Increased tissue acid mediates a progressive decline in the glomerular filtration rate of animals with reduced nephron mass

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The combination of an acid-inducing diet and reduced nephron mass is associated with a progressive decline in glomerular filtration rate (GFR) that can be corrected by dietary alkali. Here we determined whether the higher tissue acid content mediates the decline in GFR. Using Munich-Wistar rats we induced sub-total nephrectomy and measured by microdialysis the tissue acid content in the kidney cortex and in the paraspinous muscle. The GFR was lower in the rats with reduced nephron mass at 1 and 13 weeks following subtotal nephrectomy compared to the sham-operated rats. Both groups of rats ate the same acid-inducing casein-based diet and had similar plasma acid–base parameters and net urine acid excretion. However, rats with reduced nephron mass had higher tissue acid content compared to control animals and had a lower GFR at week 13 compared to that measured at week 1. Adding dietary acid to the casein diet led to an even higher tissue acid and lower GFR by week 13. By contrast, adding alkali to the casein diet or placing animals with reduced nephron mass on a soy-based diet led to a lower tissue acid content and no decline in GFR. Animals with reduced nephron mass on a soy-based diet given dietary acid had a higher tissue acid content and a decline in GFR. These studies show that dietary maneuvers that increase the tissue acid content reduce GFR, whereas diets that lower the tissue acid level preserve GFR during chronic kidney failure.

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Animals with reduced nephron mass and eating an acid (H^+)-inducing diet have progressive glomerular filtration rate (GFR) decline that is ameliorated by dietary alkali, but GFR is better preserved when these animals eat less H^+-inducing diets.[1] Similarly, animals with intact nephron mass and eating an H^+-inducing diet have progressive kidney interstitial injury that is exacerbated by additional dietary H^+, ameliorated by dietary alkali, and is less apparent when such animals eat less H^+-producing diets.[2] Because animals with reduced nephron mass that eat a standard, H^+-inducing diet[3] and those with intact nephron mass given additional dietary H^+[4] can have plasma acid–base parameters that are not different from comparable controls, the described maneuvers in these respective settings might alter a non-plasma parameter of acid–base status that mediates these untoward changes in kidney pathology and/or function. Adding H^+ to a standard H^+-inducing diet of animals with intact nephron mass increases per nephron acidification, which is associated with increased kidney cortical H^+ content.[4] Similarly, animals with reduced nephron mass eating the same H^+-inducing diet as those with intact nephron mass also have increased per nephron acidification[3] in their effort to effect net acid excretion (NAE) necessary to maintain H^+ balance (that is, acid in = acid out) despite lower nephron mass. Indeed, animals with reduced and those with intact nephron mass eating the same diet can have comparable urine NAE.[3] Animals with reduced nephron mass eating the same dietary H^+ as animals with intact nephron mass might therefore be qualitatively similar to animals with intact nephron mass challenged with added H^+ with respect to effect on tissue H^+ content. The present studies tested the hypothesis that animals with reduced compared with intact nephron mass eating the same dietary H^+ have greater tissue H^+ content that contributes to progressive GFR decline.

RESULTS

Effect of kidney mass reduction and diets

Figure 1 shows lower GFR in Nx than sham at week 1 (2625 ± 239 vs 4265 ± 368 μl/min, $P < 0.003$) and week 13 (2261 ± 210 vs 4087 ± 354 μl/min, $P < 0.001$). Table 1 shows

Nx and sham with similar arterial and stellate vessel pH/plasma total CO_2 ($PTCO_2$), but respective stellate vessel compared with arterial pH was lower and stellate vessel compared with arterial $PTCO_2$ was higher. Nx and sham had similar urine NAE but Nx had higher distal nephron J_{HCO_3}. Nx eating dietary H^+ as $(NH_4)_2SO_4$ had lower arterial $PTCO_2$ and lower stellate vessel $pH/PTCO_2$ with higher urine NAE and distal nephron J_{HCO_3}. By contrast, Nx eating dietary alkali as $CaHCO_3$ had similar arterial and stellate vessel $pH/PTCO_2$, but lower urine NAE and distal nephron J_{HCO_3}. Nx eating dietary protein as soy, labeled Nx(Soy), had similar arterial and stellate vessel pH but Nx(Soy) had higher arterial and stellate vessel $PTCO_2$, and lower urine NAE and distal nephron J_{HCO_3} than Nx. Nx(Soy) eating $(NH_4)_2SO_4$ had lower stellate vessel $pH/PTCO_2$ but had higher urine NAE and distal nephron J_{HCO_3}. By contrast, Nx(Soy) eating $CaHCO_3$ had similar arterial and stellate vessel $pH/PTCO_2$, but had lower urine NAE and distal nephron J_{HCO_3} than Nx(Soy) not eating $CaHCO_3$.

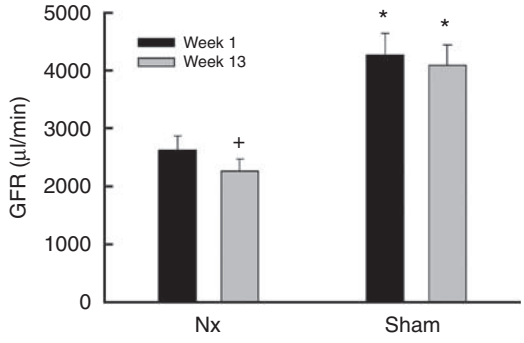

Figure 1 | GFR (µl/min) of casein-eating, conscious, 2/3 nephrectomized (Nx) compared with sham-operated (sham) animals 1 or 13 weeks after nephrectomy or sham surgery. $^*P<0.05$ vs Nx; $^+P<0.05$ vs respective 1-week value, paired *t*; $n=8$ animals for each group.

