Long-Term Effect of Partial Nephrectomy on Biological Parameters, Kidney Histology, and Guanidino Compound Levels in Mice

Mumna Al Banchaabouchi, Bart Marescau, Eric Van Marck, Rudi D'Hooge, and Peter Paul De Deyn

The long-term adverse consequences of early renal mass reduction in mice have not yet been investigated. The effects of partial surgical nephrectomy (NX) in 2-month-old mice on some biological parameters, on histopathologic and morphometric features of the kidney, and on urea and guanidino compound (GC) levels in plasma, urine, and brain were examined at 10 days, and 1, 2, 4, and 12 months postsurgery. Body weight, urinary volume, and plasma urea were most affected at 10 days and 12 months post-NX. NX-induced changes in the remaining renal tissue (including hypertrophy, glomerular mesangial expansion, and presence of protein casts) increased with age. As in human renal insufficiency, NX mice showed significantly higher plasma guanidinosuccinic acid (GSA) and creatinine (CTN) levels at all studied periods. The same tendency could be seen for most other plasma GCs examined, except for arginine (Arg), guanidinoacetic acid (GAA), and homoarginine (HA). As seen in human pathobiochemistry, the latter 2 compounds tended to be lower in NX mice in our follow-up study. Remarkably, and also similar to humans, NX mice excreted less GAA and more GSA than controls during the entire follow-up study. During the follow-up, excretion levels of GAA were unchanged in NX and sham-operated mice. In brain, GAA and γ-guanidinobutyric acid (GBA) levels were always higher in NX mice with a tendency to respectively increase or decrease over time in NX as well as sham-operated mice. Although urea and GC metabolism were influenced by time post-NX and aging, the model was confirmed to display a mild stable chronic impairment of renal function. Histopathologic and morphometric changes of the kidney increased with age.

WITH AGING, kidney function and morphology slowly deteriorate.[1,2] Loss of renal mass leads to hemodynamic alterations and hyperfiltration in the residual nephrons, which in turn leads to glomerular damage.[3-5] Furthermore, nephrectomy (NX) at young age may have dramatic pathobiochemical and pathohistological consequences on the remaining kidney later in life. Only a few studies have described the evolution of renal function in laboratory mice, with the longest follow-up lasting only 4 months.[6,7]

The biochemistry of guanidino compounds (GCs), metabolites of proteins and amino acids, is related to nitrogen metabolism. They are metabolized in kidney and other tissues[8], and are cleared primarily by the kidney. Renal failure (RF) is one of the pathological conditions where GC levels are altered, and GCs accumulate in the body fluids and brain of patients and animals.[9-12] It was suggested that some GCs, such as guanidinosuccinic acid (GSA), γ-guanidinobutyric acid (GBA), and methylguanidine (MG), may act as uremic toxins.[13-17] Several investigators reported on the concentration of GCs in serum and urine of patients and animals with chronic RF, and their relationship to the degree of RF.[8-12] However, a long-term follow-up of GC metabolism alterations after NX is not available in the literature.

The aim of the present study was to investigate whether NX at young age may cause insidious deterioration of renal function and morphology with aging. In addition, we studied the long-term effect of NX on the course of GC tissue and urine levels in mice nephrectomized at 2 months of age.

METHODS AND MATERIALS

Animals and Surgery

In all experiments, 2-month-old male mice (Swiss-Webster x C57BL hybrids, bred in our laboratory facilities) were used and observations were made 10 days, and 1, 2, 4, and 12 months following surgery. For each period of time, the experiments considered 2 groups: a group that underwent NX and a sham-operated control group (Sham). The surgical procedure was performed under Hypnorm®/midazolam anesthesia (1 part Hypnorm, 1 part Dormicum®, and 2 parts saline for injection, dose 4 mL/kg intraperitoneally).[18] Via a small bilateral dorsal flank incision to expose the kidneys, the Sham group was subjected to manipulation and exteriorization of the kidneys. In the NX group, removal of 75% renal mass in mice was performed in a single-step procedure. Before right NX, the contralateral kidney was partially infarcted by ligating the anterior renal branch of its main artery. The operated animals were then housed under standard laboratory conditions with constant temperature and humidity, a 12-hour light/dark cycle, and free access to water and food. Approval of the local ethics committee was obtained for the use of animals in this study.

Light Microscopic Examination

At each testing period, kidney(s) was (were) removed, weighed, and fixed in 10% buffered formalin. The fixed renal tissue was transsected lengthwise, and both halves were embedded in parrafin and evaluated on a 5-μm section stained with hematoxylin/eosin or Schiff periodic acid. Structural changes of the glomeruli and tubules and presence or absence of components such as mesangial expansion, segmental sclerosis, hypercellularity, and protein casts were evaluated using a semiquantitative score.

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Morphometric Evaluation

After light microscopic examination, glomerular volumes (V_G) were estimated by morphometric assessment using point-sampled intercepts (PSI).[19] Juxtamedullary glomeruli are considerably larger than cortical glomeruli. Therefore, calculations were restricted to volume evaluation of cortical glomeruli only. In all studies, the glomerulus was defined as the minimal convex polygon circumscribing the capillary tuft. Briefly, the image of the section was obtained with a Leitz 25x (NA 1.30) lens on a Leitz Orthoplan microscope (Leitz, Wetzlar, Germany) using a Pulnix TM-765 monochrome camera (Pulnix Video Division, Basingstoke, UK). The image was viewed on a computer screen simultaneous with a software-generated grid of randomly placed points. The points hitting a glomerulus were selected by the operator after which the computer draws a randomly oriented line through the selected points. The operator marks the intersections of this line with the boundaries of the glomerulus (from Bowman's capsule), resulting in a PSI. This process was repeated a number of times (at least 13), and V_G was calculated.

Plasma and Urine Sampling

Before the mice were killed, urine was collected over a 48-hour period in a metabolic cage adapted for mice and frozen at –70°C until analysis. After decapitation, blood samples were collected through a funnel into heparinized vacutainer tubes. Plasma was obtained after centrifugation at 700 × *g* for 10 minutes at 6°C. A portion of 5 μL was taken for urea determination. The remaining plasma was used for GC analysis.

Collection and Preparation of Brain Samples

Brains were removed and stored at -70°C until preparation for determination of GCs.

