The ratio of animal protein intake to potassium intake is a predictor of bone resorption in space flight analogues and in ambulatory subjects

Sara R Zwart, Alan R Hargens, and Scott M Smith

ABSTRACT
Background: Bone loss is a critical concern for space travelers, and a dietary countermeasure would be of great benefit. Dietary protein and potassium-associated bicarbonate precursors may have opposing effects on the acid-base balance in the body and therefore on bone loss.
Objective: In 2 studies, we examined the ability of dietary protein and potassium to predict markers of bone metabolism.
Design: In the first study, 8 pairs of male identical twins were assigned to 1 of 2 groups: bed rest (sedentary, or SED, group) or bed rest with supine treadmill exercise in a lower-body negative pressure chamber (EX group). In a second study, groups of 4 subjects lived in a closed chamber for 60 or 91 d, and dietary data were collected for two or three 5-d sessions. Urinary calcium, N-telopeptide, and pyridinium cross-links were measured before bed rest; on bed rest days 5–6, 12–13, 19–20, and 26–27; and daily during the chamber study. Data were analyzed by Pearson’s correlation (P < 0.05).
Results: The ratio of animal protein intake to potassium intake was significantly correlated with N-telopeptide in the SED group during bed rest weeks 3 and 4 (r = 0.77 and 0.80) and during the 91-d chamber study (r = 0.75). The ratio of animal protein intake to potassium intake was positively correlated with pyridinium cross-links before bed rest in the EX group (r = 0.83), in the EX group during bed rest week 1 (r = 0.84), and in the SED group during bed rest week 2 (r = 0.72) but not during either chamber study. In both studies, these relations were not significant with the ratio of vegetable protein intake to potassium intake.
Conclusions: The ratio of animal protein intake to potassium intake may affect bone in ambulatory and bed-rest subjects. Changing this ratio may help to prevent bone loss on Earth and during space flight.

KEY WORDS Food intake, dietary protein, dietary potassium, bones, collagen cross-links, bone markers, calcium, humans

INTRODUCTION
The role of specific nutrients in bone health is obvious in some cases (such as calcium and vitamin D), but the ability of other nutrients and dietary patterns (for example, type of protein) to influence bone health is not as clear, and the results of studies often seem contradictory. Clarification of the role of these other nutrients and dietary patterns would be beneficial for everyone, including astronauts, for whom bone loss is a significant concern.
Because bone is a substantial reservoir for ions that can titrate excess acid loads, chronic perturbations of acid-base balance in the body may induce prominent changes in the chemical makeup of bone (1, 2). Chronic low-grade metabolic acidosis can occur when the diet contains more acid precursors than base precursors (3, 4). Although respiratory acidosis can also alter systemic pH, its effects on bone are small (2, 5, 6).

The dissolution of calcium and bicarbonate from bone during acute metabolic acidosis has been documented from in vitro studies to occur mainly by a physicochemical reaction (7, 8), whereas the response during chronic metabolic acidosis also involves a cell-mediated effect (9). Other in vitro studies show that osteoclast activity and resorption pit formation are highly dependent on extracellular pH (10, 11), with increased activity at lower pH and decreased activity during metabolic alkalosis (11, 12).

If the kidney is functioning properly and excess acid (from endogenous acid production or incomplete oxidation of organic acids) is excreted in the urine, the diet can influence urine pH (13–16). Remer and Manz (16) found that renal net acid excretion can be estimated from dietary intake data. The estimation is based on the intake of sulfur-containing proteins, corrects for the intestinal absorption of minerals, and assumes a rate of urinary excretion of organic acids proportional to body surface area. Sebastian et al (17) refined the procedure of Remer and Manz to account for the specific sulfur content of each food item and to account for the effect of diet composition on the urinary excretion of organic acid anions. More recently, Frassetto et al (18) found that net endogenous noncarboxyl acid production in humans can be predicted solely from the intake of dietary potassium and protein, where dietary potassium acts as a proxy for dietary bicarbonate precursors (18).

