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Dietary protein as a modulator of the renal net acid excretion capacity: Evidence that an increased protein intake improves the capability of the kidney to excrete ammonium

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To test the hypothesis that increments in protein intake increase the capacity of the kidney to excrete ammonium (NH_4^+), two strictly controlled diet studies (both repeated-measures design with n = 6 healthy adult subjects) were performed. In study I, comprising two 5-day diet periods (A and B, protein intake: 120 and 95 g/day, respectively), the urine pH was adjusted to a low pH level by administration of L-methionine (20 mmol/day) along with diet B. In study II, comprising three 4-day diet periods (C1–C3: C1, basic diet containing 50 g protein/day; C2, basic diet + 32 g of egg white protein/day; C3, basic diet + 10 mmol L-methionine/day), the urine pH was adjusted to a high pH level by sodium citrate (6.67 mmol/day) administered with diets C2 and C3. Urine pH constancy, which guarantees a constant tubular NH_4^+ transfer, was achieved for either study (diets A, B: 5.5 ± 0.2, 5.4 ± 0.2; diets C1, C2, C3: 6.7 ± 0.1, 6.8 ± 0.1, 6.7 ± 0.1), thus allowing assessment of urinary NH_4^+ excretion rates as an index of the renal NH_4^+ production capacity. With the higher protein intake on diet A compared with diet B, a significantly elevated NH_4^+ excretion (82.8 ± 11.4 vs. 73.6 ± 7.2 mmol/day; $P < 0.01$) was observed. NH_4^+ excretion also increased from 29.4 ± 5.5 mmol/day (C1) to 34.8 ± 5.1 mmol/day (C2) in study II ($P < 0.05$) after the protein intake was raised with egg protein. When methionine was administered instead of egg protein as a different source of sulfur (C3), no effect on renal NH_4^+ output occurred, thus demonstrating that protein as a whole has a specific impact. It is concluded that the renal capacity to excrete excess acidity as NH_4^+ is modulated (independently of the underlying level of the respective renal acid load) by the amount of protein ingested. (J. Nutr. Biochem. 6:431–437, 1995.)

Keywords: ammonium; protein intake; urine pH; renal net acid excretion; methionine; acid–base metabolism

Introduction

The kidney plays a central role in the regulation of acid–base metabolism. In response to acid loads, for example induced by an elevated protein intake, the kidney increases net acid excretion,[1,2] which means the urinary output of bicarbonate falls and that of ammonium (NH_4^+) and titratable acid (TA) rises. Since the major constituent of TA in the urine is phosphate,[3] an increase in "nonphosphorus" renal acid load (e.g., sulfate from protein degradation) must be primarily managed by an elevation in NH_4^+ output. Thus, as a well known fact, NH_4^+ excretion is clearly increased in the well-nourished state after an elevation of protein intake when no simultaneous rise in food-derived alkali load (especially from potassium-rich fruits and vegetables) occurs. Recent findings[4] in male bodybuilders and normal healthy controls on freely composed protein-rich diets suggested that the degree of stimulation of renal NH_4^+ excretion seems not to be exclusively determined by the

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Table 1 Experimental characteristics of study I and study II

Study	Diet	Target urine pH	Protein intake (g/day)	Enrichment or supplement	
				For diet modification	For pH adjustment*
I	A	<5.9	120		
	B	<5.9	95		L-methionine†
II	C1	>6.7	50		
	C2	>6.7	50 (+32)‡	Egg white protein‡	Na-citrate§
	C3	>6.7	50	L-methionine‖	Na-citrate§

*pH adjustment was performed by estimating the renal net acid excretion from nutrient intake data according to our calculation model[2] and by assigning this estimated net acid excretion to the corresponding pH values. Data on the relationship between pH and renal net acid excretion obtained from healthy adults are presented elsewhere.[5]
†20 mmol (equivalent to 40 mmol H^+).
‡32 g of egg white protein/day, isoenergetically exchanged with fat.
§6.67 mmol (equivalent to 20 mmol Na^+).
‖10 mmol (equivalent to 20 mmol H^+).

