Effect of an Acute Oral Protein Load on Renal Acidification in Healthy Humans and in Patients with Chronic Renal Failure

NATALE G. DE SANTO,* GIOVAMBATTISTA CAPASSO,* GERHARD MALNIC,[†]
PIETRO ANASTASIO,* LUCIA SPITALI,* and ANGELA D'ANGELO[‡]

*Nephrology Department, Second University of Naples, Naples, Italy; [†]Institute of Biological Sciences, University of Sao Paulo, Sao Paulo, Brazil; [‡]Nephrology Department, University of Padua, Padua, Italy.

Abstract. The effect of a meat load on the renal handling of acid-base balance was studied in ten healthy subjects (GFR by inulin clearance = 98.5 ± 8.14 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}) and in ten patients affected by chronic renal failure (CRF) (GFR = 39.9 ± 5.3 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}). After the meat load (2 g $\cdot$ kg^{-1} body weight of cooked unsalted red meat), GFR increased by 26.9% (peak value) over baseline in healthy subjects and by 32% in CRF patients. The acid-base status of the healthy subjects was in the normal range, whereas the CRF patients disclosed a slight metabolic acidosis. After a meat load, there was, in the healthy subjects, an increase in the filtered load of bicarbonate coupled to an enhanced tubular reabsorption and urinary excretion. The time course between bicarbonate load and urinary excretion was coincident. In CRF patients, the increase of bicarbonate tubular load after the meal was associated with an increase in tubular reabsorption but not in urinary excretion of this anion. The relationship between bicarbonate load and reabsorption was linear in both groups up to the highest filtered loads. Baseline titratable acidity (TA) and ammonium (NH$_4^+$) excretion (expressed per ml GFR) were increased in CRF patients as compared with control subjects, but no changes were found after the meat load in both groups in these experimental conditions. The data indicate that the renal tubules contribute to the maintenance of acid-base balance both in healthy subjects and in CRF patients by reabsorbing most of the additional bicarbonate load. The transient, but significant, increase in bicarbonate excretion observed in healthy subjects could be related to the increased tubular load of bicarbonate. In CRF patients, tubular bicarbonate reabsorption was more complete, possibly because of the stimulation of H$^+$ secretion by the mild metabolic acidosis. TA and NH$_4^+$ did not participate in tubular compensation of the increased buffer load. (J Am Soc Nephrol 8: 784–792, 1997)

It is well known that an acute protein load increases GFR (1). This response has been addressed in recent years by many studies that have contributed to our understanding of the factors related to the enhancement in renal hemodynamics (2). The ensuing hyperfiltration has been related to the establishment and progression of glomerular sclerosis (3). In contrast, only few investigators have explored tubular function under these conditions (4–9). In particular, very little is known about the role of the tubule to maintain an acid-base balance in healthy subjects and in patients with chronic renal failure (CRF) after the hemodynamic and acid ash effects of a meat load. These informations might be important for at least two reasons: first, in the presence of hyperfiltration, the tubules undergo a larger filtered buffer load, which is a stimulus to increased reabsorption of bicarbonate and other filtered buffer

molecules (10,11). Therefore, an alteration of glomerular-tubular balance may lead to a modification of the acid-base balance. Second, the development of an acid medullary interstitial pH, which has been linked to an impairment of tubular acidification, has been proposed as one of the factors leading to interstitial fibrosis and, consequently, to the progression of renal disease (12,13). Therefore, experiments investigating the behavior of renal acid-base transport under conditions of a meat load would be of potential interest in the understanding of the factors responsible for progression of renal disease.

The study presented here was devised to explore renal acid-base handling after an acute protein load. This was performed in healthy subjects and in patients with CRF. Data showing that the renal tubule adapts to acute changes in GFR by modulating H$^+$ secretion and HCO$_3^-$ reabsorption is provided; this adaptation is independent of the magnitude of baseline GFR. On the other hand, this short-term modification of the filtered load of buffer molecules is not able to affect the excretion of titratable acidity (TA) and the renal formation and excretion of ammonium (NH$_4^+$).

Materials and Methods

Study Population

A group of ten healthy subjects participated in the study. All subjects were within 20% of ideal body weight and on free diets, and

all volunteered for this study. None of them was taking any medication and none had a past/present history of renal, cardiovascular, or other organ system disease. A group of ten CRF patients, matched to the healthy subjects by age, sex (six males and four females), and urinary sodium, was also studied. The CRF patients were aged 39 ± 4.7 yr and were studied while they were under free living conditions and ingesting the usual homemade foods. The purpose, the nature, and the potential risks of the study were explained to all subjects, and their consent was obtained before their admission to the study. The experimental procedure was reviewed and approved by the Health Board of the Medical Faculty. In Table 1, the data describing the characteristics of the two groups of study subjects are reported.

