A Grape-Enriched Diet Increases Bone Calcium Retention and Cortical Bone Properties in Ovariectomized Rats1–3

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Abstract

Background: Grapes and their associated phytochemicals have been investigated for beneficial effects on cardiovascular health, cancer prevention, and other chronic diseases, but the effect of grape consumption on bone health has not been fully determined. We previously found short-term benefits of grape products on reducing bone turnover in ovariectomized rats.

Objective: The objective of this study was to determine the long-term benefits of a grape-enriched diet on bone in ovariectomized rats.

Methods: Rats were ovariectomized at 3 mo of age and were administered a single dose of $^{45}\text{Ca}$ to prelabel bones at 4 mo of age. After a 1-mo equilibration period, baseline urinary $^{45}\text{Ca}$ excretion was determined. Rats ($n = 22$/group) were then randomly assigned to a modified AIN93M diet containing 25% freeze-dried grape powder or to a control diet for 8 wk. Urinary $^{45}\text{Ca}$ excretion was monitored throughout the study to determine changes in bone $^{45}\text{Ca}$ retention. Calcium balance was assessed after 1 and 8 wk with the experimental diets, and a calcium kinetic study was performed at 8 wk. After 8 wk, femurs were collected for micro-computed tomographic imaging, 3-point bending, and reference point indentation.

Results: Rats fed the grape-enriched diet had 44% greater net bone calcium retention than did rats fed the control diet. There were no differences in calcium balance due to diet at either week 1 or week 8, but there was a significant increase in net calcium absorption (10.6%) and retention (5.7%) from week 1 to week 8 in the grape-enriched diet group only. Grape-enriched diet–fed rats had 3% greater cortical thickness and 11% greater breaking strength. There were no differences in femur bone mineral density, trabecular microarchitecture, or reference point indentation variables due to diet.

Conclusion: The consumption of grape products may improve calcium utilization and suppress bone turnover, resulting in improvements in bone quality.

Keywords: bone, grape, osteoporosis, calcium, rats

Introduction

Osteoporosis is a disease of low bone mass and increased fracture risk that affects 10.2 million Americans. Another 43 million are at risk of osteoporosis due to low bone mass (1). Current osteoporosis therapies, such as hormone replacement therapy, bisphosphonates, and teriparatide, are effective in reducing fracture risk (2), but compliance with these drugs is poor (3) and links to severe potential adverse events has called the long-term safety of these therapies into question (4–7). New treatment strategies, including dietary and botanical interventions, are greatly needed. Grape (Vitis vinifera) and grape product consumption has been linked to a number of health benefits, including cardiovascular health, cancer prevention, and neurocognitive protection (8). Although the health benefits of grape and grape product consumption have been well established in other systems, the skeletal effects of grape consumption have not been well characterized.

Grapes contain a number of polyphenols, including quercetin, resveratrol, catechins, anthocyanins, and proanthocyanidins (9, 10). Grape and grape polyphenols have been linked epidemiologically to improved bone health. In Australian men, red wine consumption was positively associated with change in lumbar spine bone mineral density (BMD)4 over 2 y (11). In women aged 18–79 y, total flavonoid, anthocyanin, flavonol, and flavonoid polymer intakes were positively associated with spine bone mineral density, trabecular microarchitecture, or reference point indentation variables due to diet.

1Supported by the California Table Grape Commission.
2Author disclosures: EE Hohman, no conflicts of interest. CM Weaver received research funding for this study from the California Table Grape Commission.
3Supplemental Table 1 is available from the ‘Online Supporting Material’ link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
4Abbreviations used: BMD, bone mineral density; L(i,j), fractional transfer rate to compartment $i$ from compartment $j$; microCT, microcomputed tomography; NTX, N-terminal telopeptide; RPI, reference point indentation; $\mu$Ci, microcuries.

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BMD, and anthocyanin intake was positively associated with hip BMD (12). In postmenopausal women, flavonol, catechin, and total flavonoid intakes were positively associated with femoral neck BMD (13). Several of the constituent polyphenols in grapes were shown to benefit skeletal health in animal models, Quercetin (14), kaempferol (15), resveratrol (16), and catechins (17) protected against bone loss in ovariectomized rats. In calcium-depleted rats, the addition of grape seed proanthocyanidin extract to a calcium-replete diet restored bone mineral content to a greater extent than did a calcium-replete diet alone (18). Mühlbauer et al. (19) showed that dealkoholized red wine significantly reduced bone turnover in rats.