actual acid load that has to be eliminated via the kidney. Bodybuilders ingesting higher amounts of protein than controls revealed an increased renal NH_4^+ output without showing the clear decrease in urine pH, which is commonly observed when net acid (and NH_4^+) excretion is raised accordingly.[4]

The urine pH reflects the state of renal acid stimulation. Thus, the capacity to excrete net acid (determinable as the renal NH_4^+ output at a given urine pH) appears to be improved under a higher protein intake. To test and further substantiate this hypothesis, renal 24-hr NH_4^+ excretion was quantified along with additional metabolically relevant urine parameters in healthy adults under strictly controlled dietary conditions, i.e., in diet experiments with definite levels of protein intake and adjusted urine pH values. Short-term experiments (of 4 or 5 days length) were chosen since preliminary studies with constant daily acid loads (protein or methionine supplements) administered for 5 days revealed that the corresponding NAE increase is fully established within 3 days. The excretion of NH_4^+, TA, and bicarbonate (i.e., NAE) was no longer distinguishable between the experimental days 3, 4, and 5.*

Methods and materials

Subjects

Ten healthy adult volunteers were recruited from the members of the medical research staff of the research institute of child nutrition or from a group of students doing a period of practical training in the institute. Two separate diet studies (I and II, see below) with six participants each were performed. Two subjects participated in both studies. In study I three volunteers were females (24–25 years old) and three were males (31–49 years). In study II all subjects were males, aged 23–51 years. Except oral contraceptives (all females of study I), no medication was taken during either study (contraceptives are known not to interfere with the acid–base metabolism). No subject had a past medical history of renal, endocrine, or cardiovascular disease. The study protocols were approved by the institutional review board at the Forschungsinstitut für Kinderernährung Dortmund and written informed consent was obtained from all subjects after the experimental protocol was explained in detail.

Diet study I (higher protein intake): Adjustment of the urine pH to a low level

This study was initially carried out to investigate whether it is possible to estimate reliably the renal net acid excretion produced by different natural food diets. The results have been published recently.[2] The current paper presents additional hitherto unpublished findings on the relationship between protein intake, urine pH, and the acid load–induced NH_4^+ excretion. The present results were obtained from those two (out of four) separate dietary periods that provided the highest sulfur intake and yielded a similar net acid excretion.[2]

In brief, a high-protein natural food diet (diet A; energy, 8,439 kJ/70 kg/day; protein (intake identical for all subjects), 120 g/day; fruits and vegetables, 230 g/day) was fed for 5 days. This was followed by a 5-day period during which a moderate protein diet (diet B; energy, 8263 kJ/70 kg/day; protein, 95 g/day; fruits and vegetables, 700 g/day) was ingested along with 3.0 g (20 mmol) of L-methionine (Acimethin, Gry-Pharma, Kirchzarten, Germany). L-methionine was administered to adjust the urine pH to the greatest possible extent to the urine pH level attained under diet A (*Table 1*). Both dietary periods (A, B) were separated by a 23-day period consisting of two 9-day intervals without dietary restrictions; one of these 9-day periods preceded and the other followed a 5-day lacto vegetarian diet regimen[2] (not relevant in this context). The only beverage allowed was a mineral water with a very low mineral content (Volvic; Puy-De-Dome, France). This water was available ad libitum.

Diet study II (lower protein intake): Adjustment of the urine pH to a high level

All subjects took part in three separate, slightly modified dietary regimens (C1–C3) each lasting 4 days. C1 was a low-protein (lacto vegetarian) basic diet, (diet composition identical for all subjects; energy, 9,139.8 kJ/day; protein, 50 g/day; fat, 101 g/day; carbohydrate, 282 g/day; fruits and vegetables, 1740 g/day), C2 consisted of the low-protein basic diet in which 14.4 g of fat were isoenergetically replaced with 32 g of egg white protein to yield a

* Remer, T. and Manz, F., unpublished data.

total protein content of 82 g. C3 was the low-protein basic diet supplemented with 10 mmol L-methionine (Acimethin).