At the time of analysis, the left hemisphere was homogenized in 1 mL water at 0°C with a Tissue Tearor model 985 (Biospec Products, Bartlesville, USA). The probe was washed immediately with 1 mL of a 300-g/L trichloroacetic acid solution at 0°C, which was added to the homogenate, resulting in protein precipitation after vortex-mixing. After centrifugation (100,000 × *g* for 30 minutes at 4°C), the clear supernatant was used for GC analysis. GCs analyzed were as follows: α-keto-δ-guanidinovaleric acid (GVA), GSA, creatine (CT), guanidinoacetic acid (GAA); α-*N*-acetylarginine (NAA), argininic acid (AA), β-guanidinopropionic acid (GPA), creatinine (CTN), GBA, arginine (Arg), homoarginine (HA), guanidine (G), and MG.

Biochemical Determination

For GC analysis, plasma and urine were deproteinized by mixing equal volumes of a 200-g/L trichloroacetic acid solution with plasma or urine. The proteins were centrifuged in a Beckman microfuge E (Beckman Instruments, Palo Alto, CA) at 15,850 × *g*. The supernatant was utilized for GC concentration determination using a Biotronic LC 5001 (Biotronik, Maintal, Germany) amino acid analyzer adapted for GC determination. GCs were separated over a cation-exchange column using sodium citrate buffers and detected with the fluorescence ninhydrin method as reported earlier.[8] Urea nitrogen was determined with diacetylmonoxime as described by Ceriotti.[20] Standard GCs were purchased from Sigma Chemical Co (St Louis, MO), CT and CTN from Merck (Darmstadt, Germany). GVA was synthesized enzymatically as described earlier.[8] All other chemicals used were obtained from Merck and were of analytical grade.

Statistical Analysis

Values are expressed as mean ± SD. A pairwise comparison, protected least-significant difference (Fisher's PLSD) test, was performed when 2-way analysis of variance (ANOVA) was significant, with significance defined as $P < .05$.

RESULTS

Biological Parameters and Biochemical Analyses

In the 5 postsurgery periods, mice with induced renal insufficiency demonstrated changes in the biological parameters as shown in Table 1. Two-way ANOVA detected significant effect of NX on final body weight (BW_f; $F_{1,123} = 5.7$; $P = .0018$), left side kidney weight (KW_i; $F_{1,108} = 14.4$; $P < .001$), and urinary volume (V_{ur}; $F_{1,130} = 96.2$; $P < .001$). The decline in BW_f and the increase in V_{ur} after NX is most obvious in the experimental animals tested at the early and the late stages (10 days and 12 months postsurgery, respectively). Significant differences were also found in plasma concentrations of urea ($F_{1,137} = 117$; $P < .001$) and CTN ($F_{1,138} = 117$; $P < .001$). Plasma urea and CTN levels were higher in the NX than in the Sham groups. However, fluctuations in their levels were appeared to be due to maturation of the animals as well as to the development of RF. Plasma urea levels in NX mice were 3-fold higher compared with Sham mice at day 10; 2-fold higher from month 1 until month 4; and 2.5-fold higher at month 12.

Table 1. Biological and Renal Function Parameters of Sham-Operated and Nephrectomized Mice, 10 Days, and 1, 2, 4, and 12 Months Postsurgery

	10 Days		1 Month		2 Months		4 Months		12 Months	
	Sham (n = 14)	NX (n = 12)	Sham (n = 18)	NX (n = 22)	Sham (n = 10)	NX (n = 9)	Sham (n = 15)	NX (n = 17)	Sham (n = 17)	NX (n = 12)
BW_f (g)	21.4 ± 2.8	18.3 ± 2.9*	26.9 ± 2.7	26.9 ± 2.4	31 ± 4	30.6 ± 2.4	34 ± 5	33.2 ± 2.5	34 ± 8	29 ± 4*
KW_i (mg)	182 ± 29	207 ± 46*	206 ± 11	225 ± 34	211 ± 25	251 ± 32*	207 ± 22	263 ± 34*	273 ± 87	297 ± 62
V_{ur} (mL/48 h)	1.4 ± 0.6	4.8 ± 1.8*	1.8 ± 0.9	3.7 ± 1.8*	2.7 ± 1.8	4.3 ± 1.8	1.9 ± 0.6	6.7 ± 2.7*	2.8 ± 1.8	7.6 ± 2.4*
P_{urea} (mmol/L)	8.0 ± 1.1	23 ± 12*	9.0 ± 1.6	15 ± 4*	8.2 ± 1.3	17 ± 5*	7.9 ± 1.9	17 ± 4*	9 ± 5	24 ± 12*
P_{CTN} (μmol/L)	25 ± 4	35 ± 12*	30 ± 6	38 ± 6*	28 ± 4	46 ± 6*	29.4 ± 2.6	43 ± 6*	24 ± 6	31 ± 5*
CTN_{Cl} (mL/min)	0.48 ± 0.12	0.32 ± 0.14*	0.39 ± 0.13	0.34 ± 0.08	0.47 ± 0.19	0.22 ± 0.07*	0.40 ± 0.08	0.29 ± 0.08*	0.45 ± 0.18	0.36 ± 0.11

NOTE. Results are means ±SD of Sham and NX groups.

Abbreviations: BW_f, final body weight; KW_i, left kidney weight; V_{ur}, volume of urine; P, plasma; CTN, creatinine; CTN_{Cl}, creatinine clearance. Concentrations were statistically analyzed by 2-way ANOVA with Fisher's PLSD test: *$P < .05$, NX *v* control.

Consistently, an age-dependent effect of NX was found on plasma levels of CTN ($F_{4,138} = 3.106$; $P = .017$) and urea ($F_{4,137} = 3.38$; $P = .011$). Compared to the Sham groups, the NX groups also showed significant differences in creatinine clearances (CTN_{Cl} ; $F_{1,134} = 40.6$; $P < .001$). Although CTN_{Cl} was lower in NX groups at all ages studied, levels did not significantly change in time.

Light Microscopic Morphometric Assessment

Histologic examination of the remaining kidneys through the 5 studied periods revealed progressive changes in their structural characteristics (Table 2). At day 10 postsurgery, kidney tissue appeared normal in both Sham and NX groups, and did not show any changes (excepting the presence of some tubular dilatation in the latter group). From month 1 through 12 postsurgery, Sham animals presented normal glomeruli and tubuli (Fig 1A), but a few of them manifested hyalinosis on the afferent arterioles. Histopathologic changes were more obvious in the NX groups with a progressive increase in number of affected mice as well as severity of morphologic changes. The main changes were mesangial expansion, presence of hyalinosis particularly on the afferent arterioles, and presence of some protein casts in the tubules. Segmental sclerosis was observed in some NX mice tested 2 months postoperatively (Fig 1B). At month 12, light microscopic examination revealed lesions commonly seen in aged animals. Mesangial expansion and hyalinosis were observed in several old Sham animals (Fig 1C), and 2 of them presented sclerosis on the afferent arterioles. In the NX groups, observations included severe mesangial expansion, focal segmental sclerosis, hyalinosis, and protein casts in the tubules (Fig 1D).

