Guanidino Compounds in Serum and Urine of Nondialyzed Patients With Chronic Renal Insufficiency

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Levels of 15 guanidino compounds and urea were determined in serum and urine of nondialyzed patients with chronic renal insufficiency subdivided according to etiology and creatinine clearances. No significantly different guanidino compound levels in serum and urine were found for the interstitial nephritis, glomerulonephritis, nephrangiosclerosis, and diabetic nephropathy subgroups. Subdividing the patients according to creatinine clearance yields the following results: (1) Serum guanidinosuccinic acid (GSA) and methylguanidine levels of patients with end-stage renal failure (creatinine clearance <10 mL/min) are up to 100 and 35 times higher than control levels, while guanidine, creatinine, and symmetrical dimethylarginine (SDMA) are increased about 10 times. Serum levels of asymmetrical dimethylarginine (ADMA) are only doubled in end-stage renal failure. Serum levels of guanidinoacetic acid (GAA) and homoarginine are significantly decreased. (2) Urinary excretion levels of most guanidino compounds decrease with decreasing creatinine clearance except for GSA and methylguanidine. (3) Greater than 90% of patients with creatinine clearance ranging from subnormal to 40 mL/min have serum SDMA levels higher than the upper-normal limit; up to 80% have increased GSA levels. (4) The clearance rates of some of the guanidino compounds could be calculated: with the exception of arginine, they decrease with decreasing creatinine clearance. This study shows specific abnormal guanidino compound levels in serum and urine of nondialyzed patients with chronic renal insufficiency that can be used as complementary diagnostic parameters. The best correlation between serum guanidino compound levels and the degree of renal insufficiency is found for GSA, SDMA, methylguanidine, and guanidine. Urinary excretion levels of ADMA correlate best with decreasing creatinine clearance. Serum levels of GSA and especially SDMA are candidate indicators for the onset of renal failure.

THE PATHOPHYSIOLOGY of the uremic syndrome results in a variety of clinical symptoms. Most striking are the neurological complications (fatigue, encephalopathy, the "uremic twitch-convulsive syndrome," and polyneuropathy), gastrointestinal problems (anorexia, hiccups, stomatitis, gastritis, parotitis, and pancreatitis), cardiovascular symptoms (heart failure, hypertension, pericarditis, and atherosclerosis), hematological signs (anemia and bleeding diathesis), and changes in the immunological system. Along with several other organic compounds, guanidines rank high on the list of candidate uremic toxins.[1,2] Many are experimentally proven toxins[3,4] and are reported at higher than normal levels in uremic body fluids[5,6] and brain.[7]

We determined guanidino compound levels in the serum and urine of a large group of nondialyzed patients with chronic renal insufficiency. Patients were subdivided according to etiology to investigate whether particular guanidino compound abnormalities could be identified in different etiological groups. In addition, patients were subdivided according to creatinine clearance to correlate guanidino compound levels with the degree of renal insufficiency.

To our knowledge, this study is the first dealing with such a large number of nondialyzed patients with chronic renal failure. Former studies included mostly dialyzed and nondialyzed patients, as well as chronic and acute renal insufficiency patients.[4,8] In addition, the large number of patients included in this study made it possible to correlate adequately the different guanidino compound levels found in serum and urine with the severity of renal failure. Former studies examined only particular guanidino compounds like methylguanidine.[4]

SUBJECTS AND METHODS

Patients

This study considers 135 nondialyzed patients with varying degrees of chronic renal insufficiency (65 men and 70 women). The age range was 23 to 86 years. Serum urea concentrations were 5.40 to 68.2 mmol/L. The etiological diagnoses were as follows: interstitial nephritis (n = 40), glomerulonephritis (n = 16), nephrangiosclerosis (n = 24), polycystic renal disease (n = 6), diabetic nephropathy (n = 21), pyelonephritis (n = 3), nephrolithiasis (n = 2), focal glomerulosclerosis (n = 4), amyloid kidney (n = 2), periarteritis nodosa (n = 1), renal insufficiency of prerenal (n = 5) and postrenal (n = 4) origin, thrombosis of the renal artery (n = 1), renal insufficiency due to malignancy (n = 1), Alport's syndrome (n = 1), hepatorenal syndrome (n = 1), and kidney failure of unknown origin (n = 3). Diagnoses were based on medical history, clinical findings, biochemical data, and radiological examinations, and in some cases histological data.

In addition to the etiological classification, the patients were also classified according to creatinine clearance. Glomerular filtration was estimated by calculating predicted creatinine clearance according to the method used by Cockcroft and Gault.[9]

Age-matched control samples were obtained from healthy volunteers and individuals presenting with transient neurological complaints in

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Submitted June 27, 1996; accepted February 7, 1997.

Supported by the University of Antwerp, National Fonds voor Wetenschapppelÿk Onderzoek (3.0044.92 and 3.0064.93), the Born-Bunge Foundation, the United Fund of Belgium, the Openbaar Centrum voor Maatschappelÿk Welzÿn Medical Research Foundation, and Neurosearch Antwerp.

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0026-0495/97/4609-0010$03.00/0

whom, after performing a series of clinical and chemical diagnostic tests, no neurologic, renal, hepatic, or metabolic disease was diagnosed.

