Osteoporos Int (2007) 18:955–961
DOI 10.1007/s00198-006-0320-x

ORIGINAL ARTICLE

# Comparative effects of oral aromatic and branched-chain amino acids on urine calcium excretion in humans

**B. Dawson-Hughes · S. S. Harris · H. M. Rasmussen · G. E. Dallal**

Received: 25 September 2006 / Accepted: 20 December 2006 / Published online: 25 January 2007


**Abstract**
*Summary* In 30 adults, increasing intake of aromatic amino acids increased calcium excretion and serum IGF-1, but not indices of bone turnover, when compared with similar increases in intake of branched-chain amino acids. The mechanisms involved are not certain but these findings suggest a role for the calcium sensor receptor.
*Introduction* In contrast to branched-chain amino acids (BCAAs), aromatic amino acids (AAAs) bind to the calcium sensing receptor (CaR) and thus have an increased potential to affect calcium homeostasis. In this study we compare the effects of increased intake of AAAs versus BCAAs on calcium excretion, serum IGF-1, markers of bone turnover, and 4-hr calcium excretion after an oral calcium load.
*Methods* After two weeks on low-protein metabolic diets, 30 healthy subjects were randomized to a fivefold increase in intake of AAAs or BCAAs for two weeks. Changes in calcium excretion and other measures were compared in the two groups.
*Results* With the increase in amino acid intake, 24-hr calcium excretion (P=0.027), IGF-1 (P=0.022), and 4-hr calcium excretion after an oral load (P=0.023) increased significantly in the AAA relative to the BCAA group. Group changes in turnover markers did not differ significantly.
*Conclusion* In comparison with BCAAs, AAAs promoted calcium excretion. The calciuria does not appear to result from increases in bone resorption and may occur by increasing calcium absorption. The AAAs also increased circulating levels of IGF-1. Collectively these findings raise the possibility that AAAs may selectively influence calcium homeostasis through their interactions with the CaR.

**Keywords** Aromatic amino acids · Bone turnover · Branched-chain amino acids · Calcium excretion · IGF-1

Presented at the American Society of Bone and Mineral Research meeting in Nashville, Tenn., September 2005.

This material is based on work supported by a Discovery Grant from Dairy Management, Inc. and by the U.S. Department of Agriculture under agreement No. 59-1950-9001. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors, and do not necessarily reflect the view of the U.S. Department of Agriculture.

B. Dawson-Hughes · S. S. Harris · H. M. Rasmussen · G. E. Dallal
Jean Mayer USDA Human Nutrition Research Center
on Aging at Tufts University,
Boston, MA, USA

S. S. Harris
e-mail: Susan.Harris@Tufts.edu

H. M. Rasmussen
e-mail: Helen.Rasmussen@Tufts.edu

G. E. Dallal
e-mail: Jerry.Dallal@Tufts.edu

B. Dawson-Hughes (✉)
Bone Metabolism Laboratory at the Jean Mayer,
USDA Human Nutrition Research Center
on Aging at Tufts University,
711 Washington St.,
Boston, MA 02111, USA
e-mail: Bess.Dawson-Hughes@Tufts.edu

## Introduction

Dietary protein has varied and complex effects on calcium metabolism. It increases urinary calcium excretion and this generally occurs in proportion to the accompanying acid load [1]. Chronic ingestion of a dietary acid load leads to a

mild metabolic acidosis in the elderly as their renal function declines and they become less able to excrete hydrogen ions [2]. Metabolic acidosis reduces osteoblastic activity [3] and increases osteoclastic activity [4]. In addition, it promotes bone resorption through a direct physicochemical effect on bone [5]. Dietary protein has also been reported to increase calcium absorption [6, 7] although this has not been a consistent finding. Thus the calciuria observed on high protein intakes is thought to result from a combination of increased bone resorption and increased calcium absorption.

A potential mechanism by which protein may influence calcium absorption and calcium excretion involves the calcium sensing receptor (CaR). Conigrave et al. observed that aromatic but not branched-chain amino acids activated the CaRs in embryonic kidney cells transfected with human CaRs [8]. CaRs are present on many tissues including the kidney and parietal cells [9, 10] and antral G cells [11]. In rat whole stomachs studied ex vivo, the aromatic amino acid phenylalanine activated CaRs located on parietal cells and significantly lowered gastric luminal pH, whereas the branched-chain amino acid, leucine did not [12]. The effects of individual amino acids, taken orally, on calcium excretion, markers of bone turnover, and calcium absorption are unknown.