We recently used bone 45Ca retention to compare a number of botanical products, including several grape preparations, in ovariectomized rats. In this study, rats were administered a dose of 45Ca to label their bones. After a 1-mo period to allow the 45Ca to equilibrate with bone calcium, we measured baseline urinary 45Ca excretion to determine baseline bone turnover. Following the protocol of Mühlbauer et al., rats were then treated with botanical preparations for 10 d at a time, and changes in urinary 45Ca excretion were measured to determine the change in bone 45Ca retention in response to the botanical treatments. We found that resveratrol significantly improved bone 45Ca retention, whereas grape extract and grapeseed extract decreased urinary N-terminal telopeptide (NTX), a marker of bone resorption (20). To follow up on these findings, we conducted a 2-mo feeding trial in ovariectomized rats to determine the effect of grape consumption on bone turnover, bone density, microarchitecture, and material properties. To explore potential mechanisms for the effect of grape on bone, we also investigated the effects of a grape-enriched diet on calcium absorption and calcium balance.

Methods

Study design. Forty-four 3-mo-old ovariectomized Sprague-Dawley rats (Harlan Laboratories) were individually housed in stainless steel wire-bottom cages with a 12-h light cycle and were fed a modified AIN93M diet (Research Diets) and deionized water ad libitum. One month after ovariotomy, during which rats were acclimated to our facility, rats were injected with 50 µg microcuries (µCi) 45Ca dissolved in sterile saline. After allowing another month for 45Ca to clear from the serum (compartment 1), blood (compartment 6), urine (compartment 7), and feces (compartment 9), blood, urine, and feces samples were diluted in 0.5 N HCl/0.5% lanthanum and analyzed by atomic absorption spectrometry (AAnalyst 300; Perkin Elmer).

45Ca and total calcium analysis. Before analysis, feces were heated at 600°C, solubilized in nitric acid, and diluted in ultrapure water. To determine 45Ca counts, urine, serum, and fecal samples were diluted with Ecolite scintillation fluid (MP Biomedicals) and counted via liquid scintillation counting (Beckman LS 6500; Beckman Coulter). To determine total calcium, urine and fecal samples were diluted in 0.5 N HCl/0.5% lanthanum and analyzed by atomic absorption spectrometry (AAnalyst 300; Perkin Elmer).

Diets. All diet formulations were based on the AIN93M diet (21). Grape powder was provided by the California Table Grape Commission. The grape powder is a freeze-dried composite of seeded and seedless varieties of fresh red, green, and blue-black grapes. The powder contains 4950 mg/kg total polyphenols, including cyanidin (264 mg/kg), malvidin (194 mg/kg), peonidin (46 mg/kg), catechin (24 mg/kg), epicatechin (23 mg/kg), quercetin (71 mg/kg), kaempferol (5 mg/kg), isorhamnetin (12 mg/kg), and resveratrol (0.7 mg/kg). Polyphenol analysis was provided by the California Table Grape Commission. In the grape-enriched diet, grape powder was substituted for sucrose and cornstarch and comprised 25% of the diet by weight. Sucrose was replaced with fructose and dextrose in the control diet to match the sugar content of the grape-enriched diet. Details of the diet composition are presented in Supplemental Table 1.

Calcium balance and kinetics. During weeks 1 and 8 with the experimental diets, 24-h urine and feces were collected for 3 d to determine calcium balance. Calcium balance variables were calculated by using the following equations:

\[ \text{Net Ca absorption} = \text{Ca intake} - \text{fecal Ca} \]

\[ \text{Net Ca retention} = \text{Ca intake} - (\text{fecal Ca} + \text{urine Ca}) \]

\[ \text{Net Ca absorption efficiency} = (\text{Net Ca absorption}/\text{Ca intake}) \times 100 \]

