Fasting and Energy Intake Influence Bone Turnover in Lightweight Male Rowers

*Shawn M. Talbott and Sue A. Shapses*

The purpose of this study was to investigate the influence of an acute 24-hr fast versus usual 24-hr dietary intake on markers of bone turnover in collegiate lightweight male rowers. Bone turnover was measured by serum osteocalcin (OC) and urinary excretion of pyridinium cross-links (pyridinoline [PYD] and deoxypyridinoline [DPD]). Fasting subjects (F) (*n* = 14) reduced body weight by 1.7 ± 0.5 kg but there was no significant change among nonfasting subjects (NF) (*n* = 13). Following 24 hr of fasting, PYD and DPD were lower in F (14.1 ± 2.2 and 5.2 ± 0.7 nmol/mmol creatinine, respectively) compared to NF (16.4 ± 3.6 and 6.0 ± 0.8 nmol/mmol creatinine) (*p* < .05). Fasting also reduced OC levels (4.8 ± 0.4 ng/ml) compared to NF (6.1 ± 0.9 ng/ml) (*p* < .01). Stepwise regression analysis of NF dietary intake indicated that energy intake explained a greater portion of the variation in bone turnover for PYD (34%), DPD (36%), and osteocalcin (46%) compared to other nutrients (*p* < .05). These results indicate that bone turnover is reduced by 24 hr of fasting and suggest a role for dietary energy intake in regulating bone turnover.

**Key Words:** athletes, nutrition, osteocalcin, pyridinium cross-links, starvation

Bone turnover is known to be influenced by dietary intake and by change in body weight. In particular, dietary intakes of calcium (13), vitamin D (6, 15), protein (27), and total energy (21, 24) have been related to bone turnover and bone mass. For example, low calcium intake stimulates osteoclast recruitment and increases bone resorption (32, 39), whereas chronic undernutrition may decrease bone turnover and result in bone loss (29, 33, 36). Grinspoon et al. (11) recently showed reduced bone formation, but no change in bone resorption, subsequent to 4 days of complete fasting in healthy young women. This suggests that fasting may overwhelm the complex nutrient interactions that normally regulate bone turnover. It is unclear, however, whether males respond to fasting in a similar manner to the females previously investigated (11) or whether shorter periods of fasting (e.g., 24 hr) influence bone turnover.

Fasting is a common method of achieving rapid weight loss in weight-category sports such as wrestling and lightweight rowing. Lightweight rowers severely restrict food intake to reduce and maintain body weight within competition weight...
limits (<70 kg). Rowers use a variety of weight loss methods, including acute cycles of complete fasting and chronic periods of severe caloric restriction, to achieve target weights. To determine the acute effects of nutrient intake and fasting on bone turnover, we investigated the role of usual dietary intake and the influence of a complete 24-hr fast on markers of bone formation (serum osteocalcin) and bone resorption (urinary pyridinium cross-links, pyridinoline and deoxypyridinoline) in young male lightweight rowers.

Methods

Subjects

Collegiate lightweight male rowers (n = 27) were recruited. To avoid interfering with an athlete's normal training routine, group assignment (e.g., fasting/nonfasting) was determined by the athlete's need to lose weight before the next weigh-in. Rowers who needed to lose weight, and planned do so by fasting, were assigned to the fasting group (F). Those athletes assigned to the nonfasting group (NF) adhered to the same workout schedule as those in F but were either already at their target weight or were not competing that week and did not have to lose weight.

All subjects gave informed consent and were screened for medications or dietary supplements known to influence cortisol or bone turnover. The study was approved by the Institutional Review Board of Rutgers University.

Experimental Design

Each subject was required to provide a blood and urine sample at baseline and again after 24 hr. Samples were collected at the same time of day (5:30 p.m.). After baseline collections, subjects either adhered to a complete (water-only) fast (F) or consumed their usual dietary intake (NF) for 24 hr. Athletes in the NF group recorded their food intake during the 24-hr period between sample collections. All subjects were provided custom diet records and were instructed in their use (including how to estimate serving sizes). Subjects were asked to carry the diet records with them, enter each food and serving size, and note the meal and time of day at which each food was consumed. Subjects followed their normal training schedule but were instructed to abstain from vitamin and mineral supplements during the 24 hr between sample collections.

