A comparison of the effects of feeding sulfur amino acids and protein on urine calcium in man[1-3]

Geoffrey D. Block,[4] M.S., Richard J. Wood,[5] M.S., and Lindsay H. Allen,[6] Ph.D., R.D.

ABSTRACT It has been suggested that the sulfur amino acids in protein are responsible for the calciuria observed after protein ingestion. This hypothesis was tested by feeding meals containing either 15 g protein (control), 45 g protein (high protein), or 15 g protein plus sulfur amino acids equivalent to those in the high protein diet. Compared to the control, the high protein diet caused an increase in urinary calcium and sulfate and a decrease in the renal reabsorption of calcium. In contrast, the sulfur amino acid supplement had no effect on calcium excretion or reabsorption. Net acid excretion was unaffected by dietary treatment. *Am. J. Clin. Nutr.* 33: 2128–2136, 1980.

The consumption of dietary protein and amino acids is followed by a rapid increase in the excretion of urinary calcium (1–3). We have recently shown that this occurs because protein consumption causes a decrease in the percentage of filtered calcium that is reabsorbed by the kidney (4).

TABLE 1
Composition of diets

Ingredient	Control	Control plus sulfur amino acids	High protein
		g/meal	
Protein[a]	15.0	15.0	45.0
Sucrose[b]	20.0	20.0	20.0
Lactose[c]	0.172	0.172	0
Safflower Oil[d]	21.0	21.0	7.0
$CaCl_2 \cdot 2H_2O$[e]	0.549	0.549	0
H_3PO_4[c]	1.066	1.066	0
NaCl[e]	0.628	0.628	0.614
KCl[c]	1.052	1.052	0
MgO[e]	0.116	0.116	0.101
Zn acetate[e]	0.009	0.009	0
L(−)-Cystine[c]	0	0.429	0
L(−)-Methionine[f]	0	0.662	0

[a] Crest Foods Co., Inc., Ashton, Ill. [b] Amstar Corp; American Sugar Division, New York, N.Y. [c] Fisher Scientific Co., Fairlawn, N.J. [d] Hollywood Health Foods, Los Angeles, Calif. [e] J. T. Baker Chemical Co., Phillipsburg, N.J. [f] Eastman Kodak Co., Rochester, N.Y.

Several investigators have suggested that the sulfur amino acids in the protein might be responsible for the calciuria. Whiting and Draper (5) found that the calciuric effects of a variety of proteins fed to rats were in the same order as their sulfur amino acid content, i.e., lactalbumin > egg white > casein > gelatin. Supplementation of their control (18% casein) diet with methionine and cystine to levels present in a lactalbumin supplement caused a calciuric response that was less than that caused by the lactalbumin alone during the first 5 days, but similar thereafter. In a second experiment the same investigators showed that a high protein diet fed to rats caused a 176% increase in urine calcium compared to a 29% increase when the diets were supplemented with sulfur amino acids equivalent to those in the high protein diet (6).

Using human subjects in a 51 day metabolic study, Hegsted et al. (7) fed diets containing 8 g N, 24 g N, and 8 g N plus sulfur amino acids equivalent to those in the 24 g N diet. The added sulfur amino acids caused an increase in urinary calcium and a decrease in

[1] From the Department of Nutritional Sciences, University of Connecticut, Storrs, Connecticut 06268.

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[4] In partial fulfillment of M.S. degree. Present address: School of Public Health, University of North Carolina, Chapel Hill, North Carolina 27514. [5] Research Assistant. [6] Associate Professor of Nutritional Sciences. Author to whom requests for reprints should be addressed.

calcium balance, but not to the same extent as the high protein intake. Lemann et al. (8) found that urinary calcium increased rapidly from control values of 1 to 3 to 4 to 7 mEq/day when a 13.9 g supplement of DL-methionine was fed to three males for 5 days (8). In contrast, the data of Margen et al. (1) showed no relationship between urinary calcium and the sulfur amino acid content of various amino acid mixtures fed to humans.

All of the above studies were conducted over periods of at least several days in duration. Some metabolic adjustments to a high protein intake would occur over this time. However, humans do not typically consume meals with a consistently high protein content. In the experiment described here we have followed a similar procedure to that used previously in our laboratory, where the events following consumption of a single meal can be evaluated (4).

