Consumption of Green Tea Extract Results in Osteopenia in Growing Male Mice\textsuperscript{1–3}

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Abstract
Consumption of green tea may reduce body weight gain. Although many disorders are related to obesity, bone mass is positively correlated with body mass. Therefore, our purpose in this study was to determine the effects of green tea extract (GTE) on bone mass and architecture in rapidly growing lean (C57BL/6 wild type (WT)) and genetically obese, leptin-deficient (ob/ob) male mice. Five-week-old lean and ob/ob mice were assigned to diets containing GTE at 0, 1, or 2\% for 6 wk. Femoral and lumbar vertebral bone volume and architecture were evaluated by micro-computed tomography (\(\mu\)CT). Following \(\mu\)CT analysis, femora were ashed to determine bone mineral content and density. Compared with WT mice, ob/ob mice had shorter femora (\(P < 0.001\)), lower femoral bone volume (\(P < 0.001\)), and lower femoral bone mineral content (\(P < 0.001\)), but higher cancellous bone volume in lumbar vertebrae (\(P < 001\)). Neither genotype nor treatment affected femoral bone mineral density, indicating normal mineralization. GTE consumption resulted in lower femur length, volume, mineral content, cortical volume, and cortical thickness (\(P < 0.001\)), as well as lower cancellous bone volume/tissue volume (\(P < 0.008\)) and trabecular thickness (\(P < 0.004\)) in lumbar vertebrae. The results indicate that leptin is not essential for the reduced gains in body weight and bone mass due to GTE in growing mice and suggest that consumption of large quantities of green tea may reduce the rate of bone accumulation during growth. \textit{J. Nutr. 139: 1914–1919, 2009.}

Introduction
The prevalence of obesity has increased dramatically to become an important public health concern (1–3). The causes contributing to the incidence of obesity are multifactorial and are strongly associated with increased energy intake and reduced physical activity (4–6). Excess body weight contributes to cardiovascular disease, certain cancers, and increased morbidity and mortality (7). Paradoxically, excessive body weight may afford a protective effect on bone mass and partially mitigate the development of age-related osteoporosis (8,9).

Dietary interventions that reduce energy intake, inhibit nutrient absorption, and/or increase energy expenditure influence body weight (10–12). Animal studies indicate that green tea extract (GTE)\textsuperscript{6} decreases intestinal lipid absorption (13) and reduces body weight gain without affecting energy intake (14,15). In addition, consumption of green tea or its bioactive components (e.g. catechins and caffeine) may have antiobesity activities in part by increasing thermogenesis and fat oxidation (11,16). Indeed, studies suggest that GTE stimulates sympathetically driven thermogenesis (10,17).

Reduced nutrient intake and increased sympathetic signaling is also associated with decreased bone formation and bone loss in animal models (18). Studies in mice suggest that body weight has direct positive effects on bone mass as well as indirect effects that are mediated by hormonal factors, including leptin (19). A limited number of studies suggest that green tea also affects skeletal health. However, the reported effects of green tea on bone range from beneficial to detrimental and, as is the case for many bioactive components, appear to be context-dependent. Thus, the interactive effects of green tea consumption on energy expenditure, body mass, and bone health require further investigation. The goal of the present study was to determine the effects of green tea on bone mass and architecture in lean (C57BL/6) and obese (ob/ob) mice. We chose ob/ob mice, because they do not produce leptin, an important regulator of energy homeostasis and a potential mediator of the effects of fat mass on bone mass. The beneficial effects of GTE on body fat accumulation, hepatic steatosis and injury, hepatic antioxidants, and serum lipids and adiponectin in this cohort of mice were reported previously (15).

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\textsuperscript{3} Supplemental Figure 1 is available with the online posting of this paper at jn.nutrition.org.

\textsuperscript{4} Abbreviations used: ECGG, epigallocatechin gallate; GTE, green tea extract; \(\mu\)CT, micro-computed tomography; WT, wild type.

