Reduced renal plasma clearance does not explain increased plasma asymmetric dimethylarginine in hypertensive subjects with mild to moderate renal insufficiency

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IN PATIENTS with chronic kidney disease (CKD), the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) and its structural isomer symmetric dimethylarginine (SDMA) build up in plasma even in the early stages of renal insufficiency (10, 19, 21). Moreover, ADMA already rises in incipient CKD. The exact mechanisms responsible for this increase of ADMA and SDMA at these early stages of CKD have not been totally clarified but are likely to involve changes in the regulation of the plasma concentration by the kidneys’ renal plasma clearance (RPCL) (37, 49).

The kidneys play an important role in the plasma concentration regulation of ADMA and SDMA. By measuring arteriovenous concentration differences, a previous study (37) demonstrated that the kidneys clear substantial amounts of ADMA and SDMA from the circulation. Furthermore, both dimethylarginines are excreted in the urine in amounts, which are dependent on the glomerular filtration rate (GFR) (2, 30, 31, 49). However, the main elimination pathway for ADMA is intracellular degradation into citrulline and dimethylamine by the enzyme Nω,Nω-dimethylarginine dimethylaminohydrolase (DDAH), which is highly expressed in the kidney (17, 22, 24, 47). Nevertheless, DDAH is also expressed in other organs, such as the pancreas, liver, lungs, and endothelium. Contrary to ADMA, SDMA is almost entirely eliminated from the plasma by excretion into the urine and cannot be eliminated by DDAH (21). Therefore, it has been proposed that the plasma concentration of SDMA is more affected by reduced GFR and changes in RPCL than the plasma concentration of ADMA (20).

On the other hand, small amino acids, like these dimethylarginines (molecular weight: 0.2 kDa), are usually able to freely pass the glomerular barrier, and normally their RPCL does not decrease until glomerular filtration has fallen substantially (moderate to severe CKD) (9, 16, 41). Furthermore, based on their identical molecular weight (a main determinant of RPCL), the dependence of RPCL on GFR should be similar for both dimethylarginines (29).

Although some studies have addressed the RPCL (37, 43) or urinary clearance (2, 30, 31, 49) of ADMA and SDMA, these studies have only been performed in subjects with either “severe” CKD or “normal” renal function. Moreover, the studies that measured RPCL by arteriovenous concentration differences did not investigate the relationship with GFR. Accordingly, we performed the present study in a large cohort of hypertensive patients with mild to moderate renal insufficiency to unravel the mechanistic relationship among GFR, RPCL (measured by renal arteriovenous concentration differences), and (arterial) plasma concentrations of ADMA and SDMA in the early phases of renal impairment.

METHODS

Patients and study protocol. Between May 2002 and July 2008, 326 Caucasian patients underwent diagnostic renal angiography for the exclusion of renal artery stenosis according to a standardized...
clinical protocol. This protocol includes selective renal blood sampling (for the assessment of ADMA and SDMA) of the aorta, left and right renal veins, selective mean renal blood flow (MRBF) measurements, and renal angiography. Suspicion for renal artery stenosis was based on the following clinical clues: hypertension despite treatment with at least three antihypertensive agents, accelerated hypertension, evident peripheral vascular disease, and the presence of an abdominal bruit or unexplained renal function deterioration, e.g., in response to antihypertensive treatment.

For reasons of standardization, all antihypertensive medication was discontinued for 3 wk, and patients were requested to adhere to a sodium-restricted (55 mmol/24 h) diet during the 1 wk before renal angiography. In patients with diabetes mellitus (DM), the use of metformin was discontinued 2 days before renal angiography to lower the risk of contrast medium-induced nephropathy. Other medication, including statins, was allowed. One day before renal angiography, a noninvasive ambulatory 24-h blood pressure measurement was performed (ambulatory blood pressure monitor, SpaceLabs, Redmond, WA), and 24-h urine was collected for the assessment of sodium and urinary albumin excretion (UAES).