Ingestion of protein also stimulates the production of the growth factor, IGF-1 [13, 14]. Little is known, however, about the effect of specific amino acids on the hepatic production of IGF-1.

The objective of this study was to determine and compare the effects of equimolar increases of aromatic and branched-chain amino acids on changes in 24-hr calcium excretion and serum IGF-1 in healthy older men and women. To explore potential mechanisms for any effect on calcium excretion, we assessed changes in biochemical markers of bone turnover and 4-hr calcium excretion after an oral calcium load in these subjects.

## Materials and methods

### Subjects

The subjects were healthy men and postmenopausal women, aged 51 yr and older. They were recruited through direct mailings and local newspaper advertisements. Telephone prescreening was used to identify subjects with low usual protein intakes and to determine general eligibility. Exclusion criteria included protein intakes >0.75 g/kg/d (to facilitate compliance with the low protein diets during the study), any history or disorder known to alter calcium metabolism; therapy with a glucocorticoid for >10 days in the last 3 months, growth hormone, hormone replacement therapy, or other drugs to treat osteoporosis in the last 6 months, current use of insulin or diuretics, renal disease including kidney stones in the past 5 years or renal tubular acidosis, hyperparathyroidism, untreated thyroid disease, adrenal disease, rheumatoid arthritis, lupus or other autoimmune disorder, unstable angina or myocardial infarction in past 6 months, active malignancy or cancer therapy in the last year. Screening evaluation included blood and urine tests. Subjects were excluded if their screening 24-hr urine calcium was >300 mg, femoral neck bone mineral density Z score was below −2.0, or a Chemscreen value was >10% outside of the reference range.

Fifty-three subjects were screened, 34 were eligible, 4 subjects dropped out (reasons: one did not like the food, one started a diuretic, one had a family crisis, and one gave no reason), and 30 subjects completed the 24-day study. The human investigation review board at Tufts University approved the study, and written informed consent was obtained from each subject.

### Experimental methods

All subjects were asked to stop taking their own calcium and vitamin D supplements from 1 wk before the screening visit to the end of the study. Subjects were randomly assigned to the BCAA or AAA groups. All subjects consumed the same low-protein metabolic diets throughout the study. Low protein diets were used in order to achieve a low intake of the test amino acids. On days 13–24, subjects in the BCAA group consumed a fivefold g-molar increase in the amino acids leucine (leu) and isoleucine (ileu) and subjects in the AAA group consumed a fivefold increase in intake of the amino acids phenylalanine (phe) and histidine (his). These two AAAs were selected because Conigrave found them to have the highest potency as agonists of the CaR [8]. The two BCAAs with the lowest potency were selected for the comparison group [8]. The low and high amino acid intakes were set to represent the first and 99th percentiles of the test amino acids usually consumed in the U.S., according to NHANES III [15].

All subjects took three tablets of calcium citrate, each containing 200 mg of elemental calcium, daily, two with breakfast and one with the evening meal. They also took one multivitamin daily containing 400 IU of vitamin $D_3$. The supplements were purchased as single lots from CVS Pharmacy. Fasting blood and 24-hr urine specimens were collected at entry, on day 13 (baseline), and on day 24 (end of the high amino acid period). The change in urine calcium excretion over 4-hrs after an oral calcium load was assessed on days 13 and 24. Compliance was assessed at all visits by pill count and by compliance calendar and food diary checks, as recorded by the subjects.

### Diets

All food and caloric beverages were provided by the Metabolic Research Unit. The contents of the base diets, calculated with use of version 4.05 of the University of Minnesota Food and Nutrient Database 34, released in May, 2003, are shown in Table 2. Subjects received the same constant base diet, as a 3-day cycle menu, on days 1–24. On days 13–24, the amino acids were added isocalorically (by reducing the fat and carbohydrate content of the base diet) and without changing the sodium content of the diet. The amino acids were incorporated into casseroles, vegetable spreads, fruit shakes, and desserts for palatability. They were consumed in roughly equal portions at the three meals each day. Amino acids were synthesized by Ajinomoto in Raleigh, NC, were food grade, and were purchased from Sigma-Aldrich Co., St. Louis, MO. The aromatic and branched-chain amino acid samples were analyzed independently by Covance Laboratories, Inc, in Madison, WI and found to contain 95 to 99 % of the expected amounts. Subjects came in at least three times per week to be weighed and to pick up their food. Subjects completed daily food intake diaries that were reviewed by the research dietitian at each visit.