Microdialysis data

Table 2 shows no $pH/PCO_2/TCO_2$ differences between collected and infused dialysate of kidney cortex of sham with net H^+ addition not different from zero. Changes in collected-to-infused kidney cortex microdialysate H^+ content in remaining groups were mediated mostly by changes in pH/TCO_2 and less so by PCO_2. Nx had greater net H^+ addition to dialysate than sham. $Nx+(NH_4)_2SO_4$, but not Na_2SO_4, had even greater net H^+ dialysate addition. By contrast, $Nx+CaHCO_3$, but not Ca^{2+} gluconate (CaGlu), had lower net H^+ dialysate addition. Nx eating soy protein, labeled Nx(Soy), had lower net H^+ dialysate addition than Nx eating casein. $Nx(Soy)+(NH_4)_2SO_4$, but not Na_2SO_4, had higher net H^+ dialysate addition. By contrast, net H^+ dialysate addition was not different in $Nx(Soy)+CaHCO_3$ or CaGlu. Similar to that described for microdialysis of kidney cortex, Table 3 shows no differences in pH, PCO_2, or TCO_2 between collected and infused dialysate of microdialysed paraspinous muscle of sham, indicating no net H^+ addition. The findings were qualitatively the same as for kidney cortex in Table 2. Nx had greater net H^+ addition to dialysate than sham, $Nx+(NH_4)_2SO_4$ but not Na_2SO_4 had even greater net H^+ dialysate addition, and $Nx+CaHCO_3$ but not CaGlu had lower net H^+ dialysate addition. Similarly, Nx(Soy) had lower net H^+ dialysate addition than Nx + casein. $Nx(Soy)+(NH_4)_2SO_4$, but not Na_2SO_4, had higher net H^+ dialysate addition, and net H^+ dialysate addition by paraspinous muscle was not different when comparing $Nx(Soy)+CaHCO_3$ or Nx(Soy) + CaGlu.

Table 1 | Arterial and stellate vessel plasma pH/total CO_2 (TCO_2), urine net acid excretion (NAE), and distal nephron net HCO_3 reabsorption (J_{HCO_3}) 5 weeks after kidney mass reduction

	Arterial pH	Stellate vessel pH (µM/ml)	Arterial $PTCO_2$ (mM)	Stellate vessel $PTCO_2$ (mM)	NAE (mM/d)	Distal nephron J_{HCO_3}
Nx	7.41 ± 0.02	7.26^+ ± 0.02	24.8 ± 0.5	28.7^+ ± 0.5	2.9 ± 0.4	22.4* ± 2.2
Sham	7.42 ± 0.03	7.30^+ ± 0.02	25.0 ± 0.5	29.7^+ ± 0.6	3.2 ± 0.5	14.0 ± 1.4
Nx	7.40 ± 0.02	7.25^+ ± 0.02	24.8 ± 0.4	28.1^+ ± 0.7	3.0 ± 0.4	22.0 ± 2.1
$Nx+(NH_4)_2SO_4$	7.38 ± 0.02	$7.18^{*,+}$ ± 0.02	22.6* ± 0.4	$25.1^{*,+}$ ± 0.7	4.7* ± 0.4	32.3* ± 2.6
$Nx+Na_2SO_4$	7.41 ± 0.03	7.27^+ ± 0.02	25.0 ± 0.4	28.8^+ ± 0.7	3.1 ± 0.4	22.5 ± 2.0
Nx	7.40 ± 0.03	7.26^+ ± 0.02	25.4 ± 0.5	29.0^+ ± 0.6	3.0 ± 0.3	22.9 ± 1.9
$Nx+CaHCO_3$	7.41 ± 0.02	7.28^+ ± 0.02	27.2 ± 0.6	30.8^+ ± 0.7	1.6* ± 0.2	14.9* ± 1.3
Nx+CaGlu	7.40 ± 0.03	7.26^+ ± 0.03	25.5 ± 0.6	28.6^+ ± 0.6	3.1 ± 0.3	22.6 ± 1.9
Nx	7.39 ± 0.03	7.25^+ ± 0.03	24.9 ± 0.5	28.6^+ ± 0.5	3.0 ± 0.3	22.3 ± 2.1
Nx(Soy)	7.42 ± 0.03	7.28^+ ± 0.03	26.7* ± 0.6	$31.2^{*,+}$ ± 0.5	1.7* ± 0.2	11.2* ± 1.0
Nx(Soy)	7.39 ± 0.03	7.25^+ ± 0.02	25.2 ± 0.5	28.6^+ ± 0.6	1.7 ± 0.2	11.9 ± 1.1
$Nx(Soy)+(NH_4)_2SO_4$	7.36 ± 0.03	$7.16^{*,+}$ ± 0.02	23.0 ± 0.4	$24.9^{*,+}$ ± 0.5	2.5* ± 0.2	18.0* ± 1.6
$Nx(Soy)+Na_2SO_4$	7.40 ± 0.04	7.26^+ ± 0.02	25.5 ± 0.7	28.4^+ ± 0.6	1.8 ± 0.2	12.1 ± 1.0
Nx(Soy)	7.39 ± 0.03	7.28^+ ± 0.02	26.8 ± 0.5	29.0^+ ± 0.6	1.7 ± 0.2	10.9 ± 1.1
$Nx(Soy)+CaHCO_3$	7.41 ± 0.03	7.30^+ ± 0.02	28.0 ± 0.6	30.6^+ ± 0.6	1.0* ± 0.2	7.0* ± 0.8
Nx(Soy)+CaGlu	7.40 ± 0.04	7.27^+ ± 0.02	27.0 ± 0.6	28.6^+ ± 0.6	1.7 ± 0.2	12.0 ± 1.2

n=8 animals in each group.
Values are means ± s.e. $(NH_4)_2SO_4$, Na_2SO_4, $Ca(HCO_3)_2$, and calcium gluconate (CaGlu) were added to diets of the indicated groups. All animals ingested diets containing casein as protein except for the indicated group whose dietary protein was soy.
$^*P<0.05$ vs respective Nx.
$^+P<0.05$ vs respective arterial value.

