abnormal ABPM. Systolic BP positively correlated with biomarkers AAR, HCY, and CYS. LV mass positively correlated with AAR, HCY, and CYS.

Conclusions: BP abnormalities were prevalent and associated with AAR, HCY, and CYS in children with early CKD. Our data highlighted the effect of NO and the HCY pathway on CKD-related hypertension.

Key Words: Asymmetric dimethylarginine; Chronic kidney disease; Homocysteine; Hypertension; Nitric oxide
enzyme protein, arginine methyltransferase (PRMT), which methylates ARG to form ADMA and concurrently demethylates methionine to generate homocysteine (HCY). Like ADMA, HCY is considered as a risk factor for CVD. HCY can be metabolized to L-cysteine (CYS) and glutathione (GSH). Because HCY and ADMA are biochemically linked in several ways and control BP, we propose that simultaneous analysis of ARG metabolites in the NO pathway, HCY metabolites, and their combined ratios may serve as markers to predict CVD in children with early CKD.

Next, arterial stiffness, the rigidity of the arterial walls, has become important in the development of CVD, especially in patients with CKD. Arterial stiffness not only relates to HT but also left ventricular hypertrophy (LVH), a manifestation of hypertensive target organ damage. Several noninvasive assessments of subclinical CVD have been established in adults, including pulse wave velocity (PWV), augmentation index (AI), and ABPM-derived arterial stiffness index (AASI). With limited pediatric data, the use of these assessments in children and adolescents awaits further validation. Thus we wanted to determine whether ARG metabolites, HCY metabolites, and arterial stiffness parameters are interrelated with BP abnormalities on ABPM and LVH in children and adolescents with early CKD.

**Methods**

**Study Population**

We enrolled 57 children and adolescents attending the pediatric clinic at Kaohsiung Chang Gung Memorial Hospital from January 2014 to February 2015. Informed consent was given and the research protocol was approved by the Chang Gung Memorial Hospital Institutional Review Board (102-4131C), and followed the 1964 Declaration of Helsinki. Renal function and the research protocol was approved by the Chang Gung Memorial Hospital Institutional Review Board (102-4131C), and followed the 1964 Declaration of Helsinki. Renal function was determined by estimated glomerular filtration rate (eGFR) using the Schwartz formula on the basis of body height and creatinine (Cr) level. The CKD staging was defined by the K/DOQI guideline. Stage 1 CKD was defined as eGFR ≥90 ml/min/1.73 m². All participants were assigned to CKD stage 1 or CKD stages 2–3 (eGFR 30–90 ml/min/1.73 m²). Both groups of patients were followed by the study protocol for the clinical, biomedical, and cardiovascular measures. The exclusion criteria included current pregnancy, renal transplantation, inability to complete major data collection procedures (eg, blood sampling), and history of congenital heart disease.

**ABPM**

In our analysis, 3 consecutive seated BP readings were recorded and the clinical BP was the mean of the last 2 readings. The 24-h ABPM data were collected for subjects aged 6–18 years using an Oscar II monitoring device (SunTech Medical, Morrisville, NC, USA) as previously reported. A single, experienced nurse specialist applied the monitor and a single investigator (Y.-L.T.) analyzed the ABPM data. Patient diary cards were used to document participant’s times of sleep and waking and daily activities. The ABPM was set to record the BP and pulse rate at 20-min intervals over 24h. The BP readings exceeding 25% of any individual recordings were likely erroneous and excluded from analysis. Abnormal ABPM profile was determined based on: (1) average 24-h, average daytime, or average nighttime BP exceeding 95th percentile stratified by sex and height using ABPM reference data; (2) 24-h systolic or diastolic BP load ≥25%; and (3) nocturnal decrease of BP by <10% compared with average daytime BP. Next, diastolic BP was plotted against systolic BP using the individual 24-h ABPM readings, to calculate the linear regression slope. The AASI was defined as 1 minus the regression slope.