The study was a fully balanced crossover design with two subjects on regimen C1, two on C2, and two on C3 at each experimental period. A 3-day interval separated each experimental period to avoid carryover effects. During the dietary periods with diets C2 and C3, the urine pH was adjusted with daily doses of 6.67 mmol of trisodium citrate-dihydrate (E. Merck, Darmstadt, Germany; for foodstuffs; equivalent to 20 mmol Na^+, *Table 1*). Total daily amounts of sodium citrate were given in three equally divided doses of 66.7 ml each as a 1:10 (vol/vol) diluted (with deionized water) Shohl's solution.[6] The respective volumes of the preparation were drunk along with the second breakfast, with lunch, and with dinner (i.e., around the same time when the respective third of the daily egg white protein or the daily methionine portion was ingested).

As in study I, all meals were prepared and/or cooked by an experienced dietician. Apart from the early breakfast and the dinner (for which the food was weighed or measured into disposable containers for consumption at home) the second breakfast, lunch, and the afternoon meal were eaten together with all participants in the research institute of child nutrition. No dietary supplements were administered during the experimental diet periods. The only beverage allowed ad libitum was a special mineral water (Haltern Quelle; Blau Grün Quelle GmbH, Haltern, Germany; mineral content [mmol/L]: Na, 0.3; K, 0.02; Ca, 0.21; Mg, 0.09; Cl, 0.34; PO_4, 0.0; SO_4, 0.24). One cup of coffee of constant volume and constant composition was additionally ingested each morning.

Urine sampling, measurements, and statistical analysis

During the last 2 days of each diet period (study I and study II), timed 24-hr urine samples were collected and immediately stored below −20°C.

Immediately after thawing and thoroughly mixing the 24-hr urine samples, pH, TA, NH_4^+, and bicarbonate were measured as described by Lüthy et al.[7] Net acid excretion was calculated from the analytical data in the conventional manner as the sum of TA plus NH_4^+ concentration minus bicarbonate concentration. Aliquots of all 24-hr urine samples were stored (< −20°C) for subsequent analysis. Quantification of urea was carried out with a Beckman BUN Analyzer 2 (Beckman Instruments, Inc., Fullerton, CA, USA) utilizing the Beckman-developed enzymatic (urease-based) conductivity rate method. Phosphorus and sulfate were measured with a Dionex 2000 i/SP Ion-Chromatograph (Dionex GmbH, Idstein, Germany) with an IonPac AS4A column. Creatinine measurements were carried out with a Beckman-2 creatinine analyzer (Beckman Instruments, Inc.).

All results are reported as the mean ± SD. Differences between means were tested by the paired Student's *t*-test (Study I: diet A vs. diet B). A repeated-measures analysis of variance approach (one-way ANOVA) was applied to study the effects of the diets on each of the measurement variables in study II. When the *F* value of the ANOVA was significant, comparisons between the means of paired observations were evaluated by linear contrasts. The statistical procedures were conducted with the SPSS/PC$^+$ software package (version 4.0, SPSS Inc, Chicago, IL, USA). Results were regarded to be statistically significant when $P < 0.05$.

Results

The effects of the diets A and B (study I: higher protein intake) and of the diets C1–C3 (study II: lower protein intake) on 24-hr urine pH and on the renal excretion of urea, phosphorus, and sulfate are shown in *Figure 1*. In both studies the scheduled pH adjustments to the respective target values of either <5.9 or >6.7 succeeded. However, for study II, ANOVA revealed a significant effect of diet on urine pH ($P < 0.05$ for the *F* value of the ANOVA). Evaluation by linear contrasts indicated that the urine pH was slightly, but significantly ($P < 0.05$) elevated on C2 compared with C3 (*Figure 1*). As expected urea as well as sulfate excretion was highest on A (study I) and on C2 (study II) due to the fact that these diet regimens provided the highest protein (and consequently sulfur) intake of either study. Renal urea output on C1 and C3 was almost identical as was phosphorus excretion with C1, C2, C3, and B.