After admission to our ward and an overnight fast, we cannulated the aorta and both renal veins via the femoral route. Subsequently, we drew blood samples simultaneously from the aorta and both renal veins for the assessment of ADMA and SDMA. After centrifugation, plasma samples were immediately stored at −80°C until analysis. In addition, we sampled blood for the determination of fasting glucose, serum creatinine, and lipid profile. Subsequently, we measured selective MRBF using the 133Xe washout technique, as previously described (12, 23, 51). After an intra-arterial administration of 133Xe (IBD Holland, Baarle-Nassau, The Netherlands) into the renal artery, we measured the disappearance of 133Xe from the kidney by an extracorporeal scintillation counter during 180 s. Subsequently, the obtained 133Xe washout curves of the left and right kidney were analyzed offline (using Graphpad Prism 5, Graphpad Software, San Diego, CA). In brief, after the subtraction of background radiation, curves were analyzed mathematically by means of a two-phase exponential decay. MRBF was calculated as the weighted average of the fast and slow component (16a). Occasionally, a monophasic decline in activity was observed, and the curve was analyzed accordingly.

During MRBF measurements, heart rate and intra-arterial blood pressure were monitored continuously. MRBF and mean renal plasma flow (MRPF), calculated as MRBF × (1 − hematocrit), were expressed as milliliters per minute per 100 g of kidney. No contrast agents were administered before the blood sampling and flow measurements were completed. Eventually, intra-arterial renal angiography was performed using a digital subtraction system. An experienced radiologist reported renal (artery) anomalies. The investigations were performed in accordance with the principles outlined in the Declaration of Helsinki. All patients gave their informed consent before inclusion in the study, and the local ethical committee approved the study.

Biochemical analyses. In the blood samples drawn from the aorta and both renal veins, we assessed plasma concentrations of ADMA and SDMA using an Acquity ultraperformance liquid chromatography separation module coupled to a Quattro Premier Electrospray Ionization Tandem Mass Spectrometry (Waters, Ettten-Leur, The Netherlands) (32, 50). Intra- and interassay variations at physiological concentrations were <5% for ADMA and SDMA. Standard clinical methods were used for the assessment of fasting glucose, serum creatinine, and lipid profile.

Calculations. GFR was estimated by the CKD Epidemiology Collaboration equation (26). Patients were divided into three estimated GFR (eGFR) groups (eGFR ≥ 90, eGFR 60–89, and eGFR 30–59).

RPCL in ml·min⁻¹·100 g kidney⁻¹ of both kidneys of ADMA and SDMA was calculated as follows: [MRPF right × [(A − V right) / A]] + [MRPF left × [(A − V left)/A]], where A and V are the arterial and renal vein plasma concentrations, respectively. This calculation corrects a possible overestimation of the fractional renal extraction [(calculated as (A − V)/A × 100%) (37, 43, 53)] in cases of a low renal plasma flow (3). A positive RPCL indicates net clearance of the substance from the blood, whereas a negative RPCL indicates net release into the blood.

Statistical analysis. Normally distributed variables are expressed as mean ± SD; variables with a skewed distribution are given as medians and interquartile ranges. Variables with a skewed distribution were log transformed before further analyses (UAES and RPCL of ADMA and SDMA). Multiple linear regression analyses were used to investigate: 1) the association between eGFR and arterial plasma concentrations of ADMA and SDMA, 2) the association between eGFR and RPCL of both dimethylarginines, and 3) between RPCL of both dimethylarginines and arterial plasma concentrations of ADMA and SDMA. All associations were first analyzed without adjustments and then with adjustments for potential confounders: age, sex, history of cardiovascular events, DM, LDL-cholesterol, smoking, intra-arterial mean arterial pressure (MAP), and UAES. Finally, analyses were adjusted for total renal blood flow (RBF; left MRBF + right MRBF) and/or eGFR, as appropriate. All results are expressed in standardized regression coefficients (95% confidence intervals [CIs]) to allow comparison of the strength of the association between different variables. Two-tailed P values of <0.05 were considered significant. Analyses were performed using the SPSS statistical software package (version 15.0, SPSS, Chicago, IL).

RESULTS

From the 326 patients who had undergone renal angiography, 171 patients were judged eligible for this study by fulfilling the following criteria: essential hypertension (no renovascular abnormalities), complete selective blood sampling for the assessment of ADMA and SDMA, and complete RBF measurements. Baseline characteristics of the included patients did not differ significantly from the total patient group (data not shown). The renal function of the study population ranged between “normal” (eGFR ≥ 90 ml·min⁻¹·1.73 m⁻² and no microalbuminuria) and mild to moderate renal insufficiency (stage 2 and 3 CKD according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative CKD classification) (25).