Most studies of the association of dietary (or supplemental) potassium intake with bone report positive results with increased potassium intake (19–25). This is not the case, however, with studies of the association of protein intake with bone turnover.

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3 Address reprint requests to SM Smith, Nutritional Biochemistry Laboratory, Human Adaptation and Countermeasures Office, Mail Code SK3, NASA Lyndon B Johnson Space Center, Houston, TX 77058. E-mail: scott.m.smith@nasa.gov.
Many of these studies show negative effects on bone with high protein intake (15, 24, 26–30), but many others show that high protein is beneficial to bone (31–36).

Bone loss during space flight is a significant concern for the health and safety of astronauts (37). Means of counteracting bone loss during space flight have generally focused on exercise and pharmacologic treatments. A dietary countermeasure to protect bone would be a great benefit to space exploration. Although the effects of either protein or potassium intake on bone have not been reported, many studies have shown that high protein intake to potassium intake on bone metabolism has not been reported.

The purpose of the present investigation was to determine whether dietary patterns could predict biochemical markers of bone in ambulatory subjects and in subjects during bed rest, a weightlessness analogue (38). We examined the relation between protein and potassium intake and markers of bone metabolism. These relations were evaluated in both sedentary and exercising bed rest subjects and in free-living ambulatory subjects and ambulatory subjects residing in an enclosed chamber for 60 or 91 d. Although we have published the basic results of these studies (39, 40), we have not published an evaluation of the potential influence of diet on bone health.

SUBJECTS AND METHODS

Results from 2 separate studies were analyzed to determine associations among dietary patterns and biochemical markers of bone metabolism. A 30-d bed rest study was conducted at the University of California, San Diego, and 2 closed-chamber studies were completed at the Johnson Space Center in Houston. Both protocols were reviewed and approved by the Johnson Space Center Institutional Review Board, and the bed rest protocol was also reviewed by the University of California, San Diego, Institutional Review Board. All subjects provided written informed consent before participation.

Subjects

Bed rest study

The design and subjects of the bed rest study were as previously described (40). Briefly, the subjects were 8 pairs of male

### TABLE 2

Dietary intakes for 5-d recorded dietary intake sessions

<table>
<thead>
<tr>
<th>60-d Chamber study</th>
<th>91-d Chamber study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Session 1</strong></td>
<td><strong>Session 2</strong></td>
</tr>
<tr>
<td><strong>Energy (kcal)</strong></td>
<td>2480 ± 181</td>
</tr>
<tr>
<td><strong>Calcium (mg)</strong></td>
<td>1082 ± 176</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>72.3 ± 5.9</td>
</tr>
<tr>
<td><strong>Animal</strong></td>
<td>43.0 ± 7.3</td>
</tr>
<tr>
<td><strong>Vegetable</strong></td>
<td>29.1 ± 3.3</td>
</tr>
<tr>
<td><strong>Potassium (mg)</strong></td>
<td>2919 ± 238</td>
</tr>
<tr>
<td>TPro/K (g/mEq)</td>
<td>0.98 ± 0.05</td>
</tr>
<tr>
<td>APro/K (g/mEq)</td>
<td>0.57 ± 0.08</td>
</tr>
<tr>
<td>VPro/K (g/mEq)</td>
<td>0.40 ± 0.04</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. TPro/K, ratio of total protein intake to potassium intake; APro/K, ratio of animal protein intake to potassium intake; VPro/K, ratio of vegetable protein intake to potassium intake. Dietary intakes for the 91-d chamber study (n = 4) were analyzed by using two-way repeated-measures ANOVA, comparing the 3 weekly sessions for which dietary intake was recorded. The 2 weekly recorded dietary intake sessions for the 60-d (n = 4) chamber study were compared by using a paired Student’s t test.