Table 2 shows that urinary creatinine and bicarbonate excretion did not differ significantly between the diets of either study. In study I bicarbonate excretion was even undetectable with both diets due to their high renal acid loads. Renal excretion of TA and net acid (NAE) differed ($P < 0.05$) between diets A and B in study I (*Table 2*) but was comparable with all diets of study II.

The individual responses of renal NH_4^+ excretion to the variations in protein intake are presented in *Figure 2*. With the lower protein intake on B compared with A (study I) a clear decrease in NH_4^+ excretion was observed. The mean difference (ΔNH_4^+) was 9.2 mmol/day and thus accounted for 40% of the total NAE difference between diets A and B (*Table 2*). The other 60% (of the NAE difference) is explainable by the higher phosphorus-dependent TA excretion on diet A (compare renal TA [phosphorus] output on A vs. B; *Table 2* and *Figure 1*).

In accordance with the results on renal NH_4^+ output obtained in study I the excretion of NH_4^+ increased significantly following the elevation of protein intake (with egg white protein) from the relatively low daily intake of 50 g of protein (C1) to 82 g (C2). However, the NH_4^+ increase (ΔNH_4^+ >5 mmol/day) occurred despite the fact that the NAE was kept constant (*Table 2*). When methionine was given instead of egg white protein NH_4^+ excretion was found nearly unchanged, i.e., at the base line level of diet C1, in all but one participant (who was obviously an outlier; *Figure 2*).

Discussion

Renal ammoniagenesis is stimulated as a physiological response to maintain acid–base balance, when an increasing amount of nonvolatile acids (such as sulfate or phosphate) must be excreted. In consequence, on the diets with the lowest alkali intake (diet A; potassium intake only ≈ 45 mmol/day) or the highest sulfur intake (diet B; see sulfate excretion, shown in *Figure 1*) the overall level of renal NH_4^+ output was elevated in comparison to C1, C2, and C3 (*Figure 2*). This elevated NH_4^+ excretion level was associated with a low urine pH (*Figure 1;* A, B, vs. C1–C3). In this respect our results bear no substantial new information since the rate of NH_4^+ excretion is usually higher at a lower urine pH. The urine pH reflects renal excretion of free hydrogen ions and thus mirrors the intracellular H^+ concentration of tubular cells. The later is probably one of the most important signals for a selective augmentation of renal ammoniagenesis.[3] After the tubular cells have secreted H^+ ions into the collecting duct, their con-

