Supplementation with Green Tea Polyphenols Improves Bone Microstructure and Quality in Aged, Orchidectomized Rats

Chwan-Li Shen · Jay J. Cao · Raul Y. Dagda · Thomas E. Tenner Jr. · Ming-Chien Chyu · James K. Yeh

Received: 16 January 2011 / Accepted: 25 February 2011 / Published online: 23 March 2011
© Springer Science+Business Media, LLC 2011

Abstract Recent studies show that green tea polyphenols (GTPs) attenuate bone loss and microstructure deterioration in ovariectomized aged female rats, a model of postmenopausal osteoporosis. This study evaluated the efficacy of GTPs at mitigating bone loss and microstructure deterioration along with related mechanisms in androgen-deficient aged rats, a model of male osteoporosis. A 2 (sham vs. orchidectomy) × 2 (no GTP and 0.5% GTP in drinking water) factorial design was studied for 16 weeks using 40 aged male rats. An additional 10 rats (baseline group) were killed at the beginning of study to provide baseline parameters. There was no difference in femoral mineral density between baseline and the sham only group. Orchidectomy suppressed serum testosterone and tartrate-resistant acid phosphatase concentrations, liver glutathione peroxidase activity, bone mineral density, and bone strength. Orchidectomy also decreased trabecular bone volume, number, and thickness in the distal femur and proximal tibia and bone-formation rate in trabecular bone of proximal tibia but increased serum osteocalcin concentrations and bone-formation rates in the endocortical tibial shaft. GTP supplementation resulted in increased serum osteocalcin concentrations, bone mineral density, and trabecular volume, number, and strength of femur; increased trabecular volume and thickness and bone formation in both the proximal tibia and periosteal tibial shaft; decreased eroded surface in the proximal tibia and endocortical tibial shaft; and increased liver glutathione peroxidase activity. We conclude that GTP supplementation attenuates trabecular and cortical bone loss through increasing bone formation while suppressing bone resorption due to its antioxidant capacity.

Keywords Green tea · Rat · Bone quality · Bone microstructure · Male osteoporosis

M.-C. Chyu
Department of Mechanical Engineering, Texas Tech University, Lubbock, TX 79401, USA

M.-C. Chyu
Graduate Healthcare Engineering Option, Texas Tech University, Lubbock, TX 79401, USA

J. K. Yeh
Applied Bench Core Laboratory, Winthrop-University Hospital, Mineola, NY 11501, USA

The authors have stated that they have no conflict of interest.
Osteoporosis is a degenerative bone disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility [1]. Reactive oxygen species contribute to the aging process [2] and the etiology of various degenerative diseases including osteoporosis [3]. Studies in both humans [4, 5] and other animals [6] have suggested a strong positive association between excessive reactive oxygen species and bone loss/deterioration in the development of osteoporosis.

Green tea is a nonfermented and nonoxidized product that consists of a variety of polyphenolic components (i.e., epigallocatechin-3-gallate [EGCG], epigallocatechin [EGC], epicatechin [EC], epicatechin-3-gallate [ECG], gallic acid gallate, and catechin) [7]. The most widely recognized properties of green tea polyphenols (GTPs) are their antioxidant activities, arising from their ability to scavenge reactive oxygen species [8]. In our previous studies, we have demonstrated that GTP supplementation benefits skeletal remodeling in estrogen-deficient aged rats (a model of postmenopausal osteoporosis), in terms of mitigating bone loss and deterioration of bone microarchitecture [9, 10]. However, little is known about whether such an osteoprotective effect of GTPs also presents in androgen-deficient aged rats, a model of male osteoporosis. Therefore, the objective of the present study was to investigate the effect of GTPs on bone mass, microarchitecture, and strength in intact and androgen-deficient aged male rats to evaluate the possible benefits of GTPs in skeletal health and prevention of pathological bone loss during male aging.