### Biochemical measurements

Bloods were drawn after a 12-hr fast and samples from individual subjects were batched for analyses. Serum 25 (OH)D and osteocalcin were measured with radioimmunoassay kits from Diasorin (Stillwater, MN). Serum PTH was measured by immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). Urinary N-telopeptide levels were measured with competitive-inhibition enzyme-linked immunoabsorbent assay kits from Ostex International (Seattle, WA). The coefficients of variation (CV) for these assays ranged from 2.7 to 7.7%. Serum IGF-1 was measured with radioimmunoassay kits from Nichols Institute Diagnostics (San Juan Capistrano, CA) with intra- and inter-assay CVs of 3.5% and 6.8%, respectively. Serum calcium was measured by Cobas Mira centrifugal Analyzer (Roche Instruments, Belleville, NJ) and 24-hr urinary calcium on a Nova Nucleus Chemistry Analyzer (Nova Biochemical, Waltham, MA) with CV of <3%. Serum gastrin levels were measured by radioimmunoassay kits (Gastrin $I^{125}$ Double Antibody Kits, ICN Biomedical, Costa Mesa, CA). The intra- and inter-assay CVs are 5.3 and 8.8%, respectively. Urinary sodium, potassium, and calcium were measured by direct-current plasma emission spectroscopy with a Spectraspan 6 (Beckman Instruments, Palo Alto, CA). Urinary nitrogen was measured with a model FP-2000 nitrogen/protein determinator (LECO, St. Joseph, MI). This instrument employs a Dumas combustion method and detection using a thermal conductivity cell. It measures nitrogen with a precision of 15 ppm. NAE was measured in 24-hr urine collections by a modification of the Jorgensen titration method [16], as described by Chan [17].

Net acid excretion (NAE)=Titratable acid (TA) + Ammonium ($NH_4^+$) - Bicarbonate ($HCO_3^-$). Briefly, TA-$HCO_3$ was assessed after addition of HCl, boiling the sample, and then titrating the sample to neutral pH. To measure the $NH_4^+$, formol was added to the sample to release a $H^+$ from $NH_4^+$ and the sample was again titrated to neutral pH. All titrations were carried out with a TIM 900 Titration Manager by Radiometer Analytical (Hach Inc., Loveland, CO). The precision of NAE measurements in our laboratory was determined by analyzing aliquots of a single 24-hr urine collection on 15 different days. The aliquots were stored frozen at −20 degrees Celsius for 4 to 9 months and thawed only once. The CV of the NAE measurements was 10.9%.

### Calcium excretion after an oral calcium load

After a 10-hr fast, subjects collected urine from 6 to 8 AM, consumed 600 mg of calcium as the citrate at 8 AM, and collected urine from 8 AM to noon. To reduce the impact of subject-to-subject variability in bone turnover (which increases at night and contributes to morning urine calcium) and other factors that influence fasting morning urine calcium, the 4-hr calcium excretion (collected from 8 AM to noon) was adjusted for the calcium content of the earlier bout (collected from 6 AM to 8 AM). The measurements on days 13 and 24 were done on constant diets (except for the amino acid interventions) and at the same times of day, to minimize within- subject variability. We have expressed the result as the 4-hr urine response to an oral calcium load, in mg.

### Statistical analyses

Mean values of urinary calcium excretion at days 13 and 24, and the mean changes in these values from days 13 to 24 were compared across amino acid groups with t-tests for two independent samples. Two-sided P values less than 0.05 were considered to indicate statistical significance. Analysis of covariance was used to compute and compare means adjusted for covariates across amino acid groups and to investigate potential interactions. Statistical analyses were conducted with SPSS v. 13 (SPSS Inc., Chicago, IL).