A significant effect of surgery on V_G was detected ($F_{1,103} = 101$; $P < .001$), which was manifested as a hypertrophy of the glomeruli in the NX mice. Age had a significant effect on the mean V_G in Sham as well as in NX animals ($F_{4,103} = 8.84$; $P < .001$). A progressive increase in mean V_G was observed in the Sham groups, but it was more pronounced in the NX groups (Fig 2). Only in the NX groups at day 10 postsurgery, were glomeruli much larger in size, probably due to the acute effects of NX. Consistently, NX affected mean V_G in an age-dependent manner. Besides the marked hypertrophy and edema, occurring in the glomeruli of NX mice at 10 days postsurgery (acute effect of NX in the youngest animals), it appeared to be a general tendency that the older the NX animals, the more the glomeruli increased in size ($F_{4,103} = 16.8$; $P < .001$).

Plasma Concentrations of GCs

In Table 3, plasma GC concentrations of Sham and NX groups are represented at different times postsurgery. NX caused significant alterations in most plasma GC levels when compared to the Sham groups. Increased levels were observed in the NX groups for GVA ($F_{1,128} = 38.8$; $P < .001$), GSA ($F_{1,133} = 194$; $P < .001$), CT ($F_{1,134} = 22.3$; $P < .001$), GBA ($F_{1,116} = 47.9$; $P < .001$; G ($F_{1,130} = 10.5$; $P = .002$), and MG ($F_{1,104} = 42.1$; $P < .001$), whereas levels of GAA ($F_{1,133} = 40.8$; $P < .001$) and HA ($F_{1,134} = 17.4$; $P < .001$) were significantly decreased.

Further comparison of GC levels in Sham and NX groups over the 5 periods revealed time-related changes in the levels of the different compound. Whereas plasma GSA and GBA levels remained stable in the Sham groups; they showed an abrupt decrease in their levels in the NX groups from day 10 postsurgery onward, and continued decreasing slowly over time. GAA levels decreased in both Sham and NX groups with aging. The interaction effect of NX × time on GVA ($F_{4,128} = 3.69$; $P = .007$), GSA ($F_{4,133} = 3.89$; $P = .005$), CT ($F_{4,134} = 3.12$; $P = .017$), and GBA ($F_{4,116} = 8.67$; $P < .001$) concentrations demonstrated that the effect of NX on these compounds depended on the age of the animals and/or the evolution of their RF.

Urinary Excretion of GCs

Variations in GC excretion following NX are shown in Table 4. NX caused significant changes in the levels of GSA ($F_{1,135} = 38.3$; $P < .001$); GAA ($F_{1,137} = 184$; $P < .001$), AA ($F_{1,136} = 8.19$; $P = .005$), G ($F_{1,138} = 5.54$; $P = .02$), MG ($F_{1,137} = 24.1$; $P < .001$), and urea ($F_{1,138} = 14.5$; $P < .001$).

Table 2. Scores of Histologic Changes of Components in Kidneys of Sham-Operated and Nephrectomized Mice

		Observations																			
		ME				SS				H				HC				PC			
		Absence		Presence		Absence		Presence		Absence		Presence		Absence		Presence		Absence		Presence	
Time PS	Groups	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++
10 days	Sham (9)	9	0	0	0	9	0	0	0	9	0	0	0	9	0	0	0	9	0	0	0
	NX (10)	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
1 month	Sham (12)	10	2	0	0	12	0	0	0	4	8	0	0	12	0	0	0	12	0	0	0
	NX (15)	4	10	1	0	15	0	0	0	7	8	0	0	15	0	0	0	11	4	0	0
2 months	Sham (10)	10	0	0	0	10	0	0	0	7	2	0	0	10	0	0	0	10	0	0	0
	NX (9)	0	5	4	0	6	3	0	0	6	3	0	0	9	0	0	0	1	8	0	0
4 months	Sham (16)	15	1	0	0	15	1	0	0	10	6	0	0	16	0	0	0	16	0	0	0
	NX (17)	1	10	3	3	10	6	1	0	9	4	4	0	15	2	0	0	1	13	3	0
12 months	Sham (14)	5	7	1	1	11	3	0	0	6	8	0	0	14	0	0	0	14	0	0	0
	NX (14)	1	11	1	1	8	6	0	0	2	12	0	0	13	1	0	0	4	10	0	0

NOTE. −: denotes absence of observations; +: denotes the presence of observations with different degrees (+: slight; ++: moderate; +++: severe). Values are number of mice affected.

Abbreviations: ME, mesangial expansion; SS, segmental sclerosis; H, hyalinosis; HC, hypercellularity; PC, protein casts; PS, postsurgery.