Table 1 shows the characteristics of the 171 Caucasian hypertensive patients (mean age: 53 ± 13 yr, 97 men and 74 women, number of patients/group 58 75 38). Table 1 shows the characteristics of the 171 Caucasian hypertensive patients (mean age: 53 ± 13 yr, 97 men and 74 women, number of patients/group 58 75 38).
women, mean 24-h blood pressure: 160/97 mmHg without antihypertensive medication) divided into three eGFR groups. The mean values of the eGFR of the different groups (eGFR \( \geq 90 \) eGFR 60–89, and eGFR 30–59) were 101 ± 10, 79 ± 7, and 53 ± 7 ml·min\(^{-1}\)·1.73 m\(^{-2}\), respectively. The prevalence of patients with microalbuminuria (30–300 mg/24 h) was 54% in the overall population. Microalbuminuria was mainly present in patients with an eGFR of <90 ml·min\(^{-1}\)·1.73 m\(^{-2}\). The prevalence of statin use was 40%, and none of the 23 patients with type 2 DM used thiazolidinediones.

Table 2 shows arterial and venous plasma concentrations of ADMA and SDMA divided according to eGFR groups. With declining eGFR, arterial and venous (left and right kidney) plasma concentrations of both dimethylarginines increased (Table 2). Contrast tests revealed that the eGFR 60–89 group had significantly higher arterial ADMA and SDMA compared with the eGFR \( \geq 90 \) group (\( P = 0.001 \) and \( P < 0.001 \), respectively). Moreover, the eGFR 30–59 group showed significantly higher arterial ADMA and SDMA compared with the eGFR 60–89 group (\( P = 0.036 \) and \( P < 0.001 \), respectively). MRBF (left and right) decreased with declining eGFR, whereas intra-arterial MAP did not differ between the eGFR groups.

Furthermore, with declining eGFR, RPCL of both dimethylarginines decreased (Table 2). Post hoc analyses showed that RPCL of SDMA significantly decreased across different eGFR groups (eGFR \( \geq 90 \) vs. eGFR 60–89, \( P = 0.01 \); and eGFR 60–89 vs. eGFR 30–59, \( P < 0.001 \)), whereas RPCL of ADMA only differed significantly between eGFR \( \geq 90 \) and eGFR 30–59 (\( P = 0.014 \)). The RPCL of ADMA and SDMA did not differ yet in the group with eGFR \( \geq 90 \) ml·min\(^{-1}\)·1.73 m\(^{-2}\). However, in the groups of eGFR 60–89 and eGFR 30–59 ml·min\(^{-1}\)·1.73 m\(^{-2}\), a significant difference between RPCL of ADMA and SDMA was found (Table 2).

**Table 2. Renal assessments divided according to eGFR groups**

<table>
<thead>
<tr>
<th>Number of patients/group</th>
<th>eGFR ≥ 90</th>
<th>eGFR 60–89</th>
<th>eGFR 30–59</th>
<th>( P ) value (Trend)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial ADMA, µmol/l</td>
<td>0.44 ± 0.07</td>
<td>0.47 ± 0.05</td>
<td>0.49 ± 0.07</td>
<td>0.001</td>
</tr>
<tr>
<td>Left renal vein ADMA, µmol/l</td>
<td>0.49 ± 0.08</td>
<td>0.55 ± 0.09</td>
<td>0.67 ± 0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Right renal vein ADMA, µmol/l</td>
<td>0.43 ± 0.08</td>
<td>0.49 ± 0.08</td>
<td>0.61 ± 0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arterial SDMA, µmol/l</td>
<td>0.49 ± 0.08</td>
<td>0.55 ± 0.09</td>
<td>0.67 ± 0.10</td>
<td>&lt;0.001</td>
</tr>
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<td>&lt;0.001</td>
</tr>
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</table>

Data are presented as means ± SD or medians (interquartile ranges). Comparisons of renal measurements across eGFR groups (eGFR ≥ 90, eGFR 60–89, and eGFR 30–59) were performed using one-way ANOVA. ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; MRBF, mean renal blood flow; RPCL, renal plasma clearance. \(^*\)\( P < 0.05 \) by paired Student’s \( t \) test compared with RPCL of SDMA in the same eGFR group.

**eGFR and renal plasma clearance of ADMA and SDMA.**

Table 3 shows that the positive association between eGFR and RPCL of ADMA (model 1) was not statistically significant after adjustments for potential confounders (models 2 and 3). In contrast, the positive association between eGFR and RPCL of SDMA (model 1) remained significant even after adjustments for the same confounders in models 2 and 3 (Table 3, model 2, \( \beta = 0.27 \) (95% CIs: 0.08, 0.47), \( P = 0.007 \); model 3, \( \beta = 0.21 \) (95% CIs: 0.02, 0.40), \( P = 0.034 \)).