Materials and methods

The subjects were six female, and six male volunteers from 20 to 42 years of age. All subjects described themselves as healthy. Three diets were formulated to provide

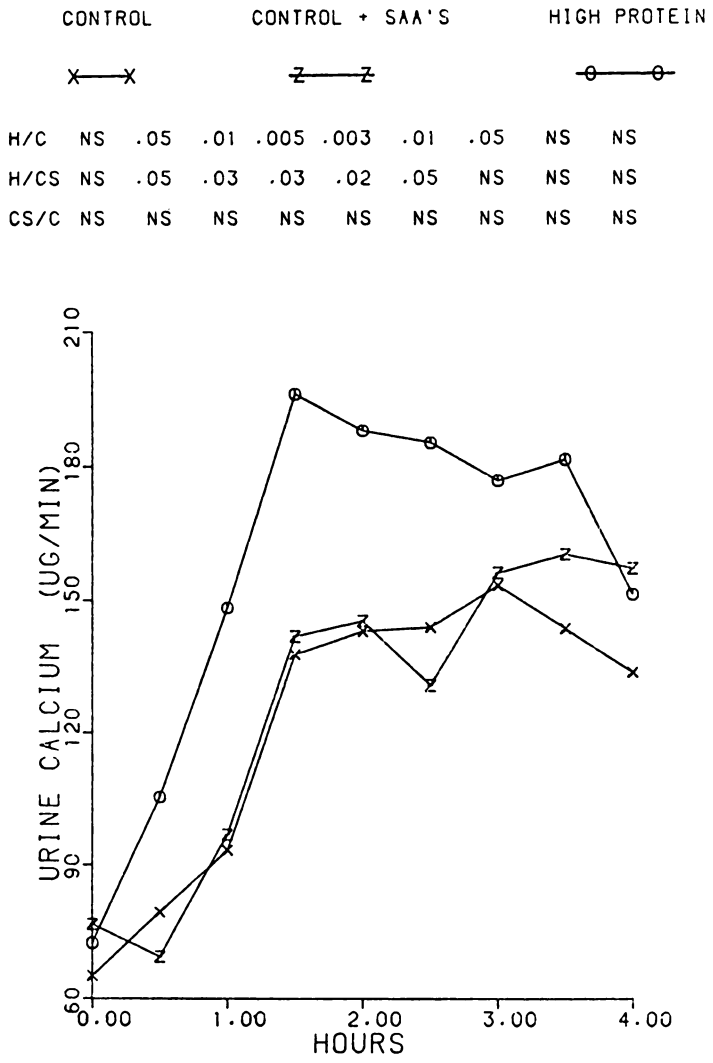

FIG. 1. Urinary excretion of calcium after consumption of meals containing 15 g protein (C, X—X), 15 g protein plus sulfur amino acids (SAA's) (CS, Z—Z), or 45 g protein (HP, O—O).

on a per meal basis either 15 g protein (control, C), 45 g protein (high protein, HP), or 15 g protein plus sulfur amino acids (CS) equivalent to those in the high protein diet. The meals were made isocaloric by adjusting the fat content.

The composition of the diets is shown in **Table 1**. The protein source was a potassium coprecipitate of bovine milk. After chemical analysis of the protein source, all meals were formulated to contain 25% of the Recommended Dietary Allowance for calcium, phosphorus, magnesium, and zinc, and similar amounts of sodium, potassium, sucrose, and lactose. After the dry ingredients were mixed, aliquots were wet-ashed (9) and analyzed for calcium (10), phosphorus (11), magnesium (12), zinc (13), nitrogen (14), and sodium and potassium by flame photometry (Corning Flame Photometer Model 450, Corning Instruments Div., Corning, N.Y.). The cysteine and methionine content was measured by amino acid liquid chromatography after acid hydrolysis (15). All measured values were within ± 5% of calculated values (Table 1). The dry ingredients for each meal were blended with water to a final volume of 350 ml.