During week 8, a subset of rats (n = 13/group) underwent a calcium kinetics study. Jugular catheters were surgically implanted by using a previously described procedure (22). After an overnight fast, 10 rats per treatment group received an oral dose of 20 µCi 45Ca as part of a 3-g test meal of the experimental diet. Three rats from each treatment group received an intravenous injection of 10 µCi 45Ca. Blood (200 µL/sample) was drawn at 0, 30, 60, 120, 240, 360, 720, 1440, 2160, and 2880 min after dosing. Urine and feces were collected in 24-h pools for 4 d. Serum, urine, and fecal 45Ca counts were corrected for background 45Ca from the previously received 45Ca dose before analysis. Kinetic modeling was performed by using WinSAAM (NIH) according to previously described methods (23). The compartmental model is shown in Figure 1. Fractional calcium absorption was calculated as the fraction of calcium entering the serum (compartment 1) from the intestine (compartment 8 and 9).

\[ \frac{d[L]}{dt} = \text{GI, gastrointestinal; L(2,1), L(2,3), L(3,2), and L(10,1) were determined from rats } \]

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Bone imaging. Left femurs were imaged with a micro-computed tomographic (microCT) scanner (μCT 40; Scanco Medical) by using previously described methods (24). Scans were performed at an isotropic resolution of 16 μm with an integration time of 190 ms and X-ray tube potential (peak) of 55 peak kVP. Distal femurs were scanned in 62 slices beginning at 18% of femur length from the distal end of the femur and proceeding distally for 1 mm. Cortical bone was assessed at the femoral midshaft in 50 slices. Segmentation values were sigma = 0.8 and support = 1, and the binarization threshold was 310. All trabecular variables were calculated by using a three-dimensional direct model. Trabecular variables evaluated included bone volume fraction, connectivity density (1/mm³), trabecular number (1/mm), trabecular thickness (μm), and trabecular separation (μm). Cortical variables included cortical area fraction, peristomial perimeter (mm), endocortical perimeter (mm), and cortical porosity. Areal BMD (g/cm²) of the whole femur was measured by using a Lunar PIXImus II (General Electric).

Bone material and mechanical testing. Tissue-level material properties were assessed by using reference point indentation (RPI; Biomet H2c; ActiveLife Scientific). Right femurs were indented with a BP2 probe with an indentation force of 5 N for 10 cycles at 2 indentations/s. RPI variables evaluated included first unloading slope (N/m), a measure of stiffness, total indentation distance (μm), and indentation distance increase (μm, a measure of toughness). Mechanical properties were assessed with a 3-point bending test by using a servohydraulic R Series Controller version 3.14.09 (Test Resources) with a speed of 100 μm/min. Frozen femurs were thawed overnight and rehydrated in physiologic saline for 24 h before mechanical testing (25).

Statistical analysis. Urinary ⁴⁵Ca excretion data were expressed as the natural log of the ratio of percentage of ⁴⁵Ca dose to total urinary calcium. Baseline values from all rats were used to generate a prediction equation with a common slope but individual intercept values for each rat. The prediction equations were extrapolated forward to the treatment period and used to generate predicted values of urinary ⁴⁵Ca retention. These values were analyzed by ANOVA with terms for diet and time, were both significantly lower in the grape-enriched diet–fed rats.

TABLE 1 Calcium balance in ovariectomized rats after 1 and 8 wk of a control or a grape-enriched diet¹

<table>
<thead>
<tr>
<th>Week</th>
<th>Control (n = 19)</th>
<th>Grape-enriched (n = 21)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Net calcium retention, mg/d</td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>7.4 ± 7.9</td>
<td>7.2 ± 6.2</td>
</tr>
<tr>
<td>Net calcium absorption efficiency, %</td>
<td>10.9 ± 8.6</td>
<td>11.0 ± 5.4</td>
</tr>
<tr>
<td>Urine calcium, mg/d</td>
<td>1.4 ± 0.6</td>
<td>0.8 ± 0.3*</td>
</tr>
<tr>
<td>Week 8</td>
<td>10.3 ± 8.0</td>
<td>12.4 ± 5.2</td>
</tr>
<tr>
<td>Net calcium absorption efficiency, %</td>
<td>18.5 ± 13.1</td>
<td>21.1 ± 8.1</td>
</tr>
<tr>
<td>Urine calcium, mg/d</td>
<td>0.64 ± 0.40</td>
<td>0.60 ± 0.34</td>
</tr>
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¹ Values are means ± SDs. *Different from control, P < 0.001.
midshaft, cortical area fraction and cortical thickness were 2.5% and 3.1% greater ($P < 0.05$), respectively, in grape-enriched diet–fed rats compared with controls, whereas cortical porosity was 3.3% lower ($P < 0.05$) in the grape-enriched diet–fed rats. The endocortical perimeter was significantly smaller ($P < 0.05$) in the grape-enriched diet fed rats, but there was no difference in periosteal perimeter.