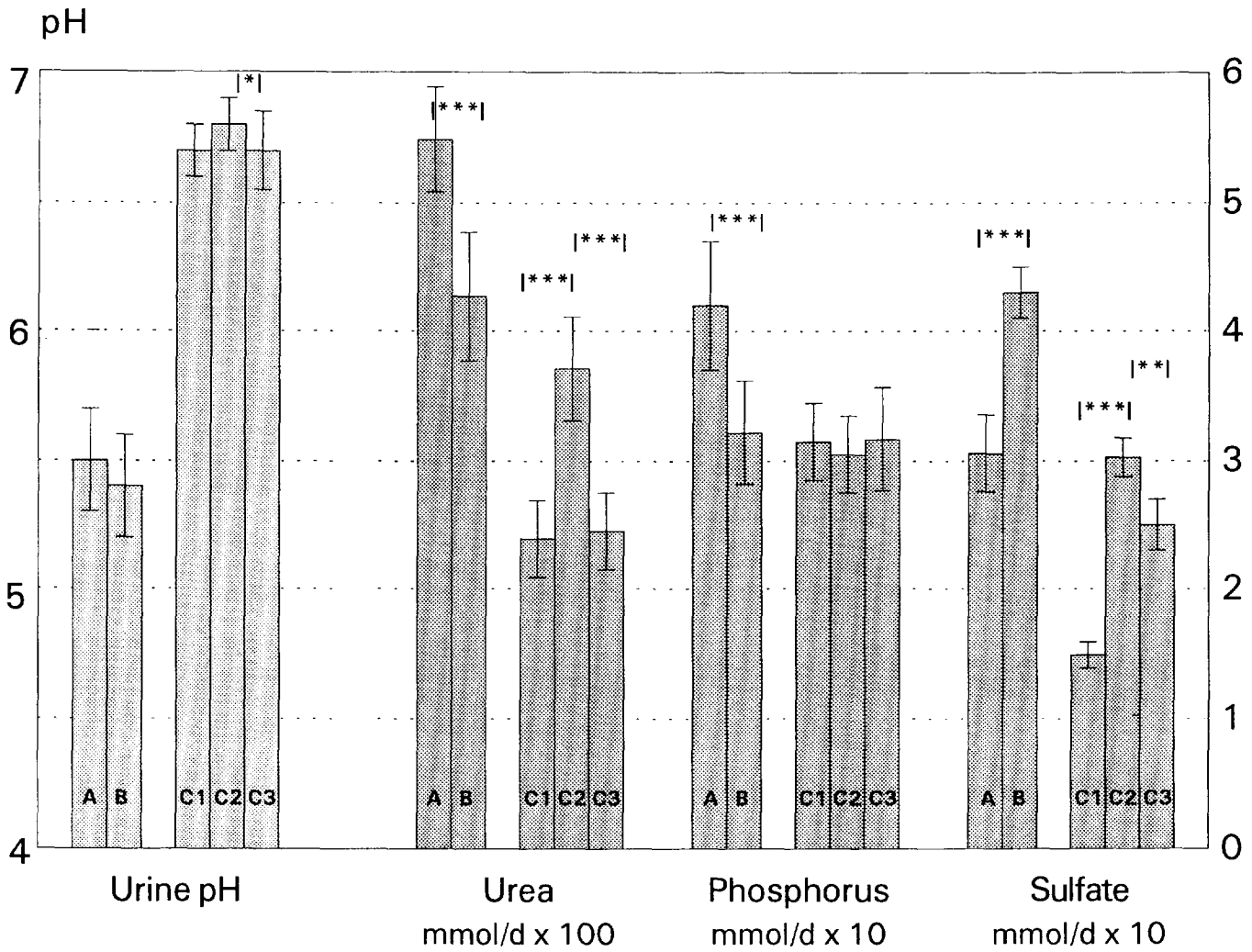

Figure 1 Urine pH and renal excretion of urea, phosphorus, and sulfate with the diets A and B (study I) and the diets C1–C3 (study II). Values shown are average daily renal excretion values; these were calculated for each subject from the urinary output of two 24-hr specimens collected successively over the last 48 hr of each diet period. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

centration determines the amount of NH_3 that is transported by nonionic diffusion from the peritubular tissue to the collecting tubules.[8] The lower the urine pH, the higher will be this nonionic diffusion.