## Results

### Main results

The clinical and biochemical characteristics of the 30 subjects, by group, are shown in Table 1. There were a

**Table 1** Clinical characteristics of the 30 subjects on day 1, by amino acid group (Mean ± SD)

| | Aromatic | Branched-chain |
|---|---|---|
| N | 15 | 15 |
| Female (N) | 11 | 9 |
| White (%)[a] | 86.7 | 80.0 |
| Age (yr) | 63.1±10.1 | 62.8±7.9 |
| Height (cm) | 163±9 | 168±8 |
| Weight (kg) | 70.0±10.8 | 72.8±10.1 |
| Femoral neck BMD ($g/cm^2$)[b] | 0.98±0.19 | 0.95±0.13 |
| Dietary protein intake (g/d)[b] | 55.7±20.8 | 54.2±17.2 |
| Dietary calcium intake (mg/d)[b] | 774±412 | 617±231 |

No group differences were statistically significant.
[a] There were 2 Hispanics and 1 black in the branched-chain group and 1 Hispanic and 1 black in the aromatic group.
[b] Measured at screening.

few more women in the AAA group but the groups did not differ significantly in any of these characteristics. The composition of the basal metabolic diet for all subjects is given in Table 2. The amino acid and total protein content of the diets in the two groups during the enrichment period, days 13–24, is shown in Table 3. Compliance with the calcium supplement exceeded 92% and with the multivitamin exceeded 97% during the periods of days 1–12 and days 13–24 and did not differ significantly by group.

Unadjusted group mean laboratory values on days 13 and 24 are shown in Table 4 and mean change in these measures (day 24-day 13), adjusted for baseline value and weight, are shown in Table 5. Mean 24-hr calcium excretion at baseline did not differ significantly in the two groups (Table 4), but the changes in the two groups in response to increased amino acid intake differed (Table 5). Calcium excretion increased by 11±9 mg in the AAA group and decreased by 20±9 mg in the BCAA group (P=0.027 for difference in change, after adjustment for baseline value and weight; see Table 5). Adjustment for sodium excretion, sex, and serum IGF-1 did not substantially alter these differences.

**Table 2** Nutrient content of the metabolic diet during the low protein diet, days 1–13, by amino acid group (Mean ± SD)

| Nutrient | Aromatic | Branched-chain |
|---|---|---|
| Energy (kcal) | 2350±330 | 2452±331 |
| Total fat (g) | 87±14 | 90±13 |
| Total carbohydrate (g) | 369±50 | 389±51 |
| Total protein (g/kg/d) | 0.50±0.00 | 0.50±0.01 |
| Total protein (g)[a] | 34.9±5.4 | 36.2±5.0 |
| Total dietary fiber (g) | 20.3±3.4 | 21±2.5 |
| Vitamin D (mcg)[b] | 2.7±0.3 | 2.6±0.1 |
| Calcium (mg)[c] | 601±4 | 595±7 |
| Phosphorus (mg) | 755±85 | 751±105 |
| Sodium (mg) | 2674±507 | 2774±475 |
| Potassium (mg) | 2089±229 | 2147±217 |

[a] Content of isoleucine, leucine, phenylalanine, and histidine was 1.6±0.2, 2.6±0.4, 1.5±0.2, and 1.1±0.4 g/d in the aromatic group and 1.6±0.2, 2.7±0.4, 1.5±0,2, and 0.9±0.1 g/d in the branched chain group, respectively.
[b] Supplemented with 10 mcg of vitamin $D_3$, as multivitamin, daily.
[c] Supplemented with 600 mg of calcium, as citrate, daily.

**Table 3** Amino acid content of the metabolic diet on days 13–24, by amino acid group (Mean ± SD)

| Nutrient, g/kg/d | Aromatic | Branched-chain |
|---|---|---|
| Isoleucine | 22.6±0.3 | 121.6±11.2 |
| Leucine | 37.7±0.6 | 214.2±20.6 |
| Phenylalanine | 106.7±0.7 | 21.8±0.3 |
| Histidine | 71.6±0.3 | 12.8±0.3 |

Serum IGF-1 levels did not differ significantly in the two groups on the low amino acid intakes (day 13) but the pattern of change differed on the higher amino acid intakes: IGF-1 increased by 11±5 ng/ml in the AAA group and decreased by 8±5 ng/ml in the BCAA group, P=0.022, after adjustment for baseline value and weight (see Table 5).