Based on the reported protective effect of antioxidant-rich GTPs on bone mass and microarchitecture in ovariectomized aged rats [9, 10], we hypothesized that supplementation with GTPs in drinking water would mitigate bone mass and microstructure and improve bone strength in both sham and orchidectomized (ORX) aged male rats via increasing antioxidant capacity. In addressing the study objectives, measurements of bone mineral density (BMD), bone histomorphometry, and micro-computed tomography (μCT) were performed. In addition, the activities of liver glutathione peroxidase were determined for a possible mechanistic property of GTP in bone remodeling. Studying the effect of GTPs on bone remodeling in intact and ORX aged rats will advance the understanding of their effects on skeletal biology to minimize bone loss in elderly men.

Materials and Methods

Animals and GTP Treatments

Fifty virgin male F344 rats (15 months old) were purchased from the National Institute on Aging/Taconic Farms (Germantown, NY). After arrival at the experimental site, all rats were acclimated for 7 days to a powdered AIM-93M diet (DYETS, Bethlehem, PA) [11] and distilled water ad libitum. After acclimation, 20 rats underwent bilateral ORX under anesthesia, taking the dorsal approach, and another 20 rats underwent sham operation (SH) under the same anesthesia conditions. The remaining 10 rats without any surgical procedures were assigned to the baseline (B) group and killed at day 0 of intervention. Values from the B group were compared to the results obtained at the end of the 16-week feeding period to determine any age effect.

Twenty ORX rats were randomly assigned to the ORX-control (ORX-C) group (n = 10), which received no GTP. The ORX+GTP group (n = 10) received GTP at 0.5% (wt/vol) in distilled drinking water. Similarly, 20 sham-operated rats were randomly assigned to the untreated sham control (SH-C) group or the SH + GTP group, receiving GTP at 0 and 0.5% (wt/v) in distilled drinking water, respectively. Dietary calcium and phosphorus were held constant at 0.54% and 0.3%, respectively. Rats had free access to distilled drinking water. Distilled water mixed with GTP was prepared fresh daily, and the amount of water consumed was recorded for each rat. Rats were housed in individual stainless steel cages under a controlled temperature of 21 ± 2°C with a 12-hour light–dark cycle. Rats were weighed bimonthly and examined daily. All procedures were approved by the Texas Tech University Health Sciences Center Animal Care and Use Committee.

GTPs were purchased from Zhejiang Yuxin Pharmaceutical (Lanxi City, China). The main GTP components were 99.25% pure, with 46.5% of EGCG, 21.25% of EGC, 10% of EC, 7.5% of ECG, 9.5% of gallic acid gallate, and 4.5% of catechin according to the HPLC-ECD and HPLC-UV analyses in our laboratory. Rats were given 0.5% concentration of GTP in drinking water daily to mimic human consumption of 4 cups of green tea a day based on our previous studies on humans [12] and other animals [9, 13].

Sample Preparation

Each animal was given an intraperitoneal injection of calcein green (10 mg/kg of body weight; Sigma, St. Louis, MO) at 14 and 4 days before being killed. The final body weight was recorded. After anesthetization, blood samples were drawn from the heart into vacutainer tubes and serum samples were isolated and stored at −80°C for later analyses. After animals were killed, femora and tibiae were harvested and cleaned of adhering soft tissues. Right tibial samples were kept in 70% ethanol and then processed for histomorphometric assays. Left femoral samples were stored in 70% ethanol for bone mass measurement and bone strength tests. Liver samples were stored at −80°C for measurement of enzymatic activities.
BMD Assessment

Total bone area, bone mineral content (BMC), and BMD of the whole left femur of each rat were determined by dual-energy X-ray absorptiometry (DEXA; QDR-2000 plus DEXA; Hologic, Waltham, MA) [9]. The machine was set at an ultrahigh-resolution mode with line spacing of 0.0254 cm, resolution of 0.0127 cm, and a collimator diameter of 0.9 cm.

Bone Microarchitecture Assessment by Histomorphometric Analysis

Preparation of right tibiae for static and dynamic bone histomorphometric analyses was described previously [10]. Undecalcified frontal sections of proximal tibia were embedded in methylmethacrylate (Eastman Organic Chemicals, Rochester, NY) and cut (5 μm thickness) using a microtome (RM 2155; Leica, Heidelberg, Germany) for metaphyseal bone histomorphometric analysis. In addition, undecalcified tibial shaft was embedded and a cross section of the proximal tibiofibular junction (8 μm thickness) was cut using a microtome for cortical bone histomorphometric analysis. All sections were coverslipped with Eukitt (Calibrated Instruments, Hawthorne, NY) for static and dynamic histomorphometric analyses using a semiautomatic image analysis system (Osteomeasure Histomorphometry System; OsteoMetrics, Atlanta, GA).