Collection and Preparation of Samples

Fasting morning serum was taken from all studied patients. From 72 patients, a corresponding 24-hour urine collection was obtained as well. After clotting, the blood was centrifuged at 2,200 × *g* at 6°C for 10 minutes. A portion of the serum was taken for urea determination. The remaining serum was stored at −75°C until analysis. During the 24-hour urine collection, urine was kept at 6°C; no additives were used. For determination of the guanidino compounds (except dimethylarginines), serum and urine samples were deproteinized by mixing equal volumes of a 200-g/L trichloroacetic acid solution with serum or urine. The proteins were centrifuged in a Beckman microfuge (Beckman Instruments International, Geneva, Switzerland). Two hundred microliters of supernatant was used for guanidino compound determination in serum. For the determination in urine, the supernatant was diluted with 0.02N HCl.

For determination of the dimethylarginines, serum and urine samples were deproteinized by mixing 200 μL sample with 50 μL 10% sulfosalicylic acid solution. The mixture was cooled for 30 minutes at 4°C. The proteins were centrifuged, and 200 μL supernatant was injected for assay of dimethylarginines in serum. For assay in urine, the supernatant was diluted 20 times with sample diluting buffer, and 200 μL of this diluted sample was injected on the column.

Guanidino Compounds and Other Chemicals

Standard guanidino compounds were purchased from Sigma Chemical (St Louis, MO), creatine and creatinine from Merck (Darmstadt, Germany), and asymmetrical dimethylarginine ([ADMA] N^GN^G-dimethylarginine) and symmetrical dimethylarginine ([SDMA] $N^GN'^G$-dimethylarginine) from Calbiochem-Novabiochem (San Diego, CA). α-Keto-δ-guanidinovaleric acid was synthesized enzymatically as described previously.[10] All other chemicals used were obtained from Merck and were of analytical grade.

Laboratory Methods

The concentration of the guanidino compounds (except dimethylarginines) was determined using a Biotronic LC 5001 (Biotronik, Maintal, Germany) amino acid analyzer adapted for guanidino compound determination. The guanidino compounds were separated over a cation-exchange column using sodium citrate buffers, and were detected with the fluorescence ninhydrin method as previously reported in detail.[10]

The concentration of ADMA and SDMA was determined using a Biotronic LC 6001 (Biotronik) amino acid analyzer. Indeed, both dimethylarginines are guanidino compounds in which two hydrogen atoms of one or two ω-amino functions of the guanidino group are substituted by two methyl groups. This substitution makes it impossible to detect both compounds with the fluorescence ninhydrin detection method or other methods based on reaction with the guanidino group. In this study, both compounds were detected with the postcolumn derivatization method with orthophthaldialdehyde (OPA). The compounds were separated on a cation-exchange resin using two lithium citrate buffers. The method used was based on the procedure described by Rawal et al.[11] The cation-exchange resin was packed in a glass column, the bed height was 160 mm, and the temperature was maintained at 50°C. The cation-exchange resin (BTC 2710; Biotronik) had a particle size of 7 μm and a 10% degree of cross-linkage. Separation of the dimethylarginines was obtained by stepwise gradient elution performed using two lithium citrate buffers with the same citrate concentration (0.07 mol/L) but with increasing lithium concentration, (1) Li^+ 0.3 mol/L, pH 4.05 (5 minutes), and (2) Li^+ 1.40 mol/L, pH 2.74 (130 minutes), at a flow rate of 18 mL/h. To the column eluent, OPA reagent was pumped at a flow rate of 18 mL/h and mixed in a reaction coil (7.5 m × 0.3 mm ID) at room temperature. The fluorophor was measured with a Gilson fluorometer (Spectra/Glo; Gilson Medical Electronics, Villiers-le-Bel, France) at excitation 360 nm; an emission filter of 455 nm was used. Under these conditions, ADMA eluted after 127 minutes and SDMA after 134 minutes. Other amino acids occurring in physiological fluids like carnosine, homocarnosine, and tryptophan eluted after SDMA.

Serum urea nitrogen was determined with diacetylmonoxime as described by Ceriotti.[12]

Statistical Analysis

Guanidino compound levels are presented as the mean ± SD for guanidino compounds present at detectable levels. If, next to detectable levels of a particular guanidino compound, levels were below the detection limit in some samples, results are given as a range from less than the detection limit to the highest level obtained in any given group. In urine, creatine levels have a large dispersion; therefore, the results were also expressed as a range of the lowest to the highest value.

Serum creatine, creatinine, guanidinoacetic acid (GAA), and homoarginine levels in controls are significantly different between men and women. Therefore, they are listed separately for men and women in controls and in patients. However, in urine, excretion levels for all the studied guanidino compounds were not significantly different between the sexes.

The results were compared using ANOVA with least-significant difference (LSD) post hoc comparison (SPSS; Microsoft, Redmond, CA). To obtain a better idea of the relationship between guanidino compound levels and the degree of renal insufficiency, the interrelationship of individual guanidino compound levels with the corresponding creatinine clearance, obtained according to the method used by Cockcroft and Gault,[9] was assessed by correlation studies (Pearson bilogarithmic correlation and bilogarithmic regression analysis).

RESULTS

Guanidino Compounds in Serum

By subdividing the nondialyzed patients with chronic renal insufficiency according to etiology and comparing the serum levels of each guanidino compound found in the interstitial nephritis, glomerulonephritis, nephrangiosclerosis, and diabetic nephropathy subgroups, we did not observe significant differences. The other etiological subgroups were not included in the ANOVA since the number of patients was too small in these subgroups.

