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Dietary protein-induced increases in urine calcium are accompanied by similar increases in urine nitrogen and urine urea: a controlled clinical trial

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Abstract

To determine the usefulness of urine urea (UU) as an index of dietary protein intake 10 postmenopausal women were enrolled and completed a randomized, double-blind, cross-over feeding trial, from September 2008 to May 2010, comparing ten days of a 45g whey supplement to ten days of a 45 g maltodextrin control. Urine nitrogen (UN), calcium (UCa), UU and bone turnover markers were measured at days 0, 7, and 10. Paired sample t tests, Pearson's correlation statistic, and simple linear regression were used to assess differences between treatments, and associations among urinary metabolites. UN/urinary creatinine (UCreat) rose from 12.3 ± 1.7 g/g (99.6 ± 13.8 mmol/mmol) to 16.8 ± 2.2 g/g (135.5 ± 17.8 mmol/mmol) with whey

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supplementation but did not change with maltodextrin. Whey supplementation caused UCa to rise by 4.76 ± 1.84 mg (1.19 ± 0.46 mmol) without a change in bone turnover markers. Since our goal was to estimate protein intake from UN/UCreat, we used our data to develop the following equation: protein intake (g/d) = 71.221 + 1.719×(UN, g)/Creat, g) ($R = 0.46$, $R^2 = 0.21$). As a more rapid and less costly alternative to UN/UCreat, we next determined if urinary urea (UU) could predict protein intake and found that protein intake (g/d) = 63.844 + 1.11×(UU, g/Creat, g) ($R = 0.58$, $R^2 = 0.34$). These data indicate that UU/UCreat is at least as good a marker of dietary protein intake as is urinary nitrogen and easier to quantitate in nutrition intervention trials.

Keywords

Urinary nitrogen; Urinary urea; Whey Protein

Introduction

The effects of dietary protein on bone health are not well characterized. An ongoing 18-month, double-blind nutrition intervention trial (clinicaltrials.gov identifier: NCT00421408) will examine the effect of a 45 g whey protein isolate or maltodextrin control supplement on bone mineral density in older men and women who have low-normal protein intakes. Assessing compliance in long-term clinical trials can be challenging, but significantly affects study outcomes[1], particularly in nutrition intervention trials. The increment in twenty-four hour urine urea is the pre-planned measure of adherence for study subjects in the above mentioned ongoing clinical trial that are randomized to the whey supplement. In an effort to precisely quantify the expected increment in urine urea, we conducted a short-term feeding study. Volunteers for this study met the same entry criteria as those recruited in our 18 month trial and were fed identical supplements using the same study protocol. Complete adherence was assured by feeding subjects all their meals, which were prepared in Yale New Haven Hospital Research Unit's metabolic kitchen. The primary objective of this study was to determine how reproducibly urinary urea (UU) and urinary nitrogen (UN) change with a fixed increment in dietary protein and how well they correlated with each other. A secondary objective was to evaluate the effect of the whey protein supplement on novel and known markers of bone turnover. Using bone turnover markers to assess elevated bone remodeling in response to a wide variety of therapies is of increasing interest, as this methodology allows for rapid, noninvasive evaluation of skeletal homeostasis[2].

Methods

Subjects and Design

We conducted a randomized, placebo-controlled, double-blind, cross-over study consisting of two, 10-day dietary interventions of a moderate protein diet to which was added either a whey protein isolate or maltodextrin control. During both 10-d experimental periods, subjects received all food from the Yale New Haven Hospital Research Unit's metabolic kitchen. These diets contained controlled levels of calcium, sodium and phosphorus. After the first 10-day experimental diet, subjects followed a 2 week washout period prior to

participating in the second intervention. As previously noted, the whey protein and maltodextrin supplements are identical to those used in the larger clinical trial.

Three timed 24-hour urines were obtained between days 0–1, 6–7 and 9–10 of each experimental period for measurement of creatinine (Creat), nitrogen (N), calcium (Ca), and urea (U). Fasting blood and urine samples were collected on days 1, 7 and 10 of each experimental period to measure serum parathyroid hormone (PTH), insulin-like growth factor 1 (IGF-1), osteocalcin and amino-terminal propeptide of type I collagen (P1NP) and urine creatinine (UCreat) and urinary markers of bone turnover.