2 Significantly higher in session 3 of the 91-d chamber study than in sessions 1 and 2, P < 0.05.

3 Significantly lower in session 2 of the 91-d chamber study than in sessions 1 and 3, P < 0.05.
identical twins (x ± SD age: 27.1 ± 5.0 y). One member of each twin pair was randomly assigned to a sedentary (SED) group, whereas one member was assigned to an exercise (EX) group. Before and during the 30-d bed rest protocol, all subjects were restricted to food and fluid intakes provided by the University of California, San Diego, General Clinical Research Center. During bed rest, all subjects remained in a 6° head-down tilt position, except during the exercise protocol, when they were horizontal.

**Chamber studies**

The design and subjects of these studies have been described elsewhere (39, 41). Briefly, 4 subjects (1 female, 3 males) were exposed to 60 d and 4 subjects (2 female, 2 male) were exposed to 91 d in an enclosed chamber facility at the Johnson Space Center in Houston. The men were aged 26–36 y, and the women were aged 28–41 y. The food system for the 60-d study was...
designed to be similar to that planned for use on the International Space Station on a 20-d cycle menu (39). For the 91-d study, the food system was designed to be similar to that planned for use on a planetary mission, also with a 20-d cycle. The 20-d cycle menu for the 91-d study consisted of a 50% vegetarian diet, defined as 4 servings of meat/wk.

Biochemical measurements

For the bed rest study, urine was collected (24-h pools) for 3 d before bed rest and on days 5–6, 12–13, 19–20, and 26–27 during bed rest (40). Collagen cross-links [N-telopeptide (NTX), pyridinium (PYD), and deoxypyridinoline (DPD)] in urine were measured by using commercially available kits (PYD and DPD kits were from Quidel Corp, Santa Clara, CA, and the NTX kit was from Ostex International Inc, Seattle), as described previously (40). Urinary calcium was measured by inductively coupled plasma emission mass spectrophotometry techniques, as described previously (40). Fasting (8 h) serum was collected for 3 d before bed rest and during bed rest on days 4 (n = 2 for each group) or 5 (n = 6 for each group), 12, 19, 26, and 31 (40). Serum bone-specific alkaline phosphatase (BSAP) was measured by using a commercially available kit (Quidel Corp).

For the chamber studies, urine was collected every day during the 60-d and 91-d studies. Urine was collected as individual voids, and 24-h pools were created as described (39). Biochemical measurements were completed as described above.

Dietary assessments

Bed rest study

All subjects were placed on an isocaloric diet consisting of 15% protein, 55% carbohydrate, and 30% fat, with 3500 mg sodium, 800–1200 mg calcium, ≥25 g dietary fiber or 1 g/100 kcal, and enough energy to maintain the subject’s pre–bed rest body weight within ±1 kg. The subjects did not participate in the study concurrently. Individual diets were modified to meet the preferences of each subject while maintaining the nutrient requirements outlined above; therefore, the foods and menus were not necessarily the same for each subject. An 8-d menu cycle was used. All foods were weighed on a Mettler balance, and the menus were adjusted daily to maintain the subjects’ pre–bed rest body weights within 1 kg. Any leftover food was weighed to determine actual intake. Energy expenditure was calculated by using the Harris-Benedict equation times an activity factor based on each individual’s reported activity level. Animal protein was grouped as dairy products, poultry, tuna, and beef. Vegetable protein included peanut butter; grains, starches, cereals, and starchy vegetables; fruit; vegetables; and other foods, including margarine, jam, salad dressing, soy sauce, and gelatin. Nutrient data for all foods were obtained by using NUTRITIONIST IV (First DataBank, The Hearst Corporation, San Bruno, CA).

Chamber studies

Five-day weighed-food records were completed for weeks 2 and 5 of the 60-d study and weeks 3, 7, and 11 of the 91-d study (39). The subjects were provided a digital scale and a log book and were taught how to weigh and record all food, fluid, and supplement intakes. Nutrient data for all foods were obtained by using the Nutrition Data System (NDS-R, version 4.01/29; developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis) Food and Nutrient Database 29, released in December 1996.