Renal Function and Protein Loading

GFR was measured as the inulin clearance. The studies, as described elsewhere (14), were started at 8.30 a.m. after an overnight fast by administering an oral water load (20 ml · kg^{-1} body wt). Subsequent water replacement was calculated according to loss so that urine volume was kept constant. The accurate collection of urine was performed by the use of an indwelling bladder catheter. Small FEP-Teflon (DuPont, Wilmington, DE) cannulae were inserted into an antecubital vein of each arm for inulin. A bolus of inulin (40 mg · kg^{-1} body wt) was administered. This was followed by a continuous inulin infusion through a constant-speed infusion pump (Braun, Melsungen, Germany) at a rate adjusted to maintain plasma concentration for inulin at about 20 mg · dl^{-1} throughout the experiment. After a 90-min equilibration period, three subsequent baseline clearance periods were performed in each subject, each of 30 min duration (C_1, C_2, C_3). Baseline inulin clearance was obtained by averaging the three baseline clearance rates (C_1, C_2, C_3). The stimulation of GFR by means of an oral protein load (2 g · kg^{-1} body wt) was achieved by administering cooked red meat (beefsteak) which was served within 20 to 25 min. The protein load has been proven sufficient to stimulate GFR maximally (15). No salt, bread, or other cereals were allowed. After the oral protein load (OPL) began, five clearance collections were carried out (C_4 through C_8), each lasting 30 min except for C_8, which lasted for 60 min. Urine and blood samples were obtained at the end of each period. Plasma clearance of inulin was calculated by the usual standard formula and corrected for 1.73 m^2 body surface.

Blood and Urine Analysis

Inulin levels was measured by a colorimetric method (16); creatinine concentration was assayed according to a rate-dependent modi-

fication of the Jaffe reaction, utilizing a Beckman creatinine analyzer (Fullerton, CA) that provides a measure of true creatinine concentration (17).

The acid-base status of the blood was explored by standard methods at baseline and at maximal GFR response after OPL by using a blood gas analyzer (Radiometer, ABL 300, Copenhagen, Denmark). The urinary TA and bicarbonate concentration were determined according to Györy et al. (18) at baseline and at the time of the maximal GFR after OPL. Ammonium ions (NH_4^+) were retained by a cationic exchange resin's being eluted thereafter, once the interfering substances were washed away. Ammonium ions were then quantified at baseline and at the time of maximal GFR by a spectrophotometric reading of the indophenol formed in the Berthelot reaction, by using the Biosystems phenol-hypochlorite kit (Barcelona, Spain). Because of technical reasons, these last measurements were done only on seven subjects in the group of CRF patients.

Statistical Analyses

Comparisons between experimental groups were performed by two-way analysis of variance (two-way ANOVA), and contrasts were evaluated by the Bonferroni test. When only two groups were compared, a paired t test was used. The regression lines of Figures 1 and 2 were compared by ANOVA, using Graphpad Prisma 2.0 (San Diego, CA) software. All data are expressed as mean ± SEM.

Results
Healthy Subjects

Table 2 describes GFR, urine flow rate, and the acid-base status of blood and urine after a 12-h night fast (baseline conditions) and at the time of the maximal GFR response after an acute OPL. The meat meal caused a statistically significant ($P < 0.01$) 26.9% increase in GFR without affecting blood pH, P_{CO2}, and bicarbonate. The urine flow rate was constant.

At the time of the maximal GFR response, urinary pH increased significantly ($P < 0.01$) over baseline values (6.83 ± 0.12 versus 6.53 ± 0.167). At the same time, urinary bicarbonate excretion more than doubled ($P < 0.005$) over baseline (58.76 ± 11.59 μmol · min^{-1} versus 25.05 ± 9.93 μmol · min^{-1}). By contrast, urinary TA and urinary ammonium excretion remained constant.

Table 3 shows the tubular handling of bicarbonate in healthy subjects in baseline conditions and after a meat meal. At

Table 1. Characteristics of the patients enrolled in the study[a]

Parameter	Control Patients	Chronic Renal Failure Patients	P Value
Age	35 ± 4.9	39 ± 4.7	
Body weight (kg)	57.8 ± 2.1	67.8 ± 2.1	<0.005
Systolic BP (mmHg)	122 ± 5.1	153 ± 3.3	<0.005
Diastolic BP (mmHg)	76 ± 2.1	100 ± 4	<0.005
Protein intake (g · kg^{-1} body wt)	0.92 ± 0.05	0.71 ± 0.07	<0.05
Urinary Na$^+$ (mmol · day^{-1})	105 ± 8.8	101 ± 3.5	
Blood urea (g · l^{-1})	0.18 ± 0.13	75 ± 12.9	<0.005
Blood creatinine (mg · dl^{-1})	0.91 ± 0.01	2.9 ± 0.5	<0.005
Creatinine clearance (ml · min^{-1} · 1.73 m^2 · BSA^{-1})	116 ± 6.8	57.4 ± 9.3	<0.005
Hb (g · dl^{-1})	12.5 ± 0.34	11.1 ± 0.9	

[a] BP, blood pressure; Hb, hemoglobin.