For these reasons it is essential to adjust the urine pH. This adjustment eliminates variations of the major stimulus for the medullary NH_3 transfer process toward the renal collecting duct. When the medullary NH_3 transfer is kept

Table 2 Renal excretion* of creatinine, titratable acid, bicarbonate, and net acid with study I and study II as well as increases of ammonium excretion† in response to the respective highest protein intake of either study

Study	Diet	Creatinine (mmol/day)	TA (mEq/day)	Bicarbonate (mmol/day)	NAE (mEq/day)	Δ-ammonium†
I	A	13.7 ± 2.31	52.7 ± 7.36‡	0.0	135.5 ± 16.4‡	9.2 ± 4.68
	B	13.3 ± 2.15	39.0 ± 5.71	0.0	112.6 ± 10.9	
II	C1	14.2 ± 1.19	15.3 ± 3.31	27.8 ± 9.48	16.9 ± 12.6	5.3 ± 1.51
	C2	13.9 ± 1.14	14.1 ± 3.03	31.2 ± 6.87	17.7 ± 12.4	5.7 ± 3.64
	C3	14.0 ± 1.34	15.9 ± 3.88	28.3 ± 11.0	18.6 ± 16.0	

Values are ± SD of average daily renal excretion values; these were calculated for each subject from the urinary output of two 24-hr specimens collected successively over the last 48 hr of each diet period.
†Mean increase (±SD) of NH_4 excretion with diet A versus diet B, or diet C2 versus C1, or diet C2 versus C3.
‡$P < 0.05$ versus diet B.