Ten healthy women (average age 64.2 ± 7 y) who were at least five years postmenopausal volunteered for this study. Exclusion criteria included known skeletal or active inflammatory bowel diseases, untreated hyperparathyroidism, diabetes, history of renal disease or kidney stones, and chronic liver disease. Additional exclusion criteria included any cancer within the past 18 months or long-term use of chemotherapeutic drugs, aromatase inhibitors or tamoxifen, current use of methotrexate, phenytoin, phenobarbital, inhaled corticosteroids (greater than 800 ug/day), active treatment for leukemia or multiple myeloma, a change in thyroid medications, use of herbal supplements with estrogenic activity, medications known to affect calcium metabolism, and the use of proton pump inhibitors twice daily. Women with dietary protein intakes less than 0.6 g/kg or greater than 1.0 g/kg, or a BMI over 32 or under 20 were also excluded. The study was approved by Investigational Review Boards at Yale University (New Haven, CT) and the University of Connecticut (Storrs, CT). All participants gave their written informed consent.

Diets

Subjects completed a 4-day food record prior to starting the study to assess their usual nutrient intake which was used to guide the design of the experimental diets. The experimental diets contained between 0.6 g protein/kg and 1 g protein/kg, and matched the subject’s usual intake of protein. The protein sources used for the experimental diets were a typical mix of animal and vegetable sources. All subjects received a multivitamin daily (One-A-Day 50 plus, Bayer Nutritionals, Morristown, NJ). Dietary calcium was kept in a range of 1200 – 1500 mg (30 – 37.5 mmol) through diet alone or when necessary, the addition of a calcium supplement (Tums ® GlaxoSmithKline, Pittsburg, PA). Other nutrients known to affect calcium metabolism (sodium and phosphorus) and energy were matched to each subject’s usual intake. Forty- five grams of whey protein, Proven 290, (Glanbia Nutritionals Inc, Twin Falls, ID) or maltodextrin, Maltrin® M100, powder (Grain Processing Corporation, Muscatine, IA) was added directly to the subject’s foods/beverages each day throughout the experimental diet. Adding 45 g of whey protein isolate to the experimental diet provided subjects with an additional 40 g of protein. The whey protein isolate and maltodextrin (placebo) supplements were formulated to achieve equivalent caloric density (160 kcal/45g) as well as equivalent content of sodium (73–81mg, 3.2–3.5 mmol), potassium (182 mg, 4.7 mmol), phosphorus (112 mg, 3.6 mmol), and calcium (236–239 mg, 5.9–6.0 mmol) expressed as per 45g powder. Distilled water was provided ad lib. Subjects were also allowed to consume 1 serving of wine or beer/d.

Biochemical Assessment and Assays

Twenty-four hour UN was determined using a micro-Kjeldahl apparatus (Tecator Kjeltec System, Hoganus, Sweden). N balance was calculated using the following equation: N Balance (g) = N Input (g) − N Output (g) + 2 g (an estimation of fecal, dermal, and miscellaneous N losses). UU was analyzed using QuantiChrom™ Urea Assay (BioAssay Systems, Hayward, CA) at an optical density of 515nm. Urinary calcium (UCa) and UCreat were measured on an AlfaWasserman ACE® analyzer (Alfa Wassermann Diagnostic Technologies, LLC, West Caldwell, NJ). Serum intact P1NP was measured using a competitive RIA (Uniq™ P1NP RIA Orion Diagnostica Oy, Espoo, Finland). Serum IGF-1 and urinary alpha and beta urine crosslaps (α-CTX and β-CTX) were measured using commercially available ELISAs (Immunodiagnostic Systems Inc., Scottsdale, AZ). Osteocalcin was measured in the laboratory of Dr. Caren Gunberg (Department of Orthopaedics, Yale School of Medicine) using a double antibody RIA[3].

To control for variability in the completeness of 24-h and 2-h urine collections, urinary excretion N, Ca, U and α-CTX and β-CTX were expressed as a ratio with Creat excretion.