A digitizing morphometric system was used to measure bone histomorphometric parameters. The system consisted of an epifluorescence microscope (Nikon E-400), an Osteomeasure High Resolution Color Subsystem coupled to an IBM computer, and a morphometry program (all from OsteoMetrics). The measured parameters for cancellous bone included total tissue volume (TV), bone volume (BV), bone surface (BS), single- and double-labeled surfaces, interlabel width, osteoclast surface, osteoclast cell number, and eroded surface. These data were used to calculate standard morphometric parameters analyzed in bone studies, including cancellous bone volume (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular number (Tb.N, n/mm), trabecular separation (Tb.Sp, μm), trabecular bone-formation rate (BFR/BS, μm3/μm2/day), and eroded surface/bone surface (ES/BS, %) according to the standard nomenclature recommended by the American Society for Bone and Mineral Research Nomenclature Committee [14]. The region of bone measured in all groups is 1–4 mm from the growth plate in the proximal tibia.

Measurements in cortical bone included periosteal mineral total bone area, periosteal perimeter, marrow area, endocortical perimeter, periosteal and endocortical single- and double-labeled parameters, interlabel widths, and endocortical eroded surface. These measures were then used to calculate cortical bone area (Ct.Ar, %), marrow area (Ma.Ar, %), periosteal mineralized surface/bone surface (Ps-MS/BS, %), periosteal mineral apposition rate (Ps-MAR, μm/day), periosteal bone-formation rate (Ps-BFR/BS, μm3/μm2/day), endocortical mineralized surface/bone surface (Ec-MS/BS, %), endocortical mineral apposition rate (Ec-MAR, μm/day), endocortical bone-formation rate (Ec-BFR/BS, μm3/μm2/day), and endocortical eroded surface/bone surface (Ec-ES/BS, %) [10].

Bone Microarchitecture Assessment by μCT

Bone microarchitecture in femora was assessed using μCT (MicroCT40; Scanco Medical, Bassersdorf, Switzerland) according to the procedure of Shen et al. [10].

Trabecular bone of the femur was scanned so that 250 images were acquired. The volume of interest (VOI) comprised the secondary spongiosa in 100 cross-sectional slices of the distal femur beginning 25 slices from the growth plate region. All scans were performed in a 1,024 × 1,024 matrix, resulting in an isotropic voxel resolution of 16 μm3. An integration time of 150 milliseconds per projection was used. Trabecular parameters in the femur included BV/TV, Tb.N, Tb.Th, and Tb.Sp. Coefficients of variation (CVs) were 2.0% (BV/TV), 1.1% (Tb.N), 0.66% (Tb.Th), and 1.30% (Tb.Sp) for morphometric parameters.

Bone Strength Assessment

Femoral strength was determined by a three-point bending test using a custom-designed and -built apparatus according to the procedures of Nielsen [15]. The terms used for assessment of bone strength have been described previously [16]. Maximum force (N) and yield point force (N) to break bones as well as modulus of elasticity were assessed.

Blood Analysis

Serum testosterone concentration was quantified by an EIA kit (Testosterone Assay, catalog KGE-010; R&D Systems, Minneapolis, MN) following the manufacturer’s instructions. The concentrations of osteocalcin (OC) and tartrate-resistant acid phosphatase (TRAP) in serum were measured by ELISA kits from Biomedical Technologies (Stoughton, MA) and Immunodiagnostic System (Fountain Hills, AZ), respectively, according to the manufacturers’ instructions.

Analysis of Liver Enzymatic Activities

Liver samples were minced and homogenized (100 mg/1 mL) at 4°C in 0.1 M Tris-HCl buffer (pH 7.4), then centrifuged at 10,000 × g for 15 minutes. Supernatants were
collected and stored at −80°C for glutathione peroxidase (GPX) enzymatic activity assays. GPX activity was coupled to NADPH utilization, and NADP production was measured spectrophotometrically at 340 nm [17].

Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). All data were analyzed with SigmaStat software (version 2.03; Systat Software, San Jose, CA). Differences between the baseline and each dietary treatment group were analyzed by t-test to determine age or age-plus-androgen-deficient effect (α = 0.05). Data on body weight were analyzed by three-way analysis of variance (ANOVA) (ORX × GTP dose × time) followed by Fisher’s protected least significant difference (LSD) post hoc test to evaluate the effects of ORX, GTP levels, time (week), and interaction. Other outcome parameters were analyzed by two-way ANOVA (ORX × GTP dose) followed by Fisher’s LSD post hoc test to evaluate the effect of ORX, GTP dose, or interaction (P < 0.05).

Results

Body Weight

There was no difference in initial body weight among all treatment groups (data not shown). Over the course of the 16-week study, all animals gained body weight in a time-dependent pattern, regardless of treatment group. Neither ORX (P = 0.895) nor GTP supplementation (P = 0.458) affected final body weight. Regarding water consumption, animals in the GTP-supplemented groups (SH-C group 36.2 mL/day, ORX-C group 33.1 mL/day) consumed less water than those without GTP in the drinking water (SH-C group 37.8 mL/day, ORX-C group 37.1 mL/day) throughout the study.

Serum Testosterone Concentration

Relative to the B group, the ORX-C group had a lower testosterone concentration in serum of aged male rats (Fig. 1). Castration suppressed serum testosterone concentration. GTP supplementation did not affect serum testosterone concentration. There was no interaction between ORX and GTP supplementation in serum testosterone levels.

Serum Bone Biomarker Concentrations

Compared to the B group, both the SH-C and ORX-C groups increased serum OC concentrations in aged male rats (Fig. 2a). Based on the results of two-way ANOVA, (1) both castration and GTP supplementation significantly increased serum OC levels (P = 0.005 for castration effect, P = 0.004 for GTP effect) and (2) there was no interaction between ORX and GTP in serum OC levels (Fig. 2a). In terms of serum TRAP concentrations, both SH-C and ORX-C groups had lower serum TRAP levels than the B group (Fig. 2b). Castration significantly increased serum TRAP concentrations (P < 0.001). However, the impact of GTP supplementation on suppression of serum TRAP was observed only in ORX-treated animals (ORX-C vs. ORX-GTP, P = 0.016) (Fig. 2b).

BMD of Femur

There was no difference in femoral bone area among all treatments (data not shown). The ORX-C group had a significantly lower BMD than the B group (Fig. 3). Based on two-way ANOVA results, (1) castration led to a significant decrease in BMD of aged male rats; (2) GTP supplementation in the drinking water mitigated bone loss, as determined by BMD, in both SH and ORX rats (Fig. 3); and (3) there was no interaction between ORX and GTP dose affecting the BMD of rats.

Histomorphometric Changes in Proximal Tibia

Relative to the B group, the ORX-C group showed lower values for BV/TV, Tb.Th, and Tb.N but higher values for Tb.Sp, BFR/BS, MAR, and ES/BS at the proximal tibia
Table 1. ORX resulted in a significant decrease in BV/TV, Tb.Th, and Tb.N and a significant increase in Tb.Sp, BFR/BS, MAR, and ES/BS at the proximal tibia in aged male rats. GTP supplementation in the drinking water significantly increased BV/TV, Tb.Th, BFR/BS, and MAR; significantly suppressed ES/BS; but had no effect on Tb.N and Tb.Sp. There was no interaction between ORX and GTP in any these parameters (Table 1).

Alteration in Dynamic Parameters in Cortical Bone of Tibial Shaft

Compared to baseline, both SH-C and ORX-C groups had lower values for Ct.Ar and periosteal parameters including Ps-MS/BS, Ps-MAR, and Ps-BFR/BS but higher values for endocortical parameters including Ec-MS/BS, Ec-MAR, Ec-BFR/BS, and Ec-ES/BS. GTP supplementation caused a significant increase in Ps-MS/BS, Ps-BFR/BS, and Ec-MS/BS; a significant decrease in Ec-ES/BS; but no effect on Ct.Ar, Ps-MAR, Ec-MAR, and Ec-BFR/BS (Table 2). No interaction between ORX and GTP supplementation was found (Table 2).