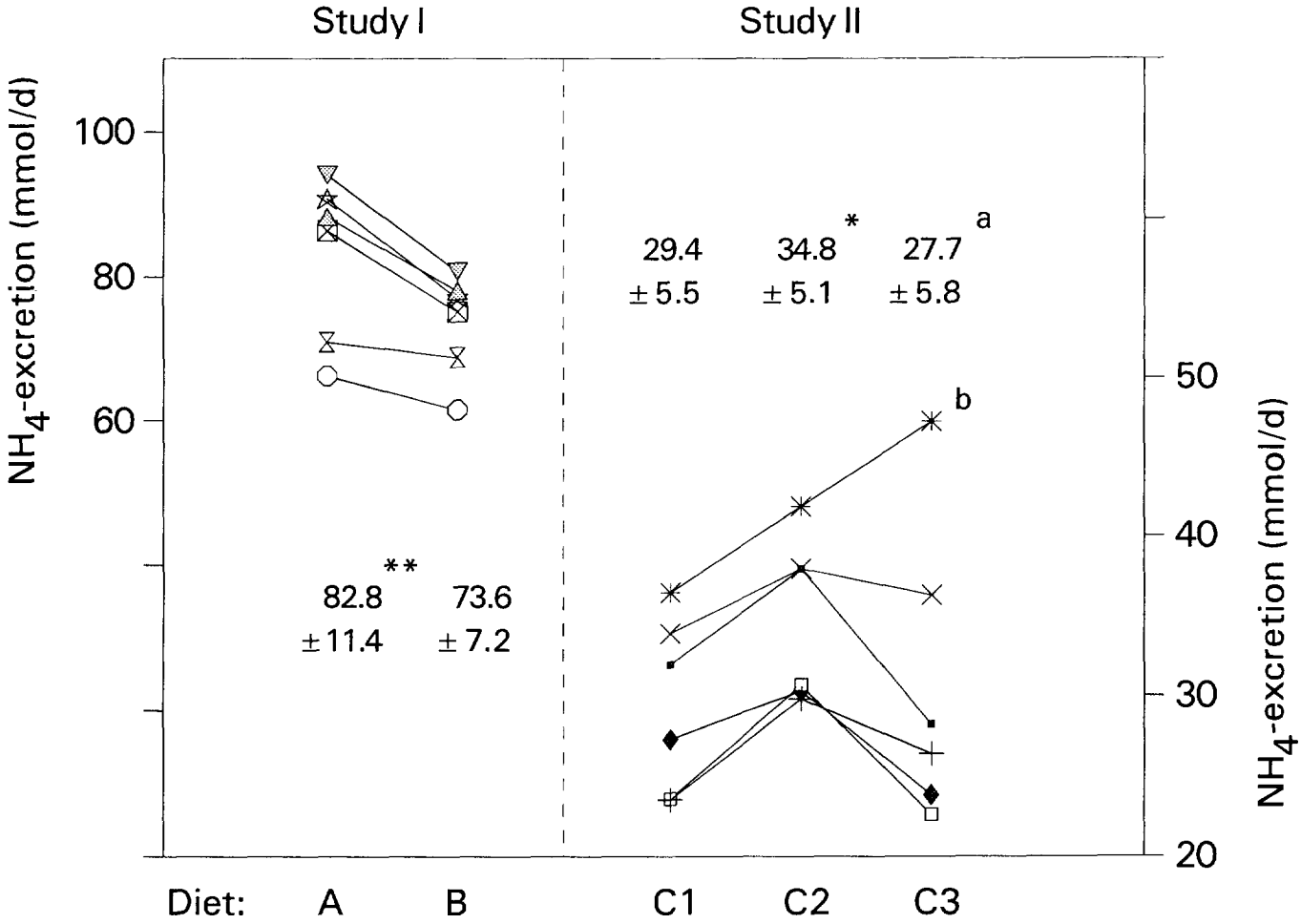

Figure 2 Individual renal NH_4^+ excretion with each diet of study I and study II (values shown are average daily renal excretion values, see legend of *Figure 1*). [a]$\bar{x}$ ± SD of n = 5 subjects (without the outlier[b]); NH_4^+ excretion for all n = 6 subjects was: 30.9 ± 9.5. [b]This subject (on diet C3) is regarded as an outlier and his NH_4^+ excretion value is not included in $\bar{x}$ ± SD[a] given in the figure. *$P < 0.05$ vs. diet C1 and vs. diet C3; **$P < 0.01$.

constant, the urinary NH_4^+ excretion rates are an index of NH_4^+ production. In other words, when no definite acid loading tests are performed, the capacity to excrete NH_4^+ can only be determined reliably for a given (constant) urine pH level. The latter has been obtained with reasonable success with diets A and B (low pH level of study I) and with the diets C1, C2, and C3 (high pH level of study II). This again confirms that our calculation model proposed for the prediction of the renal net acid excretion[2,5] and used for the present pH setting works adequately.

The minimally but significantly elevated mean urine pH on C2 (*Figure 1*) does by no means limit the interpretation of the NH_4^+ excretion data. On the contrary, it further substantiates the pecularity of the present findings, because usually an increase in urine pH is associated with a decreased NH_4^+ excretion rate and a decreased net acid excretion. However, in response to the elevated protein intake on C2, NH_4^+ excretion increased in parallel with the urine pH. This means that even a stronger rise in the NH_4^+ excretion capacity did occur than would have been present with the same NH_4^+ excretion values but without that slight urine pH elevation. Thus the data indicate a protein-induced stimulation of NH_4^+ production even on the basis of a reduced stimulus for the medullary NH_3 transfer to the final urine.

The constant net acid excretion found during all three diet periods of study II indicates that renal ammoniagenesis (ΔNH_4^+: 5.3 or 5.7 mmol/day; *Table 2*) must have been augmented specifically by the addition of egg white protein to the basic diet. Apart from the constant net acid excretion there are two additional reasons to assume a specific effect of protein on renal NH_4^+ output (when consuming diet C2). First, egg white protein has an extremely low phosphorus content, as was confirmed by virtually equal phosphorus excretion rates on C1, C2, and C3 (*Figure 1*). Consequently, possible confounding effects on NH_4^+ excretion due to changes in TA can be excluded (TA was constant; *Table 2*). Second, administration of methionine, i.e., of the major source of sulfate in protein, did not reproduce the stimulating effect of C2 on NH_4^+ excretion.

Corresponding findings turned out for the diet experiments of study I, covering an overall higher protein intake than the experiments of study II. The higher protein intake of diet A compared with diet B was associated with an elevated renal NH_4^+ output despite a constant signal (i.e., a constant urine pH) for the augmentation of NH_4^+ transfer.