Table 2. GFR, urine flow rate, and acid-base status after 12-h night fast (baseline) and at the time of the maximal GFR response (peak) after an acute oral protein load in healthy subjects[a]

Parameter	Baseline	Peak	P Value
GFR (ml · min^{-1} · 1.73 m^2)	98.5 ± 8.14	125 ± 6.6	<0.01
Urine flow (ml · min^{-1})	10.59 ± 0.89	10.61 ± 1.05	
Blood			
pH	7.37 ± 0.01	7.38 ± 0.01	
P_{CO2} (mmHg)	39 ± 1.59	39.69 ± 1.63	
HCO_3^- (mmol · l^{-1})	22.7 ± 0.71	23.52 ± 0.92	
Urine			
pH	6.53 ± 0.167	6.83 ± 0.12	<0.01
HCO_3^- (μmol · min^{-1})	25.09 ± 9.93	58.76 ± 11.59	<0.005
TA (μmol · min^{-1})	18.21 ± 3.51	16 ± 2.31	
NH_4^+ (μmol · min^{-1})	39.4 ± 7.0	45.0 ± 12	

[a] TA, titratable acidity.

Table 3. Tubular handling of bicarbonate in healthy subjects at baseline and after meat meal[a]

Parameter	Baseline	Peak	P Value
HCO_3^- filtered (μmol · min^{-1})	2284 ± 113	2941 ± 185	<0.005
HCO_3^- excreted (μmol · min^{-1})	25.1 ± 9.93	58.8 ± 11.59	<0.005
HCO_3^- reabsorbed (μmol · min^{-1})	2258 ± 117	2899 ± 178	<0.005
HCO_3^- fractional reabsorption (%)	98.88 ± 0.35	98.11 ± 0.38	<0.05
HCO_3^- fractional excretion (%)	1.11 ± 0.35	1.89 ± 0.38	<0.05

[a] Data are expressed as mean ± SEM.

maximal GFR response after OPL, the filtered bicarbonate load increased significantly ($P < 0.005$) over baseline (2941 ± 185 μmol · min^{-1} versus 2284 ± 113 μmol · min^{-1}). The increase of the bicarbonate load is coupled to a significant increase in absolute ($P < 0.005$) and fractional ($P < 0.05$) bicarbonate reabsorption. Nevertheless, the absolute and fractional bicarbonate excretion after OPL was significantly ($P < 0.05$) higher than that at baseline conditions (58.8 ± 11.59 μmol · min^{-1} versus 25.1 ± 9.93 μmol · min^{-1}, 1.89 ± 0.38% versus 1.11 ± 0.35%, respectively).

In Figure 1, urinary bicarbonate excretion is plotted versus serum bicarbonate. When these data are analyzed by the linear regression technique, it is concluded that during the control period, the slope of bicarbonate excretion versus serum bicarbonate is not statistically significant ($P = 0.0786$), whereas in the OPL period, this slope is significant ($P = 0.0305$), showing that bicarbonate excretion correlates significantly with serum bicarbonate ($r = 0.68$) under the latter condition and during the control period. In addition, the intercept of the OPL regression line was significantly higher in CRF patients than in control subject ($P = 0.0498$), indicating that bicarbonate excretion at a given plasma level was higher after OPL.

Patients with Chronic Renal Disease

Table 4 shows that in patients with CRF, after an OPL, there was also a statistically significant ($P < 0.001$) 32% increase in

GFR (52.7 ± 9.3 ml · min^{-1} · 1.73 m^2 body wt), although the absolute level of GFR was markedly lower than that of healthy subjects. The urine flow rate did not change after the meat load; however, when compared (two-way ANOVA) with the corresponding urine flow rates in healthy subjects, a significant decrease ($P < 0.01$) was found in CRF patients. In general, these patients show a slight reduction in blood P_{CO2} and bicarbonate levels that is compatible with the moderate metabolic acidosis of CRF. As observed by other investigators, the absolute excretion of ammonium in CRF patients is not increased when compared with that of healthy subjects, whereas the bicarbonate excretion is significantly reduced ($P < 0.01$ by two-way ANOVA). The acid-base status of blood and urine was not affected by the meat load.