Statistical analyses

Dietary intakes from the bed rest and 91-d chamber study were analyzed by using two-way repeated-measures analysis of variance. Mean animal protein intakes for the 60-d chamber studies

FIGURE 3. Correlation between deoxypyridinoline (DPD) excretion and the ratio of animal protein intake to potassium intake (APro/K) in ambulatory subjects before bed rest (Pre) and for both sedentary (SED) and exercising (EX) subjects during weeks 1 through 4 of bed rest. Each data point represents the data for 1 subject. Each set of data was analyzed by simple linear regression, and a Pearson’s correlation coefficient (r) was determined. *P < 0.05.
TABLE 3
Pearson’s correlation coefficients, before and during bed rest, for the association between markers of bone metabolism and the ratio of protein to potassium intake in the sedentary (SED) and exercise (EX) groups

<table>
<thead>
<tr>
<th></th>
<th>SED group (n = 8)</th>
<th>EX group (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Week 1</td>
</tr>
<tr>
<td>VPro/K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTX</td>
<td>0.74</td>
<td>0.12</td>
</tr>
<tr>
<td>PYD</td>
<td>0.69</td>
<td>−0.02</td>
</tr>
<tr>
<td>DPD</td>
<td>0.81</td>
<td>−0.27</td>
</tr>
<tr>
<td>Urinary calcium</td>
<td>0.83</td>
<td>0.57</td>
</tr>
<tr>
<td>TPro/K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTX</td>
<td>0.49</td>
<td>0.83</td>
</tr>
<tr>
<td>PYD</td>
<td>0.68</td>
<td>0.66</td>
</tr>
<tr>
<td>DPD</td>
<td>0.55</td>
<td>0.70</td>
</tr>
<tr>
<td>Urinary calcium</td>
<td>0.78</td>
<td>0.86</td>
</tr>
</tbody>
</table>

1 Pre, before bed rest; VPro/K, ratio of vegetable protein intake to potassium intake; TPro/K, ratio of total protein intake to potassium intake; NTX, urinary N-telopeptide excretion; PYD, urinary pyridinium cross-link excretion; DPD, urinary deoxypyridinoline excretion. Linear associations between VPro/K or TPro/K and urinary calcium or collagen cross-links were described with Pearson’s correlation coefficients. In the bed rest study, 2 subjects were missing for 1 data collection session (urinary data for bed rest day 5–6 for both SED and EX groups). Statistical analyses were performed with this reduced data set (n = 6) for this time point.

2 *P < 0.05.

RESULTS

Dietary intake

Mean (±SD) dietary intakes are reported for each week of bed rest (Table 1) and for each weekly dietary intake session from the chamber studies (2 sessions for the 60-d chamber study and 3 sessions for the 91-d chamber study; Table 2). There were no significant changes in these variables over time in the bed rest study or the 60-d chamber study. However, the ratio of total protein intake to potassium intake was higher during the third session and the ratio of vegetable protein intake to potassium intake was lower during the second session of the 91-d chamber study.

Biochemical analysis

Bed rest study

The ratio of animal protein intake to potassium intake was significantly correlated with NTX excretion only during weeks 3 and 4 of bed rest in the SED group and during week 1 of bed rest in the EX group (Figure 1). The ratio of animal protein intake to potassium intake was also significantly correlated with PYD excretion during week 2 of bed rest in the SED group and before bed rest and during week 1 of bed rest in the EX group (Figure 2). The ratio of animal protein intake to potassium intake was significantly correlated with DPD excretion in weeks 2-4 of bed rest in the SED group but in week 4 only in the EX group (Figure 3).

The ratio of vegetable protein intake to potassium intake had significant positive correlations with NTX and DPD excretion before bed rest in the SED group (Table 3). The ratio of total protein intake to potassium intake was significantly correlated with NTX excretion during week 1 in both the SED and the EX groups (Table 3) and with PYD excretion before bed rest in the EX group (Table 3).

The ratio of animal protein intake to potassium intake was significantly correlated with urinary calcium during weeks 2-4 of bed rest for both the SED and the EX groups (Figure 4). The ratio of total protein intake to potassium intake was significantly correlated with urinary calcium before bed rest and during all bed rest weeks for the SED group only (Table 3).