Each subject was asked to consume foods from a standardized menu on the day prior to the experiment. They then fasted from 1930 hr until the next morning, when they emptied their bladders, recorded the time of urination, and drank 300 ml water. At 0845 hr, fasting blood and urine samples were obtained, followed by ingestion of a meal at 0900 hr. A steady diuresis was maintained by having each subject ingest 200 ml distilled water immediately after the meal and every 30 min for the next 4 hr. Blood samples were drawn at 0.5, 1.0, 1.5,

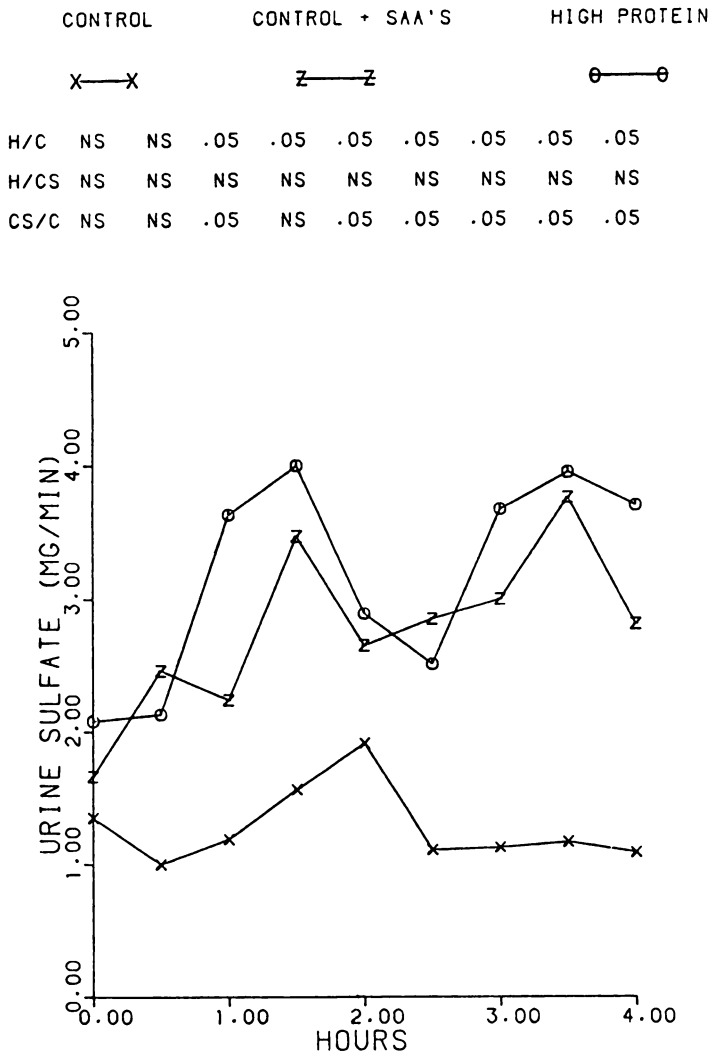

FIG. 2. Urinary excretion of sulfate after consumption of meals containing 15 g protein (C, X—X), 15 g protein plus sulfur amino acids (SAA's) (CS, Z—Z), or 45 g protein (HP, O—O).

2.5, and 3.5 hr, and urine samples were collected at every 0.5 hr after meal ingestion. Each subject consumed the three meals on separate occasions, in a random sequence, with 1 week elapsing between meals.

Urine volume was determined, and aliquots taken for immediate measurement of ionic calcium with an ion specific electrode (Orion Calcium Ion Electrode Model 93-20, Orion Research, Inc., Cambridge, Mass.), pH, titratable acid and ammonium ion after the removal of bicarbonate (16). On the same day, analysis was made for total calcium (17), creatinine (18), and sulfate (19). Serum was analyzed on the day of the experiment for total calcium (17), filterable calcium (20), and creatinine (18), using an autoanalyzer.

The paired *t* test (21) was used to compare HP versus C (HP/C), HP versus CS (HP/CS), and CS versus C (CS/C) for all parameters measured. Each subject served as his or her own control. Levels of significance are shown at the *top* of each figure.

Results

Urine flow rate increased rapidly from pre-meal values of 1.5 ml/min to stabilize at approximately 9 ml/min by 1 hr after a meal. There was no significant effect of dietary treatment on the rate of urine production.