Table 2 | Kidney cortical dialysate acid–base parameters

	pH	PCO_2	TCO_2	pH	PCO_2	TCO_2	pH	PCO_2	TCO_2
	Nx			Sham					
Infused	7.30 ± 0.02	54.6 ± 1.6	26.8 ± 1.4	7.30 ± 0.03	54.1 ± 1.7	26.6 ± 1.3			
Collected	7.24$^+$ ± 0.02	53.2 ± 2.0	22.2$^+$ ± 1.5	7.31 ± 0.02	52.6 ± 2.3	27.0 ± 1.5			
Net H^+ addition (fmol)	446 ± 78			−69* ± 59					
	Nx			Nx+$(NH_4)_2SO_4$			Nx+Na_2SO_4		
Infused	7.29 ± 0.02	53.2 ± 1.5	26.5 ± 1.2	7.29 ± 0.02	55.0 ± 1.7	25.9 ± 1.3	7.30 ± 0.02	54.2 ± 1.4	26.2 ± 1.5
Collected	7.22$^+$ ± 0.02	51.8 ± 1.3	21.2$^+$ ± 1.1	7.14$^+$ ± 0.02	53.8 ± 2.0	18.1$^+$ ± 1.2	7.25$^+$ ± 0.02	52.0 ± 2.0	21.6$^+$ ± 1.3
Net H^+ addition (fmol)	538 ± 94			1269* ± 236			367 ± 67		
	Nx			Nx+$CaHCO_3$			Nx+CaGlu		
Infused	7.30 ± 0.02	53.0 ± 1.4	26.4 ± 1.3	7.30 ± 0.02	56.2 ± 1.7	26.8 ± 1.4	7.29 ± 0.02	55.1 ± 1.6	26.3 ± 1.5
Collected	7.23$^+$ ± 0.02	51.8 ± 2.3	22.2$^+$ ± 1.2	7.27 ± 0.02	55.1 ± 2.2	25.0 ± 1.5	7.23$^+$ ± 0.02	53.7 ± 2.1	22.0$^+$ ± 1.4
Net H^+ addition (fmol)	526 ± 99			215* ± 54			456 ± 71		
	Nx			Nx(Soy)					
Infused	7.29 ± 0.02	54.7 ± 1.4	26.3 ± 1.3	7.30 ± 0.02	55.7 ± 1.4	26.6 ± 1.4			
Collected	7.22$^+$ ± 0.02	53.2 ± 2.0	22.4$^+$ ± 1.6	7.29 ± 0.02	53.2 ± 1.8	25.0 ± 1.6			
Net H^+ addition (fmol)	538 ± 103			70* ± 25					
	Nx(Soy)			Nx(Soy)+$(NH_4)_2SO_4$			Nx(Soy)+Na_2SO_4		
Infused	7.30 ± 0.02	53.1 ± 1.5	26.4 ± 1.2	7.30 ± 0.02	53.9 ± 1.6	25.8 ± 1.1	7.29 ± 0.02	53.5 ± 1.3	26.1 ± 1.3
Collected	7.28 ± 0.02	51.0 ± 1.3	25.5 ± 1.1	7.24$^+$ ± 0.02	53.0 ± 1.7	21.6$^+$ ± 1.0	7.27 ± 0.02	5.21 ± 1.8	25.2 ± 1.2
Net H^+ addition (fmol)	142 ± 20			446* ± 89			145 ± 23		
	Nx(Soy)			Nx(Soy)+$CaHCO_3$			Nx(Soy)+CaGlu		
Infused	7.31 ± 0.02	54.0 ± 1.3	26.3 ± 1.2	7.30 ± 0.02	54.2 ± 1.7	26.8 ± 1.3	7.29 ± 0.02	54.2 ± 1.4	26.6 ± 1.5
Collected	7.29 ± 0.02	52.7 ± 2.1	25.0 ± 1.1	7.29 ± 0.02	51.6 ± 2.0	26.0 ± 1.3	7.28 ± 0.02	52.4 ± 2.0	25.2 ± 1.4
Net H^+ addition (fmol)	139 ± 18			70 ± 18			72 ± 16		

Values are means ± s.e. $(NH_4)_2SO_4$, Na_2SO_4, $Ca(HCO_3)_2$, and calcium gluconate (CaGlu) were added to diets of the indicated groups. All animals ingested diets containing casein as protein except for the indicated group whose dietary protein was soy.
*$P<0.05$ vs respective Nx.
$^+P<0.05$ vs respective infused value.