**Material and mechanical testing.** Because of a testing error, valid results from 3-point bending were obtained for 14 of 22 rats in each group. Femurs from rats fed the grape-enriched diet had a significantly greater breaking strength in the 3-point bending test than femurs from the control rats. There were no differences due to diet in any of the RPI outcomes (Table 4).

**Discussion**

In this 2-mo feeding trial in ovariectomized rats, we found that a grape-enriched diet containing 25% dried grapes increased bone calcium retention as well as cortical bone structure and strength. These results are in accordance with our previous finding that 10-d treatment with dietary grape extract and grapeseed extract reduced the bone resorption marker NTX and that resveratrol improved bone calcium retention (20). We did not observe any significant effects of a grape-enriched diet on femoral BMD, trabecular bone microarchitecture, or tissue-level material properties.

Rendina et al. (26) recently reported an 8-wk feeding trial of grape, plum, apple, apricot, or mango in ovariectomized C57BL/6 mice. At 2 wk postovariectomy, mice were randomly assigned to diets containing 25% (wt:wt) dried fruit or to a control diet for 8 wk. Mice fed the grape diet had greater total body and spine BMD than did mice fed the control diet. We did not assess BMD at the total body or spine, but we observed no significant difference in femoral BMD between control and grape-enriched diet–fed rats. In accordance with our distal femur microCT results, these authors observed no effect of the grape diet on trabecular microarchitecture at the proximal tibial metaphysis. In contrast with our results, they observed no effect of dietary grape on cortical thickness or mechanical properties with the use of finite element analysis. In general, these results agree with our findings that a grape-enriched diet has modest beneficial effects on bone health. Differences in specific outcomes may be due to species differences between mice and rats or differences in the composition of the grape powder.

In rats fed the grape-enriched diet, we observed a significant decrease in the urinary $^{45}$Ca:total Ca ratio, indicating an increase in net bone calcium retention. To further explore this finding, we looked at the 2 components of the $^{45}$Ca:total Ca ratio. Urinary excretion of $^{45}$Ca decreased significantly in rats fed the grape-enriched diet, indicating a decrease in net bone turnover, whereas urinary $^{45}$Ca excretion was unchanged in the control group. Total urinary calcium excretion increased during treatment compared with the baseline period in both the control and grape-enriched diet groups, but the increase was larger in the control group, resulting in significantly greater total urinary calcium excretion during the treatment period in the control group compared with the grape-enriched diet group. These data suggest that a grape-enriched diet improves net bone calcium retention by both reducing net bone turnover and improving renal calcium conservation.

Although we did not observe a significant difference in calcium balance between the 2 groups at either week 1 or week 8, there was a significant increase in net calcium absorption and retention from week 1 to week 8 in the grape-enriched diet group but not in the control group. This finding was unexpected, because many previous studies of functional foods found that positive effects on calcium absorption tend to disappear over time, suggesting intestinal adaption (27–29). Longer studies will be needed to determine if such adaptation eventually occurs with a grape-enriched diet. We also observed a trend toward increased fractional calcium absorption in our kinetic modeling study. The addition of a second route of absorption from the lower gastrointestinal tract improved the fit of our model, although the contribution of calcium absorption at this site was small, accounting for an average 4.5% of total fractional calcium absorption in the control group and 10.4% in the grape-enriched diet group. These values are similar to reported estimates of the contribution of calcium absorption in the colon to total calcium absorption (30, 31).