Urinary calcium excretion data are shown in **Figure 1**. The excretion of calcium was increased by consuming all meals, and when

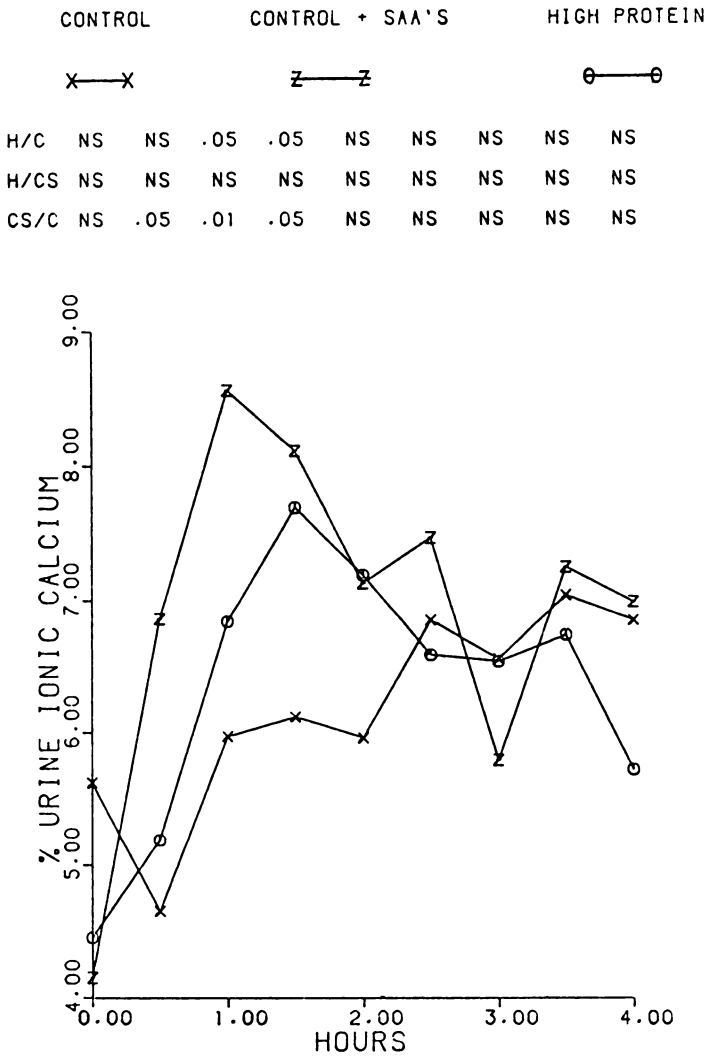

FIG. 3. Percentage of urine calcium in the ionic form after consumption of meals containing 15 g protein (C, X—X) 15 g protein plus sulfur amino acids (SAA's) (CS, Z—Z), or 45 g protein (HP, O—O).

compared to the control diet it was significantly higher between 0.5 and 3.0 hr after the high protein meal. A comparison of the C with the CS diet showed that ingestion of the added sulfur amino acids had no significant effect on urinary calcium excretion.

Compared to the control, the urinary excretion of sulfate was significantly increased between 1.0 and 4.0 hr after the HP and CS diets (**Fig. 2**).

The amount of calcium in the ionic form was measured in order to evaluate the effect of each diet on the ratio of complexed to ionic calcium in urine. As shown in **Figure 3**, consumption of either the CS of the HP diets resulted in a higher percentage of calcium being excreted in the ionic form between 0.5 and 1.5 hr.

Neither the total nor the filterable (**Fig. 4**) calcium in serum was significantly affected by dietary treatment. Glomerular filtration rate (creatinine clearance) was also unaffected by diet (**Fig. 5**). The product of serum filterable calcium and glomerular filtration rate was calculated to provide an estimate of the amount of calcium per minute filtered by