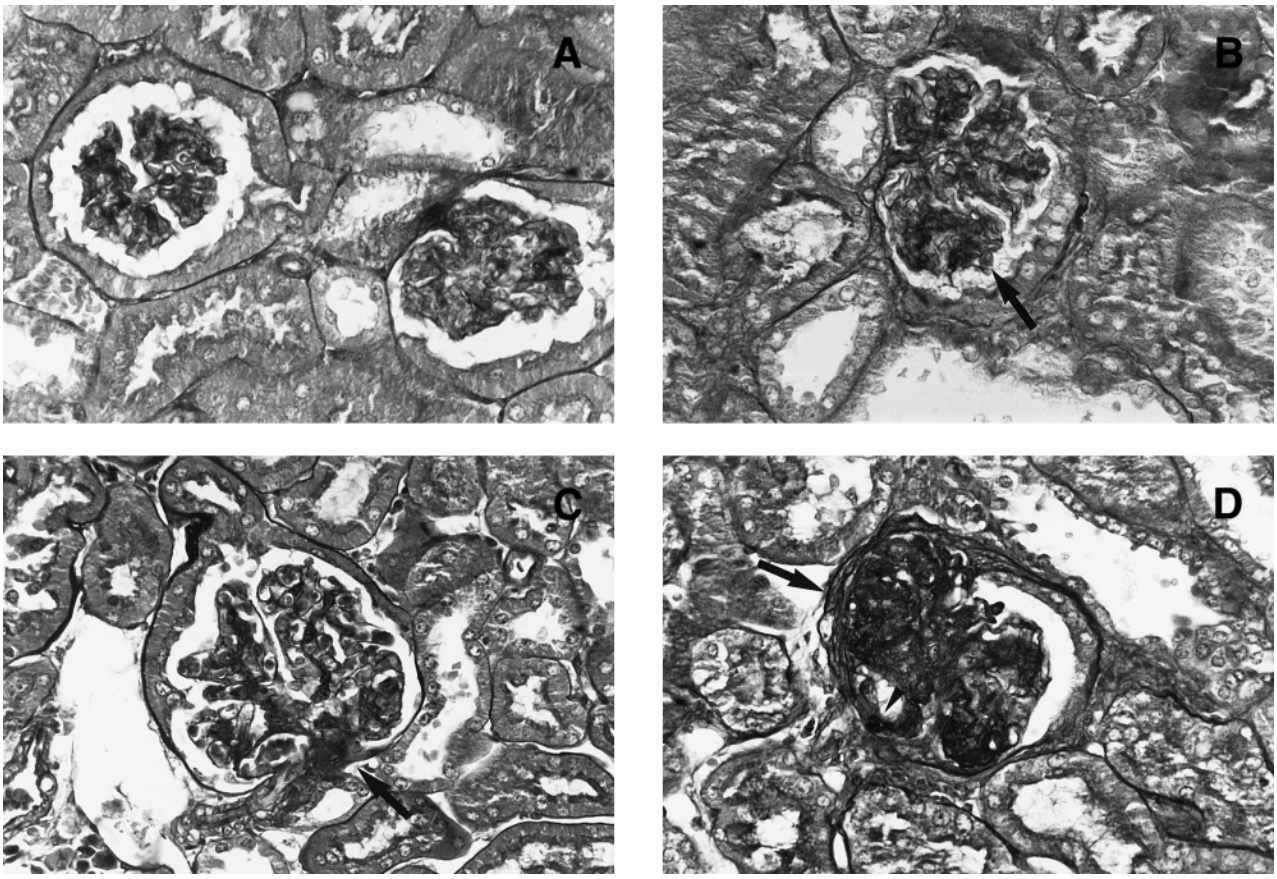

Fig 1. Effects of nephrectomy on kidney histology with some details of glomerular changes (periodic acid Schiff stain; original magnification × 448). (A) Micrograph of a kidney of a Sham mouse, 1 month postsurgery, showing normal glomeruli and tubuli. (B) Micrograph of a kidney of a 75% nephrectomized mouse, 2 months postsurgery, showing segmental mesangial expansion and focal sclerosis (arrow). (C) Micrograph of a kidney of a Sham mouse, 1 year postsurgery, showing slight mesangial expansion and hyalinosis on the afferent arteriole (arrow). (D) Micrograph of a kidney of a 75% nephrectomized mouse, 1 year postsurgery, showing pronounced segmental sclerosis, adhesion of the glomerular tufft to Bowman's capsule (arrow) and hyalinosis on the afferent arteriole (arrowhead).

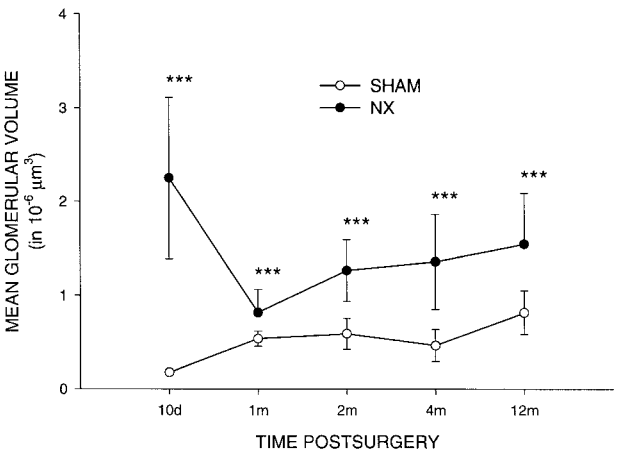

Fig 2. Mean glomerular volumes (× 10^{-6} μm^3) in kidneys of Sham and 75% nephrectomized mice examined at different periods postsurgery. Error bars indicate SD. ***$P < .001$, Sham *v* NX.

At all 5 time points, urinary GAA excretion in NX mice was 2-fold lower than the amount excreted by Sham mice.

Brain Concentrations of GCs

Table 5 lists cerebral levels of all GCs analyzed. As shown by 2-way ANOVA, NX induced significant changes in cerebral levels of GSA ($F_{1,126} = 84.8$; $P < .001$), NAA ($F_{1,124} = 7.25$; $P = .011$), AA ($F_{1,121} = 5.82$; $P = .031$), and GBA ($F_{1,124} = 55.8$; $P < .001$). Cerebral GSA and GBA concentrations were significantly higher in NX animals in all studied periods. Fluctuation in other GC levels were observed in both Sham and NX groups. High levels of GVA, CTN, and MG were measured at 10 days postsurgery in both Sham and NX groups, which decreased abruptly at month 2, and either increased (GVA and CTN) or remained unchanged (MG) thenceforth.

DISCUSSION

In this report, we followed through time the function and the adaptation of the remaining kidney in mice nephrectomized at 2 months of age. Until month 4 postnephrectomy, hypertrophy was demonstrated by the increase in the remaining kidney

Table 3. Plasma Concentration of Guanidino Compounds and Urea in Sham-Operated and Nephrectomized Mice 10 days, and 1, 2, 4, and 12 Months Postsurgery