Renal plasma clearance of ADMA and SDMA and arterial plasma concentrations of ADMA and SDMA. Figure 2A and Table 4 show that there was no significant association between RPCL and the arterial plasma concentration of ADMA. Adjustments for age, sex, history of cardiovascular events, DM, LDL-cholesterol, smoking, intra-arterial MAP, and UAE in model 2 did not change these results (Table 4). However, additional adjustments for total RBF and GFR in models 3 and 4.
enhanced the association to some extent, but the association remained statistically nonsignificant.

Crude analysis revealed an inverse association between RPCL and the arterial plasma concentration of SDMA (Table 4, model 1, and Fig. 2B). Adjustments for potential confounders in model 2 did not materially change the strength of the association (Table 4). Finally, after additional adjustments for total RBF and eGFR, the association between RPCL of SDMA and the arterial plasma concentration of SDMA diminished to a large extent (Table 4, β from −0.20 (95% CIs: −0.35, −0.05), \( P = 0.009 \), in model 2 to −0.08 (95% CIs: −0.21, 0.05), \( P = 0.239 \), in model 4).

Additional analyses. In the aforementioned analyses, we showed an independent association between eGFR and RPCL of SDMA (Table 3, model 3), and we showed that the association between RPCL and the arterial plasma concentration of SDMA could be explained (mediated) to a large extent by eGFR and total RBF in this population (Table 4, models 3 and 4). Therefore, we additionally investigated the extent to which the association between eGFR and the arterial plasma concentration of SDMA could be explained (mediated) by RPCL of SDMA. Additional adjustment for RPCL of SDMA (after adjustments for potential confounders) in the association between eGFR and the arterial plasma concentration of SDMA attenuated the association to some extent (\( \beta \) from = −0.66 (95% CIs: −0.83, −0.49), \( P < 0.001 \), to −0.48 (95% CIs: −0.73, −0.22), \( P < 0.001 \)). RPCL of SDMA explained ~27% (change of \( \beta \) from −0.66 to −0.48) of the association between eGFR and the arterial concentration of SDMA.

The association between eGFR and plasma concentrations of ADMA and SDMA did not differ according sex (\( P = 0.304 \) and \( P = 0.226 \) for sex interaction, respectively). Furthermore, we found no effect modification by sex in any of the other investigated associations, i.e., in the associations between eGFR and RPCL of both dimethylarginines and in the associations between RPCL of both dimethylarginines and their plasma concentrations.

**DISCUSSION**

The present study is the first in a large group of hypertensive patients with mild to moderate renal insufficiency that directly
measured arteriovenous ADMA and SDMA concentration differences of both kidneys in combination with selective MRBF measurements to investigate the relationship between renal function (eGFR), RPCL, and (arterial) plasma concentrations of both dimethylarginines. We found that plasma concentrations of ADMA and SDMA increased with declining renal function. However, the RPCL of ADMA was independent of renal function in this population. Moreover, a lower RPCL of ADMA was not associated with a higher arterial concentration of ADMA. On the other hand, reduced renal function was indeed associated with lower RPCL of SDMA, and the association between lower RPCL of SDMA and higher plasma concentration of SDMA could be explained by the combination of renal function and RBF. Taken together, these data suggest, first, that reduced renal function does not necessarily result in decreased RPCL of ADMA, whereas it does result in decreased RPCL of SDMA at these stages of renal insufficiency. Second, decreased RPCL of ADMA does not explain the increase in plasma concentration of ADMA in this hypertensive population with mild to moderate renal insufficiency, whereas a decreased RPCL of SDMA does so for the increase in the plasma concentration of SDMA.

Increased ADMA plasma concentration has been reported in a wide range of cardiovascular disorders (7, 34, 44, 55). Among these conditions, ADMA plasma concentration is particularly high in patients with CKD (49). Although the association between reduced renal function and accumulation of ADMA in CKD has been addressed in several studies (2, 15, 31, 35), findings have been variable and studies could not always confirm the existence of an independent association (14, 19, 37). This is not surprising considering the fact that ADMA is >80% eliminated via intracellular enzymatic degradation by DDAH (1) and not primary by urinary excretion, which is probably more GFR dependent. Notably, DDAH activity is not confined to the kidney but also present in the liver and other organs. Moreover, it is conceivable that depending on the pathophysiological cause and severity of renal insufficiency, the decline in renal excretory function is not paralleled by a reduction in renal DDAH activity. Finally, other mechanisms involved in the plasma regulation of ADMA, synthesis of ADMA by posttranscriptional methylation of proteins catalyzed by protein arginine methyltransferases (PRMT) (33), protein turnover, transcellular transport of ADMA by cationic amino acid transporters (CAT) (46), and metabolism by alanine-glyoxylate aminotransferase 2 (39, 42) may or may not be affected and contribute to the accumulation of ADMA in a particular population of CKD (48, 54).