After 8 wk of being fed the experimental diets, rats fed the grape-enriched diet had significant increases in femoral cortical area fraction and cortical thickness and breaking strength and a significant decrease in cortical porosity compared with rats fed...
the control diet. Although there was no difference due to diet in periosteal perimeter, endocortical perimeter was significantly lower in rats fed the grape-enriched diet. These data suggest that the grape-enriched diet improved cortical bone properties by protecting against endocortical resorption, which is elevated after ovariectomy in rats (32) and in postmenopausal women (33). Despite the improvements in cortical microarchitecture and strength in rats fed the grape-enriched diet, there were no differences due to diet in trabecular bone, and thus no difference in total femur BMD, which includes both cortical and trabecular regions. A longitudinal microCT study of bone loss in rats ovariectomized at 2 mo of age showed that trabecular bone loss was most rapid between 4 and 8 wk postovariectomy, reaching a plateau after 8 wk (34). In contrast, a study in rats ovariec-
tomized at 6 mo of age found that femoral cross-sectional area and thickness were not different from intact rats at 3 mo postovariectomy but were significantly reduced compared with sham-operated rats at 6 mo postovariectomy (32). The timing of our intervention, which started 2 mo postovariectomy, may explain why only cortical bone was preserved, because the majority of trabecular bone loss had presumably occurred by this point. We designed our intervention to begin at 2 mo postovariectomy to represent the later, more stable phase of human menopause, which is longer than the initial rapid bone loss phase and thus represents a longer window of opportunity for intervention. Bone loss in early menopause may be dominated by hormonal changes and be less responsive to nutritional intervention. Previous research in rats (35) and humans (36) demonstrated positive effects of calcium supplementation on bone in late, but not early, menopause. In addition, the abrupt ablation of estrogen in the ovariec-
tomized rat model does not reflect natural menopause well in the early phase. Future studies with grape powder administered earlier in estrogen deficiency are needed to further clarify if a grape-enriched diet can prevent against trabecular bone loss.

There are several possible mechanisms through which grapes may act on calcium metabolism and bone health. Red wine, resveratrol, kaempferol, anthocyanins, and quercetin all have reported effects on estrogen receptors (37–42). Thus, grape polyphenols may improve bone health by acting as phytoestrogens. Many cell culture studies showed that resveratrol (43, 44), quercetin (42, 45, 46), kaempferol (45, 47), proanthocyanidins (48), and catechins (49) can directly affect bone cells. Grape polyphenols may also act by reducing inflammation, a risk factor for bone loss (50). In cell culture, resveratrol (51), quercetin, anthocyanins (52), and proanthocyanidins (53) reduced inflammatory response.

Our previous study (20) demonstrated that resveratrol, but not a resveratrol-devoid grape extract, improved bone calcium retention. Although our grape-enriched diet did contain some resveratrol (~0.18 mg/kg diet), the amount was several orders of magnitude lower than the effective dose (2000 mg/kg) in our previous study, making it unlikely that resveratrol alone is responsible for the bone-sparing effects observed in this study. In addition to polyphenols, whole grape powder contains other components including carbohydrates, fiber, and micronutrients, which may act synergistically with polyphenols to promote bone health.

In addition to polyphenols, grapes are rich in a number of vitamins and minerals that may positively contribute to bone health. The grape-enriched diet provided an estimated 63% more potassium, 21% more vitamin A (as β-carotene), 16% more magnesium, and 15% more copper than the control diet. Intake of these nutrients has been positively associated with bone health (54–61). These increases may have contributed to the effects of the grape-enriched diet on calcium metabolism and bone properties.

One limitation of our study was that the amount of grape powder that we were able to incorporate into the diet provided a considerably lower polyphenolic content than was used in our previous study with grape extract and grapeseed extract (20) or in previous studies on the individual phenolic compounds found in grapes (14–17). However, we still observed beneficial effects on bone and mineral metabolism, suggesting that the effective doses of grape polyphenols may be lower than previously thought or that the combination of many polyphenols and nutrients found in whole grapes is more effective than the individual components. Further research is needed to determine the relative efficacy of whole grapes, which can be incorporated into the diet, vs. individual grape components, which would more likely be administered as dietary supplements. The amount of grape powder used in the diet in this proof-of-concept study was chosen to maximize polyphenol intake and is comparable to