	10 Days		1 Month		2 Months		4 Months		12 Months	
	Sham (n = 13)	NX (n = 10)	Sham (n = 18)	NX (n = 22)	Sham (n = 10)	NX (n = 9)	Sham (n = 15)	NX (n = 17)	Sham (n = 18)	NX (n = 13)
GVA	0.24 ± 0.08	0.53 ± 0.15*	0.39 ± 0.10	0.45 ± 0.17	0.41 ± 0.13	0.6 ± 0.3	0.26 ± 0.09	0.42 ± 0.17*	0.27 ± 0.16	0.8 ± 0.5*
GSA	0.18 ± 0.04	0.74 ± 0.24*	0.15 ± 0.03	0.41 ± 0.19*	0.143 ± 0.027	0.50 ± 0.23*	0.13 ± 0.05	0.44 ± 0.18*	0.15 ± 0.05	0.41 ± 0.19*
CT	304 ± 77	293 ± 94	383 ± 67	409 ± 62	323 ± 81	450 ± 75*	343 ± 62	421 ± 70*	310 ± 61	398 ± 109*
GAA	2.1 ± 0.5	1.8 ± 0.4	2.2 ± 0.4	1.5 ± 0.3*	2.0 ± 0.4	1.34 ± 0.28*	1.8 ± 0.3	1.3 ± 0.4*	1.4 ± 0.4	1.3 ± 0.6
NAA	<0.015–0.1	<0.015–0.31	ND–<0.015	ND–0.25	ND	ND	ND	ND	ND	ND
AA	<0.015–0.014	0.24 ± 0.12	ND–0.13	ND	ND	ND	0.17 ± 0.12	0.22 ± 0.09	0.27 ± 0.19	0.40 ± 0.17
GPA	<0.013–0.04	<0.013–0.09	ND–<0.013	ND	ND	ND	ND	ND	ND–0.02	ND–0.025
CTN	25 ± 4	35 ± 12*	30 ± 6	38 ± 6*	28 ± 4	46 ± 6*	29.4 ± 2.6	43 ± 6*	24 ± 6	31 ± 5*
GBA	0.35 ± 0.28	1.5 ± 0.7*	0.37 ± 0.21	0.7 ± 0.4*	0.35 ± 0.22	0.5 ± 0.4	0.25 ± 0.10	0.43 ± 0.22*	0.20 ± 0.07	0.6 ± 0.3*
Arg	123 ± 50	191 ± 98*	185 ± 114	173 ± 84	295 ± 210	412 ± 337	145 ± 110	143 ± 74	143 ± 75	112 ± 30
HA	0.39 ± 0.09	0.31 ± 0.06	0.38 ± 0.10	0.30 ± 0.06*	0.39 ± 0.08	0.28 ± 0.07*	0.37 ± 0.07	0.26 ± 0.05*	0.39 ± 0.16	0.37 ± 0.20
G	0.29 ± 0.07	0.46 ± 0.16*	0.36 ± 0.18	0.44 ± 0.18	0.34 ± 0.13	0.40 ± 0.14	0.33 ± 0.09	0.34 ± 0.17	0.20 ± 0.07	0.27 ± 0.09*
MG	0.063 ± 0.015	0.14 ± 0.05*	0.08 ± 0.03	0.13 ± 0.06*	0.085 ± 0.029	0.17 ± 0.07	0.050 ± 0.017	0.11 ± 0.06*	0.042 ± 0.009	0.071 ± 0.029*
Urea	8.0 ± 1.1	23 ± 12*	9.0 ± 1.6	15 ± 4*	8.2 ± 1.3	17 ± 5*	7.9 ± 1.9	17 ± 4*	9 ± 5	24 ± 12*

NOTE. Results are means ± SD. Concentrations are expressed as μmol/L except for urea as mmol/L.

Abbreviations: GVA, α-keto-δ-guanidinovaleric acid; GSA, guanidinosuccinic acid; CT, creatine; GAA, guanidinoacetic acid; NAA, α-*N*-acetylarginine; AA, argininic acid; GPA, β-guanidinopropionic acid; CTN, creatinine; GBA, γ-guanidinobutyric acid; Arg, arginine; HA, homoarginine; G, Guanidine; MG, methylguanidine; ND, not determined.

Results were statistically analyzed by 2-way ANOVA with Fisher's PLSD test: *$P < .05$, NX *v* Sham.

weight of our experimental mice. It is known that reduction in renal mass results in structural and functional compensatory growth of the remaining nephrons.[3,21] This compensatory renal hypertrophy is characterized structurally by an increase in size of tubules and glomeruli. Persistant hypertrophy at month 12 postsurgery was illustrated by the mean V_G. Microscopically, the kidneys of both Sham and NX mice exhibit age-related structural alterations, including expansion of the glomerular mesangial cells and marked thickening of the glomerular basement membrane. These observations were also reported by previous investigators.[22,23]

Appearance of focal and segmental glomerular sclerosis and hyalinization detected in the older Sham animals is consistent with insidious age-dependent changes (Table 2). However, early renal mass reduction and/or hypertrophy might accelerate and worsen these processes. As observed in our older NX animals, the remnant kidney was particularly more vulnerable to develop severe morphologic changes. This was demonstrated by the larger increase in the mean V_G when compared to those of old Sham animals. NX at young age may anticipate the histopathologic changes of the glomeruli due to aging, thus accelerating to functional failure and final loss.

As a consequence of chronic hyperfiltration in the residual glomeruli, resulting in altered intraglomerular transcapillary pressures, glomerular distension may play a role in the pathogenesis of progressive glomerulosclerosis.[3,24,25] Moreover, it has been shown that renal ablation accelerates the spontaneous development of focal glomerulosclerosis.[26,27] The latter leads

Table 4. Urinary Excretion of Guanidino Compounds and Urea in Sham-Operated and Nephrectomized Mice 10 Days, and 1, 2, 4, and 12 Months Postsurgery

	10 Days		1 Month		2 Months		4 Months		12 Months	
	Sham (n = 14)	NX (n = 14)	Sham (n = 17)	NX (n = 22)	Sham (n = 10)	NX (n = 9)	Sham (n = 15)	NX (n = 17)	Sham (n = 17)	NX (n = 13)
GVA	0.15 ± 0.04	0.12 ± 0.04	0.13 ± 0.04	0.10 ± 0.04*	0.14 ± 0.08	0.10 ± 0.03	0.14 ± 0.05	0.14 ± 0.05	0.16 ± 0.08	0.17 ± 0.06
GSA	0.057 ± 0.014	0.11 ± 0.04*	0.071 ± 0.021	0.084 ± 0.019*	0.12 ± 0.06	0.16 ± 0.05	0.079 ± 0.028	0.12 ± 0.04*	0.078 ± 0.024	0.103 ± 0.024*
CT	7.5 ± 1.8	6.9 ± 1.9	11 ± 3	8.0 ± 2.4*	15 ± 6	11.2 ± 2.5	15 ± 4	13 ± 3	15 ± 5	17 ± 3
GAA	2.0 ± 0.6	0.9 ± 0.4*	2.0 ± 0.5	0.97 ± 0.26*	2.4 ± 1.2	0.91 ± 0.24*	2.2 ± 0.6	1.0 ± 0.3*	2.1 ± 0.6	1.1 ± 0.4*
NAA	0.039 ± 0.010	0.045 ± 0.010	0.036 ± 0.010	0.034 ± 0.010	0.042 ± 0.019	0.027 ± 0.011	0.038 ± 0.016	0.043 ± 0.012	0.052 ± 0.018	0.061 ± 0.018
AA	0.048 ± 0.011	0.056 ± 0.017	0.064 ± 0.024	0.064 ± 0.019	0.08 ± 0.04	0.060 ± 0.012	0.061 ± 0.023	0.096 ± 0.029*	0.055 ± 0.022	0.083 ± 0.025*
GPA	0.011 ± 0.002	0.014 ± 0.011	0.011 ± 0.003	0.012 ± 0.003	0.015 ± 0.006	0.014 ± 0.003	0.012 ± 0.002	0.014 ± 0.002	0.010 ± 0.002	0.012 ± 0.002
CTN	16.8 ± 2.8	15 ± 3	16 ± 4	18 ± 4	18 ± 5	14 ± 4	16.7 ± 2.9	18 ± 3	15 ± 4	15.5 ± 2.9
GBA	0.8 ± 0.3	0.93 ± 0.26	0.78 ± 0.24	0.72 ± 0.20	0.7 ± 0.3	0.64 ± 0.18	0.70 ± 0.20	0.78 ± 0.19	0.63 ± 0.24	0.79 ± 0.27
Arg	0.30 ± 0.15	0.28 ± 0.11	0.33 ± 0.28	0.25 ± 0.09	0.42 ± 0.23	0.19 ± 0.09*	0.31 ± 0.07	0.28 ± 0.09	0.33 ± 0.10	0.29 ± 0.09
HA	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL–0.024	<DL–0.033
G	0.27 ± 0.07	0.28 ± 0.07	0.23 ± 0.06	0.25 ± 0.06	0.23 ± 0.09	0.22 ± 0.05	0.21 ± 0.05	0.28 ± 0.05*	0.17 ± 0.07	0.22 ± 0.08*
MG	0.089 ± 0.023	0.101 ± 0.026	0.104 ± 0.029	0.117 ± 0.026	0.10 ± 0.04	0.132 ± 0.025	0.098 ± 0.019	0.133 ± 0.029*	0.079 ± 0.026	0.104 ± 0.024*
Urea	2.0 ± 0.4	2.3 ± 0.5	1.8 ± 0.5	2.0 ± 0.3	1.4 ± 0.5	1.4 ± 0.4	2.0 ± 0.4	2.6 ± 0.6*	2.0 ± 0.8	2.5 ± 0.5*