Table 1 shows serum guanidino compound levels in both controls and nondialyzed patients with chronic renal insufficiency subdivided according to creatinine clearance. The guanidino compounds in serum that have the highest concentration in controls and in nondialyzed patients with chronic renal insufficiency are arginine, creatinine, and creatine. Serum levels of most guanidino compounds increase or decrease with decreasing creatinine clearance.

Nondialyzed chronic renal insufficiency patients with a creatinine clearance less than 10 mL/min have serum methylguanidine and guanidinosuccinic acid (GSA) levels that are 35 and 100 times higher than control levels. Serum guanidine, creatinine, and SDMA levels in patients of the same group increase approximately 10-fold. The increase of ADMA in nondialyzed patients with chronic renal insufficiency is only 100%.

Levels of GAA and homoarginine are significantly decreased in the serum of nondialyzed chronic renal insufficiency patients.

Table 1. Serum Guanidino Compound (μmol/L) and Urea (mmol/L) Levels in Controls and Nondialyzed Patients With Chronic Renal Insufficiency Subdivided According to Creatinine Clearance (mL/min)

		Patients			
Guanidino Compound	Controls (n = 66)	Subnormal-40 (n = 19)	40-20 (n = 36)	20-10 (n = 42)	<10 (n = 38)
α-K-δ-GVA	<0.035-0.200	<0.035-0.280	<0.035-0.310	<0.035-0.860	<0.035-0.930
GSA	0.259 ± 0.096	0.821 ± 0.424	5.08 ± 7.03†	10.0 ± 7.34§	26.8 ± 14.3§
CT					
Men	30.1 ± 12.3	31.3 ± 15.1	34.4 ± 37.6	24.6 ± 23.6	36.4 ± 62.2
Women	54.8 ± 21.0	46.9 ± 20.0	68.4 ± 72.9	113 ± 118*	95.3 ± 124
GAA					
Men	2.61 ± 0.517	1.99 ± 0.406†	1.76 ± 0.874§	1.68 ± 0.606§	1.37 ± 0.578§
Women	2.01 ± 0.572	1.28 ± 0.189†	1.54 ± 0.313†	1.40 ± 0.479‡	1.91 ± 0.772
α-N-AA	<0.019-0.620	<0.019-1.62	<0.019-1.02	<0.019-1.46	<0.019-1.57
ArgA	<0.019-0.440	<0.019-1.13	<0.019-0.540	<0.019-0.750	<0.019-1.13
CTN					
Men	80.8 ± 17.7	131 ± 23.3	246 ± 113§	517 ± 150§	862 ± 193§
Women	65.3 ± 19.7	129 ± 37.3	190 ± 36.3†	350 ± 176§	710 ± 221§
γ-GBA	<0.019-0.055	<0.019-0.090	<0.019-0.140	<0.019-0.130	<0.019-0.07
ADMA	0.409 ± 0.088	0.601 ± 0.102§	0.717 ± 0.224§	0.839 ± 0.154§	0.803 ± 0.144§
SDMA	0.377 ± 0.104	0.825 ± 0.217†	1.41 ± 0.631§	2.24 ± 0.738§	3.17 ± 1.05§
ARG	110 ± 23.9	111 ± 24.1	119 ± 33.4	122 ± 35.8	129 ± 37.0†
HArg					
Men	1.98 ± 0.634	1.28 ± 0.413‡	0.914 ± 0.547§	1.03 ± 0.457§	0.846 ± 0.470§
Women	1.51 ± 0.609	0.898 ± 0.400†	0.870 ± 0.370§	0.768 ± 0.378§	0.920 ± 0.514§
G	<0.130-0.210	<0.130-0.740	<0.130-4.53	<0.130-3.15	2.05 ± 0.857
MG	<0.06	<0.06	<0.06-0.810	<0.06-2.29	2.13 ± 2.16
Urea	4.42 ± 1.10	7.59 ± 1.65	16.2 ± 7.50§	22.3 ± 6.95§	34.8 ± 12.1§

NOTE. The levels of β-guanidinopropionic acid were lower than the detection limit (<0.019 μmol/L). The control group consisted of 33 men and 33 women, the subnormal-40 group 13 and 6, the 40-20 group 17 and 19, the 20-10 group 22 and 20, and the group with creatinine clearance <10 mL/min 13 and 25. The data were compared using ANOVA with LSD post hoc comparison (ANOVA, SPSS).

Abbreviations: α-K-δ-GVA, α-keto-δ-guanidinovaleric acid; CT, creatine; α-N-AA, α-*N*-acetylarginine; ArgA, argininic acid; CTN, creatinine; γ-GBA, γ-guanidinobutyric acid; ARG, arginine; HArg, homoarginine; G, guanidine; MG, methylguanidine.

*$P < .05$, †$P < .01$, ‡$P < .001$, §$P < .0001$: *v* control.

Some individuals in our control group and some of the nondialyzed patients with chronic renal insufficiency have α-keto-δ-guanidinovaleric acid, α-*N*-acetylarginine, argininic acid, γ-guanidinobutyric acid, guanidine, and methylguanidine in serum at levels lower than our analytical detection limit. To obtain some idea about the relationship between these guanidino compounds and the degree of renal insufficiency, we substituted the guanidino compound levels less than the detection limit by a value corresponding to half the respective detection limits.