There were no significant group differences in baseline 24-hr urinary sodium, potassium, or in nitrogen or net acid excretion and no significant differences in changes in these measures during the study.

### Exploration of mechanisms

There were no group differences in the biochemical markers of bone turnover, 24-hr urinary NTX or serum osteocalcin, on day 13 and no statistically significant differences in the changes in any of these measures when amino acid intake was increased (Tables 4 and 5).

Calcium excretion over the 4-hr period after a 600 mg oral calcium load, adjusted for preload calcium excretion, didn't differ significantly between the two groups on day 13 but on day 24, it was higher in the AAA group than in the BCAA group (P=0.038, see Fig. 1).

Serum PTH tended to be higher in the branched-chain group at entry (data not shown) and throughout the study. Increasing intake of the two types of amino acids had no significant effect on serum levels of PTH, calcium, or gastrin.

## Discussion

Increasing from low to high intake of aromatic and branched-chain amino acids had significantly different effects on calcium excretion, the primary outcome of this study in healthy older men and women. The change in calcium excretion was significantly more positive in the AAA than in the BCAA group. We purposely avoided

**Table 4** laboratory values on days 13 (low protein diet) and 24 (enriched amino acid) by amino acid group (Mean ± SD)

| | Day 13 aromatic | Day 13 branched-chain | Day 24 aromatic | Day 24 branched-chain |
|---|---|---|---|---|
| N | 15 | 15 | 15 | 15 |
| Serum[a] | | | | |
| Osteocalcin (ng/ml) | 7.50±4.79 | 7.85±2.48 | 7.10±4.37 | 7.51±2.47 |
| 25(OH)D (ng/ml) | 23.2±7.1 | 21.1±5.7 | 22.7±6.5 | 21.6±4.9 |
| PTH (pg/ml) | 41.9±23.2 | 54.4±16.6 | 42.1±18.4[c] | 56.5±19.9 |
| IGF-1 (ng/ml) | 117±48 | 115±35 | 128±61 | 107±35 |
| Total calcium (mg/dl) | 8.95±0.44 | 8.87±0.41 | 9.11±0.54 | 8.87±0.32 |
| Gastrin (pg/ml) | 69.0±88.7[d] | 37.9±8.2 | 60.2±55.5[d] | 38.0±7.7 |
| 24-Hr Urine[b] | | | | |
| Calcium (mg) | 150±68 | 136±77 | 160.0±79.9 | 117.8±77.4 |
| N-telopeptide (nmol) | 107±43 | 145±103 | 104±35 | 146±101 |
| Net acid excretion (mmol/l) | 2.22±4.80 | 4.18±6.79 | 2.45±4.48 | 3.10±6.75 |
| Sodium (mg) | 1730±452 | 2098±1155 | 1826±596 | 1687±625 |
| Potassium (mg) | 1274±303 | 1371±494 | 1357±372 | 1336±474 |
| Nitrogen (g) | 5.29±1.25 | 7.16±3.50 | 6.7±1.8 | 8.0±3.2 |
| Nitrogen (g/kg/d) | 0.08±0.02 | 0.10±0.05 | 0.10±0.03 | 0.11±0.05 |

[a] Metric to SI conversions: osteocalcin ng/ml×0.172=nmol/L; 25(OH)D ng/ml×2.496=nmol/L; PTH pg/ml×0.106=pmol/L; IGF-1 ng/ml×7.649=nmol/L; albumin g/dl; total calcium mg/dl×0.250; gastrin pg/ml×1=ng/L

[b] Metric to SI conversions: calcium mg/24 h×0.025=mmol/d; sodium mg/24 h×0.044=mmol/d; potassium mg/24 h=0.026 mmol/d; nitrogen g/24 h×71.38=mmol/d.

[c] Groups differ at P<0.05.

[d] Values after an outlier was removed are 47.2±28.5 (day 13; P for group difference=0.007) and 47.2±28.5 (day 24; P for group difference=0.01).