Figure 1 shows lower week 13 than week 1 GFR for Nx (2261 ± 210 vs 2625 ± 239 µl/min, $P<0.04$, paired t) but not sham (4087 ± 354 vs 4265 ± 368 µl/min, $P=0.24$, paired t). Figure 2 shows lower week 13 than week 1 GFR in Nx (2369 ± 184 vs 2589 ± 191 µl/min, $P<0.04$, paired t), Nx + $(NH_4)_2SO_4$ (1670 ± 142 vs 2493 ± 199 µl/min, $P<0.002$, paired t), and Nx + Na_2SO_4 (2310 ± 188 vs 2516 ± 178 µl/min, $P<0.05$, paired t). Week 13 GFR in Nx + $(NH_4)_2SO_4$ was lower than the respective week 13 value for Nx ($P<0.03$, ANOVA). By contrast, Figure 3 shows that week 13 and week 1 GFRs were not different in Nx + $CaHCO_3$ (2571 ± 202 vs 2602 ± 215 µl/min, $P=0.89$, paired t), but these respective values were lower in Nx without additional salt (2322 ± 197 vs 2662 ± 250 µl/min, $P<0.04$, paired t) and Nx + CaGlu (2219 ± 187 vs 2514 ± 191 µl/min, $P<0.04$, paired t). Figure 4 shows that unlike Nx eating casein in which week 13 GFR was lower than at week 1, GFR at week 13 and week 1 were no different in Nx(Soy) (1985 ± 177 vs 1979 ± 167 µl/min, $P=0.98$, paired t). Figure 4 also shows that week 1 GFR was lower in Nx(Soy) than Nx. Figure 5 shows that week 13 GFR was lower than at week 1 in Nx(Soy) + $(NH_4)_2SO_4$ (1677 ± 146 vs 1958 ± 154 µl/min, $P<0.05$, paired t) but were similar in Nx(Soy) + Na_2SO_4 and in Nx(Soy) without additional salt. By contrast, Figure 6 shows that week 13 and week 1 GFRs were not different in Nx(Soy) + $CaHCO_3$ and also were not different in Nx(Soy) + CaGlu or in Nx(soy) without additional salt.

DISCUSSION

Nx eating H^+-inducing diets have progressive GFR decline yet might have plasma acid–base parameters similar to sham[3,5] or reflect only mild metabolic acidosis.[1] Consequently, H^+-inducing diets might cause progressive GFR decline through acid–base changes that are not reflected in plasma. The studies described tested the hypothesis that progressive GFR decline of animals with reduced nephron mass is mediated through higher tissue H^+ content. These studies show that Nx eating the same H^+-inducing diet as sham have similar plasma acid–base parameters and urine NAE yet have higher H^+ content in kidney cortex and skeletal muscle by microdialysis, consistent with greater overall tissue H^+ content. Furthermore, dietary maneuvers that increased tissue H^+ content in Nx led to GFR decline after 12 weeks but those maneuvers that decreased tissue H^+

Table 3 | Paraspinous muscle dialysate acid–base parameters

	pH	PCO_2	TCO_2	pH	PCO_2	TCO_2	pH	PCO_2	TCO_2
	Nx			Sham					
Infused	7.37 ± 0.02	42.4 ± 1.3	25.7 ± 1.3	7.37 ± 0.03	42.1 ± 1.3	25.8 ± 1.3			
Collected	7.28$^+$ ± 0.02	41.0 ± 1.6	20.0$^+$ ± 1.4	7.36 ± 0.02	40.9 ± 1.7	25.1 ± 1.5			
Net H^+ addition (fmol)	589 ± 112			60* ± 13					
	Nx			Nx+$(NH_4)_2SO_4$			Nx+Na_2SO_4		
Infused	7.37 ± 0.02	42.1 ± 1.4	25.5 ± 1.3	7.36 ± 0.02	42.6 ± 1.7	25.6 ± 1.2	7.37 ± 0.02	42.0 ± 1.3	25.5 ± 1.5
Collected	7.29$^+$ ± 0.02	41.4 ± 1.3	19.7$^+$ ± 1.3	7.22$^+$ ± 0.02	41.5 ± 2.0	17.5$^+$ ± 1.1	7.28$^+$ ± 0.02	40.9 ± 1.2	19.8$^+$ ± 1.3
Net H^+ addition (fmol)	518 ± 86			996* ± 119			589 ± 90		
	Nx			Nx+$CaHCO_3$			Nx+CaGlu		
Infused	7.36 ± 0.02	42.8 ± 1.3	25.4 ± 1.2	7.37 ± 0.02	41.7 ± 1.4	25.8 ± 1.3	7.36 ± 0.02	42.0 ± 1.4	25.6 ± 1.4
Collected	7.28$^+$ ± 0.02	41.7 ± 1.5	19.6$^+$ ± 1.2	7.34 ± 0.02	40.3 ± 1.8	22.5 ± 1.3	7.29$^+$ ± 0.02	40.5 ± 1.8	19.2$^+$ ± 1.3
Net H^+ addition (fmol)	530 ± 80			183* ± 28			458 ± 73		
	Nx			Nx(Soy)					
Infused	7.37 ± 0.02	42.4 ± 1.3	25.6 ± 1.3	7.37 ± 0.02	42.0 ± 1.3	25.7 ± 1.3			
Collected	7.29$^+$ ± 0.02	41.0 ± 1.7	20.3$^+$ ± 1.3	7.35 ± 0.02	40.3 ± 1.7	22.9 ± 1.3			
Net H^+ addition (fmol)	518 ± 81			121* ± 16					
	Nx(Soy)			Nx(Soy)+$(NH_4)_2SO_4$			Nx(Soy)+Na_2SO_4		
Infused	7.37 ± 0.02	42.5 ± 1.4	25.3 ± 1.2	7.36 ± 0.02	42.8 ± 1.2	25.7 ± 1.3	7.36 ± 0.02	42.5 ± 1.4	25.4 ± 1.4
Collected	7.35 ± 0.02	41.7 ± 1.5	22.8 ± 1.3	7.28$^+$ ± 0.02	41.5 ± 1.5	20.2$^+$ ± 1.2	7.34 ± 0.02	41.9 ± 1.8	23.3 ± 1.3
Net H^+ addition (fmol)	121 ± 23			530* ± 93			123 ± 16		
	Nx(Soy)			Nx(Soy)+$CaHCO_3$			Nx(Soy)+CaGlu		
Infused	7.36 ± 0.02	43.0 ± 1.3	25.6 ± 1.3	7.37 ± 0.02	42.1 ± 1.3	25.8 ± 1.4	7.37 ± 0.02	42.5 ± 1.4	25.5 ± 1.4
Collected	7.33 ± 0.02	41.9 ± 1.7	22.6 ± 1.2	7.35 ± 0.02	41.2 ± 1.7	23.2 ± 1.3	7.34 ± 0.02	41.1 ± 1.8	22.8 ± 1.3
Net H^+ addition (fmol)	187 ± 35			121 ± 19			183 ± 25		

Values are means ± s.e. $(NH_4)_2SO_4$, Na_2SO_4, $Ca(HCO_3)_2$, and calcium gluconate (CaGlu) were added to diets of the indicated groups. All animals ingested diets containing casein as protein except for the indicated group whose dietary protein was soy.
*$P<0.05$ vs respective Nx.
$^+P<0.05$ vs respective infused value.