**Biochemistry and HPLC Analysis**

Fasting plasma specimens and spot urine samples were divided into aliquots and stored at −80°C until analysis. Uric acid, glucose, total cholesterol, low-density lipoprotein, triglyceride, sodium, potassium, calcium, phosphate and hemoglobin levels, and the urinary total protein-to-creatinine ratio were measured by standard laboratory assays. We directed the family to avoid their children having a dietary intake of foods rich with L-arginine (eg, peanut and gelatin), nitrates (eg, sausage), and nitrates (eg, green leafy vegetables) for 1 week before blood and urine sampling. Participants were classified based on the international BMI cutoff values as normal weight, overweight, or obese. Hyperuricemia was defined as exceeding the normal range for age- and sex-specific values: 5.9 mg/dl for 6–8 years (both sexes), 6.1 mg/dl for 9–11 years (both sexes), 7.0 mg/dl for ≥12-year-old males, and 6.2 mg/dl for ≥12-year-old females.

The levels of ARG, ADMA, and symmetric dimethylarginine (SDMA, an isomer of ADMA) in the plasma were measured using high-performance liquid chromatography (HPLC: HP series 1100, Agilent Technologies, Inc) with the o-phthalaldehyde-3-mercaptopropionic acid (OPA-3MPA) derivatization reagent as described previously. The standards contained ARG, ADMA, and SDMA at 1–100 μmol/L, 0.5–5 μmol/L, and 0.5–5 μmol/L, respectively. The recovery rate was approximatively 85–105%. Plasma HCY, CYS, and GSH levels were also measured using HPLC (HP series 1100, Agilent Technologies, Inc).

**Cardiovascular Assessments**

The LV mass (LVM) was calculated using images obtained in the parasternal long-axis or short-axis view of the left ventricle by M-mode echocardiography. The LVM index (LVMi) was obtained by correcting the value of LVM for the body surface area. LVMi was defined as LVM ≥95th percentile using age-specific reference intervals for normal children. The carotid ultrasound assessment was performed by 2 experienced pediatric cardiologists (S.-J.C. & I.-C.L.) as previously reported. The patients were supine for at least 10 min in a quiet room prior to examination. With their neck hyperextended and turned 30–45 degrees contralaterally to the probe, the bilateral mid common carotid artery was imaged using a 5- to 12-MHz linear array transducer. We used the distance between the leading edges of the luminal-intimal interface and the medial-adventitial interface for the measurement of carotid intima-media thickness (cIMT). The cIMT was measured during end-diastole as determined by the R wave on the electrocardiogram. These images were obtained by the ProSound α7 ultrasound coupled to computer-assisted analysis software (e-TRACKING system; Aloka Co, Tokyo, Japan). For reproducibility of cIMT measurements, our coefficient of variation was 4.1%. Next, arterial stiffness parameters, PWV and AI, were determined by echo-tracking methods (e-TRACKING system; Aloka Co).

**Statistical Analysis**

Data are expressed as median (interquartile range). The independent t-test or the Mann-Whitney U-test was used to test the differences in variables between children with CKD stage 1 and those who with CKD stages 2–3. Logistic or linear regression was performed to further investigate the effects of
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blood urea nitrogen, creatinine, and uric acid but lower eGFR compared with those with CKD stage 1. Approximately 24.6% (14/57) of patients were diagnosed as overweight/obese and 22.8% of participants had LVH (13/57). There was no statistical difference for overweight/obese patients or LVH between the 2 groups. A total of 27 patients (47.4%) were diagnosed as having hyperuricemia, significantly greater numbers of patients with CKD stages 2–3 compared with stage 1.

Next, of the 57 patients, 43 (75.4%) underwent the 24-h ABPM study (Table 2), 20.9% (10/43) were diagnosed as having HT by office BP measurement. However, we found up to 69.8% (30/43) of children and adolescents with CKD stages 1–3 had at least 1 BP abnormality on ABPM in this study. The ABPM identified 13 patients (30.2%) with 24-h HT, 12 (27.9%) with daytime HT, 17 (39.5%) with nighttime HT, 26 (60.5%) with increased BP load, and 15 (34.9%) with nocturnal BP non-dipping. A total of 8 patients received antihypertensive agents, and there was no difference between patients with CKD stages 2–3 and stage 1.