Data Analysis

Analyses were performed using SPSS (version 12.0 for Windows, 2003, SPSS Inc., Chicago, IL). Graphical summaries were generated using Prism (version 4.0, GraphPad Software, La Jolla, CA). The baseline and intervention data are presented as mean ± SEM. An initial paired t-test showed no significant difference in any metabolites between day 7 and 10 (with the exception for IGF-1). Thus, data from day 7 and 10 were averaged and these mean data used when analyzing the effects of the two dietary interventions. Paired sample t tests, Pearson's correlation statistic, and simple linear regression were used to assess differences between treatments, and associations among urinary metabolites. A probability level of $P < 0.05$ was statistically significant and 0.05–0.10 was suggestive of a trend.

Results and Discussion

Participants

Ten subjects completed and tolerated the study without difficulty. Two subjects did not complete the study, one due to distaste for study food and one because of newly diagnosed type 2 diabetes. Body weight (71.3 ± 8.4 kg) remained constant in all subjects throughout the study. The average nutrient content of the experimental diets (exclusive of the study supplements) were as follows: protein: 80.4 ± 19.4 g, calcium: 1156 ± 124 mg (28.9 ± 3.1 mmol), phosphorus: 1206 ± 240 mg (38 ± 8 mmol), sodium: 2528 ± 628 mg (110 ± 27 mmol), magnesium: 281 ± 48 mg (12 ± 2 mmol), potassium: 2800 ± 632 mg (72 ± 16 mmol) and fiber: 19.6 ± 5.5 g. Within each subject there were no significant differences in the nutrient content of the experimental food sources between the two interventions. The small sample size, which can be considered a study limitation, allowed the feasibility of the tightly controlled dietary intervention, a primary strength of the study.

Urinary Markers of Protein Intake

Baseline and intervention measures of urine metabolites, markers of bone turnover and N balance are presented in the Table. As expected baseline urinary metabolites did not differ between interventions. UN/Creat rose with whey supplementation in comparison to baseline (from 12.3 ± 1.7 g/g (99.6 ± 13.8 mmol/mmol) to 16.8 ± 2.2 g/g (135.5 ± 17.8 mmol/mmol), P = 0.004), but did not change with maltodextrin. A similar trend was observed for UU/Creat excretion during the protein intervention (from 24.9 ± 3.5 g/g (100.73 ± 14.09 mmol/mmol) to 34.0 ± 4.0 g/g (137.2 ± 16.1 mmol/mmol), P = 0 .069). UN/Creat (16.8 ± 2.2 g/g (135.5 ± 17.8 mmol/mmol)) and UU/Creat (34.0 ± 4.0 g/g (137.2 ± 16.1 mmol/mmol)) were higher during the protein supplemented diet compared to the maltodextrin control (10.6 ± 1.1g/g (85.5 ± 9.1 mmol/mmol), P = 0.008, 21.0 ± 2.2 g/g (84.8 ± 8.9 mmol/mmol), P = 0 .007, respectively). Our finding that 24-hr UN increased with increased dietary protein is consistent with several previously published reports[4–8]. As noted, we measured 24-hr UU to determine if it could serve as a convenient measure of adherence in our larger intervention. In women UN faithfully tracks with UU, with UU representing a constant fraction of UN except at very low levels of protein intake[9]. The relationship between total N and urea nitrogen has also been examined[9–11]. For individuals consuming greater than adequate amounts of dietary protein, 82 ± 2% of total UN is in the form of urea[9]. However, if individuals consume a lower protein diet, the other N sources, such as Creat, contribute relatively more N, and urea may not accurately depict total UN to the same extent[11]. Currently, urea is regularly measured by clinicians because it is simpler and faster than the Kjeldahl method of measuring total UN[11].

In our study, we observed a statically significant positive relationship between UN and UU ($P < 0.0001$, $r = 0.73$). To determine if UN and/or UU could be used as indices of dietary protein intake, we pooled baseline and intervention data to develop the following equations relating protein intake to these two urine parameters. First, using UN, we developed the following equation: protein intake (g/d) = 71.221 + 1.719 × (UN, g)/Creat, g) ($R = 0.46$, $R^2 = 0.21$). For UU, the formula was: protein intake (g/d) = 63.844 + 1.11 × (UU, g/Creat, g) ($R = 0.58$, $R^2 = 0.34$). As can be seen from these two equations UU proved to correlate at least as well if not better than UN with dietary protein. Measuring UN is cumbersome, time consuming and requires considerable operator experience to ensure reasonable interassy coefficients of variation. In contrast measuring UU is rapid and simple and uses a highly reliable ELISA methodology. Because our larger clinical trial employs dietary protein intakes that are considered moderate, along with the ease and greater precision of UU measurement, our findings support the conclusion that UU is a reliable index for protein intakes in this range. Consequently, UU is an appropriate measure of compliance for the ongoing larger clinical trial.