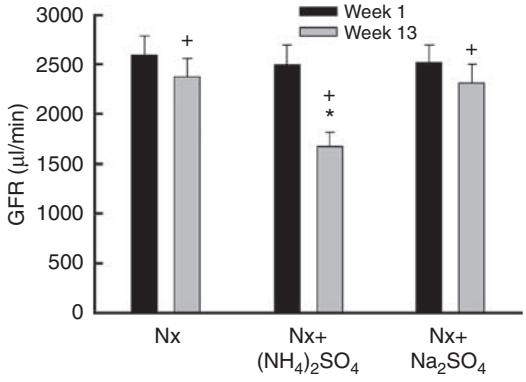

Figure 2 | GFR (µl/min) of casein-eating, conscious Nx given dietary $(NH_4)_2SO_4$ to increase intrinsic H^+ production or Na_2SO_4 as dietary SO_4 control compared with Nx without added salt. Nx, 2/3 nephrectomized animals. *$P<0.05$ vs Nx; $^+P<0.05$ vs respective 1-week value, paired t; $n=8$ animals for each group.

content led to no measurable decline in GFR. The data support that increased tissue H^+ content mediates GFR decline in animals with reduced nephron mass.

Humans with chronically reduced GFR might have progressive GFR decline despite improved blood pressure control and angiotensin-converting enzyme inhibition.[6] Most humans in industrialized societies eat H^+-inducing diets[7] so greater tissue H^+ content might contribute to progressive GFR decline in human nephropathy. Because added dietary H^+ induces urine NAE excretion in humans that is less than the dietary H^+-induced increase in intrinsic acid production,[8] this maneuver might induce a steady-state increase in tissue H^+ content as in animals with intact nephron mass eating added dietary H^+[4] and in Nx of the present studies. Added dietary H^+ caused GFR decline in Nx of the present studies and as shown previously,[1] but this maneuver caused kidney interstitial injury without measurable GFR decline in animals with intact nephron mass.[2] Consequently, H^+-inducing diets have greater propensity to cause GFR decline in animals with reduced compared with intact nephron mass. Humans with chronically reduced GFR, similar to Nx, must mount the same NAE as those with intact nephron mass to maintain H^+ balance when each eats diets with the same acid–base content.[9,10] Humans with reduced GFR can indeed achieve NAE equivalent to intrinsic acid production[10] and

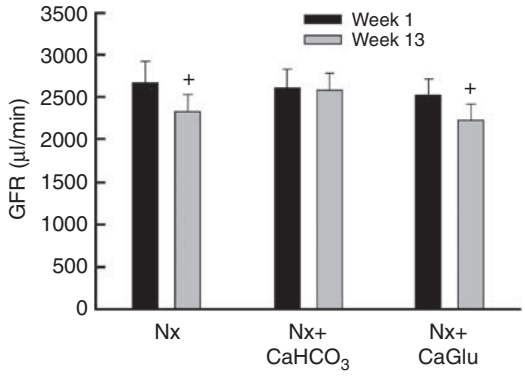

Figure 3 | GFR (µl/min) of casein-eating, conscious Nx given dietary $CaHCO_3$ to decrease intrinsic H^+ production or Ca^{++} gluconate (CaGlu) as dietary Ca^{++} control compared with Nx without added salt. Nx, 2/3 nephrectomized animals. $^+P<0.05$ vs respective 1-week value, paired *t*; $n=8$ animals for each group.

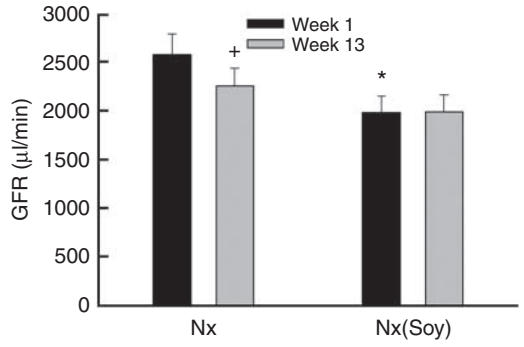

Figure 4 | GFR (µl/min) of casein-eating, conscious Nx compared with Nx eating dietary protein as soy, labeled Nx(Soy). Nx, 2/3 nephrectomized animals. $*P<0.05$ vs Nx; $^+P<0.05$ vs respective 1-week value, paired *t*; $n=8$ animals for each group.