As shown in Table 3, children with CKD stages 2–3 had biomarkers as predictors of more severe BP abnormalities in ABPM. The association between variables was examined by the Pearson correlation coefficient. A value of P<0.05 was considered statistically significant. All analyses were performed using SPSS 14.0 (SPSS Inc, Chicago, IL, USA).

### Results

**Table 1** shows the basic characteristics of the patient population. Our study population was slightly predominantly male (60% M vs. 40% F) and 70% of participants had a congenital anomaly of the kidney and urinary tract (CAKUT); renal agenesis/dysgenesis, 19 patients (33.3%); renal cystic disease, 8 patients (14%); obstructive nephropathy, 5 patients (8.8%); reflux nephropathy, 5 patients (8.8%); and neurogenic bladder 3 patients (5.3%). Among the non-CAKUT group, nephrotic syndrome was present in 11 patients (19.3%); systemic lupus erythematosus, 4 patients (7%); IgA nephropathy, 1 patient (1.8%); and genetic disease, 1 patient (1.8%). Children with CKD stages 2–3 were older, and had higher plasma levels of blood urea nitrogen, creatinine, and uric acid but lower eGFR compared with those with CKD stage 1. Approximately 24.6% (14/57) of patients were diagnosed as overweight/obese and 22.8% of participants had LVH (13/57). There was no statistical difference for overweight/obese patients or LVH between the two groups. A total of 27 patients (47.4%) were diagnosed as having hyperuricemia, significantly greater numbers of patients with CKD stages 2–3 compared with stage 1.

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Discussion

In this study of children with CKD stages 1–3, we documented a remarkably high prevalence of BP abnormalities accessed by ABPM. Previous studies indicated that children with CKD stages 2–4 are prone to develop HT, which is not associated with their GFR and is frequently masked when office BP is measured. These findings are supported by the current data showing that two-thirds of children with CKD, even in CKD stage 1, exhibit BP abnormalities, including daytime and nighttime HT, increased BP load, and nocturnal BP non-dipping. The detection of HT has significantly improved with the use of ABPM. Approximately one-half of the present children had hyperuricemia and one-quarter were overweight/obese. These findings provide further evidence that children, even in the early stage of CKD, are at increased cardiovascular risk.

Importantly, components of the NO pathway and HCY metabolites are closely related to each other. Among them, higher levels of plasma HCY and AAR were associated with increasing systolic BP and LVM. We have previously demonstrated that ARG metabolites in the NO pathway correlate with abnormal ABPM profiles in children with CKD stages 1–3, and to further elucidate the roles of NO and the HCY pathway in cardiovascular outcomes in these children, we extended our study to analyze HCY metabolites, carotid ultrasound, echocardiography, and arterial stiffness in the current study.

higher plasma levels of HCY, but lower CYS, compared with those with CKD stage 1 after adjustment. However, plasma levels of ARG metabolites and their combined ratios were not different between children/adolescents with different stages of CKD.

Using data pooled from all subjects (CKD stages 1–3), correlations of LV mass, aortic stiffness parameters, and biomarkers were analyzed (Figure 1). We observed that systolic BP positively correlated with biomarkers AAR, HCY, and CYS, but negatively with ASR. The LVM positively correlated with AAR, HCY, and CYS levels. The AI, an aortic stiffness parameter, correlated with 3 biomarkers in the NO pathway: ADMA, SDMA, and AAR. In contrast, neither PWV nor AASI correlated with an elevation of any biomarker. It is noteworthy that several components of the NO pathway and HCY metabolites correlated with each other, indicating a close link between these pathways in early CKD. Furthermore, systolic BP positively correlated with PWV and LVM. Also, there was a positive correlation between LVM and AASI.

In the logistic regression, a high level of HCY remained significantly associated with LVH (β=0.239, P=0.015) and abnormal ABPM profile, including 24-h HT (β=0.204, P=0.03), daytime HT (β=0.214, P=0.027), and nocturnal HT (β=0.492, P=0.006). We also found that elevation of the CYS level was significantly associated with LVH (β=0.016, P=0.025) and nocturnal HT (β=0.023, P=0.023).