Measurements

Urine samples were assayed for pyridinium cross-links. Total pyridinium cross-links (PYDX), pyridinoline (PYD), and deoxypyridinoline (DPD) were measured using high-performance liquid chromatography by a modified method of Eyre et al. (8). A 0.5-ml aliquot of urine was hydrolyzed in 6 mol/L HCl and applied to a CF-1 cellulose (Whatman, Maidstone, Kent, UK) column for fractionation according to the methods of Black et al. (4). The expected normal range for healthy adult males is 15–30 nmol/mmol for PYD and 3–8 nmol/mmol for DPD. All samples were analyzed in duplicate. Assay reproducibility was good, as indicated by coefficients of variation of 3.8% and 5.9% for PYD and DPD, respectively, measured in 4 young healthy subjects on three consecutive days. Cross-link values are expressed per creatinine excretion (nmol/mmol). Creatinine was measured in urine with a
colorimetric method using alkaline picrate solution (Sigma Diagnostics, St. Louis, MO) and a spectrophotometer at a wavelength of 500 nm.

Blood serum was analyzed for cortisol and osteocalcin concentration by standard radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX). Cortisol was detected using a specific rabbit anticortisol antibody. Osteocalcin was detected using a double antibody system with bovine osteocalcin as a standard, rabbit antiovine osteocalcin antiserum, and goat antirabbit gamma globulin as the precipitating reagent. The expected normal range for healthy adults is 9–23 μg/dl for serum cortisol and 4–12 ng/ml for serum osteocalcin. In our laboratory, intra- and interassay variability is 5.3–11.1% and 8.9–11.5%, respectively, for cortisol and 5.4–8.4% and 5.5–14.7%, respectively, for osteocalcin.

Nutrient analysis of 24-hr dietary recalls was performed for nonfasting athletes using nutrition analysis software (Nutritionist IV, First DataBank, San Bruno, CA). The same individual entered all diet records into the database, and all nutrient data were cross-checked for errors before analysis.

**Statistical Analysis**

Data were analyzed by repeated-measures ANOVA (SuperANOVA Version 1.11, Abacus Concepts, Berkeley, CA) with group (F/NF) and time (pre/post) as independent factors and osteocalcin, pyridinium cross-links, and cortisol as dependent variables. Simple linear regression analysis was performed (Statview Version 4.5, Abacus Concepts) in NF subjects to examine the relationship between dietary intake, bone turnover, and cortisol. Significant correlations were further analyzed by stepwise regression. Data are reported in text as mean ± SD.

**Results**

**Food Intake and Body Weight**

Subjects' average age was 19.5 ± 1.2 years and average body mass index was 22.1 ± 2.2 kg/m². Height, initial weight, and age were not significantly different between the F and NF subjects (Table 1). Intake of selected nutrients is reported for NF subjects in Table 2. Compared to the recommended dietary allowances (9), mean intake of selected nutrients was within recommended levels for protein (127 ± 62%) and phosphorous (98 ± 47%); low for total energy (71 ± 36%), calcium (89 ± 51%), and vitamin D (34 ± 34%); and extremely high for vitamin C (520 ± 322%). Estimated daily energy requirement for all subjects was 3,903 ± 969, with NF subjects reporting a wide range of energy intakes (54–173% of estimated requirements).

Athletes assigned to F consumed no food for 24 hr and reduced body weight by 2.4 ± 0.3% (p < .05). Fasting subjects were initially at 93 ± 2% of ideal body weight (40) before fasting and lost 1.7 ± 0.5 kg to reach their target weight goal. NF subjects were at 91 ± 3% of ideal body weight and had no significant change between measurements.

**Bone Turnover and Cortisol**

At baseline, PYD and DPD averaged 17.8 ± 3.1 and 6.1 ± 1.0 (nmol/mmol cr), respectively, and were not significantly different between groups (Table 1). Following
Table 1  Subject Characteristics and Responses to 24 hr of Fasting

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fasting (n = 14)</th>
<th>Nonfasting (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>24 hr</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>19.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.9</td>
<td>5.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Pyridinoline (nmol/mmol cr)</td>
<td>18.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Deoxypyridinoline (nmol/mmol cr)</td>
<td>6.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>5.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Cortisol (μg/dL)</td>
<td>16.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Differs from baseline, p < .05. **Differs from NF, p < .05.