Table 5 shows that both filtered and reabsorbed bicarbonate were significantly increased by the meat load. Contrary to what had been observed in healthy subjects, fractional bicarbonate reabsorption and excretion were not significantly affected by the meat load.

In Figure 2, urinary bicarbonate excretion is plotted as function of serum bicarbonate. Contrary to what had been observed in healthy subjects, none of the slopes of bicarbonate excretion versus serum bicarbonate was significant ($P = 0.22$ for control subjects and 0.61 for OPL subjects). The intercepts of these lines were also not significantly different from each other ($P = 0.23$). These results indicate that the tubules of CRF
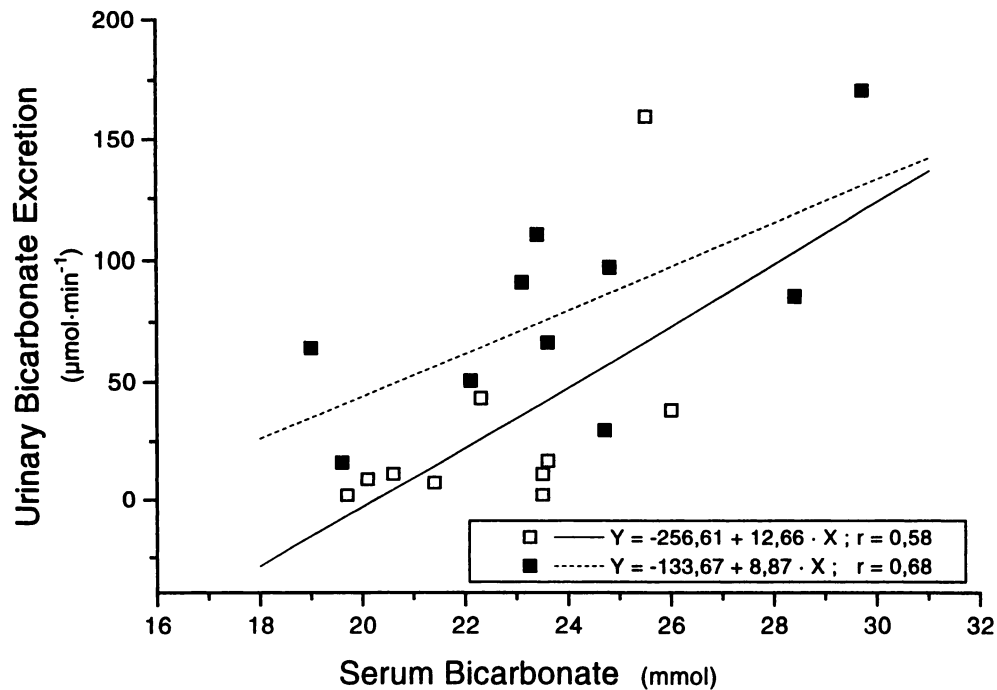
Figure 1. Urinary bicarbonate excretion plotted as a function of serum bicarbonate in healthy volunteers (Controls) under fasting condition (open squares) and at the peak (closed squares) of the hyperfiltering renal response induced by a meat meal (2 g of protein per kg body wt).

Table 4. GFR, urine flow rate, and acid-base status after 12-h night fast (baseline) and at the time of the maximal GFR response (peak) after an acute oral protein load to patients with chronic renal failure

Parameter	Baseline	Peak	*P* Value
GFR (ml · min^{-1} · 1.73 m^2)	39.9 ± 5.3	52.7 ± 9.3	<0.001
Urine flow (ml · min^{-1})	5.62 ± 0.43	7.15 ± 0.78	
Blood			
pH	7.36 ± 0.03	7.36 ± 0.02	
P_{CO2} (mmHg)	35.46 ± 2.01	34.8 ± 1.29	
HCO_3^- (mmol · l^{-1})	20.19 ± 1.21	19.65 ± 0.80	
Urine			
pH	6.38 ± 0.187	6.38 ± 0.17	
HCO_3^- (μmol · min^{-1})	8.39 ± 1.65	18.73 ± 8.21	
TA (μmol · min^{-1})	18.29 ± 3.51	16.62 ± 3.90	
NH_4^+ (μmol · min^{-1})	21.9 ± 5.2	36.0 ± 4.0	

patients are able to reabsorb a greater proportion of the filtered bicarbonate than are those of healthy subjects.

The difference between the two groups is better illustrated in Figure 3, where the fractional excretion of bicarbonate is plotted as a function of filtered bicarbonate. Although a 29% increase in filtered bicarbonate induces a significant stimulation of FEHCO$_3$ in control subjects, the same increase of bicarbonate load (29%) does not affect urinary bicarbonate excretion in CRF patients.