Table 2. BP in Children With CKD Stages 1–3

<table>
<thead>
<tr>
<th>Hypertension (by office BP), n (%)</th>
<th>CKD stage 1 (n=25)</th>
<th>CKD stages 2–3 (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average 24-h BP &gt;95th percentile</td>
<td>7 (28)</td>
<td>6 (33)</td>
</tr>
<tr>
<td>Average daytime BP &gt;95th percentile</td>
<td>6 (24)</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Average nighttime &gt;95th percentile</td>
<td>9 (36)</td>
<td>8 (27)</td>
</tr>
<tr>
<td>BP load ≥25%</td>
<td>15 (60)</td>
<td>11 (61)</td>
</tr>
<tr>
<td>Nocturnal decrease of BP &lt;10%</td>
<td>8 (32)</td>
<td>7 (39)</td>
</tr>
<tr>
<td>On antihypertensive drugs</td>
<td>4 (16)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>No. of medications</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Type of medication</td>
<td>3</td>
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</tbody>
</table>

Table 3. Plasma Levels of CIT, ARG, ADMA, SDMA, and DMA and Their Combined Ratios in Children With CKD Stages 1–3

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<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>CKD stage 1 (n=34)</td>
</tr>
<tr>
<td>ARG</td>
</tr>
<tr>
<td>ADMA</td>
</tr>
<tr>
<td>SDMA</td>
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<td>HCY</td>
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<td>CYS</td>
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<td>GSH</td>
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<tr>
<td>ARG-to-ADMA ratio</td>
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<tr>
<td>ADMA-to-SDMA ratio</td>
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*P<0.05 by the Mann-Whitney U test. Adjusted OR, each OR of the variable was adjusted by the other factors listed in the table in the logistic regression model. ADMA, asymmetric dimethylarginine; ARG, L-arginine; CYS, L-cysteine; GSH, glutathione; HCY, homocysteine; OR, odds ratio; SDMA, symmetric dimethylarginine. Other abbreviations as in Table 1.
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Figure 1. Schematic diagram of the inter-relationship of ARG metabolites in the NO pathway, HCY metabolites, systolic BP, LV mass, and arterial stiffness (PWV, AI, and AASI). Double-headed arrows indicate significant associations. *P<0.05, **P<0.01. AAR, ARG-to-ADMA ratio; AASI, ABPM-derived arterial stiffness index; ABPM, ambulatory blood pressure monitoring; ADMA, asymmetric dimethylarginine; AI, augmentation index; ARG, L-arginine; SBP, systolic blood pressure; CYS, L-cysteine; HCY, homocysteine; NO, nitric oxide; PWV, pulse wave velocity; SDMA, symmetric dimethylarginine.

Figure 2. Schematic diagram of how the nitric oxide (NO) and homocysteine (HCY) metabolic pathways are closely linked but differently related to arterial stiffness, systolic blood pressure (SBP), and hypertensive target organ damage in chronic kidney disease (CKD). Asymmetric and symmetric dimethylarginine (ADMA and SDMA, respectively) are synthesized by the enzyme protein arginine methyltransferase PRMT, which concurrently methylates L-arginine to form the dimethylarginines and demethylates methionine to generate HCY. ADMA can compete with L-arginine to inhibit NO synthase (NOS) to generate NO. ADMA is metabolized by DDAH, which can be inhibited by HCY. HCY is metabolized to generate L-cysteine (CYS), glutathione (GSH), and hydrogen sulfide (H2S). Both H2S and NO are vasodilators. High HCY and low CYS are related to CKD progression. We hypothesize that impaired HCY metabolic pathway is inter-connected with a compensatory NO pathway to increase arterial stiffness, elevate SBP, and induce hypertensive target organ damage in CKD.
Figure 2 is a simplified schematic that attempts to summarize our results into a potential pathological framework linking biomarkers, arterial stiffness, HT, and target organ damage.