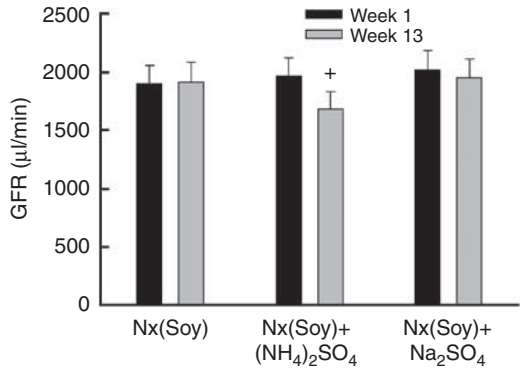

Figure 5 | GFR (µl/min) of Nx(Soy) given dietary $(NH_4)_2SO_4$ to increase intrinsic H^+ production or Na_2SO_4 as dietary SO_4 control compared with Nx(Soy) without added salt. Nx, 2/3 nephrectomized animals. $^+P<0.05$ vs respective 1-week value, paired *t*; $n=8$ animals for each group.

maintain normal plasma acid–base parameters[11] similar to Nx of the present studies but might do so in the setting of increased tissue H^+ content similar to Nx. Whether humans

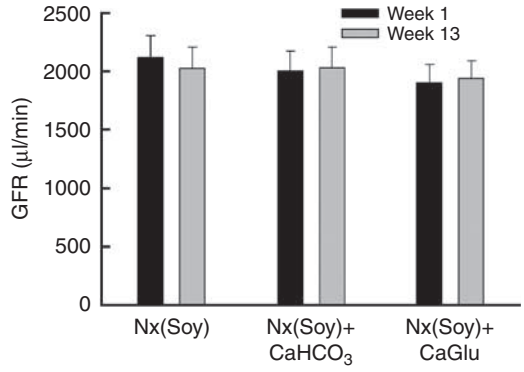

Figure 6 | GFR (µl/min) of Nx(Soy) given dietary $CaHCO_3$ to decrease intrinsic H^+ production or Ca^{++} gluconate (CaGlu) as dietary Ca^{++} control compared with Nx(Soy) without added salt. Nx, 2/3 nephrectomized animals. $n=8$ animals for each group.

with reduced GFR also have increased tissue H^+ content awaits determination by future studies.

The studies examining tissue H^+ content in casein-eating or soy-eating animals additionally given dietary H^+ or alkali support the importance of dietary H^+ in influencing the level of tissue H^+ content. Nx animals given dietary H^+ and those given dietary alkali had more and less tissue H^+ content, respectively. Furthermore, Nx eating soy diet, one that is less H^+-inducing than casein,[7] had lower kidney tissue H^+ content than Nx eating casein. The data support that reduced GFR alone does not determine the level of tissue H^+ content but the level of the systemic H^+ challenge also makes an important contribution. In addition, these data show that tissue H^+ content can be changed by either ingesting a diet of different H^+ content or by adding acid or alkali salts.

In summary, the present studies support that higher tissue H^+ content mediates progressive GFR decline in animals with reduced nephron mass. Animals with reduced nephron mass that eat H^+-inducing diets and/or ingest H^+-inducing salts have higher tissue H^+ content and progressive GFR decline. By contrast, animals with reduced nephron mass that eat less H^+-inducing diets and/or ingest salts that reduce intrinsic H^+ production have lower tissue H^+ content and ameliorated GFR decline. Further studies will determine whether increased tissue H^+ content contributes to GFR decline in human nephropathy.

MATERIALS AND METHODS

Animals, diet, and study protocol

Male and female Munich–Wistar rats (Harlan Sprague–Dawley, Houston, TX, USA) of 180–211 g were used to investigate the influence of kidney mass reduction on tissue H^+ content measured by kidney cortical and skeletal muscle H^+ content (see below) and the influence of tissue H^+ content on GFR decline. Animals ate standard rat chow (Prolab RMH 2500 with 23% protein of various sources; Purina Labs, St Louis, MO, USA) prior to kidney mass reduction surgery. Earlier studies showed that arterial $PTCO_2$

calculated from blood gases of rats with 5/6 nephrectomy and ate a 20% casein diet was comparable to sham.[3] Other studies in which rats had 5/6 nephrectomy and ate the same diet but in which $PTCO_2$ was measured directly with ultrafluorometry[12] showed that $PTCO_2$ was slightly less than sham.[1] Preliminary studies that compared $PTCO_2$ in animals with 2/3 rather than 5/6 kidney mass reduction with sham eating identical 20% casein diets showed similar $PTCO_2$ (24.7 ± 0.7 vs 24.9 ± 0.6 mM, $n = 4$, $P = 0.84$). Consequently, we used 2/3 kidney mass reduction for nephrectomized (Nx) animals. Following kidney mass reduction, animals ate minimum electrolyte diets with 20% protein as casein or soy (ICN Nutritional Biochemicals, Cleveland, OH, USA) and drank distilled H_2O *ad libitum*. Some were given $(NH_4)_2SO_4$ (75 μM/g diet) or $Ca_2(HCO_3)_2$ (75 μM/g diet) after kidney mass reduction as H^+ or alkali challenge, respectively. $(NH_4)_2SO_4$ was used for H^+ challenge because it does not stimulate distal nephron HCO_3 secretion[13] and $Ca_2(HCO_3)_2$ was used as the alkali challenge because it does so without increasing blood pressure.[1] Additional animals eating Na_2SO_4 (75 μM/g diet) were studied to control for SO_4 ingestion. To control for dietary Ca^{2+}, animals eating equivalent amounts of CaGlu (75 μM/g diet) and $Ca_2(HCO_3)_2$ were compared, as done previously.[1] In preliminary studies, Nx and similar weight controls ate 17.8 ± 0.9 vs 20.4 ± 0.8 g/day, respectively, ($n = 4$, $P = 0.07$) and so all animals received 17 g/day to assure similar diet intake.