According to the degree of correlation between the serum level of any given guanidino compound and the creatinine clearance, the determined guanidino compounds could be divided into three groups: in the first group, a good correlation between serum level and corresponding creatinine clearance can be observed; in the second group, there is a lower correlation; and a minor or no correlation is found in the third group. Serum guanidino compounds that are increased most in nondialyzed patients with chronic renal insufficiency are the ones that show the best correlation with the creatinine clearance values: GSA ($r = -.926, P < .0001$; Fig 1), SDMA ($r = -.916, P < .0001$; Fig 2), methylguanidine ($r = -.871, P < .0001$; Fig 3), and guanidine ($r = -.827, P < .0001$; Fig 4). A less pronounced correlation coefficient was found for ADMA ($r = -.700, P < .0001$) and GAA in men ($r = .505, P < .0001$). The other guanidino compound levels correlate poorly or not significantly with the creatinine clearance values: homoarginine in men ($r = .326, P = .003$) and women ($r = .319, P = .0025$), argininic acid ($r = -.348, P < .0001$), α-keto-δ-guanidinovaleric acid ($r = -.313, P < .0001$), α-*N*-acetylarginine ($r = -.227, P = .0057$), γ-guanidinobutyric acid ($r = -.184, P = .024$), arginine ($r = -.185, P = .0167$), and creatine in men ($r = .0602$,

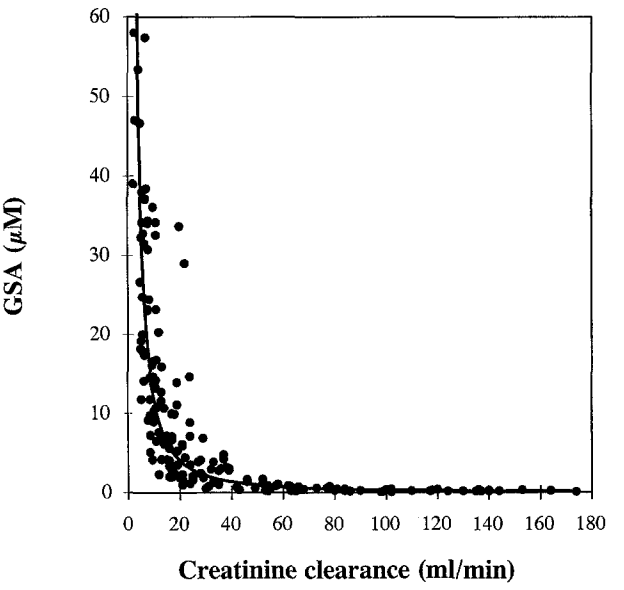

Fig 1. Relationship between serum GSA levels and creatinine clearance.

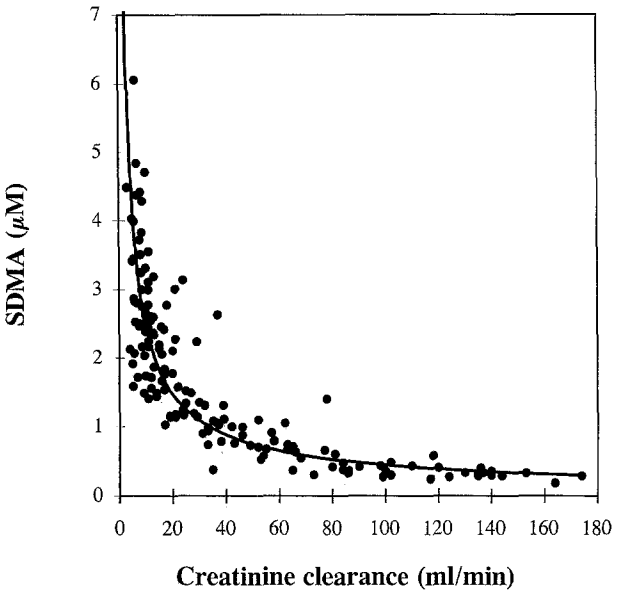

Fig 2. Relationship between serum SDMA levels and creatinine clearance.

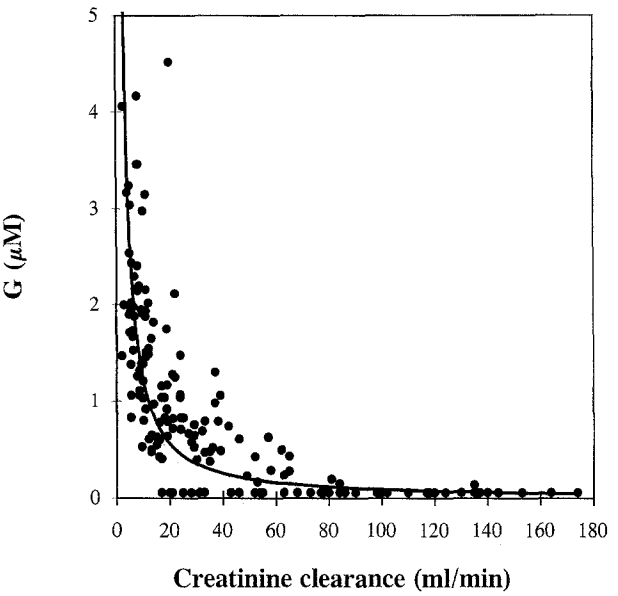

Fig 4. Relationship between serum guanidine (G) levels and creatinine clearance.