**TABLE 3** Femur BMD and microarchitecture in ovariectomized rats after 8 wk of a control or a grape-enriched diet1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Grape-enriched</th>
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<tbody>
<tr>
<td><strong>Whole femur</strong></td>
<td></td>
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<tr>
<td>aBMD, g/cm²</td>
<td>0.176 ± 0.006</td>
<td>0.174 ± 0.007</td>
</tr>
<tr>
<td><strong>Distal femur</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV/TV</td>
<td>0.061 ± 0.02</td>
<td>0.058 ± 0.02</td>
</tr>
<tr>
<td>Conn.D,1/mm³</td>
<td>15.1 ± 7.1</td>
<td>14.2 ± 7.8</td>
</tr>
<tr>
<td>Tb.N,1/mm</td>
<td>2.47 ± 0.25</td>
<td>2.47 ± 0.30</td>
</tr>
<tr>
<td>Tb.Th, mm</td>
<td>0.073 ± 0.007</td>
<td>0.071 ± 0.006</td>
</tr>
<tr>
<td>Tb.Sp, mm</td>
<td>0.41 ± 0.05</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td><strong>Midshaft femur</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl.Ar/Tt.Ar</td>
<td>0.606 ± 0.02</td>
<td>0.621 ± 0.02*</td>
</tr>
<tr>
<td>Cl.Th, mm</td>
<td>0.609 ± 0.03</td>
<td>0.628 ± 0.03*</td>
</tr>
<tr>
<td>Ps.Pm, mm</td>
<td>11.7 ± 0.99</td>
<td>11.3 ± 0.98</td>
</tr>
<tr>
<td>Ec.Pm, mm</td>
<td>7.42 ± 0.34</td>
<td>7.17 ± 0.36*</td>
</tr>
<tr>
<td>Cl.Po,%</td>
<td>0.0279 ± 0.001</td>
<td>0.0270 ± 0.001*</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs, n = 22/group. *Different from control, P < 0.05. aBMD, areal bone mineral density; BMD, bone mineral density; BV/TV, bone volume to total volume fraction; Conn.D, connectivity density; Cl.Ar/Tt.Ar, cortical area to total area fraction; Cl.Po, cortical porosity; Cl.Th, cortical thickness; Ec.Pm, endocortical perimeter; Ps.Pm, periosteal perimeter; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness.

**TABLE 4** Femur tissue and whole-bone mechanical properties in ovariectomized rats after 8 wk of a control or a grape-enriched diet1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Grape-Enriched</th>
</tr>
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<tbody>
<tr>
<td><strong>Reference point indentation</strong></td>
<td></td>
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</tr>
<tr>
<td>US1st, N/μm</td>
<td>0.65 ± 0.03</td>
<td>0.61 ± 0.03</td>
</tr>
<tr>
<td>TID, μm</td>
<td>32.3 ± 4.0</td>
<td>34.1 ± 5.8</td>
</tr>
<tr>
<td>IDI, μm</td>
<td>3.97 ± 0.54</td>
<td>4.08 ± 0.64</td>
</tr>
<tr>
<td><strong>2-Point bending</strong></td>
<td></td>
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</tr>
<tr>
<td>Breaking force, N</td>
<td>101 ± 14.0</td>
<td>112 ± 9.8*</td>
</tr>
<tr>
<td>Stiffness, N/mm</td>
<td>297 ± 48.2</td>
<td>306 ± 29.9</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs. IDI, indentation distance increase; TID, total indentation distance; US1st, first unloading slope. *Different from control, P < 0.05.

2 n = 22/group.

3 n = 14/group.
that in other animal studies evaluating the effect of fruit powders on bone health (20, 26, 62). With the use of a body surface area conversion (63), the amount of grape powder in our rodent diet translates to ~5 servings (0.75 cups/serving) of fresh grapes per day, which may be difficult to achieve in the human diet. Further study is needed to determine if a lower intake of fresh grapes, or a concentrated extract, can affect bone health and calcium metabolism in humans.

In summary, the data in this study suggest that a grape-enriched diet bestows benefits to bone health and calcium metabolism in the postmenopausal state. Future studies are needed to determine if the trend toward improved calcium metabolism with a grape-enriched diet continues to increase over time, to elucidate the mechanisms through which grapes exert these benefits, and ultimately to replicate these findings in humans.

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