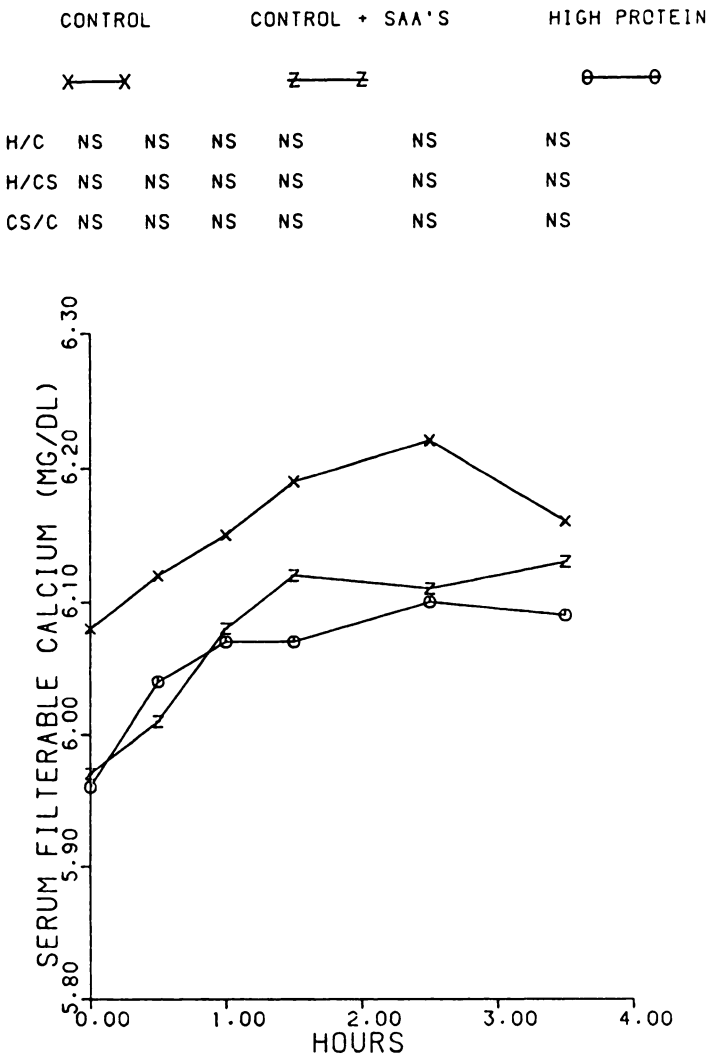

FIG. 4. Serum filterable calcium after consumption of meals containing 15 g protein (C, X—X), 15 g protein plus sulfur amino acids (SAA's) (CS, Z—Z), or 45 g protein (HP, O—O).

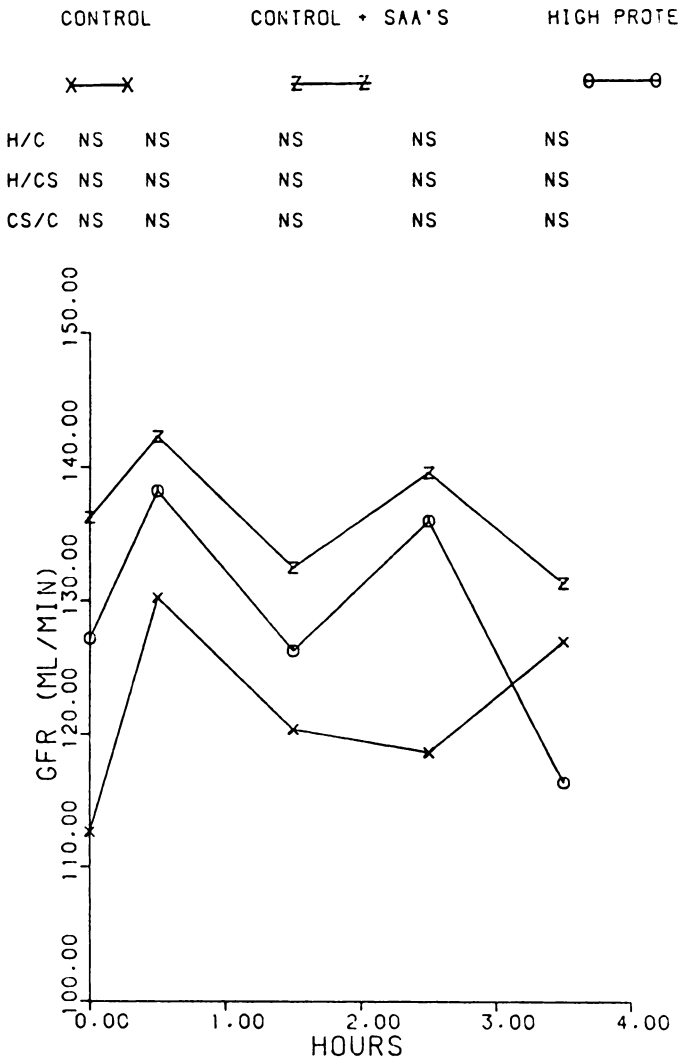

FIG. 5. Glomerular filtration rate after consumption of meals containing 15 g protein, (C, X—X), 15 g protein plus sulfur amino acids (SAA's) (CS, Z—Z), or 45 g protein (HP, O—O).