Dietary Assessment Methods

A number of current dietary assessment techniques rely on various methods of dietary recall, tracking and recording which depend heavily on an individual's memory and level of motivation[12, 13]. Commonly used assessment methods tend to require prior knowledge of nutrition and portion sizes, literacy and/or time, thus burdening the patient or subject and decreasing the likelihood of obtaining reliable dietary intake data[13–15]. Our method relies on

a quantifiable bioindex of dietary protein intake thus circumventing limitations seen with a number of current more subjective dietary assessment tools[12–15].

Urinary Calcium and Dietary Protein

As summarized in the Table, UCa/Creat tended to increase during the protein intervention (0.35 ± 0.06 mg/mg (1.00 ± 0.18 mmol/mmol)) compared to protein baseline (0.29 ± 0.06 mg/mg (0.83 ± 0.18 mmol/mmol), P = 0 .064). There were also strong, significant, positive correlations between UCa/Creat and UN/Creat (P < 0.0001, r = 0.66) and UCa/Creat and UU/Creat (P < 0.0001, r = 0.58; Figure). These findings are consistent with the well-established observation that increasing dietary protein results in an increase in UCa. A review of over 20 clinical trials found a strong positive linear association between protein intake and UCa[16]. On average, for every 50 g increase in dietary protein, there is approximately a 6.4 mg (1.6 mmol) increase in 24-h UCa[16]. In this current study, a modest increase of only 40 g of protein resulted in a 4.76 ± 1.84 mg (1.19 ± 0.46 mmol) average increase in UCa. The magnitude of change in UCa between the two studies is remarkably close, particularly given the fact that the Kerstetter review included many studies of varying lengths, interventions and subjects. These findings underscore the fact that dietary protein has an important and measurable impact on UCa[17, 18]. Consistent with our previous work, there were no short-term changes in bone turnover with protein supplementation (Table), indicating that the increase in UCa we observed, is more than likely primarily due to improved intestinal Ca efficiency[19, 20].

Conclusion

In summary urinary urea holds promise as a more convenient and equally reliable index of dietary protein consumption as compared to urinary nitrogen. Larger and longer clinical trials should provide more robust estimates of this relationship.