NOTE. Results are means ± SD. Concentrations are expressed as μmol/24 hours, except for urea as mmol/24 hours.

Abbreviation: <DL, under detection limit.

Results were statistically analyzed by 2-way ANOVA and Fisher's PLSD test; *$P < .05$, NX *v* Sham.

Table 5. Brain Concentration of Guanidino Compounds in Sham-Operated and Nephrectomized Mice 10 Days, and 1, 2, 4, and 12 Months Postsurgery

	10 Days		1 Month		2 Months		4 Months		12 Months	
	Sham (n = 11)	NX (n = 9)	Sham (n = 18)	NX (n = 22)	Sham (n = 10)	NX (n = 9)	Sham (n = 15)	NX (n = 13)	Sham (n = 17)	NX (n = 13)
GVA	1.19 ± 0.22	1.24 ± 0.21	0.55 ± 0.13	0.55 ± 0.19	0.48 ± 0.15	0.56 ± 0.12	0.47 ± 0.12	0.58 ± 0.18	0.65 ± 0.21	1.0 ± 0.5*
GSA	0.15 ± 0.04	0.38 ± 0.13*	0.30 ± 0.18	0.42 ± 0.09*	0.24 ± 0.06	0.41 ± 0.08*	0.32 ± 0.09	0.56 ± 0.11*	0.36 ± 0.16	0.64 ± 0.20*
CT	8506 ± 1025	8159 ± 979	9254 ± 586	10280 ± 2146	8641 ± 308	8601 ± 514	8729 ± 559	8576 ± 597	8952 ± 577	9139 ± 678
GAA	8.6 ± 1.1	9.4 ± 1.8	9.2 ± 2.1	9.3 ± 1.7	8.3 ± 1.0	8.9 ± 1.6	8.3 ± 1.2	8.0 ± 1.0	9.1 ± 2.6	8.7 ± 1.3
NAA	1.08 ± 0.24	1.0 ± 0.3	0.91 ± 0.22	0.99 ± 0.19	0.66 ± 0.15	0.82 ± 0.18*	0.94 ± 0.28	1.00 ± 0.24	0.91 ± 0.17	1.16 ± 0.22*
AA	2.8 ± 0.5	2.9 ± 0.5	3.1 ± 0.4	3.2 ± 0.4	3.0 ± 0.7	2.9 ± 0.4	3.7 ± 0.9	4.3 ± 0.7*	2.8 ± 0.5	3.2 ± 0.7*
GPA	0.21 ± 0.08	0.23 ± 0.06	0.15 ± 0.04	0.16 ± 0.04	0.17 ± 0.05	0.161 ± 0.024	0.15 ± 0.04	<DL	0.179 ± 0.024	0.21 ± 0.05
CTN	240 ± 53	262 ± 62	137 ± 29	127 ± 14	118 ± 27	184 ± 73	233 ± 56	229 ± 32	201 ± 49	203 ± 51
GBA	1.60 ± 0.23	2.3 ± 0.9*	1.43 ± 0.22	1.80 ± 0.28*	1.16 ± 0.23	1.48 ± 0.25*	1.32 ± 0.15	1.622 ± 0.14*	1.21 ± 0.26	1.64 ± 0.31*
Arg	151 ± 20	171 ± 28	299 ± 89	216 ± 55*	149 ± 29	154 ± 23	292 ± 110	418 ± 128	126 ± 19	142 ± 28
HA	0.60 ± 0.14	0.65 ± 0.27	0.62 ± 0.22	0.58 ± 0.12	0.52 ± 0.08	0.46 ± 0.08	0.58 ± 0.09	0.49 ± 0.09	0.62 ± 0.15	0.491 ± 0.078
G	1.2 ± 1.0	3.1 ± 2.3*	2.8 ± 0.4	0.88 ± 0.27*	0.9 ± 0.5	1.1 ± 0.4	2.11 ± 0.06	2.1 ± 0.5	0.81 ± 0.29	0.80 ± 0.20
MG	2.3 ± 1.0	2.4 ± 1.2	0.43 ± 0.13	0.35 ± 0.08	0.49 ± 0.20	0.6 ± 0.3	0.45 ± 0.24	0.32 ± 0.07	0.9 ± 0.3	0.89 ± 0.26

NOTE. Results are means ± SD of Sham and NX groups. Concentrations are expressed as nmol/g tissue.
Results were statistically analyzed by 2-way ANOVA and Fisher's PLSD test: *$P < .05$, NX *v* Sham.

to disruption of glomerular endothelium and epithelium with extravasation of plasma-derived proteins into the subendothelial space, accumulation of hyaline material, and capillary occlusion and tuft adhesion to Bowman's capsule.[28] Some of these changes were in accordance with those observed in our mice of both groups used at month 12 postsurgery.