the kidney: the amount of filtered calcium was the same after three diets. The percentage of filtered calcium that was reabsorbed by the kidney was calculated as (filtered calcium minus urinary calcium divided by filtered calcium) in each time period. As shown in **Figure 6**, compared to the control, the HP diet caused a significant reduction (between 0.5 and 1.5 hr) in the percentage of filtered calcium which was reabsorbed, whereas the CS diet had no significant effect on this parameter.

Net renal acid excretion (**Fig. 7**) calculated from the sum of titratable acid plus ammonium excretion in each time period was highest at 1.0 hr after the HP meal, but due to a large variance in this parameter diet had no significant effect. Neither titratable acid, pH, nor ammonium was significantly higher after the CS or HP when compared to the control diet.

Discussion

By measuring urinary calcium excretion postprandially, we have confirmed our previous observation that consumption of dietary protein reduces the renal fractional reab-

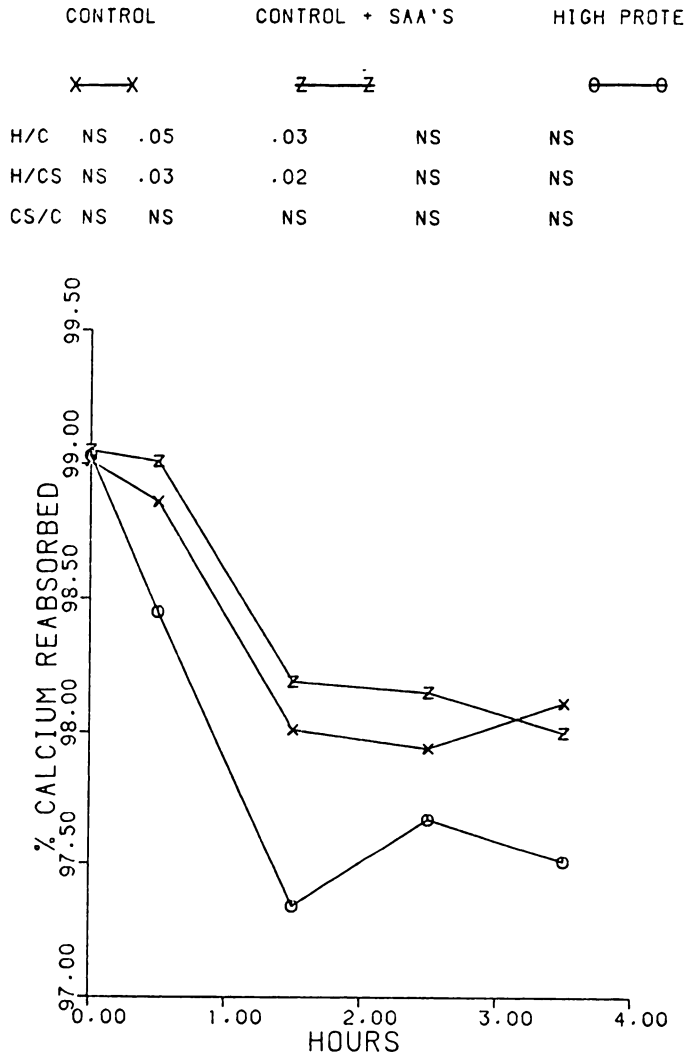

FIG. 6. Percentage of filtered calcium reabsorbed after consumption of meals containing 15 g protein (C, X—X), 15 g protein plus sulfur amino acids (SAA's) (CS, Z—Z) or 45 g protein (HP, O—O).

sorption of calcium. In contrast, consumption of sulfur amino acids did not affect the excretion of calcium, or its reabsorption by the kidney.

The sulfur in amino acids is oxidized during metabolism, and the sulfur is primarily excreted as inorganic sulfate in urine (8). On average, approximately 12% of urine calcium is bound to sulfate (22). It is not clear to what extent the complexing of calcium with anions, such as sulfate, affects the renal reabsorption of calcium. However, in general it is assumed that reabsorption of complexes is less than that of ionized calcium (23). In the experiment reported here, the maximum reduction in the renal reabsorption of calcium occurred at 1.5 hr after protein ingestion, and at this time point, urinary sulfate was highest. However, although urinary sulfate was increased to the same level by the HP and CS diets, calcium reabsorption was only reduced by the HP diet. We therefore conclude that calcium reabsorption was unaffected by urinary sulfate level.