Kidney mass reduction

Nx was induced by surgical removal of approximately 2/3 of kidney mass in two stages using modification of the technique used previously.[3] Briefly, the left kidney of anesthetized animals was exposed through a flank incision, the main renal artery and vein temporarily occluded, and the inferior kidney pole was removed with scissors to leave about 2/3 of the single kidney mass. Bleeding was controlled with thrombin applied to the cut surface, the remnant kidney was returned to the abdominal cavity, and the animal was allowed to recover. The right kidney was removed 1 week later through a flank incision and the animal allowed to recover. Shams had left kidney exteriorization followed in 1 week by exteriorization of the right kidney and its return to the abdomen. Heparinized polyethylene tubes (PE 50) were placed and secured in the left jugular vein for vascular access and in the right carotid artery for blood sampling. These vascular lines were flushed daily with 10% heparin in 5% dextrose in water and then capped with a metal plug after the animal had been placed in a comfortable restraining device. At 1 week following the second surgery during which animals ate the described experimental diet, GFR was measured in conscious, Nx and sham by slope of the decrease in plasma concentration of intravenously infused ^{3}H-inulin over 180 min.[14]

Microdialysis technique to compare kidney cortical and skeletal muscle H^+ content

At 11 weeks after the second surgery during which animals ate the described experimental diet, Nx and sham had surgery to insert the microdialysis catheter. Relative tissue H^+ content among sham, Nx, H^+-ingesting Nx, and alkali-ingesting Nx was determined by comparing the difference in H^+ content ($[H^+]$ times dialysate volume) between collected and infused dialysate using microdialysis of the kidney cortex[13] and paraspinal muscles.[15] Changes in dialysate PCO_2 and total CO_2 (TCO_2) were measured to distinguish the $[H^+]$ determinant that changed to mediate changes in tissue H^+ content. A microdialysis apparatus was constructed as described previously.[13] The left kidney was exposed through a flank incision in rats anesthetized with ketamine (100 mg/kg; Park Davis, Morris Plains, NJ, USA). The kidney capsule was penetrated with a 31-gauge needle that was tunneled in the outer kidney cortex ~1 mm from the kidney surface for ~0.5 mm before exiting by penetrating the kidney capsule again. The needle tip was inserted into one end of the dialysis probe and the needle was pulled together with the dialysis tube until the dialysis fiber was situated within the kidney cortex. The inflow and outflow tubes of the dialysis probe were tunneled subcutaneously through a bevel-tipped tube and exteriorized near the interscapular region. The incision was extended posteriorly to expose a paraspinous muscle for insertion of the same apparatus through the fascia for 0.5 mm as described for the kidney cortex. Inflow and outflow tubes of the dialysis probe were exteriorized as described and marked to distinguish them from the kidney probe. Subcutaneous tissue was closed with 3-0 prolene and the skin with clips. Exterior ends of the dialysis tubes and arterial line were sutured to a skin site on the animal's back from which its hair had been sheared. Exteriorized portions of the tubes were placed in a stainless steel spring to prevent the animal from damaging them. Determination of reliability of the microdialysis apparatus to assess kidney cortical H^+ content was done previously.[13] We compared *in vitro* and *in vivo* ^{3}H-inulin recovery to test the reliability of microdialysis of paraspinous muscle. *In vitro* ^{3}H-inulin recovery, evaluated by immersing dialysis membranes of four identically constructed probes into a beaker without [^{3}H]-inulin, was 91%. *In vivo* ^{3}H-inulin recovery in microdialysis of paraspinous muscle was 89%, consistent with minimal to no leakage.

Urine NAE

At 6 days after insertion of the microdialysis catheter, urine NAE[16] was measured in a 24-h sample in eight animals each of control and experimental groups kept in metabolic cages.

Microdialysis of kidney cortex and paraspinous muscle was carried out in comfortably restrained, conscious animals 7 days after microdialysis catheter insertion (12 weeks after kidney mass reduction surgery). Inflow tubes were connected to a gas-tight syringe filled with a modified (below) Ringer's HCO_3 solution. The solution for the kidney cortex was equilibrated with 6.7% CO_2, chosen to approximate PCO_2 in rat kidney cortex,[17] recognizing that the precise kidney cortical PCO_2 level is controversial.[18] The solution for the paraspinous muscle was equilibrated with 5% CO_2 to approximate systemic PCO_2. The kidney cortex solution was infused after CO_2 equilibration at 3 μl/min (Harvard Apparatus, Saint-Laurent, QC, Canada), a rate found to be optimal.[4] The paraspinous solution was perfused at 2.5 μl/min, a flow rate found to be optimal for this tissue.[15] Preliminary studies yielded dialysate that when perfused in sham yielded no change in H^+ content (that is, no difference between collected and infused dialysate). We reasoned that such a solution would gain H^+ when dialysed against tissue with higher-than-sham H^+ content and would lose H^+ if tissue H^+ content were less than control. Preliminary studies showed that this was achieved using Ringer's HCO_3 with $[HCO_3] = 26$ meq/l for the kidney and 25 meq/l for paraspinous muscle. Three 20-min collection periods were done in eight animals in each group. Volume of collected tissue dialysate was not different from an identically timed infusion onto a glass slide under H_2O-equilibrated mineral oil among groups (~60 μl). Anaerobically obtained collected and infused dialysate were analyzed for pH (micro flow through pH monitor; Lazar Research Labs, Los Angeles, CA, USA), PCO_2 (micro flow through CO_2 probe; Lazar Research Labs), TCO_2 by flow-through ultrafluorometry.[12]

Whole blood and plasma parameters

Immediately after microdialysis (12 weeks after kidney mass reduction), 0.35 ml of carotid arterial blood for arterial blood gases and plasma $PTCO_2$ (the latter by flow-through ultrafluorometry) was slowly removed from awake, gently restrained, and calm animals and was replaced with an equivalent blood volume from a paired, identically treated animal. The animal was returned to its metabolic cage for an additional 1 week. Measurement of GFR was repeated as described, now 12 weeks after initial GFR measurement and 13 weeks after kidney reduction surgery.