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Materials and Methods

**Mice.** Male, 4-week-old, leptin-deficient (ob/ob) obese mice and their lean C57BL/6 wild type (WT) littermates (n = 24/genotype) were purchased from the Jackson Laboratory. Males were used because they gain weight and increase bone mass more rapidly than females. The mice were housed individually in a temperature- and humidity-controlled room with a 12-h-light/dark cycle. All mice were acclimated to the facility for 1 wk prior to study initiation. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Connecticut where the study was conducted.

**Experimental design.** The experimental design and diet composition have been previously described (15). In brief, equal numbers of lean and ob/ob mice were assigned randomly to 1 of 3 dietary treatments for 6 wk (n = 8 per genotype per dietary treatment): 1) 0% GTE (control); 2) 1% GTE; or 3) 2% GTE. Diets and water were available ad libitum. The GTE was mixed homogenously into the powdered diet as appropriate for each treatment. The catechin and caffeine content of GTE was evaluated by HPLC-UV (20); the GTE contained 30% (wt:wt) catechins [consisting of 48% epigallocatechin gallate (EGCG), 31% epigallocatechin, 13% epicatechin gallate, and 8% epicatechin] and 5.6 mg caffeine/100 mg GTE. Dietary GTE at 1% is equivalent to ~7 servings/d (1 serving = 120 mL). This amount is commonly consumed by Japanese adults and is associated with decreased mortality from cardiovascular disease (21). Body weights were recorded weekly and food intake was measured daily for each mouse by determining pre- and post-weights of food jars. At the end of the 6-wk dietary intervention, the mice were killed under anesthesia by cervical dislocation. The duration of treatment was based on time course studies showing that the interval between 6 and 12 wk of age in mice corresponds to the pubertal growth spurt in humans (22).

**Tissue collection and analyses.** Left femora and 3rd lumbar vertebrae were excised, cleaned of soft tissue, and placed in 70% ethanol. Micro-computed tomography (μCT) was used for nondestructive 3-dimensional evaluation of bone volume and architecture. Femora and lumbar vertebrae were scanned using a Scanco μCT40 scanner (Scanco Medical) at a voxel size of 12 μm × 12 μm × 12 μm. The threshold value for evaluation was determined empirically and set at 265 (gray scale, 0–1000). Entire femora (cancellous + cortical bone) were evaluated followed by evaluation of cortical bone at the femoral midshaft and cancellous bone in the distal femoral metaphysis (Supplemental Fig. 1A). For the femoral midshaft, 20 slices (240 μm in length) of bone were evaluated and total cross-sectional volume (cortical and marrow volume, mm³), cortical volume (mm³), marrow volume (mm³), and cortical thickness (μm) were measured. For the femoral metaphysis, 125 slices (1.5 mm in length) of cancellous bone (secondary sponiosa only) were measured. Following μCT analysis, femora were dried and then ashed in an oven (Fisher Scientific) at 550°C. The ashed bones were weighed (Mettler AE 200) to determine total femur bone mineral content (mg). True mineral density was calculated for the femur as bone mineral content divided by total bone volume (mg/mm³). Midshaft femur volumetric mineral density was calculated as bone mineral density × (cortical volume/cross-sectional volume). Analysis of the lumbar vertebra included the entire region of secondary sponiosa between the cranial and caudal growth plates (Supplemental Fig. 1B). Direct cancellous bone measurements in the distal femur and lumbar vertebra included cancellous bone volume/tissue volume (volume of total tissue occupied by cancellous bone, %), connectivity density (number of redundant connections per unit volume, 1/mm³; this index detects defects in cancellous architecture), structure model index (quantifies the plate vs. rod characteristics of cancellous bone on a scale from 0 to 3, with 0 representing purely plate-like structures and 3 representing purely rod-like structures), trabecular thickness (mean thickness of individual trabeculae, μm), trabecular number (no. of trabeculae within the sample tissue, 1/mm³), and trabecular separation (the distance between trabeculae, μm) (23).