To better compare the response of individual tubules in health and CRF subjects, we factored the urinary data by the respective GFR and analyzed the data by two-way ANOVA and Bonferroni contrasts. The results are shown in Table 6. Bicarbonate excretion is larger after a meat meal in healthy

subjects (P < 0.01) but not in CRF patients. On the other hand, baseline bicarbonate excretion is not different in healthy *versus* CRF subjects.

TA per ml GFR was not different at baseline and after a meat load in both groups of subjects. However, it was markedly larger (P < 0.01) in CRF patients than in healthy subjects. The same holds for ammonium excretion. This could indicate a more rapid adaptation of renal bicarbonate handling as compared with TA and ammonium excretion in CRF patients.

Discussion

In this study, the administration of a meat meal induced an increase of 27% in GFR in healthy subjects and 32% in patients with CRF. We have previously found similar increases in GFR

Table 5. Tubular handling of bicarbonate at baseline and after meat meal in ten patients with chronic renal failure[a]

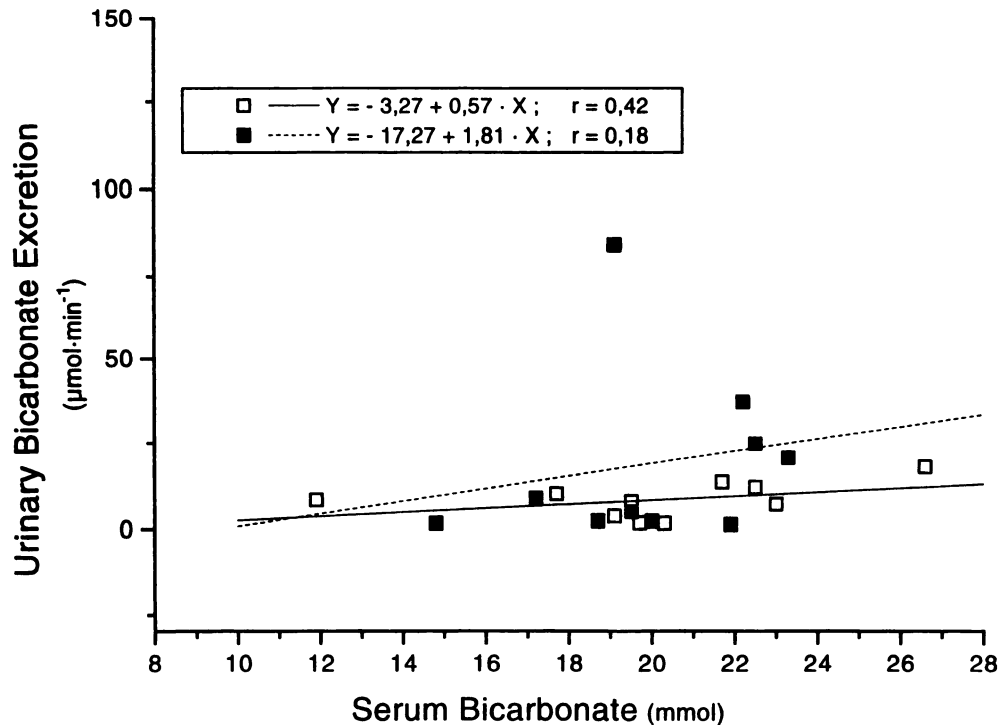| Parameter	Baseline	Peak	*P* Value
HCO_3^- filtered (μmol $\cdot$ min^{-1})	820 $\pm$ 157	1078 $\pm$ 199	<0.01
HCO_3^- excreted (μmol $\cdot$ min^{-1})	8.39 $\pm$ 1.65	18.73 $\pm$ 8.21	
HCO_3^- reabsorbed (μmol $\cdot$ min^{-1})	812 $\pm$ 156	1063 $\pm$ 197	<0.01
HCO_3^- fractional reabsorption (%)	98.20 $\pm$ 0.68	98.38 $\pm$ 0.49	
HCO_3^- fractional excretion (%)	1.81 $\pm$ 0.68	1.61 $\pm$ 0.47	

[a] Data are expressed as mean $\pm$ SEM.

Figure 2. Urinary bicarbonate excretion plotted as a function of serum bicarbonate in patients with chronic renal disease (IRC) under fasting conditions (baseline, open squares) and at the peak (closed squares) of the hyperfiltering renal response induced by a meat meal (2 g of protein per kg body wt).