**Table 5** Mean change in laboratory values from day 13 to day 24, by amino acid group (Mean ± SEM)

| | Δ day 24-day 13[a] | |
|---|---|---|
| | Aromatic | Branched-chain |
| N | 15 | 15 |
| Serum[b] | | |
| Osteocalcin (ng/ml) | −0.43±0.26 | −0.31±0.26 |
| 25(OH)D (ng/ml) | −0.23±0.56 | 0.46±0.58 |
| PTH (pg/ml) | −2.05±3.12 | 4.32±3.12 |
| IGF-1 (ng/ml) | 11±5 | −8±5[c] |
| Total calcium (mg/dl) | 0.16±0.09 | −0.01±0.09 |
| Gastrin (pg/ml) | −3.0±2.9 | −5.6±2.9 |
| 24-Hr Urine[b] | | |
| Calcium (mg) | 11±9 | −20±9[d] |
| N-telopeptide (nmol) | −5±8 | 3±8 |
| Sodium (mg) | −36±153 | −280±153 |
| Potassium (mg) | 56±89 | −7±89 |
| Nitrogen (g) | 1.24±0.36 | 0.97±0.36 |
| Nitrogen (g/kg/d) | 0.02±0.018 | 0.01±0.023 |
| Net acid excretion | −0.22±1.36 | −0.63±1.36 |

[a] Adjusted for weight and baseline (day 13) value.

[b] Metric to SI conversions: osteocalcin ng/ml×0.172=nmol/L; 25(OH)D ng/ml×2.496=nmol/L; PTH pg/ml×0.106=pmol/L; IGF-1 ng/ml×7.649=nmol/L; albumin g/dl; total calcium mg/dl×0.250; gastrin pg/ml×1=ng/L. Urine calcium mg/24 h×0.025=mmol/d; sodium mg/24 h×0.044=mmol/d; potassium mg/24 h=0.026 mmol/d; nitrogen g/24 h×71.38=mmol/d.

[c] Change in groups differs at P=0.022.

[d] Change in groups differs at P=0.027.

using sulfur-containing amino acids in this study because of the link between sulfur content and calcium excretion.

The observed difference in change in calcium excretion could have originated from group differences in dietary compliance particularly with calcium or sodium rich foods (determinants of calcium excretion), from differential

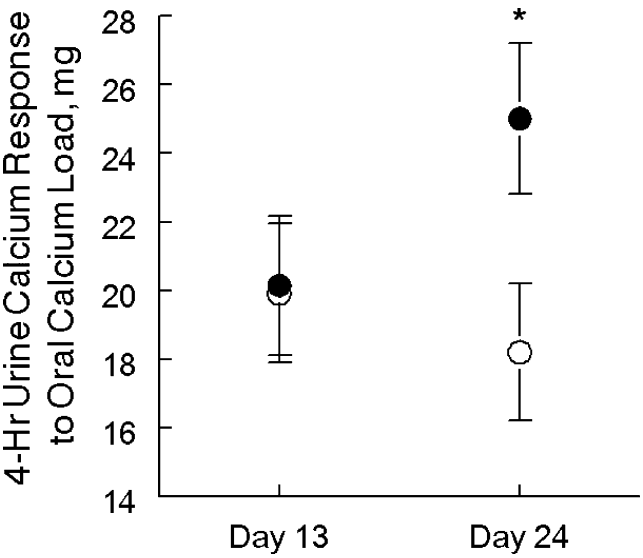


**Fig. 1** 4-hr urine calcium response to a 600 mg calcium load at baseline (day 13) and after 10 days on the increased amino acid intake (day 24) in the branched-chain (open circles) and aromatic amino acid (closed circles) groups. * indicates significant group difference on day 24 (P=0.038). Unadjusted 4-hr urine calcium values for the AAA and BCAA groups on days 13 and 24 were similar but more variable and not statistically significant (p=0.389)

effects of the two types of amino acids on bone turnover, from differential effects on calcium absorption or renal handling of calcium (CaRs are known to be present in the gut and kidney), or it could have occurred by chance. Of note, changes in urinary calcium excretion can occur without affecting the overall calcium economy. Group differences in degree of compliance with calcium supplements and with the metabolic diets seem unlikely to account for the observed difference in change in calcium excretion. Compliance with the calcium supplements and with the diets was high by self report and adjustment for differences in sodium excretion did not alter the results. There was no significant difference in change (and no change) in serum osteocalcin or urinary N-telopeptide in either group, making it somewhat unlikely that the two types of amino acids had differential effects on bone turnover. Although 12 days is not sufficient time to see maximal changes, dramatic changes in biochemical markers of bone turnover have been documented in similar short intervals [18, 19]. A primary effect of the AAAs on renal handling of calcium can not be excluded.