**Statistical analysis.** The effect of genotype (with 2 levels: lean WT and obese ob/ob), GTE treatment (with 3 levels: 0, 1, and 2% GTE), and their interaction (genotype × GTE treatment) on each dependent variable were analyzed using 2-way ANOVA. When the interaction between genotype and treatment was significant, a separate analysis was conducted for each fixed level of one factor while varying the other factors. A Bonferroni post hoc test was used to evaluate differences among the 3 treatment groups. Differences were considered significant at an α-level of P < 0.05. All data are reported as mean ± SEM.

### Results

**Body weight.** As expected, ob/ob mice weighed more than their lean WT littermates (Fig. 1). GTE-fed mice in both genotypes weighed less than their respective controls. A significant interaction between genotype and GTE treatment occurred such that the effects of GTE consumption on body weight were more pronounced in the ob/ob mice (15).

**Femur.** Femoral length (Fig. 2A), bone volume (Fig. 2B), and bone mineral content (Fig. 2C) were lower in ob/ob than in WT mice. True mineral density of the femur did not differ among the groups (Fig. 2D). Midshaft femur cross-sectional volume, cortical volume, marrow volume, cortical thickness, and volumetric bone density were also lower in ob/ob than in lean WT mice (Table 1). Genotype did not affect cancellous bone volume/tissue volume, connectivity density, or trabecular thickness in the distal femur (Table 1). However, trabecular number was lower and trabecular spacing was higher in ob/ob mice compared with lean littermates. GTE consumption resulted in shorter femora and lower total femur volume and mineral content, irrespective of genotype; genotype and GTE did not interact significantly (Fig. 2). Furthermore, GTE intake resulted in lower midshaft femur cross-sectional volume, cortical volume, marrow volume, cortical thickness, and volumetric bone density in both WT and ob/ob mice (Table 1). However, GTE consumption did not affect distal femur cancellous bone volume and architecture (Table 1). An interaction between genotype and GTE affected trabecular spacing; WT mice did not differ, but trabecular spacing was greater in ob/ob mice consuming 1% GTE than in those consuming either 0 or 2% GTE.

**Lumbar vertebra.** As expected, vertebral cancellous bone volume and architecture were dramatically altered in ob/ob mice compared with lean WT littermates (24–26). Specifically, ob/ob mice had higher vertebral cancellous bone volume/tissue.
volume (Table 2). This was associated with higher connectivity density, trabecular number, and trabecular thickness and lower structure model index and trabecular spacing. GTE consumption resulted in lower vertebral cancellous bone volume/tissue volume and trabecular thickness and higher structure model index in both genotypes. Connectivity density, trabecular number, and trabecular separation were not affected by consumption of GTE. Genotype and GTE consumption did not interact significantly for any of the vertebral endpoints evaluated.

**Discussion**

Consumption of GTE decreased body weight gain in mice regardless of genotype. GTE has been shown to protect against nonalcoholic fatty liver disease in this cohort of *ob/ob* mice by

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**Table 1**

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Lean WT mice</th>
<th>Obese <em>ob/ob</em> mice</th>
<th>ANOVA (P &lt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% GTE</td>
<td>1% GTE</td>
<td>2% GTE</td>
</tr>
<tr>
<td>Midshaft femur (cortical bone)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-sectional volume, mm$^3$</td>
<td>0.43 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>Cortical volume, mm$^2$</td>
<td>0.19 ± 0.00</td>
<td>0.18 ± 0.00</td>
<td>0.16 ± 0.00</td>
</tr>
<tr>
<td>Marrow volume, mm$^3$</td>
<td>0.24 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Cortical thickness, μm</td>
<td>198 ± 2</td>
<td>192 ± 3</td>
<td>178 ± 2</td>
</tr>
<tr>
<td>Volumetric bone density, mg/mm$^3$</td>
<td>0.52 ± 0.01</td>
<td>0.51 ± 0.01</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>Distal femur (cancellous bone)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone volume/tissue volume, %</td>
<td>6.9 ± 0.5</td>
<td>7.4 ± 0.6</td>
<td>6.2 ± 0.6</td>
</tr>
<tr>
<td>Connectivity density</td>
<td>68 ± 5</td>
<td>73 ± 12</td>
<td>53 ± 13</td>
</tr>
<tr>
<td>Trabecular number, 1/mm$^2$</td>
<td>4.4 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Trabecular thickness, μm</td>
<td>37 ± 1</td>
<td>38 ± 1</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>Trabecular spacing, μm$^2$</td>
<td>227 ± 8</td>
<td>226 ± 9</td>
<td>236 ± 11</td>
</tr>
</tbody>
</table>