(14), as have other investigators, under similar conditions (1,2). The aim of the study presented here was to investigate the role of this increase in GFR in the renal regulation of the acid-base balance. For the group of healthy subject, the overall acid-base status of the studied individuals was in the normal range, although their plasma bicarbonate level was in the lower range of normal, possibly because of the overnight fast preceding the study. In the CRF patients, however, the blood acid-base status indicates a tendency toward a mild metabolic acidosis, as expected in this pathological condition (19).

HCO_3^- Excretion

Among the mechanisms responsible for urine acidification, renal bicarbonate handling was most affected by the meat meal. Both absolute and fractional bicarbonate excretion rates are enhanced by the meat meal in healthy subjects (Figure 1 and 3), thus indicating that tubular H^+ secretion has likely increased. This enhancement is also apparent when bicarbonate excretion is expressed per ml GFR (Table 6). It may be related directly to the increased filtered load of bicarbonate found after the meat meal in these subjects, without a complete compensation by tubular reabsorption. On the other hand, it is interesting to compare these data with those obtained in CRF patients. First, the general level of absolute bicarbonate excretion is lower in CRF patients. However, when this excretion rate is expressed per ml GFR, urine bicarbonate is not significantly different in healthy and in CRF patients. The bicarbonate excretion rate did not increase significantly after the meat meal in the CRF group (Figure 2 and 3), as was observed in healthy subjects. This might be because of an enhanced rate of tubule H^+ secretion in these patients, possibly stimulated by the mild metabolic acidosis. In fact, several previous reports support this view (20). The reduced filtered load of bicarbonate found in CRF patients may participate in this phenomenon; nevertheless, when we correct the data per ml GFR, the increased bicarbonate excretion was also not apparent (Table 6).
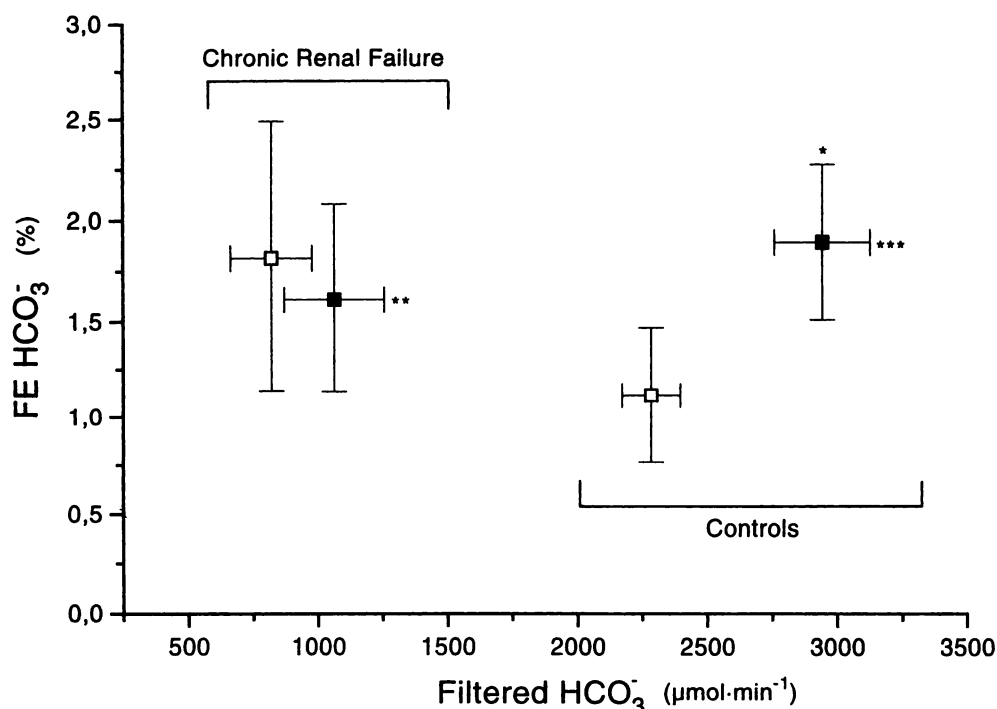
Figure 3. Fractional bicarbonate excretion *versus* filtered bicarbonate in patients with chronic renal failure and in control subjects under fasting conditions (baseline, open squares) and at the peak (closed squares) of the hyperfiltering renal response induced by a meat meal (2 g of protein per kg body wt). $*P < 0.05$; $**P < 0.01$; $***P < 005$ *versus* baseline.