We did not measure calcium absorption, but to the extent that the increment in calcium excretion after the 600-mg oral calcium load given after a 10-hr fast reflects absorption, the greater increment in the AAA group is consistent with the possibility that the AAAs promoted calcium absorption, relative to the BCAAs. If so, this provides a potential explanation for the mechanism by which the AAAs could selectively promote calcium excretion. The urine calcium excretion after an oral load has been validated as an index of calcium absorption in other contexts (different calcium loads, different populations, with and without concurrent renal function assessments) [20, 21], but the specific variant we used has not been validated.

Increasing the intake of AAAs, when compared to increasing intake of BCAAs, induced an increase in serum IGF-1 levels in this study. Hepatic production of IGF-1 is under the influence of protein intake [13, 22]. However the effects of aromatic versus branched-chain amino acids on circulating levels of IGF-1 in humans have not to our knowledge been examined. Based on recent evidence, the effect of dietary amino acids on IGF-1 seems to be independent of insulin [23]. Currently there is no solid basis for relating the observed difference in change in IGF-1 to the CaR, but this is a possibility. Canaff and colleagues have demonstrated that CaRs are present in the rat hepatocyte, the cell where IGF-1 is produced and that activation of these CaRs promotes the flow of bile [24]. These authors noted the clinical correlation that subjects with familial hypocalciuric hypercalcemia (FHH), a condition resulting from a genetic defect in the CaR, have an increased incidence of gall stones [25]. Notably, in a small group of six subjects from two kindreds with FHH, basal levels of IGF-1 were low in two and below the midpoint of the reference range in the other four subjects [26]. These patients also had an impaired growth hormone response to provocative stimulation, which could also have influenced their IGF-1 levels. Our findings are consistent with the possibility that AAAs stimulate IGF-1 relative to BCAAs.

This study has limitations and a larger and longer study is needed to confirm the findings. A low protein only group would be useful to define more clearly the individual effects of aromatic and branched-chain amino acids on calcium metabolism. A measurement of true calcium absorption is also needed. The use of metabolic diets represented an effort to standardize intake. The lack of significant group differences in net acid excretion and in urinary excretion of sodium, potassium, and nitrogen provide some evidence that compliance with the diet was similar in the two groups. However, it is notable that urinary potassium was lower than expected in both groups suggesting some degree of non-compliance, most likely the subjects avoided some of the fruits and vegetables that were on their diets. The low levels of nitrogen excretion in the two groups suggest that subjects did not consume significant amounts of protein on their own. Compliance with low protein diets is plausible because a low usual protein intake was one of the entry criteria for the study. Despite these limitations, the study does suggest that further investigation of the role of individual amino acids in calcium and bone metabolism may be productive.

In conclusion, when compared with BCAAs, increasing intake of AAAs caused greater urinary calcium excretion. The increase does not appear to have resulted from increased bone resorption and may have resulted from increased calcium absorption. Compared with BCAAs, the AAAs also promoted an increase in serum IGF-1, suggesting that selected amino acids may be responsible for the well-known trophic effects of dietary protein on IGF-1 levels. The selective effects of the AAAs observed in this study may be operating through the CaR but this remains to be demonstrated.

**Acknowledgments** We are grateful to the staff of the Metabolic Research Unit at Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University for assistance in carrying out this study. BD-H, SH, HMR, and GED contributed to the design, analysis, and writing of the manuscript. BD-H obtained the funding. None of the authors had any conflicts of interest.