Metabolic acidosis, induced by ammonium chloride feeding, is known to inhibit calcium reabsorption in the distal nephron (24, 25). Consumption of sulfur amino acids might cause a metabolic acidosis, since two equivalents of hydrogen ion are produced per mole

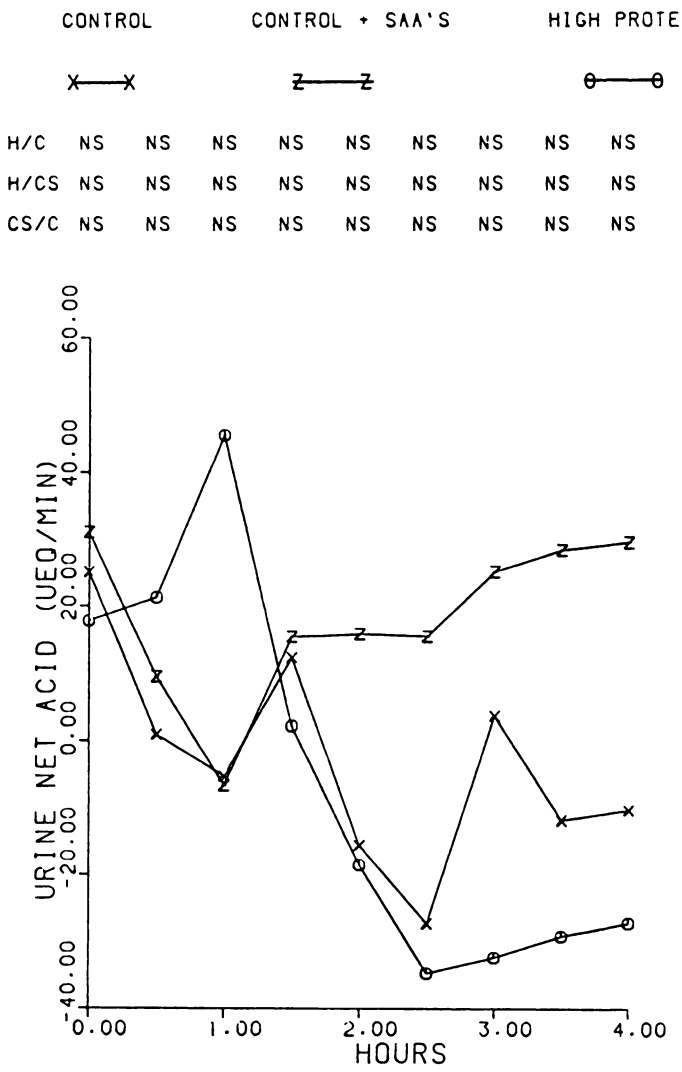

FIG. 7. Urinary net acid excretion after consumption of meals containing 15 g protein (C, X—X), 15 g protein plus sulfur amino acids (SAA's) (CS, Z—Z) or 45 g protein (HP, O—O).

of oxidized sulfur when sulfur amino acids are metabolized. In an experiment reported by Lemann et al. (8), who fed a large amount of DL-methionine (14 g/day) to adult males, there was a decrease in serum carbon dioxide, and an increase in urinary calcium, inorganic sulfate, net acid, and ammonium ion excretion within 24 hr. The excretion of net acid and ammonium ions increased steadily for several days during methionine feeding. In contrast, in our experiment we found that the sulfur amino acid content of the diet had no significant effect on urinary pH, titratable acid, ammonium ion, or net acid excretion. If our subjects had consumed four meals per day, the total amount of sulfur amino acids from the HP or CS diets would have only been one-fourth of that ingested by Lemann's subjects. Our inability to demonstrate a significant effect of the sulfur amino acids on acid-base balance may have been due to the smaller amounts of amino acids ingested by our subjects, and the relatively brief experimental period. However, our experimental procedure was designed to evaluate the effects of an amount of protein or sulfur amino acids which might realistically be consumed in a single meal.

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