However, in parallel with the progressive structural deterioration of the remaining kidney, RF is also associated with the accumulation in blood of substances that are related to nitrogen metabolism, such as urea, CTN, and other GCs.[9-11] GCs are primarily cleared by the kidney and the accumulation of some GCs in uremic patients was shown not to be only due to reduced clearance, but also to an increase in the production of some compounds, eg, GSA.[29,30,31] As found in our mice, at the early stage (10 days postoperatively), NX in mice seems to have induced an acute RF as manifested biochemically by a high increase in plasma urea, CTN, and other GC levels when compared to the Sham group over the same period. Plasma urea was higher at 10 days postnephrectomy (3-fold) but showed also an increase in level from month 1 through 12. Acute RF may also be associated with increased Arg utilization and increased urea synthesis as there was evidence that the turnover rate of the urea cycle is accelerated in experimental acute renal failure.[32,33] Note that Arg, which is a key amino acid, is involved in the production of several GCs.[34] The high urea level at 12 months postnephrectomy might be due to the functional decline of the remaining viable renal tissue accelerated by aging. Furthermore, the NX groups showed a consistent low level in CTN_{Cl} from day 10 postnephrectomy onward, suggesting a stable renal insufficiency in these animals. However, the duration of follow-up was associated with a small, progressive increase in CTN_{Cl}, possibly the result of late, compensatory hypertrophy.

Levels of GSA were higher in plasma, urine, and brain of our NX mice compared to Sham at all studied time points. Increased GSA levels in plasma and urinary output were also reported in patients and rats with RF.[31] Moreover, it was demonstrated that GSA synthesis in liver was increased in rats with acute RF.[32]

Noteworthy is also the abrupt decline of urinary GAA excretion observed in our mice after NX. This persists during the long-term follow-up and is in accordance with earlier findings.[35] The decline might be due to renal mass reduction since GAA, the precursor of CT and therefore an essential substrate for muscle energy formation, is synthesized mainly in the kidney.[36]

GBA showed significant increase in plasma and brain levels in our NX mice. The blood increase might be due to its increased retention, whereas the brain increase could arise from a local synthesis through the transamidination reaction between arginine and GAA[37-39] since Pisano et al[39] suggested that GBA apparently does not cross the blood-brain barrier. However, a possibility that GBA might cross the blood-brain barrier cannot be excluded since blood-brain barrier disturbances were observed in uremic encephalopathy.[40]

From month 1 onward, the experimental mice seem to present a state of chronic RF. The fact is that most GC concentrations remained higher (or lower, eg, GAA) in NX mice compared to Sham groups. However the levels during the chronic phase of RF seem to be lower compared to the acute phase. It is more likely that adaptive changes occurred in the remaining kidney, in the early stage of RF, resulting in its enhanced function.[41,42] However, Sham groups also manifested changes in GC levels in body fluids and in brain over time. These changes might represent age-related alterations in protein metabolism or food intake. It was found that GC metabolism is affected by maturation and aging in the normal condition as well.[43,44]

These biochemical and histologic data lead us to 2 interesting obervations. First, that nitrogen and GC metabolism are more disturbed in the acute phase of RF (10 days postsurgery), and remain disturbed but partially reestablished during the chronic phase of RF (from month 1 postsurgery onward). Second, due to NX at young age, the histologic alterations seem to worsen with aging, as observed at month 12. Noteworthy is that NX at young age seems to affect with time primarily the structure of the remnant kidney by accelerating the aging process and changing characteristic renal structures. The ab-

sence of more severe functional deterioration of the remnant kidney in our experimental animals may be due to several factors. Possibly, the long-term follow-up was not long enough. Alternatively, the RF induced was not severe enough or the tested strain of mice present a genetic background allowing them to develop adaptive mechanisms in case of changes in the environmental conditions (eg, physical stress). Otherwise, studies in humans show that the surgical loss of renal functional mass in the presence of a normal remnant kidney rarely leads to renal insufficiency.[45-47] Although it is common knowledge that it is possible for man to survive more than 30 years with only 1 remnant kidney, and to maintain normal and stable serum CTN,[48] one has to consider that the decrease in functioning glomeruli which occurs with advancing age is an unavoidable gradual process despite absence of any superimposed disease. On the other hand, the observed structural deteriorations in our experimental animals may predict increased susceptibility to develop RF when NX is accompanied by diseases such as atherosclerosis, diabetes, or hypertension. After subtotal NX at a young age, and with the process of aging, the issue will be to preserve the intact residual nephrons to function adequately in the absence or presence of disease. This model can serve as a tool to investigate the age-dependent progression of renal insufficiency. In addition, combined models could be developed in the presence or absence of comorbidity and other risk factors.