The results of previous studies (2, 6, 15, 19, 21, 31, 35) regarding the association between reduced GFR and higher plasma SDMA concentration are more consistent and generally demonstrated, similar to our study, a much stronger association between GFR and SDMA compared with the association with ADMA. A meta-analysis (21) of these studies even suggested that SDMA is a reliable endogenous marker of renal function. However, considering the fact that SDMA is almost entirely eliminated from the plasma by excretion in the urine, our finding that reduced RPCL explains only 27% of the rise in plasma SDMA concentration in this population is less than we expected. This indicates that the plasma clearance of SDMA is not exclusively assigned to the kidney and implies that the plasma concentration of SDMA is not solely regulated by plasma clearance, as the plasma concentration is the result of the balance between production and clearance. Siroen et al. (43) showed that the human liver also takes up substantial amounts of SDMA (and ADMA) from the portal and systemic circulation, which shows that the liver also contributes to the plasma clearance of SDMA. However, the results of a study (27) in subjects with hepatorenal syndrome suggested that renal dysfunction is the main determinant of increased SDMA concentration. Accordingly, the increased synthesis of SDMA by type II PRMT in our study may explain that the increased plasma concentration in this population is not exclusively assigned to the kidneys and RPCL. Unfortunately, the specific contribution of increased PRMT activity on increasing plasma concentration of SDMA in humans with CKD is unknown.

The first key finding of the present study is that in contrast to the RPCL of SDMA, the RPCL of ADMA was independent of renal function in our population. From a theoretical point of view, these findings seem surprising. Assuming that the RPCL of both ADMA and SDMA is determined mainly by their molecular weight (similar weight of 0.2 kDa), one would expect an equal dependency of the RPCL on renal function (29). Indeed, previous studies (9, 16) showed that the RPCL of comparable small proteins is independent of renal function until glomerular filtration has fallen substantially (moderate to severe CKD). On the other hand, steric or electrostatic factors could play a role in determining a difference in RPCL between ADMA and SDMA (29). Indeed, some studies (4, 5, 18, 30) in patients with end-stage renal disease showed that SDMA, although of similar molecular weight, could be much more easily removed by dialysis than ADMA. We must also bear in mind that the calculation of RPCL using arteriovenous concentration differences represents the net amount of substance extracted from the plasma by both kidneys. The outcome of the RPCL calculation is a result of the net balance between all possible mechanisms in the kidney contributing to the regulation of the plasma concentration of a substance. This includes not only the three main routes of renal ADMA clearance from the plasma by 1) uptake by cells via transport by CAT followed by intracellular degradation by DDAH (45, 46), 2) metabolism by alanine-glyoxylate aminotransferase 2 (39, 42), and 3) urinary excretion but also the release of ADMA into the plasma. Our results, although indirectly, indicate that the net effect of all these renal plasma ADMA-regulating mechanisms is independent from renal function in hypertensive patients with mild to moderate renal insufficiency, whereas RPCL of SDMA is renal function dependent at these stages of renal insufficiency. Perhaps RPCL of ADMA will be dependent of renal function at more advanced stages of renal insufficiency.

Our second key finding contradicts the commonly held notion that impaired renal (plasma) clearance of ADMA accounts, at least in part, for the increase in plasma ADMA concentration in patients with mild to moderate renal insufficiency. The combination of the frequently reported inverse correlation between impaired renal function and elevated ADMA plasma concentration and substantially decreased urinary excretion of ADMA suggested a causal relation between reduced renal function and elevated ADMA plasma concentration. However, the present study shows that re-
duced RPCL of ADMA cannot explain the increase of plasma ADMA concentration in our population. Previous studies in subnephrectomized (33) and totally nephrectomized (11) rats are in line with our data, lending further credibility to our results. First, plasma concentration of ADMA increased in proportion to the degree of nephrectomy despite a marked increased RPCL of ADMA (33). Contrary to ADMA, RPCL of SDMA was indeed impaired in subnephrectomized rats. Second, both liver and kidney gene expression of PRMT increased, whereas DDAH protein expression decreased in subnephrectomized rats (33). These results indicate that probably the enzymatic changes rather than decreased GFR and RPCL caused the increased plasma concentration of ADMA. Finally, the study in totally nephrectomized rats demonstrated that total plasma clearance of ADMA did not change after total nephrectomy and the plasma concentration of ADMA even decreased, which suggests a role for (compensatory) systemic ADMA metabolism in this acute condition (11). Therefore, the increase in the plasma concentration of ADMA may be a marker (or consequence) of systemic endothelial dysfunction, increased oxidative stress, or atherosclerosis in our population with mild to moderate renal insufficiency rather than the result of reduced RPCL (40, 52).