Micropuncture protocol

At 1 day after the second GFR measurement, animals underwent *in vivo* microperfusion micropuncture of accessible distal nephron epithelia as described.[19] Net HCO_3 transport was measured in about 1 mm of tubule with the tip of the infusion and collection pipette occupying 5–7 μm of tubule length proximal to and distal to, respectively, the segment in which HCO_3 transport occurred. The perfusate contained 5 mM $[HCO_3]$ to approximate *in situ* $[HCO_3]$.[3] Earlier studies showed that Nx had higher early distal nephron flow rates than sham but that there were no qualitative differences in net distal tubule HCO_3 reabsorption (J_{HCO_3}) between the two perfusion rates when comparing Nx and sham.[3] Consequently, surface distal nephron epithelia were perfused at the *in situ* rate of sham, 6 nl/min.

Analytical methods

Collected and infused dialysate, stellate vessel,[18] and arterial plasma, microdialysate were immediately analyzed for TCO_2 using flow-through ultrafluorometry.[12]

Calculations

Urine NAE was the mean for each animal group. Net J_{HCO_3} was calculated as described.[19] Net dialysate H^+ addition was calculated as described[4] by multiplying the $[H^+]$ difference between collected and infused dialysate (calculated from the measured pH) times the total volume of collected dialysate ($3\,\mu l/min \times 20\,min = \sim 60\,\mu l$). A positive value for net H^+ addition indicated greater H^+ content in collected compared with infused dialysate (that is, H^+ gain) and a negative value indicated lower H^+ content in collected dialysate (that is, H^+ loss). Net H^+ addition for each of three collection periods was averaged for a single animal value. This value was then averaged for each animal for a group value.

Statistical analysis

The data were expressed as means ± s.e. Paired perfusions of the same tubule were compared using paired *t*-test; otherwise, ANOVA was used for multiple group comparisons. We used the Bonferroni method for multiple comparisons ($P<0.05$) of the same parameter among groups.

DISCLOSURE

All the authors declared no competing interests.

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REFERENCES

1. Phisitkul S, Hacker C, Simoni J *et al.* Dietary protein causes a decline in the glomerular filtration rate of the remnant kidney mediated by metabolic acidosis and endothelin receptors. *Kid Int* 2008; **73**: 192–199.
2. Wesson DE, Nathan T, Rose T *et al.* Dietary protein induces endothelin-mediated kidney injury through enhanced intrinsic acid production. *Kid Int* 2007; **71**: 210–217.
3. Wesson DE. Endogenous endothelins mediate augmented acidification in remnant kidneys. *J Am Soc Nephrol* 2001; **12**: 1826–1835.
4. Wesson DE. Dietary acid increases blood and renal cortical acid content in rats. *Am J Physiol* 1998; **274**(Renal Physiol 43): F97–F103.
5. Kunau RT, Walker KA. Distal tubular acidification in the remnant kidney. *Am J Physiol* 1990; **258**(Renal Fluid Electrolyte Physiol 27): F69–F74.
6. Appel LJ, Wright Jr JT, Greene T *et al.* Long-term effects of rennin–angiotensin-system-blocking therapy and a low blood pressure goal on progression of hypertensive chronic kidney disease in African Americans. *Arch Int Med* 2008; **168**: 832–839.
7. Remer T. Influence of nutrition on acid–base balance–metabolic aspects. *Eur J Nutr* 2001; **40**: 214–220.
8. Lemann Jr J, Bushinsky DA, Hamm LL. Bone buffering of acid and base in humans. *Am J Physiol* 2003; **285**: F811–F832.
9. MacClean AJ, Hayslett JP. Adaptive change in ammonia excretion in renal insufficiency. *Kid Int* 1980; **17**: 595-606.
10. Goodman AD, Lemann Jr J, Lennon EJ *et al.* Production, excretion, and net balance of fixed acid in patients with renal failure. *J Clin Invest* 1980; **17**: 595–606.
11. Widmer B, Gerhardt RE, Harrington JT *et al.* Serum electrolyte and acid base composition: The influence of graded degrees of chronic renal failure. *Arch Int Med* 1979; **139**: 1099–1102.
12. Wesson DE. Dietary HCO_3 reduces distal tubule acidification by increasing cellular HCO_3 secretion. *Am J Physiol* 1996; **271**(1 Pt. 2): F132–F142.
13. Wesson DE. Endogenous endothelins mediate increased distal tubule acidification induced by dietary acid in rats. *J Clin Invest* 1997; **99**: 2203–2211.
14. Blaufox MD, Aurell M, Bubeck B *et al.* Report of the Radionuclides in Nephrourology Committee on renal clearance. *J Nucl Med* 1996; **37**: 1883–1890.
15. Kim TJ, Freml L, Park SS *et al.* Lactate concentrations in incisions indicate ischemic-like conditions may contribute to postoperative pain. *J Pain* 2007; **8**: 59–66.
16. Wesson DE. Dietary bicarbonate reduces rat distal nephron acidification evaluated *in situ*. *Am J Physiol* 1990; **258**(Renal Fluid Electrolyte Physiol. 27): F870–F876.
17. DuBose Jr TD, Pucacco LR, Lucci MS *et al.* Micropuncture determination of pH, PCO_2, and total CO_2 concentration of the rat renal cortex. *J Clin Invest* 1979; **64**: 476–482.
18. Dubose Jr TD, Gennari FJ, Maddox DA *et al.* Comment on PCO_2 in renal cortex. *Am J Physiol* 1991; **260**: F608–F612.
19. Wesson DE, Dolson GM. Augmented bidirectional HCO_3 transport by rat distal tubules in chronic alkalosis. *Am J Physiol* 1991; **261**(Renal Fluid Electrolyte Physiol 30): F308–F317.