In line with previous studies of pediatric CKD,21–23 we found a high prevalence of participants had LVH, which is related to high systolic BP but not GFR or ABPM abnormalities. Interestingly, increasing LVM and systolic BP both strongly correlated with elevated HCY, CYS, and AAR. We found that children with CKD stages 2–3 have higher plasma levels of HCY and lower CYS compared with those with CKD stage 1. These findings suggest either increasing HCY synthesis or impairment of its metabolism in CKD progression. During HCY synthesis, it is presumed that ARG is methylated to ADMA, consequently the AAR is decreased. However, we observed a tendency for AAR to be elevated in children with CKD stages 2–3 and AAR positively correlated with BP. Because elevated AAR represents increased NO bioactivity,4 thus AAR is likely to reflect a compensatory response to elevation of BP in children with early CKD. This finding is consistent with our previous report showing that high NOx (the metabolites of NO) levels relate to high BP load and tend to a high office BP in children with CKD stages 1–3 despite NOx levels not being associated with CKD stage.8 On the other hand, HCY metabolizes to generate CYS and hydrogen sulfide (H2S), which is a vasodilator involved in BP control. Given that hyperhomocysteinemia and dysregulation of H2S production have been reported in CKD,27 and that hyperhomocysteinemia plays a role in HT,28 our data support impaired HCY metabolism as possibly the major factor contributing to HT in patients with CKD. Notably, several components in the NO pathway and HCY metabolic pathway correlated with each other in this study. We found a negative association between ASR and HCY. In animal models, our previous study showed high ASR reflects low dimethylarginine dimethylaminohydrolase (DDAH) activity,29 while HCY is reported to inhibit DDAH activity,30 therefore, HCY might regulate DDAH activity to modulate the ADMA/NO pathway, leading to CVD in CKD.

In addition to biomarkers, we examined arterial stiffness parameters in this study. Measurement of arterial stiffness enables evaluation of arterial dysfunction, which may precede structural vascular changes evaluated by cIMT. Although PWV, AI, and AASI have been used in children, reproducibility studies and age-specific reference ranges are still lacking.2 We found no association between arterial stiffness parameters and the CKD stages. However, PWV positively correlated with systolic BP. In addition, there was a positive correlation between AASI and LVM. Our data support the findings of previous reports that BP, not renal function, is the major determinant of arterial stiffness in adult CKD.31 Although cIMT was reported to be increased in children with CKD stages 2–4 compared with normal controls,32 we found cIMT was not statistically different between CKD stages and cIMT did not correlate with arterial stenosis parameters or biomarkers in the NO and HCY pathways. It is noteworthy that we observed most risk factors were not associated with CKD staging in children with early CKD. However, hyperuricemia was present in nearly one-half of participant with CKD stages 1–3, which closely correlated with eGFR, LVM, office BP, ABPM abnormalities, AAR, and HCY. Our data support the findings of previous studies showing that hyperuricemia is common in pediatric CKD and related to high BP.33–35

**Study Limitations**

Firstly, small number of study subjects might not be sufficient for statistical comparisons to show the true associations. Secondly, this cross-sectional study does not allow us to surmise causality. Thirdly, we used ABPM and LVM reference data from studies in Germany and America, respectively.17,22 Ethnic differences should be considered. Although the ABPM reference data from German youth were used because normal clinical ranges of ABPM for Taiwanese children are unavailable, 1 small study, however, has shown no differences in ABPM data between Taiwanese and German children.36 To date, there are no reference values for PWV, AI, and AASI to define cutoff points between healthy subjects and children with CKD. A larger study population may be warranted.

**Conclusions**

BP abnormalities were highly prevalent in Taiwanese children and adolescents with early CKD, even in CKD stage 1. These BP abnormalities are associated with elevated levels of HCY and CYS, and changes in AAR, which suggested an effect of the NO and HCY metabolic pathways on CKD-related HT. It is imperative to early detect BP abnormalities, surrogate biomarkers, and arterial stiffness parameters, in order to develop an effective therapeutic approach to improving cardiovascular outcomes in children with CKD.

**Acknowledgments**

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**References**