The ratio of animal protein intake to potassium intake was significantly correlated with the bone formation marker BSAP during week 4 in the SED group (r = 0.81, P < 0.05). The ratios of vegetable protein intake to potassium intake and of total protein intake to potassium intake were significantly correlated with BSAP before bed rest (r = 0.74 and 0.72, respectively, P < 0.05).

In the EX group, animal protein intake alone was significantly associated with urinary PYD excretion during weeks 2-4 (r = 0.84, 0.91, and 0.88 for the 3 weeks, respectively, P < 0.05 for each week) and with DPD during weeks 2 and 4 (r = 0.73 and 0.81, respectively). Potassium intake was positively correlated with PYD excretion only during weeks 3 and 4 (r = 0.83 and 81, respectively, P < 0.05). Neither animal protein intake nor potassium intake alone was significantly associated with NTX before or during bed rest.

Chamber studies

In the chamber studies, in which all subjects were ambulatory (although within a relatively confined space), the only significant positive correlation found was between the ratio of animal protein intake to potassium intake and NTX in the 91-d study (Figure 5). Otherwise, the ratio of animal, vegetable, or total protein intake to potassium intake was not significantly associated with...
However, the ratios of vegetable protein intake to potassium intake and of total protein intake to potassium intake were significantly correlated with urinary calcium excretion in the 60-d and 91-d studies, respectively (Table 4). Animal protein or potassium intake alone had no significant correlation with any bone marker in either chamber study (data not shown).

**DISCUSSION**

Bed rest is a well-accepted analogue for many of the physiologic effects of space flight, especially the musculoskeletal changes, which are similar to those observed during space flight studies (42–45). Consistent with previous bed rest studies, the bed rest subjects in the present study were previously shown to have increased urinary calcium, NTX, PYD, and DPD compared with values before bed rest (40). The aim of the present investigation was to determine whether dietary intakes of protein, protein source, and potassium could predict directional changes in markers of bone metabolism. Although there were no significant differences in the group averaged dietary intakes, the individual data showed positive correlations between dietary intakes and markers of bone resorption. Our results indicate that during bed rest, a significant relation does exist between the ratio of animal protein intake to potassium intake and bone metabolism: the higher the ratio, the higher the rate of bone resorption. The relation was not significant in the same subjects before bed rest, and performing an exercise protocol during bed rest tended to blunt the relation, suggesting that the effect of diet on bone resorption may be greater for subjects whose rate of bone breakdown is increased.

The concept of evaluating the ratio of protein and potassium intake was based on a method described by Frassetto et al (18) to...
estimate net endogenous noncarbonic acid production. We ana-
lyzed the ratio of total protein intake to potassium intake on bone
markers and also examined these relations when animal or veg-
etable protein was used instead of total protein. The ratio of
animal protein intake to potassium intake more consistently pre-
dicted directional changes in collagen cross-link excretion than
either the ratio of vegetable protein intake to potassium intake or
the ratio of total protein intake to potassium intake. It is not
surprising, however, that the ratio of total protein intake to po-
tassium intake could also be predictive, because of the contribu-
tion of animal protein. Furthermore, the ratio of vegetable protein
intake to potassium intake could be predictive because of the high
variability in the sulfur content in vegetable protein, if in fact the
predictions in directional changes of bone resorption markers are
due to changes in acid production in the body. Certain vegetable
proteins (eg, oatmeal, walnuts, whole wheat, white rice, and
barley) have more sulfur per gram of protein than do tuna,
chicken, or beef (27, 46, 47). In our data, grains were grouped with
vegetables, starches, and cereals, many of which have more sulfur
per gram of protein than does animal protein. Therefore,
differences in the specific foods making up the vegetable protein
in the different studies (eg, if the vegetable protein was from
wheat, the sulfur content would be higher than if the vegetable
protein was from soy) could explain why only the ratio of animal
protein intake to potassium intake had a significant relation with
resorption markers in some populations but only the ratio of total
protein intake to potassium intake had such a relation in others.