Table 6. Renal acid-base excretion expressed per ml GFR after a meat meal in healthy subjects and in patients with chronic renal failure[a]

Parameter	Baseline	Peak	*P* Value
Healthy subjects			
HCO_3^- (μmol $\cdot$ min^{-1} GFR $\cdot$ min)	0.278 ± 0.008	0.459 ± 0.009	<0.01
TA (μmol $\cdot$ min^{-1} GFR $\cdot$ min)	0.164 ± 0.005	0.150 ± 0.002	
NH_4^+ (μmol $\cdot$ min^{-1} GFR $\cdot$ min)	0.456 ± 0.159	0.416 ± 0.115	
Chronic renal failure			
HCO_3^- (μmol $\cdot$ min^{-1} GFR $\cdot$ min)	0.309 ± 0.009	0.327 ± 0.010	
TA (μmol $\cdot$ min^{-1} GFR $\cdot$ min)	0.475 ± 0.188	0.421 ± 0.108	
NH_4^+ (μmol $\cdot$ min^{-1} GFR $\cdot$ min)	1.440 ± 0.601	1.180 ± 0.386	

[a] Data are expressed as mean $\pm$ SEM.

The urinary spillover of bicarbonate found in healthy individuals may be attributed to extracellular volume (ECV) expansion as a result of a bicarbonate load rather than to saturation of tubular bicarbonate reabsorption *per se* (21). Although no salt was added to the food and the hematocrit value did not change (14), it is still possible that the meat load may have induced a moderate degree of ECV expansion in the healthy subjects. In CRF patients, on the other hand, it is known that there is already a chronic state of moderate ECV expansion as a result of the reduction in the number of active nephrons (22). Therefore, the meat meal may not enhance the ECV expansion in these patients and thus may not affect bicarbonate excretion.

It is interesting to note that the renal tubule of the diseased kidney is still able to reabsorb the additional bicarbonate load

consequent to the increase in GFR after a protein load at a constant systemic acid-base balance (Figure 3). Such a mechanism will contribute to maintenance of an acid-base balance in equilibrium during CRF by avoiding an excess of urinary bicarbonate loss during physiological oscillations of GFR.

The increase in tubular bicarbonate reabsorption that we have shown both in control and CRF subjects after a meat meal could also be explained by the increased flow rate of tubular fluid as result of the increased filtered load. The flow-dependent stimulation of bicarbonate reabsorption is a well-described phenomenon that has been shown along the proximal tubule (23), the loop of Henle (24), and the distal tubule (25,26). This stimulation has been attributed to two possible mechanisms: (*1*) stimulation of an unsaturated bicarbonate
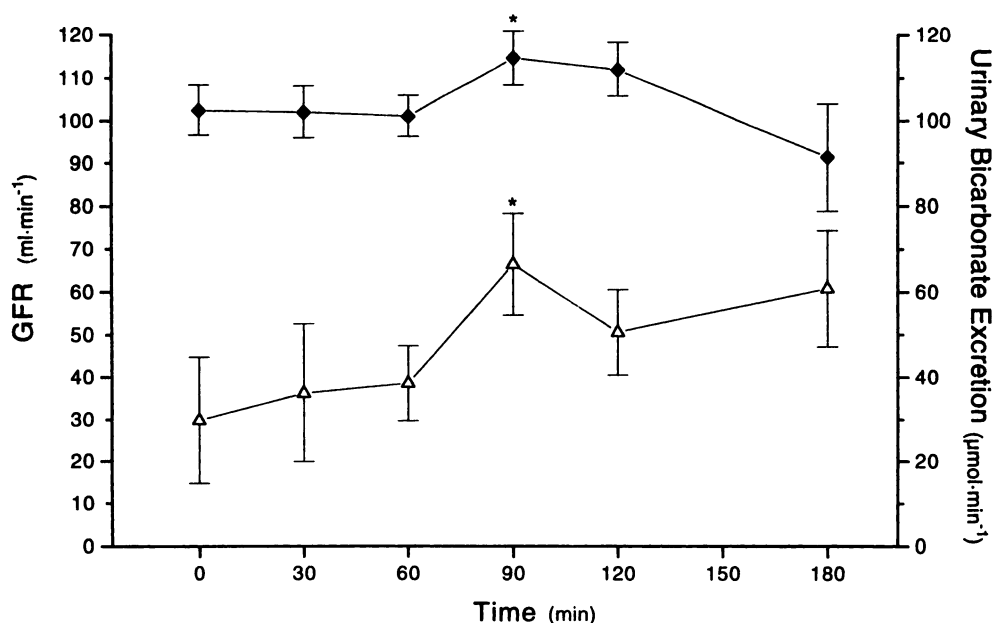
Figure 4. Time course of GFR (closed circles) and urinary bicarbonate excretion (open triangles) in healthy subjects under fasting conditions (baseline, time 0) and after a meat meal (2 g of protein per kg body wt). $*P < 0.05$ versus baseline (time 0).

transport system by an alteration of the luminal diffusion barrier; (2) attenuation of the fall of intraluminal bicarbonate concentration and, thus, stimulation of proton secretion.