1 Data are mean ± SEM, n = 7–8. Within a genotype, means with superscripts without a common letter differ, P < 0.05.

2 NS, Not significant, P > 0.05.
limiting hepatic lipid accumulation and injury without affecting hepatic antioxidant status and adiponectin-mediated lipid metabolism (15). However, we now show that these beneficial effects of GTE are accompanied by potentially detrimental effects on the growing skeleton. Specifically, GTE consumption decreased total and cortical bone volume in the femur and cancellous bone volume in vertebrae of both lean WT and genetically obese, leptin-deficient ob/ob mice compared with their respective controls fed no GTE. In part, these skeletal changes are attributable to a decrease in bone size that accompanied the decrease in body weight gain. However, changes in volumetric bone mineral density and bone architecture adjusted for bone size that reflect decreased bone quality, were also observed in mice consuming GTE compared with controls.

Few studies have been conducted to investigate the relationship between green tea consumption and bone metabolism. In vitro findings indicate that green tea catechins have the potential to have direct stimulatory effects on bone cancer cells and stimulatory or inhibitory effects on immortalized and primary osteoblast and osteoclast lineage cells (27–29). However, they provide little insight regarding the physiological effects of green tea consumption on the skeleton.

In vivo studies have been inconclusive as to whether tea influences bone metabolism. Cross-sectional cohort studies in humans have reported positive and negative associations between green tea consumption and bone mineral density (14,30,31). Green tea catechins enhanced chondrogenesis and suppressed osteogenesis in a rat model for ectopic bone formation (32) and attenuated skeletal abnormalities in cadmium-poisoned, rapidly growing rats (33). Green tea polyphenols have also been reported to reduce age-related and ovariectomy-induced bone loss in rats (34,35). In the present study, we observed decreases in bone length as well as cross sectional bone volume, implying that green tea consumption inhibited chondrogenesis as well as osteogenesis in normal and genetically obese mice. Thus, the effects of green tea on bone metabolism may be context dependent and differ during growth and aging. High levels of green tea consumption may reduce peak bone mass by slowing bone growth during adolescence, but in adults may prevent excessive bone loss during aging by attenuating elevated bone turnover.

In addition to catechins, green tea contains caffeine. Caffeine in green tea could have independent effects on bone or potentially interact with catechins or other bioactive compounds of the tea. The effects of caffeine on the skeleton have been investigated (36,37). In calcium-replete individuals, caffeine does not appear to have negative effects on bone status or calcium economy. Because mice in the present study were fed a diet in which calcium exceeded requirements, it is unlikely that the reduced bone growth in mice fed GTE is attributable to caffeine.

In the present study, bone mineral content and volumetric bone mineral density were reduced by GTE consumption. However, GTE did not affect true bone mineral density, indicating that it did not result in a mineralization defect. Although we did not measure bone mechanical properties, the magnitude of reduction in size in mice fed GTE would be expected to result in a large decrease in strength. Additionally, GTE altered important indices of bone quality in vertebra, including increasing structure model index and decreasing trabecular thickness and connectivity density. The observed increase in structure model index indicates a transition of the individual trabeculae from a more biomechanically competent plate-like structure to a rod-like structure. Trabecular thinning and a reduction in connectivity density, a measure of the number of connections per unit volume (38), also suggest that GTE consumption reduced bone quality.

The present studies were performed in young, rapidly growing mice. GTE decreased bone size in part by decreasing bone length. In humans, lifestyle factors (e.g., physical activity and diet) are more likely to influence bone size prior to skeletal maturation than later in life (39). As a result, heavy green tea consumption during growth has the potential to irreversibly reduce peak bone mass.