$P = .5932$) and women ($r = -.105$, $P = .331$), and GAA in women ($r = .0921$, $P = .3932$).

Guanidino Compounds in Urine

As in serum, no significant differences were found for the urinary excretion of guanidino compounds in the interstitial nephritis, glomerulonephritis, nephrangiosclerosis, and diabetic nephropathy subgroups.

Table 2 illustrates that urinary excretion is much higher for creatinine than for the other guanidino compounds in controls and in nondialyzed patients with chronic renal insufficiency irrespective of creatinine clearance. The excretion levels of most guanidino compounds decrease with decreasing creatinine clearance, except for GSA and methylguanidine, which significantly increase with decreasing creatinine clearance.

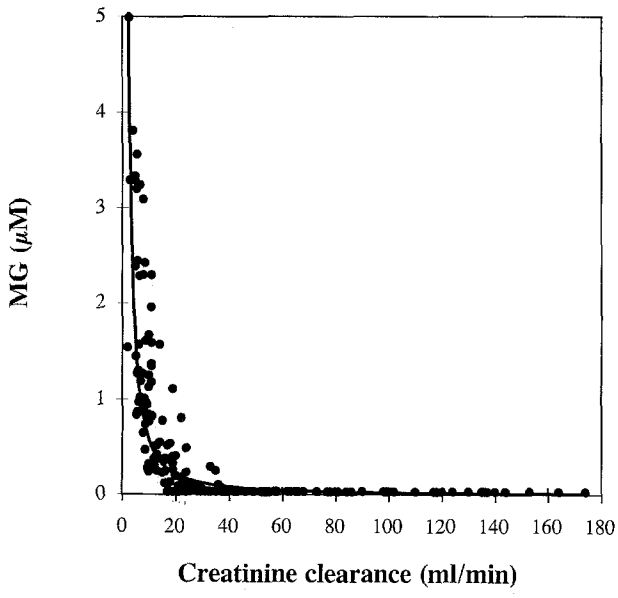

Fig 3. Relationship between serum methylguanidine (MG) levels and creatinine clearance.

To perform correlation studies between the urinary excretion level of the guanidino compounds and the corresponding creatinine clearance, it was necessary, as for serum values, to substitute values lower than the detection limit by a value equal to half of the lowest excretion level in the studied population for a particular guanidino compound. The best correlation is found for ADMA ($r = .861$, $P < .0001$; Fig 5). A second group of guanidino compounds is characterized by lower but still significant correlation coefficients: GAA ($r = .779$, $P < .0001$), methylguanidine ($r = -.770$, $P < .0001$), α-*N*-acetylarginine ($r = .721$, $P < .0001$), GSA ($r = -.679$, $P < .0001$), γ-guanidinobutyric acid ($r = .618$, $P < .0001$), SDMA ($r = .563$, $P < .0001$), argininic acid ($r = .516$, $P < .0001$), α-keto-δ-guanidinovaleric acid ($r = .476$, $P < .0001$), creatine ($r = .382$, $P < .0001$), and homoarginine ($r = .323$, $P = .0009$). However, no correlation is found between the excretion levels of arginine ($r = .116$, $P = .242$), β-guanidinopropionic acid ($r = .111$, $P = .267$), and guanidine ($r = -.088$, $P = .376$) and the degree of renal insufficiency.

Clearance of Guanidino Compounds

Endogenous clearance rates are only calculated for the compounds with detectable levels in serum and in urine for all studied individuals in controls and in the different subgroups with chronic renal insufficiency (Table 3). The clearance of most of the guanidino compounds, with the exception of arginine, decreases with increasing renal insufficiency. Guanidino compound clearances varied widely among controls depending on the guanidino compound considered, ranging from 0.120 mL/min for arginine, to about 110 mL/min for GAA, and about 113 mL/min for creatinine. It is also remarkable that the clearance rates of the dimethylarginines are practically identical in the controls and decrease equally in the patients with renal insufficiency.

Table 2. Urinary Guanidino Compound (μmol/24 h) and Urea (mmol/24 h) Levels in Controls and Nondialyzed Patients With Chronic Renal Insufficiency Subdivided According to Creatinine Clearance (mL/min)

		Patients			
Guanidino Compound	Controls (n = 33)	Subnormal-40 (n = 9)	40-20 (n = 26)	20-10 (n = 19)	<10 (n = 18)
α-K-δ-GVA	<DL-25.9	<DL-11.2	< DL-15.8	<DL-6.09	<DL-9.98
GSA	28.1 ± 10.5	42.3 ± 23.6	95.9 ± 80.5‡	108 ± 62.2§	164 ± 116§
CT	29.5-4,825	57.0-4,222	21.8-3,209	10.4-1,088	28.4-1,603
GAA	350 ± 210	189 ± 183†	45.0 ± 46.1§	24.0 ± 18.0§	22.1 ± 16.2§
α-N-AA	27.3 ± 11.6	7,63-205	14.0 ± 11.0§	7.56 ± 5.67§	<DL-18.0
ArgA	6.77 ± 2.93	6.89 ± 3.56	4.55 ± 3.29†	<DL-11.2	<DL-11.6
β-GPA	<DL-1.01	<DL	<DL-1.25	<DL	<DL
CTN	10,429 ± 3,213	8,972 ± 2,638	7,862 ± 3,378†	6,677 ± 3,096§	7,064 ± 2,846‡
γ-GBA	12.4 ± 6.97	8.19 ± 5.70	<DL-8.46	<DL-8.06	0.362-11.4
ADMA	42.3 ± 10.1	28.4 ± 12.3§	12.2 ± 5.54§	7.37 ± 2.63§	7.64 ± 4.24§
SDMA	39.3 ± 9.08	36.8 ± 15.9	23.4 ± 6.54§	20.9 ± 7.15§	23.4 ± 11.0§
ARG	18.3 ± 9.35	10.2 ± 8.19	14.2 ± 15.1	12.3 ± 11.7	0.310-112
HArg	<DL-12.2	<DL-8.15	<DL-8.92	<DL-3.33	<DL-2.32
G	12.0 ± 4.34	10.2 ± 3.57	13.3 ± 8.03	12.8 ± 6.56	17.8 ± 8.19†
MG	5.56 ± 2.89	10.5 ± 6.58	22.8 ± 22.0‡	37.5 ± 23.9§	66.3 ± 29.2§
Urea	273 ± 98.3	241 ± 79.8	231 ± 119	188 ± 97.4†	207 ± 116*