We cannot ignore the potential confounding effect of dietary
calcium intake or calcium status on the effects of dietary protein
on bone. One observational study (29) found no evidence for a
relation between hip fracture and calcium intake or protein intake
from nondairy animal sources. However, subjects in the lowest
quartile of calcium intake and highest quartile of nondairy animal
protein intake had double the risk of this type of fracture. In
another study in which subjects were supplemented with calcium
or placebo, protein intake had a different effect on bone in each
group (36). In the unsupplemented group, protein intake tended
to be associated with bone loss, whereas in the supplemented
group, protein intake had a positive effect on bone (36). In our
study, it is important to note that the bed rest model itself yields
increased bone resorption, increased urinary calcium (48, 49),
and decreased calcium absorption (50); therefore, this decreased
calcium status could be viewed as similar to decreased calcium
status from low calcium intake. Thus, it is not surprising that the
ratio of protein intake to potassium intake tended to predict
negative changes in bone resorption markers during the latter part
of bed rest when bone resorption markers and calcium excretion
were also the highest (40).

A limitation of our study is the relatively small sample size
overall, and particularly in week 1. As noted in the statistical
methods section, only 6 subjects in each of the SED and EX
groups were used in the analysis in week 1 (because of missing
sample collections). This small sample size for week 1 explains
why the Pearson correlation coefficients of 0.80 and 0.70 for
weeks 1 and 2 (Figure 1) for the SED group were not significant.
When weeks 1 and 2 were combined, the ratio of animal protein
intake to potassium intake was significantly correlated (P <
0.05) with urinary NTX in both the SED and the EX groups (r =
0.65 and 0.60, respectively). Although we saw that exercise
tended to blunt the relation between animal protein:potassium
and markers of bone resorption in the present study, a larger
sample size is necessary to establish whether these effects are
real. Another limitation of the present study involves the poten-
tial involvement of other covariates in determining relations be-
tween animal protein:potassium intake and bone resorption
markers. Although we looked at calcium intake, factors other
than protein or potassium intake, including dietary variables,
could be involved.

The ratio of animal protein intake to potassium intake was a
better predictor of urinary NTX excretion in the 91-d chamber
study (r = 0.75, P = 0.005) than in the 60-d study (r = 0.42). One
confounding factor between the studies is the type of diet. The
91-d chamber study included more vegetables and less animal
meat (91-d chamber study subjects averaged 29 g animal protein
per day, 60-d study subjects averaged 42 g animal protein per
day; P < 0.05). Animal protein intake before bed rest (59 g/d for
both the SED and the EX groups) tended to be higher than that
during the chamber studies.

It is notable that intake of animal protein or potassium alone
was generally not related to urinary NTX, PYD, or DPD excre-
tion. According to the results of this study, intake of both acid
and base precursors is required for predicting bone response, rather
than the intake of either precursor alone. This could help explain
why so many conflicting findings have resulted from studies
relating protein intake and bone loss. Most of the studies that
show a negative correlation between protein intake and bone loss
do not report potassium intake data (31–36); therefore, the sub-
jects with the highest protein intakes in those studies could also
have had the highest potassium intakes.

Bed rest is an analogue commonly used to produce physio-
logic changes similar to those that occur during space flight. A
dietary adjustment would be an ideal countermeasure to mini-
mize bone loss among astronauts, because of ease, low cost of
application, and minimal side effects. The results of the present
studies, particularly the results from the bed rest subjects, suggest
that dietary modification to reduce the ratio of animal protein
intake to potassium intake, either by decreasing animal protein
consumption or by increasing potassium intake through dietary
counseling or supplementation, may decrease bone resorption
among astronauts.

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oversaw the completion of the protocols and reviewed and contributed to the
manuscript. SMS was the principal investigator for the chamber studies,
oversaw the biochemical analyses for both studies, and contributed to the
interpretation of the data and the writing of the manuscript. None of the
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