In consequence, the renal NH_4^+ excretion capacity (determined as the renal NH_4^+ output for a given urine pH) is improved by a rise in daily protein intake. Without this improvement, the same NH_4^+ increment (mandatory for the elimination of the excess acid load of diet A) could have only been attained after a decrease in urine pH (i.e., after a higher stimulus for the medullary NH_3 transfer to the collecting duct). However, that would reduce the remaining renal capacity for the elimination of additional acid loads.

A rise in *maximum* renal NH_4^+ excretion has also been observed in adult patients with moderate to severe protein malnutrition after adequate protein repletion.[9] The authors suggested that decreased renal gluconeogenesis or decreased substrate delivery could be the underlying cause for the reduced renal NH_4^+ output in the protein-deficient state. In fact, an increase in NH_4^+ production has been observed in dogs after their glutamine levels had been raised by glutamine infusion.[10] As proposed by Williams et al.,[11] the high renal ammoniagenesis being present during sustained metabolic acidosis could be fueled directly by a catabolism-induced increase in the release of glutamine from skeletal muscle.

On the other hand there is strong evidence from diet studies in healthy males that dietary protein intake varies inversely with plasma glutamate and glutamine concentrations,[12] thus indicating that the modulating effect of protein intake on renal NH_4^+ production is probably not mediated by changes in plasma glutamine concentrations. We favor the idea that the clear effect of protein intake on the renal NH_4^+ excretion capacity could be induced by variations in the glomerular filtration rate (GFR) because increases in protein intake are known to stimulate the GFR[13] and the latter is directly associated with one of the most important energy-consuming processes in the kidney: the rate of tubular sodium reabsorption. This view is in full agreement with the concept of Carlisle et al.[8] who suggested that a reduced GFR is associated with a decreased capacity for the production of NH_4^+ due to a lowered metabolism of the important renal energy fuel, glutamine.

When the renal energy requirements are increased (for example, for an elevated sodium reabsorption after an increase in the GFR) and glutamine is metabolized at a higher rate, more NH_4^+ ions will be liberated in the respective tubular cells. Thus, renal NH_4^+ production appears to be in part dependent on the energy demands of the kidney. Definitely no such mechanism related to the renal energy metabolism exists for creatinine. The daily (nonenzymatically) produced total amount of creatinine mainly affected by the individual muscle mass is not influenced by a mere increase in the GFR. Increases of the latter would only result in temporary increments of creatinine excretion. These would subsequently be compensated for by appropriately reduced excretion rates (due to lowered serum creatinine values) leaving total daily creatinine output constant. This creatinine constancy, as confirmed in each of our studies, again emphasizes the specificity of the observed NH_4^+ increases occurring without the usual fall in the urine pH.

The present experimental results also confirm our findings in bodybuilders on self-selected diets,[4] and in addition, the conclusions drawn allow the explanation of earlier hitherto unexplained observations.[1] Lennon et al.[1] found an essentially unchanged 24-hr urine pH in adult volunteers who increased their renal net acid excretion markedly by a raised intake of soy protein or meat.

In summary, the present findings from strictly controlled diet experiments strongly suggest that in healthy subjects the final degree of the renal capacity to excrete NH_4^+ (and consequently to excrete net acid) will be modulated by the amount of protein ingested. This protein effect is obviously not limited to a certain status of "renal acid stimulation," rather it can be seen at high as well as at low 24-hr urine pH levels. The diet experiments of study II have especially clearly demonstrated that ammoniagenesis can be increased specifically, i.e., independently of the renal acid load, by dietary protein. Consequently, our data provide biochemical evidence that humans as typical omnivores have developed a separate diet-related adaptation mechanism during their phylogenesis to cope with the variations in renal acid loading caused by (partly extreme) daily or seasonal fluctuations of protein intakes. This mechanism allows the kidney to meet acid–base demands more efficiently and thus leaves a renal surplus capacity for the elimination of additional acid loads.

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