## References

1. Hegsted M, Schuette SA, Zemel MB et al (1981) Urinary calcium and calcium balance in young men as affected by level of protein and phosphorus intake. J Nutr 111:553–562
2. Frassetto LA, Todd KM, Morris RC Jr et al (1998) Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. Am J Clin Nutr 68:576–583

3. Sprague SM, Krieger NS, Bushinsky DA (1994) Greater inhibition of in vitro bone mineralization with metabolic than respiratory acidosis. Kidney Int 46:1199–1206
4. Arnett TR, Spowage M (1996) Modulation of the resorptive activity of rat osteoclasts by small changes in extracellular pH near the physiological range. Bone 18:277–279
5. Bushinsky DA (1996) Metabolic alkalosis decreases bone calcium efflux by suppressing osteoclasts and stimulating osteoblasts. Am J Physiol 271:F216–F222
6. Kerstetter JE, O'Brien KO, Insogna KL (1998) Dietary protein affects intestinal calcium absorption. Am J Clin Nutr 68:859–865
7. Kerstetter JE, O'Brien KO, Caseria DM et al (2005) The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. J Clin Endocrinol Metab 90:26–31
8. Conigrave AD, Quinn SJ, Brown EM (2000) L-amino acid sensing by the extracellular Ca2+-sensing receptor. Proc Natl Acad Sci USA 97:4814–4819
9. Cheng I, Qureshi I, Chattopadhyay N et al (1999) Expression of an extracellular calcium-sensing receptor in rat stomach. Gastroenterology 116:118–126
10. Geibel JP, Wagner CA, Caroppo R et al (2001) The stomach divalent ion-sensing receptor scar is a modulator of gastric acid secretion. J Biol Chem 276:39549–39552
11. Ray JM, Squires PE, Curtis SB et al (1997) Expression of the calcium-sensing receptor on human antral gastrin cells in culture. J Clin Invest 99:2328–2333
12. Busque SM, Kerstetter JE, Geibel JP et al (2005) L-type amino acids stimulate gastric acid secretion by activation of the calcium-sensing receptor in parietal cells. Am J Physiol-Gastrointest Liver Physiol 289:G664–G669
13. Isley WL, Underwood LE, Clemmons DR (1983) Dietary components that regulate serum somatomedin-C concentrations in humans. J Clin Investiga 71:175–182
14. Schurch MA, Rizzoli R, Slosman D et al (1998) Protein supplements increase serum insulin-like growth factor-I levels and attenuate proximal femur bone loss in patients with recent hip fracture. A randomized, double-blind, placebo-controlled trial. Ann Int Med 128:801–809
15. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (2005) Dietary reference intakes: energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Institute of Medicine-Washington, DC 1060–1061
16. Jorgensen K (1957) Titrimetric determination of the net excretion of acid/base in urine. Scand J Clin Lab Invest 9:28–291
17. Chan JC (1972) The rapid determination of urinary titratable acid and ammonium and evaluation of freezing as a method of preservation. Clin Biochem 5:94–98
18. Maurer M, Riesen W, Muser J et al (2003) Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. Am J Physiol-Renal 284:F32–F40
19. Sebastian A, Morris RC Jr (1994) Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate. New Engl J Med 330:1776–1781
20. Broadus AE, Dominguez M, Bartter FC (1978) Pathophysiological studies in idiopathic hypercalciuria: use of an oral calcium tolerance test to characterize distinctive hypercalciuric subgroups. J Clin Endocrinol Metab 47:751–760
21. Harvey JA, Zobitz MM, Pak CY (1988) Dose dependency of calcium absorption: a comparison of calcium carbonate and calcium citrate. J Bone Miner Res 3:253–258
22. Thissen JP, Ketelslegers JM, Underwood LE (1994) Nutritional regulation of the insulin-like growth factors. Endocrine Reviews 15:80–101
23. Noguchi T (2000) Protein nutrition and insulin-like growth factor system. Br J Nutr 84(Suppl 2):S241–S244
24. Canaff L, Petit JL, Kisiel M et al (2001) Extracellular calcium-sensing receptor is expressed in rat hepatocytes. coupling to intracellular calcium mobilization and stimulation of bile flow. J Biol Chem 276:4070–4079
25. Law WM Jr, Heath H III (1985) Familial benign hypercalcemia (hypocalciuric hypercalcemia). Clinical and pathogenetic studies in 21 families. Ann Int Med 102:511–519
26. Cecconi E, Bogazzi F, Cetani F et al (2003) Impaired GH secretion to provocative stimuli in two families with hypocalciuric hypercalcaemia. Clin Endocrinol 59:604–606