A low peak bone mass predisposes individuals to osteoporosis, a disease that contributes to over 2 million fractures annually in the United States alone (40). The acquisition of bone mass occurs primarily during childhood and the decade following puberty and is tightly coupled to energy metabolism. Anorexia is associated with a low bone mass and increased fracture risk, whereas obesity is associated with high bone mass and reduced fracture risk (8,9). The increased bone mass in obese individuals has been variously attributed to systemic factors or increased skeletal loading due to increased body mass. The low cortical bone mass observed in the ob/ob mouse suggests that weight is insufficient to fully account for the increased bone mass associated with obesity in humans. Because the sole genetic defect in ob/ob mice is leptin deficiency, leptin is an obvious candidate as the mediator of the low bone mass phenotype. Interestingly, consumption of the green tea catechin EGCG decreases serum leptin levels in mice and rats (41,42).

Leptin has been reported to have anabolic, antiadipogenic, and antiosteogenic effects on bone (24,43–45). However, leptin-deficient ob/ob mice have reduced bone length and reduced overall bone mass and strength compared with WT mice.

### Table 2

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Lean WT mice</th>
<th>Obese ob/ob mice</th>
<th>ANOVA (P &lt; 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% GTE</td>
<td>1% GTE</td>
<td>2% GTE</td>
</tr>
<tr>
<td>Bone volume/tissue volume, %</td>
<td>17.4 ± 0.5</td>
<td>17.3 ± 0.7</td>
<td>15.5 ± 0.6</td>
</tr>
<tr>
<td>Connectivity density, 1/mm²</td>
<td>245 ± 7</td>
<td>243 ± 13</td>
<td>234 ± 20</td>
</tr>
<tr>
<td>Structure model index</td>
<td>1.7 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>Trabecular number, 1/mm</td>
<td>5.6 ± 0.1</td>
<td>5.7 ± 0.1</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>Trabecular thickness, μm</td>
<td>42 ± 1</td>
<td>42 ± 1</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>Trabecular spacing, μm</td>
<td>175 ± 2</td>
<td>173 ± 3</td>
<td>172 ± 4</td>
</tr>
</tbody>
</table>

1 Data are mean ± SEM, n = 8.
2 NS, Not significant, P > 0.05.
(25,26,46,47). These findings indicate that leptin is required for optimal peak bone growth and quality in mice. This conclusion is strongly supported by evidence that hypothalamic leptin gene therapy restores body mass and bone architecture to normal in ob/ob mice (26). Severe dietary restriction is the most common cause of systemic leptin insufficiency in humans. It is, therefore, possible that leptin insufficiency, as well as a low body weight, may in part contribute to the low bone mass in adolescents with eating disorders (48). Bone volume is correlated with body weight in mice. In C57BL/6 mice fed nonpurified diet, body weight accounts for 35–90% of the observed variation in bone volume depending upon the skeletal site examined (19). However, the relationship between body weight and bone mass is very sensitive to genetic and environmental perturbations. For example, ob/ob mice are much heavier than WT mice but have smaller bones. Similarly, ovariectomy results in increased body weight in mice but decreased cancellous bone mass (49).

Obesity induced by the consumption of a high-fat diet is also associated with greater bone mass in mice (19). Depending upon the skeletal site, the effects of weight require or are independent of leptin (19). Leptin deficiency-induced genetic obesity is rare in humans, but dietary obesity may be the result of decreased leptin signaling at critical sites in the brain. Impaired central leptin signaling, in turn, increases food consumption and reduces nonshivering thermogenesis. Resistance to leptin has been postulated to occur due to reduced leptin entry across the blood brain barrier, despite chronic systemic hyperleptinemia (50–52) or downregulation of leptin receptor signaling (53,54). Alternatively, inadequately leptin signaling may be due to suboptimal hypothalamic leptin levels (55) or an opposing regulatory mechanism (56). In the present study, GTE decreased weight gain and bone growth in ob/ob mice as well as in WT mice. Thus, it is unlikely that the antiobesity or antiosteogenic effects of green tea in mice require leptin. However, the precise mechanisms of action of green tea on bone metabolism require further investigation.

**Literature Cited**