REFERENCES

1. O'Donnell MP, Kasiske BL, Raij L, et al: Age is a determinant of the glomerular morphologic and functional response to chronic nephron loss. J Lab Clin Med 106:308-313, 1985
2. Baylis C, Schmidt R: The aging glomerulus. Semin Nephrol 16:265-276, 1996
3. Hostetter TH, Olson JL, Rennke HG, et al: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. Am J Physiol 241:F85-F93, 1981
4. Olson JL, De Urdaneta AJ, Heptinstall RH: Glomerular hyalinosis and its relation to hyperfiltration. Lab Invest 52:387-397, 1985
5. Anderson S, Meyer T, Brenner BM, et al: The role of hemodynamic factors in the initiation and progression of renal disease. J Urol 133:363-368, 1985
6. Gabizon D, Goren E, Shaked U, et al: Induction of chronic renal failure in the mouse: A new model. Nephron 40:349-352, 1985
7. Gagnon RF, Duguid WP: A reproducible model for chronic renal failure in the mouse. Urol Res 11:11-14, 1983
8. Marescau B, Deshmukh DR, Kockx M, et al: Guanidino compounds in serum, urine, liver, kidney and brain of man and some ureotelic animals. Metabolism 41:526-532, 1992
9. De Deyn PP, Marescau B, Cuykens JJ, et al: Guanidino compounds in serum and cerebrospinal fluid of non-dialyzed patients with renal insufficiency. Clin Chim Acta 167:81-88, 1987
10. De Deyn PP, Marescau B, D'Hooge R, et al: Guanidino compounds levels in brain regions of non-dialyzed uremic patients. Neurochem Int 27:227-237, 1995
11. Matasumuto M, Kishikawa H, Mori A: Guanidino compounds in the sera of uremic patients and in the sera of and brain of experimental rabbits. Biochem Med 16:161-168, 1978
12. Mannetti S, Balestri PL, Barsotti G: Methylguanidine in uremia. Arch Intern Med 131:709-713, 1973
13. Kelly RA, Mitch WE: Creatinine, uric acid, and other nitrogenous waste products: Clinical implication of the imbalance between their production and elimination in uremia. Semin Nephrol 3:286-294, 1992
14. Vanholder R: Uremic toxins. Adv Nephrol 26:143-106, 1997
15. De Deyn PP, MacDonald RL: Guanidino compounds that are increased in uremia inhibit GABA and glycine responses on mouse neurones in cell culture. Ann Neurol 28:627-633, 1990
16. De Deyn PP, Saxena VA, Abts H, et al: Clinical and pathophysiological aspects of neurological complications in renal failure. Acta Neurol Belg 92:191-206, 1992
17. D'Hooge R, Pei Q-Y, Marescau B, et al: Convulsive action and toxicity of the uremic guanidino compounds: Behavioral assessment and relation to brain concentration in adult mice. J Neurol Sci 112:96-105, 1992
18. Bertens APMG, Booij LHDJ, Flecknell PA, et al: Anesthesia, analgesia and euthanasia, in Van Zutphen LFM, Baumans V, Beynen AC (eds): Principles of Laboratory Animal Science. Amsterdam, Netherlands. Elsevier, 1993, pp 267-298
19. Gundersen HJG, Jensen EB: Particle sizes and their distributions estimated from line- and point-sampled intercepts. Including graphical unfolding. J Microsc 131:291-310, 1983
20. Ceriotti G: Ultramicrodetermination of plasma urea by reaction with diacetylmonoxime-antipyrine without deproteinization. Clin Chem 7:400-402, 1971
21. Hayslett JP: Functionnal adaptation to reduction of renal mass. Physiol Rev 59:137-164, 1979
22. Chou SY, Lindeman RD: Structural and functional changes in the aging kidney, in Jacobson HR, Striker GE, Klahr S (eds): The Principles and Practice of Nephrology (ed 2). St Louis, MO, Mosby, 1995, pp 510-514
23. Levi M, Rowe JW: Renal function and dysfunction in aging, in Seldin DW, Giebesch G (eds): The Kidney: Physiology and Pathophysiology. New York, NY, Raven, 1992, pp 3433-3456
24. Brenner BM, Meyer TH, Hostetter TH: Dietary protein intake and the progressive nature of kidney disease. N Engl J Med 307:652-659, 1982
25. Thoner PS, Arbus GS, Celermajer DS, et al: Focal segmental glomerulosclerosis and progressive renal failure associated with a unilateral kidney. Pediatrics 73:806-810, 1984
26. Shimamura T, Morrison AB: A progressive glomerulosclerosis occuring in partial five-sixth nephrectomized rats. Am J Pathol 79:95-106, 1975
27. Grond J, Schilthuis MS, Koudstaal J, et al: Mesangial function and glomerular sclerosis in rats after unilateral nephrectomy. Kidney Int 22:338-343, 1982
28. Zatz R, Fujihara CK: Glomerular hypertrophy and progressive glomerulonephropathy: Is there a definite pathogenic correlation. Kidney Int 45:S27-S29, 1994 (suppl)
29. Orita Y, Tsubakihara Y, Ando A: The effect of arginine and creatinine administration on the urinary excretion of methylguanidine. Nephron 22:328-336, 1978
30. Cohen BD: Guanidinosuccinic acid in uremia. Arch Intern Med 126:846-850, 1970
31. Cohen BD, Stein IM, Bonas JE: Guanidinosuccinic aciduria in uremia, a possible alternate pathway for urea synthesis. Am J Med 45:63-68, 1968
32. Hopper-Seyler G: Studies on urea cycle enzymes in rat liver during acute uremia. Eur J Clin Invest 5:15-20, 1975
33. Perez GO, Rietberg B, Owens B, et al: Effects of acute uremia on arginine metabolism and urea and guanidino acid production by perfused rat liver. Pflugers Arch 372:275, 1977
34. Robin Y: Metabolism of arginine in invertebrates: Relation to urea cycle to other guanidine derivates, in Lowenthal A, Mori A,

Marescau B (eds): Urea Cycle Diseases. New York, NY, Plenum, 1982, pp 407-417

35. Sasaki M, Takahara K, Natelson S: Urinary guanidinoacetate/guanidinosuccinate ratio; an indicator of kidney dysfunction. Clin Chem 19:315-321, 1973

36. McGuire DM, Gross MD, Elde RP, et al: Localization of L-arginine-glycine amidinotransferase protein in rat tissues by immunofluorescence microscopy. J Histochem Cytochem 34:429, 1986

37. Irreverre F, Evans RL, Hayden AR, et al: Occurrence of gamma-guanidinobutyric acid. Nature 180:704-705, 1957

38. Pisano JJ, Mitoma C, Udenfriend S: Biosynthesis of γ-guanidinobutyric acid from γ-aminobutyric acid and arginine. Nature 180: 1125-1126, 1957

39. Pisano JJ, Abraham D, Udenfriend S: Biosynthesis and disposition of γ-guanidinobutyric acid in mammalian tissues. Arch Biochem Biophys 100:323-329, 1963

40. Jeppsson B, Freund HR, Gimmon Z, et al: Blood-brain barrier derangement in uremic encephalopathy. Surgery 92:30-35, 1982

41. Brenner BM: Nephron adaptation to renal injury or ablation. Am J Physiol 18:F324-F337, 1985

42. Kaufman JM, Dimeola HJ, Siegel NJ, et al: Compensatory adaptation of structure and function following progressive renal ablation. Kidney Int 6:10-17, 1974

43. Watanabe Y, Shindo SH, Akitan M: Developmental changes in guanidino compound levels in mouse organs, in Mori A, Cohen BD, Lowenthal A (eds): Guanidines. New York, NY, Plenum, 1985, pp 49-57

44. Matsumura M, Kuroda M, Itoh M, et al Guanidino compounds in the elderly. Jpn J Geriatr (Nippon Ronen Igakkai Zasshi) 29:279-284, 1992

45. Robitaille P, Mongeau JG, Lortie L, et al: Long-term follow-up of patients who underwent unilateral nephrectomy in childhood. Lancet 8:1297-1299, 1985

46. Provoost AP, Brenner BM: Long-term follow-up of human with single kidney: The need for longitudinal studies to assess true changes in renal function. Curr Opin Nephrol Hypertens 2:521, 1993

47. Provoost AP, Baudoin P, De Keijzer MH, et al: The role of nephron loss in the progression of renal failure: Experimental evidence. Am J Kidney Dis 17:27-32, 1991 (suppl 1)

48. Foster MH, Sant GR, Donohoe JF, et al: Prolonged survival with a remnant kidney. Am J Kidney Dis 17:261-265, 1991