There are limitations to our study. First, we cannot establish whether the associations are causal due to the cross-sectional design of the study. Unfortunately, the invasive nature of the selective renal blood sampling and renal plasma flow measurements prohibit a longitudinal study design and more robust conclusions. Second, in the present study, the calculated RPCL represents the net amount of substance extracted from the plasma by both kidneys. This calculation includes all possible mechanisms in the kidney contributing to the net clearance of ADMA from the plasma [metabolic mechanisms (42, 46) and urinary excretion (2, 30, 31, 49)]. Unfortunately, in this study, we were not able to differentiate between these mechanisms, partly because we did not analyze the 24-h urine for ADMA and SDMA. Furthermore, plasma concentrations of dimethylarginines are the result of many other processes at the intracellular and organ levels besides the kidneys. Third, we used arterial plasma concentrations of both dimethylarginines from the aorta for our analyses and associations, which makes comparison with plasma concentrations of venous blood derived from the antecubital vein difficult. Therefore, we cannot determine whether the range of our ADMA and SDMA plasma concentrations in this hypertensive population with mild to moderate renal insufficiency is considered “normal” or “increased” compared with “healthy” adults (13, 37, 43). Furthermore, associations made in an apparently “normal” range of ADMA plasma concentration may elicit the absence of specific associations. However, additional analyses in the highest quartile of ADMA plasma concentration still revealed no independent association between RPCL of ADMA and the plasma concentration of ADMA or eGFR and RPCL of ADMA in this population.

In conclusion, in this study of hypertensive patients with mild to moderate renal insufficiency, RPCL of ADMA is independent of renal function after correction for several cardiovascular risk factors. Moreover, the contribution of decreased RPCL appears to be of minor importance in the relationship between renal function and the increase in the plasma concentration of ADMA. This suggests that other, possibly extrarenal, mechanisms than decreased RPCL play a key role in the relationship between renal function and the increase in the plasma ADMA concentration. In contrast, reduced RPCL of SDMA was found to play a significant role in the relationship between renal function and the increase of plasma SDMA concentration in this population.

**Perspectives**

Because the plasma concentration of ADMA increases in patients with CKD (49) and the kidneys play an important role in the plasma concentration regulation of ADMA (37), reduced RPCL appears a logical explanation for the increase in this population. The present study, however, indicated that the increased plasma ADMA concentration is not a direct consequence of the kidneys failing but merely a result of extrarenal changes in the regulation of ADMA related to reduced renal function in patients with mild to moderate CKD. Indeed, in addition to the kidneys, the liver is an important plasma ADMA-clearing (and regulating) organ (36, 43). An animal study (33) has indicated that after subtotal nephrectomy, liver gene expression of type I PRMT activity (responsible for ADMA synthesis) increased, whereas liver expression levels of DDAH decreased. However, more studies in patients with CKD are needed to unravel the interaction between the kidneys and liver as plasma ADMA-regulating organs in humans.

An alternative extrarenal explanation for our finding of increased plasma ADMA is an overall increased production initiated by oxidative stress. In the presence of cardiovascular risk factors such as hypertension, hypercholesterolemia, smoking, DM, aging, and end-stage CKD, oxidative stress is increased. Interestingly, oxidative stress has already been reported in CKD stage 3 patients (38). Recent studies (8, 28, 52) investigating the interaction between ROS and ADMA showed that ROS can increase type I PRMT activity, decrease DDAH activity, and decrease CAT activity, thereby increasing cellular ADMA concentrations. Over time, this could be accompanied by an increase in the plasma concentration of ADMA, like in our study population (46). Although a role for oxidative stress in the increase in the plasma concentration of ADMA in a population of patients with mild to moderate CKD is plausible, more studies are needed to confirm this hypothesis.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

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