NOTE. The data were compared using ANOVA with LSD post hoc comparison (ANOVA, SPSS).

Abbreviations: DL, detection limit; α-K-δ-GVA, α-keto-δ-guanidinovaleric acid; CT, creatine; α-N-AA, α-*N*-acetylarginine; ArgA, argininic acid; β-GPA, β-guanidinopropionic acid; CTN, creatinine; γ-GBA, γ-guanidinobutyric acid; ARG, arginine; HArg, homoarginine; G, guanidine; MG, methylguanidine.

*$P < .05$, †$P < .01$, ‡$P < .001$, §$P < .0001$: *v* controls.

DISCUSSION

The results presented (Tables 1 and 2) clearly show specific abnormalities in the guanidino compound pattern for nondialyzed patients with chronic renal insufficiency. On one hand, GSA and methylguanidine levels are increased in serum and in urine, which suggests an increased biosynthesis. Levels of GAA and homoarginine are decreased in serum and in urine, which suggests a decreased biosynthesis of both products. On the other hand, serum levels of creatinine and dimethylarginines in nondialyzed patients with chronic renal insufficiency are increased, while the urinary excretion of these compounds is decreased.

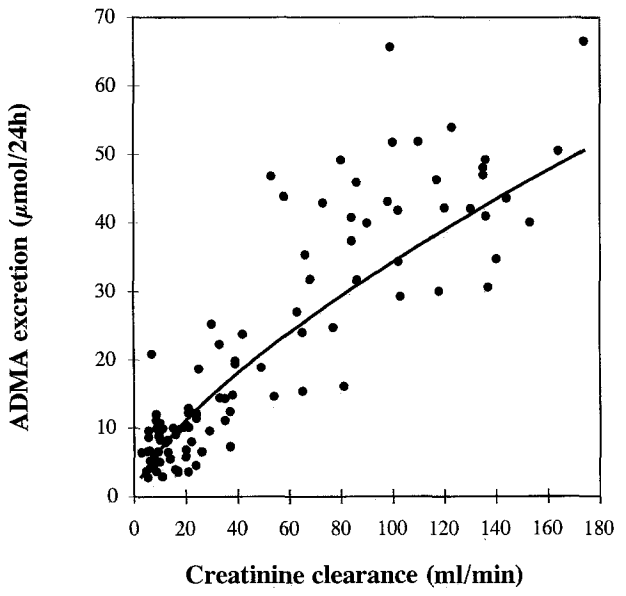

Fig 5. Relationship between urinary excretion of ADMA and creatinine clearance.

The increase of urinary excretion of GSA in patients with renal insufficiency was first demonstrated by Natelson et al.[13] Later, increased levels were shown in serum[14] and cerebrospinal fluid[5,6,8] of uremic patients. Our data are consistent with the above-mentioned results. In our patient group, we showed a good correlation between serum GSA levels and residual creatinine clearance or degree of renal failure. The biosynthesis of GSA seems to be related to urea metabolism.[15,16]

The pattern of urinary and serum levels of guanidine obtained in this study corresponds to the results obtained by Menichini and Giovannetti.[17] Little is known about the biosynthesis of guanidine. However, methylguanidine could be formed from creatinine via creatol formation,[18] and its biosynthesis is enhanced by active oxygen species.[19,20]

With a colorimetric detection method, Sasaki et al[21] already demonstrated the decreased urinary excretion of GAA. Chromatography with fluorescence detection confirmed the decreased serum levels of this compound in nondialyzed patients with chronic renal insufficiency.[22] GAA is synthesized by transamidination from arginine to glycine in the proximal convoluted tubules[23] and is further converted to creatine mainly in the liver. Tofuku et al[22] showed decreased arginine-glycine amidinotransferase activity in rabbits with chronic renal failure.