Table 2  Nutrient Intake for Nonfasting Rowers (n = 13)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2,771</td>
<td>1,429</td>
<td>1,014–6,162</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>74</td>
<td>37</td>
<td>28–153</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1,068</td>
<td>611</td>
<td>149–2,179</td>
</tr>
<tr>
<td>Phosphorous (mg)</td>
<td>1,181</td>
<td>571</td>
<td>191–1,854</td>
</tr>
<tr>
<td>Vitamin D (μg)</td>
<td>3.4</td>
<td>3.4</td>
<td>0–11.4</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>312</td>
<td>322</td>
<td>22–614</td>
</tr>
<tr>
<td>% PRO</td>
<td>11</td>
<td>4</td>
<td>6–19</td>
</tr>
<tr>
<td>% CHO</td>
<td>71</td>
<td>10</td>
<td>54–86</td>
</tr>
<tr>
<td>% Fat</td>
<td>18</td>
<td>8</td>
<td>7–29</td>
</tr>
</tbody>
</table>

24 hr of fasting, urinary excretion of PYD and DPD decreased by 27.1 ± 22.4% and 21.3 ± 22.7% (p < .05), respectively, with no significant change in bone resorption in the NF group (Figure 1). Serum osteocalcin levels averaged 6.1 ± 0.8 ng/ml at baseline and were not significantly different between groups (Table 1). Fasting reduced serum osteocalcin by 20.2 ± 15.3% (p < .05) with no change among NF subjects (Figure 1).

Serum cortisol levels averaged 16.4 ± 2.0 μg/dl at baseline and were not significantly different between groups (Table 1). Cortisol values were reduced 32.2 ± 4.4% and 25.3 ± 5.1% in F and NF, respectively, from the first measurement to the second (p < .05, Figure 1).
Dietary Analysis

Analysis of 24-hr dietary intake among NF subjects indicated a wide range of energy intake (1,014–6,162 kcal). One subject reported an energy intake that was much greater than the mean (6,162 vs. 2,771 kcal). Analysis of the correlation between nutrient intake and bone turnover, however, indicated similar results whether this subject was included or excluded from the data set. Results presented here include all NF subjects.

Simple linear regression indicated a significant negative correlation ($p < .05$) between dietary intake of calcium and energy and markers of bone resorption (PYD vs. calcium, $R = .57$; PYD vs. energy, $R = .59$; DPD vs. calcium, $R = .57$; DPD vs. energy, $R = .60$). Serum OC, however, was positively correlated with dietary intake of energy ($R = .68, p < .01$) and protein ($R = .66, p < .01$). Using the ratio between bone resorption and bone formation (PYD/OC and DPD/OC) as an index of bone turnover balance, a strong association ($R = .73, p < .01$) was found with dietary energy intake (Figure 2). Stepwise regression analysis indicated that dietary energy intake explained a greater portion of the variation in PYD (34%), DPD (36%), and osteocalcin (46%) compared to other nutrients. No influence of fasting or dietary intake was noted on cortisol levels, nor was cortisol associated with markers of bone turnover.

Discussion

In this study, we investigated the role of dietary intake and the influence of fasting on bone turnover in lightweight male rowers. Markers of bone turnover were reduced following a 24-hr fast, suggesting an overall suppression of bone turnover by complete dietary restriction. Additionally, our data in nonfasting athletes suggest an association between dietary energy intake and bone turnover whereby partial energy restriction may promote bone resorption and suppress formation.
Previously, Grinspoon and colleagues demonstrated a 40–50% reduction in serum osteocalcin (11) and urinary pyridinium cross-links (10) following 4 days of fasting in healthy young females. In the current study we found a 20–25% reduction in the same markers of bone turnover following a shorter fast (24 hr) in male athletes. To our knowledge, this is the first investigation of the effect of dietary intake on markers of bone turnover in males as well as the first to demonstrate a reduction in bone turnover following acute (24 hr) fasting. Our data in males, as those of Grinspoon et al. in females (10, 11), provide direct evidence for a relationship between dietary restriction and reduced bone turnover, which may help explain previous findings of reduced bone mass in conditions of severe chronic undernutrition (29).

Chronic nutritional deprivation, whether generalized as in protein–energy malnutrition, or specific as in vitamin, mineral, or amino acid deficiencies, can impair linear bone growth and maturation (29), collagen metabolism (27, 36), and bone mineralization and biomechanics (36). Dietary restriction may adversely influence bone metabolism through a number of mechanisms including effects on
insulin, insulin-like growth factors, calcium-regulating hormones, and sex steroids. Acute and chronic periods of food deprivation (e.g., fasting and anorexia nervosa) decrease levels of insulin and insulin-like growth factor–I (IGF-I) (10, 12), both of which have anabolic effects on bone (19). Although we did not measure serum insulin or IGF-I, it is likely that levels were reduced in our fasting athletes compared to nonfasting controls (10). Testosterone, which also has an anabolic effect on bone and increases bone turnover, is reduced in male endurance athletes, possibly due to elevated energy expenditure of exercise coupled with chronic dietary restriction (2). Finally, exercise is known to influence bone turnover, such that endurance athletes have reduced bone turnover and bone mass compared to power athletes and sedentary control subjects (5). Exercise-mediated alterations in bone turnover, which may be mediated by calcium regulating and anabolic hormones (2, 5, 19), are unlikely to have differed between our subjects, who were members of the same team and were following the same training program.