TA and NH_4^+ Excretion

Under control conditions, the absolute TA excretion did not change after the protein load; this also happened in CRF. However, when the data are corrected for GFR, the excretion of TA increased in CRF as compared with control subjects; however, the protein load did not induce any change of TA. It was expected that TA would increase when the GFR was enhanced, because this alteration could induce a larger filtered buffer load. The reason why this was not found to be true in this study is not entirely clear. However, it is possible that the increased amount of buffers filtered after the meat load might not have been titrated because of the presence of a larger amount of bicarbonate in the urine in the healthy group and of the presence of more urinary NH_3/NH_4^+ in the CRF patients (Tables 4 and 5).

The pattern of NH_4^+ excretion is very similar to that of TA: NH_4^+ excretion, when expressed as an absolute amount, is not larger in CRF patients and is not increased after the protein load; however, when the data were adjusted for GFR, more NH_4^+ was excreted in CRF patients, as shown previously in long-term balance studies (27,28). The absence of urinary NH_4^+ alterations after a meat load in healthy and CRF subjects is probably a result of the short term of the experimental procedure. It has been reported that a study period of several days is necessary for the renal excretion of NH_4^+ to be affected (29).

Combining the data obtained in this work, we are able to calculate rates of net acid excretion in absolute values as N = TA + NH_4^+ − HCO_3^-. Note that baseline absolute values are

equal in healthy humans (32.5 μmol $\cdot$ min^{-1}) and in CRF patients (39.8 μmol $\cdot$ min^{-1}). However, the acute protein load significantly reduces the acid balance in healthy subjects (2.3 μmol $\cdot$ min^{-1}) but not in patients with renal disease (33.9 μmol $\cdot$ min^{-1}). This difference appears to be a result of the increased bicarbonate excretion in the healthy subjects. When the data are quantitatively expressed per ml GFR, highly similar results are obtained.

So far we have separately discussed the data on HCO_3^- reabsorption, NH_4^+ excretion, and TA formation after an OPL; however, the three processes are closely interrelated and can be explained sequentially. As we know, NH_4^+ excretion is dependent on luminal [H^+] and interstitial NH_4^+ accumulation. The decrease in luminal pH because of the increased HCO_3^- concentration after a meat meal should decrease NH_4^+ trapping in the tubular fluid. Because the data show that NH_4^+ urinary excretion is not affected by the OPL, it is justifiable to hypothesize that medullary interstitial NH_4^+ concentrations have increased. Following the same reasoning, the higher luminal pH will explain the lack of change in TA excretion even in the presence of increased filtered load of TA.

Alkaline Tide

The phenomenon of blood and urine alkalization after a meal (alkaline tide) was first described many years ago (30), and—although the data are not univocal—has been attributed by several authors to the stimulation of gastric acid secretion (31–34). It has been postulated that because the secretion of H^+ by the parietal cells of the gastric mucosa is stimulated by food ingestion, the cell preserves pHi by promoting bicarbonate extrusion through the basolateral membrane. The data presented in this study may possibly provide a new interpretation of this old observation, at least for the part that concerns

urine. The increase in urine bicarbonate excretion after a meat meal could be the consequence of the increased filtered load of bicarbonate, related to the increased GFR and associated with the inability by the renal tubule to counteract the GFR variation adequately. Indeed, Figure 4 shows that the peaks of bicarbonate excretion and GFR of healthy subjects were coincident when plotted *versus* time. Such an explanation will explain why the alkaline tide can be demonstrated only in the final urine sample and not in blood, and why it could be identified in urinary excretion of bicarbonate and not in urine pH. It should also be stressed that when individual data were analyzed, three subjects in ten showed an increase in bicarbonate excretion that preceded the activation of GFR. The finding that the alkaline tide was observed only in control subjects, although it was undetectable in patients with CRF, is probably dependent on the mild metabolic acidosis found in the latter group, which could have obscured the phenomenon by maximizing tubular H^+ secretion.

In conclusion, the data presented in this work demonstrate that the increase in tubular buffer load after a protein meal will induce adaptive changes in H^+ secretion/HCO_3^- reabsorption of tubule cells that contribute to maintenance of the systemic acid-base balance. In healthy subjects, the increased load induces a greater bicarbonate reabsorption but also an enlarged urine bicarbonate excretion, which might possibly be mediated by a moderate ECV expansion. In CRF patients, bicarbonate reabsorption increases without significant enhancement of bicarbonate excretion because the mild metabolic acidosis is expected to maximize H^+ secretion. As expected, TA and NH_4^+ excretion per ml GFR are increased in CRF patients; however, no effect of the meat load was disclosed in either healthy or CRF subjects.

Acknowledgments

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