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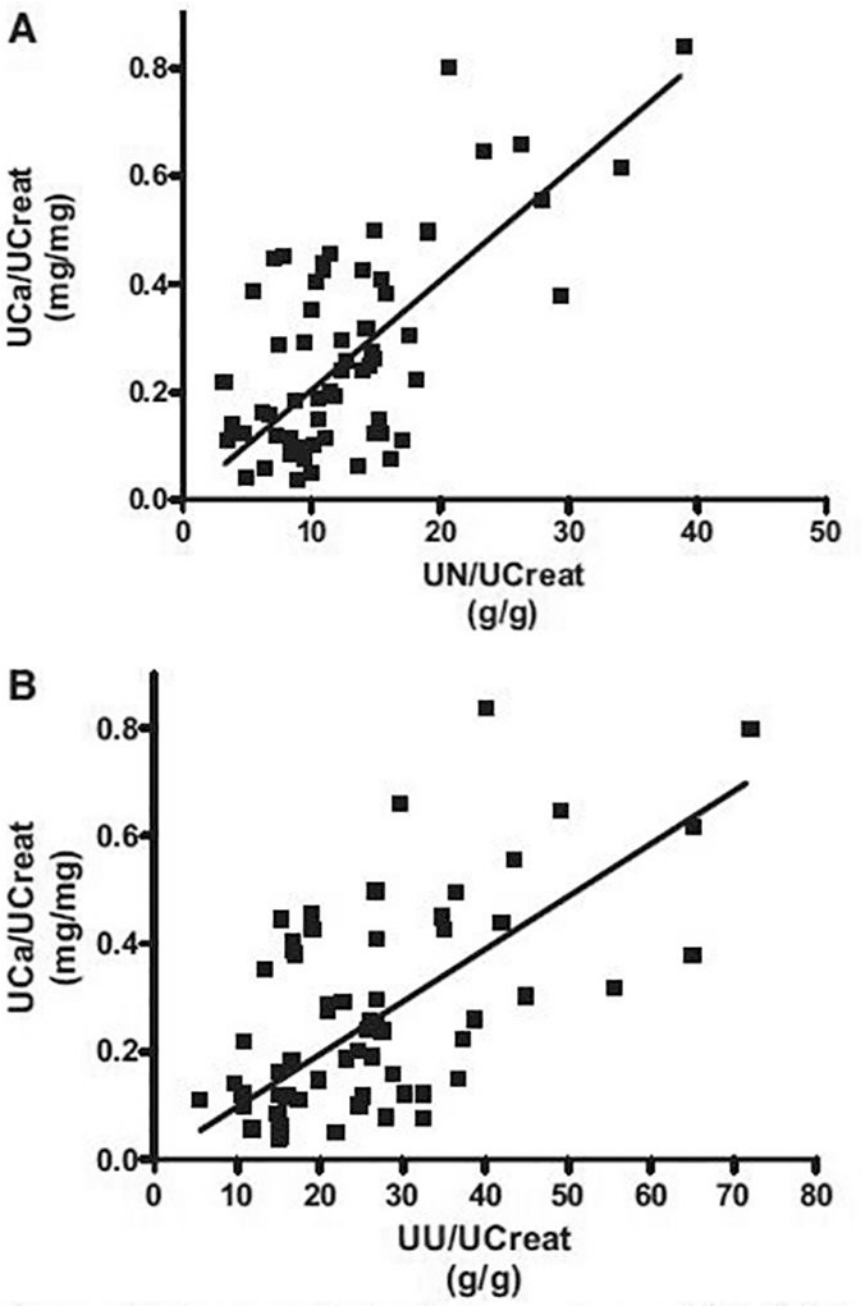

Figure 1.

Significant correlation between urinary calcium (UCa) and (A) urinary nitrogen (UN) ($P<0.0001$; $r=0.66$) and (B) urinary urea (UU) ($P<0.0001$, $r=0.58$) from 10 postmenopausal women participating in a cross-over study consisting of two 10-day interventions where dietary protein was manipulated (pooled data).

Table

Baseline and intervention measures of urine and serum markers of bone turnover and protein status of 10 postmenopausal women participating in a cross-over study consisting of two 10-day interventions where dietary protein was manipulated

	Control		Protein	
	Baseline	**Intervention**	**Baseline**	**Intervention**
Urine ratios	← mean±standard error of mean →			
24-h UN[a]/urinary creatinine (g/g)	11.7±2.4	10.6±1.1	12.3±1.7	16.8±2.2[bc]
24-h UCa[d]/urinary creatinine (mg/mg)	0.21±0.04	0.22±0.05	0.29±0.06	0.35±0.06[c]
24-h UU[e]/urinary creatinine (g/g)	26.8±6.1	21.0±2.2	24.9±3.5	34.0±4.0[c]
2-h α-CTX/urinary creatinine (μg/mmol)	0.69±0.12	0.64±0.09	0.7±0.11	0.77±0.11
2-h β-CTX/urinary creatinine (μg/mmol)	2.61±0.70	2.27±0.41	2.34±0.53	2.59±0.48
Serum				
Intact parathyroid hormone (pg/mL)	39.3±4.5	38.7±3.3	41.8±3.2	38.3±3.2
Propeptide of type I collagen (ng/mL)	55.9±4.5	55.9±5.2	56.7±4.3	54.9±4.0
Insulin-like growth factor 1 (ng/mL)	86.34±7.40	86.41±7.56	85.04±7.71	89.69±7.94
Osteocalcin (ng/mL)	7.4±0.9	7.7±1.0	7.4±0.8	7.3±0.8
Nitrogen balance (g)	4.1±1.4	2.3±1.1	3.6±1.4	6.4±1.8[cf]

[a] UN=urinary nitrogen.

[b] Significantly different from protein baseline; $P<0.01$.

[c] Significantly different from control intervention; $P<0.05$.

[d] UCa=urinary calcium.

[e] UU=urinary urea.

[f] Significantly different from protein baseline; $P<0.05$.