Cortisol has been shown to suppress serum osteocalcin (22, 25), and corticosteroid administration suppresses overall bone turnover (14, 26). This led to our hypothesis that bone turnover would be related to serum cortisol, with elevated levels of cortisol being associated with reduced rates of bone turnover. Our finding that there was no relationship between serum cortisol and bone turnover in these athletic subjects was unexpected but has been observed by others in elderly women (1). We did, however, observe a 30% reduction in serum cortisol levels in all athletes from the first to the second measurement. Although fasting is generally considered to increase cortisol levels (3), this effect may be attenuated during periods of reduced exercise intensity and duration (e.g., tapering) (7, 23, 30, 34). Additionally, reduced serum cortisol values in both groups of athletes may also be due to an adaptation of the hypothalamo-pituitary-adrenal axis. Repeated exposure to stress (e.g., chronic exercise training or repeated bouts of fasting) diminishes the adrenal cortisol response (20, 38) and may have blunted the cortisol response to fasting in our subjects. Finally, it is possible that if fasting and exercise altered the normal diurnal rhythm of serum cortisol, which peaks in the early morning and reaches its nadir in the late afternoon, a single cortisol value may not accurately characterize the body’s true cortisol levels (3, 35).

The nonrandomized design of this study allowed “self-selection” of subjects into fasting or nonfasting groups and may have biased our results if one group was more “adapted” to diet or exercise stress than the other. However, both groups were part of the same varsity team and were well matched in terms of exercise level, height, and age.

In nonfasting athletes, we expected nutrient intake, particularly vitamin C (24), vitamin D (6, 15), calcium (6, 32, 39), and protein (27, 28), to be related to bone turnover. However, we found significant relationships only for calcium, protein, and total energy intake. Our finding that dietary energy intake had the strongest relationship with bone turnover supports the results of Michaelsson et al. (21), who found an independent association of macronutrient intake (carbohydrate, protein, and fat) with bone mineral density but no significant relationship between dietary intake of isolated nutrients and serum osteocalcin. When our data are expressed as a ratio between bone resorption and formation, however, a stronger association with energy intake is noted, in which reduced energy intake is associated with increased bone resorption and decreased bone formation (see Figure 2).
Due to the small number of nonfasting subjects examined here and the well-known limitations associated with self-reported diet records, it is difficult to draw strong conclusions regarding the relationship between nutrient intake and bone turnover. Of interest is our finding that these “nonfasting” athletes had a wide range of reported energy intakes, ranging from 1,014 to 6,162 kcal/day, or 54 to 173% of their estimated daily energy requirements (9). This finding suggests that some nonfasting subjects were either restricting or underreporting their energy intake. In addition, because our examination of dietary intake covered only a single 24-hr period in nonfasting subjects and did not control for diet prior to the 1-day fast in fasting subjects, previous diet history could influence these findings. Nonetheless, because these markers of bone turnover respond acutely to changes in dietary intake (32), we feel that previous diet history played a smaller role in our findings and that the relationship between energy intake and bone turnover is interesting and warrants further study.

Because of the high correlation between intake of most nutrients and total energy, epidemiologists have recommended using energy-adjusted nutrient intakes (37) in studies of nutrition and disease. Previous use of energy-adjusted nutrient intakes by other investigators, however, has shown conflicting relationships between specific nutrients and various parameters of bone turnover and density (22, 24). Our data, after adjustment for total energy intake, show no significant relationships between isolated nutrients and markers of bone turnover. An interesting point of difference between the present study and previous investigations is that our subjects were young male athletes, whereas other researchers examined somewhat older sedentary female subjects (21, 22, 24). It is possible that the relationship between nutrient intake and bone turnover differs with age and gender. We suggest that the relationship between total energy intake and bone turnover is part of a complex interaction between several nutrients, with energy intake explaining a greater proportion of the variance. Moreover, this work supports the work of others who have found energy intake to be related to bone mineral content (18, 24) as well as a significant variable in predicting the annual rate of change in lumbar spine bone mineral density (17). Additionally, because markers of bone turnover can predict future bone mineral density (31), further investigation of the relationship between energy intake and bone mass is warranted.

This study demonstrates a rapid fall in markers of bone turnover following a 24-hr fast in young male athletes. Data of nonfasting athletes suggest an association between energy intake and bone turnover, whereby dietary energy restriction may promote bone resorption and suppress formation. The long-term effect of repeated periods of acute fasting or chronic energy deficit on bone turnover and bone mass remains to be elucidated.

References


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