Our results suggest for the first time a decreased biosynthesis of homoarginine in nondialyzed patients with chronic renal insufficiency. Homoarginine may be formed from lysine by a homologous urea cycle in which arginine is replaced by lysine.[24,25]

The present urinary control values for the dimethylarginines correspond well with those reported by Kakimoto and Akazawa[26] or Carnegie et al[27]; they are lower than those found by Vallance et al.[28] Our control levels for dimethylarginines in

Table 3. Endogenous Clearance of Guanidino Compounds and Urea (mL/min) in Controls and Nondialyzed Patients With Chronic Renal Insufficiency Subdivided According to Creatinine Clearance

Guanidino Compound	Controls (n = 33)	Patients			
		Subnormal-40 (n = 9)	40-20 (n = 26)	20-10 (n = 19)	<10 (n = 18)
GSA	76.8 ± 27.2	41.1 ± 12.6§	18.5 ± 9.13§	8.61 ± 3.47§	5.57 ± 3.11§
CT	10.8 ± 13.4	12.0 ± 14.0	3.70 ± 3.11†	2.35 ± 1.35†	3.05 ± 3.04†
GAA	106 ± 62.6	84.0 ± 97.0	18.5 ± 17.9§	10.7 ± 7.28§	9.31 ± 7.44§
ADMA	77.5 ± 27.8	36.6 ± 17.7§	13.3 ± 7.11§	6.04 ± 2.59§	7.35 ± 5.24§
SDMA	80.1 ± 31.1	36.4 ± 16.1§	13.4 ± 7.00§	6.75 ± 2.47§	5.75 ± 2.95§
ARG	0.121 ± 0.080	0.063 ± 0.050	0.098 ± 0.121	0.087 ± 0.094	0.164 ± 0.202
Urea	44.7 ± 19.1	24.3 ± 10.2§	10.7 ± 6.50§	5.66 ± 2.56§	4.54 ± 2.76§

NOTE. The data were compared using ANOVA with LSD post hoc comparison (ANOVA, SPSS).
Abbreviations: CT, creatine; CTN, creatinine.
*$P < .05$, †$P < .01$, ‡$P < .001$, §$P < .0001$: *v* controls.

serum are also slightly lower than those found by Vallance et al.[28] Approximately equal amounts of both dimethylarginines are found in serum and urine of controls. The increase of serum SDMA levels in our nondialyzed patients with residual creatinine clearance less than 10 mL/min is on the same order of magnitude as the increase found by Vallance et al[28] in patients with end-stage chronic renal failure who underwent regular hemodialysis. They found the same degree of increase for ADMA in the plasma of regularly hemodialyzed uremic patients.[28] Our results clearly show that the increase of ADMA in serum of nondialyzed patients with severe chronic renal insufficiency is moderate, with values reaching only twice the control levels. To our knowledge, no other reference values of ADMA and SDMA in serum of nondialyzed and dialyzed uremic patients have been published. Reference urinary excretion values for both compounds in patients with renal failure have not yet been published. With increasing renal failure, urinary excretion of ADMA seems to be more disrupted than for SDMA when renal failure becomes more severe. It is also worth noting that urinary ADMA excretion levels correlate better with the corresponding creatinine clearance values than do urinary SDMA excretion levels. However, the inverse can be stated for serum.

The dimethylarginines occur either protein-bound or in free states. Reports of pathophysiological abnormal levels of the free dimethylarginines are rare: ADMA and SDMA were significantly elevated in the urine of children with muscular dystrophy,[29] with a ADMA/SDMA ratio of 2.6. In the urine of patients with liver failure, a slight increase of the ADMA/SDMA ratio was observed.[27] Vallance et al[28] were the first to note increased levels of ADMA and SDMA in serum of hemodialyzed patients. Our study presents the first data for dimethylarginines in nondialyzed patients with chronic renal insufficiency.

The specific increases and decreases of some guanidino compounds in serum and urine observed in nondialyzed patients with chronic renal insufficiency convinced us to further promote those guanidino compounds as complementary (to serum creatinine values) diagnostic parameters for renal failure. Special attention should be focused on the determination of SDMA in serum to evaluate its importance as a possible indicator for the onset of renal failure. Figure 6A and B shows that greater than 90% of patients with creatinine clearance values ranging from subnormal to 40 mL/min have serum SDMA levels higher than the upper-normal limit (mean + twice the standard deviation). In the same subgroup, up to 80% of the individuals have GSA levels higher than the upper-normal limit.

The results of the clearance study suggest that some guanidino compounds like arginine are of vital interest; in controls, they are practically totally reabsorbed in the tubules. The clearance of arginine is very low. Other guanidino compounds like creatinine, GAA, GSA, and the dimethylarginines are nitrogen waste products with high clearance values. The clearly established differences in clearance rates of the guanidino

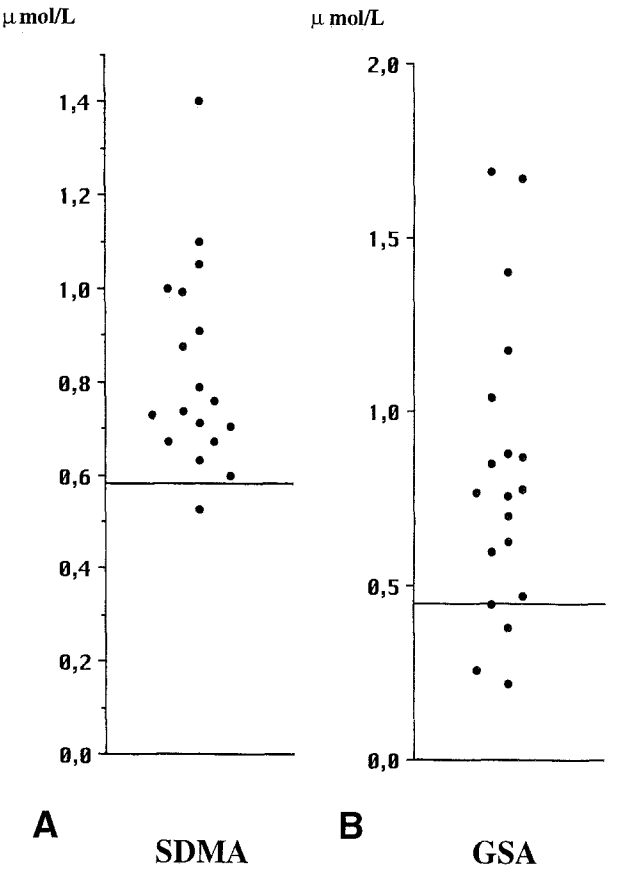

Fig 6. (A) SDMA and (B) GSA levels (µmol/L) in the serum of individual nondialyzed patients with chronic renal failure with renal creatinine clearance ranging from subnormal to 40 mL/min. The horizontal line represents the upper-normal limit (mean + twice the standard deviation).

compounds are not related to differences in molecular weight. The molecular weight of the considered guanidino compounds ranges from 117 for GAA to 204 for the dimethylarginines. The molecular weight of GSA is about the same as for arginine; however, the corresponding clearances are of another order. The clearance of the guanidino compounds, with the exception of arginine, decreases with increasing renal insufficiency.

Recently, we studied the alterations of guanidino compound levels in rats after 20% to 90% nephrectomy.[30] The alterations observed in the animal model correspond well with the abnormal pattern seen in human pathology: the increased creatinine, GSA, guanidine, and methylguanidine in serum, the increased urinary excretion of GSA and methylguanidine, and the decreased excretion of GAA are noteworthy. However, the clear decrease of GAA and homoarginine observed in serum of nondialyzed patients with chronic renal insufficiency is not seen in the animal model. These findings indicate specific differences in guanidino compound metabolism between man and rat.

Several guanidino compounds have been proposed as candidate uremic toxins. Although still controversial,[31] GSA is believed to be related to the uremic bleeding diathesis.[32,33] Methylguanidine could be related to polyneuropathy in uremic dogs[3] and has been shown experimentally to induce a catabolic state, hemolysis,[3] and epilepsy.[34] γ-Guanidinobutyric acid, methylguanidine, homoarginine, creatine, and creatinine were found to have a convulsive effect in animals when injected intracisternally.[35-38] GSA, methylguanidine, guanidine, and creatinine were suggested to be chloride channel blockers and were shown to induce myoclonic and generalized seizures after systemic and intracerebroventricular administration in mice.[39,40] Furthermore, in vitro studies have shown that GSA, creatine, GAA, guanidine, and β-guanidinopropionic acid might be factors responsible for the increased hemolysis.[41] Methylguanidine and creatinine were shown to induce hemolysis in vitro and in vivo.[3] GSA decreased erythrocyte transketolase activity,[42] and methylguanidine inhibited brain sodium-potassium adenosine triphosphatase activity.[43] Reynolds and Rothermund[44] showed that guanidine, methylguanidine, GSA, and creatinine interact with the NMDA receptor in vitro, as indicated by their ability to inhibit [^{3}H]dizocilpine binding, accelerate the dissociation of [^{3}H]dizocilpine, and increase intracellular Ca^{2+} concentrations in cultured central neurons. Guanidine, methylguanidine, and GSA decreased the synaptosomal membrane fluidity of rat cerebral cortex.[45]

Another point of interest related to the observed accumulation of some guanidino compounds as candidate uremic toxins is raised by Vallance et al[28] and MacAllister et al,[46] who suggested that endogenous ADMA could inhibit nitric oxide synthesis in patients with end-stage chronic renal failure who undergo regular hemodialysis. They suggest that ADMA might contribute to the hypertension and immune dysfunction associated with chronic renal failure.[28] Nitric oxide contributes to the regulation of blood pressure[47,48] and host defense,[49] and is recognized as a messenger in the central nervous system.[50] The same group demonstrated that methylguanidine also inhibits nitric oxide synthesis, although to a lesser extent (about 10 times).[46]

Recently, we studied guanidino compound levels in the brain of patients with renal insufficiency,[7] and the values found for GSA and creatinine were comparable to those previously observed in the brain of experimental animals with convulsions following intraperitoneal injection of the respective compounds.[40] Moreover, GSA acts as a NMDA-type glutamate receptor agonist and inhibits gamma-aminobutyric acid responses on mouse neurons in primary dissociated cell culture at presumably comparable concentrations of up to 100 μmol/L.[39,51] These compounds must be considered as candidates involved in the neurological and neuropsychological complications observed in patients with renal insufficiency.

We have shown the specific abnormal pattern of several guanidino compound levels in serum (highly increased levels of GSA, methylguanidine, guanidine, creatinine, and SDMA together with decreased homoarginine and GAA levels) and in urine (decreased urinary excretion of most guanidino compounds together with increased excretion of GSA and methylguanidine) of nondialyzed patients with chronic renal failure. This abnormal pattern can be used as a complementary diagnostic parameter. This study provides information about clearance rates of some guanidino compounds and is also the first with data concerning serum and urinary excretion levels of the dimethylarginines in nondialyzed patients with chronic renal failure. Finally, serum levels of GSA and especially SDMA have been proposed as candidate indicators for the onset of renal failure.

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