Microarchitectural Properties of Trabecular Bone of Femur

Data on trabecular bone microarchitecture in the femur (Table 3) show that (1) relative to the B group, only the ORX-C group, not the SH-C group, increased Ec-ES/BS at cortical bone of the tibial shaft (Table 2). Neither ORX nor GTP supplementation affected total area (T.Ar). Castration decreased Ct.Ar and periosteal activities (Ps-MS/BS, Ps-MAR, and Ps-BFR/BS) and increased endocortical activities (Ec-MS/BS, Ec-MAR, Ec-BFR/BS, and Ec-ES/BS). GTP supplementation caused a significant increase in Ps-MS/BS, Ps-BFR/BS, and Ec-MS/BS; a significant decrease in Ec-ES/BS; but no effect on Ct.Ar, Ps-MAR, Ec-MAR, and Ec-BFR/BS (Table 2). No interaction between ORX and GTP supplementation was found (Table 2).
Table 1 Bone histomorphometric measurements in trabecular bone of proximal tibia of sham-operated (SH) and orchidectomized (ORX) rats supplemented with green tea polyphenols (GTPs) in drinking water

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline (B group)</th>
<th>SH -GTP (SH-C)</th>
<th>+GTP (SH + GTP)</th>
<th>ORX -GTP (ORX-C)</th>
<th>+GTP (ORX + GTP)</th>
<th>Two-way ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV/TV (%)</td>
<td>21.0 ± 0.9</td>
<td>18.8 ± 1.0ab</td>
<td>22.1 ± 0.9a</td>
<td>8.9 ± 0.8a</td>
<td>10.0 ± 0.8a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Th.Th (μm)</td>
<td>72.18 ± 2.95</td>
<td>72.45 ± 3.20ab</td>
<td>79.04 ± 3.20a</td>
<td>50.88 ± 2.87ab</td>
<td>59.85 ± 2.87a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tb.N (n/mm)</td>
<td>2.95 ± 0.16</td>
<td>2.63 ± 0.16a</td>
<td>2.87 ± 0.15a</td>
<td>1.69 ± 0.14ab</td>
<td>1.74 ± 0.14b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tb.Sp (μm)</td>
<td>277.1 ± 16.6</td>
<td>317.7 ± 48.8a</td>
<td>278.4 ± 45.1a</td>
<td>582.5 ± 40.8a</td>
<td>551.3 ± 40.8a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BFR/BS (μm²/μm²/day)</td>
<td>15.64 ± 0.71</td>
<td>16.65 ± 1.60ab</td>
<td>19.45 ± 1.50ab</td>
<td>35.82 ± 1.36ab</td>
<td>49.17 ± 1.36a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAR (μm/day)</td>
<td>0.75 ± 0.02</td>
<td>0.71 ± 0.06ab</td>
<td>0.79 ± 0.03a</td>
<td>0.97 ± 0.03ab</td>
<td>1.08 ± 0.03a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ES/BS (%)</td>
<td>3.37 ± 0.23</td>
<td>4.01 ± 0.45a</td>
<td>2.76 ± 0.4a</td>
<td>8.60 ± 0.38a</td>
<td>6.40 ± 0.38ab</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± SEM, n = 10

BV/TV percent trabecular bone volume, Th.Th trabecular thickness, Tb.N trabecular number, Tb.Sp trabecular separation, BFR/BS trabecular bone-formation rate, MAR mineral apposition rate, ES/BS trabecular eroded surface/bone surface

* Significantly different from the B group (B vs. SH-C; B vs. ORX-C) based on t-test, P < 0.05. Different letters (a and b for GTP effect) are significantly different by two-way ANOVA and Fisher’s LSD test (P < 0.05)

Table 2 Bone histomorphometric measurements in cortical bone of tibial shaft of sham-operated (SH) and orchidectomized (ORX) rats supplemented with green tea polyphenols (GTPs) in drinking water

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline (B group)</th>
<th>SH -GTP (SH-C)</th>
<th>+GTP (SH + GTP)</th>
<th>ORX -GTP (ORX-C)</th>
<th>+GTP (ORX + GTP)</th>
<th>Two-way ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.Ar (mm²)</td>
<td>4.81 ± 0.06</td>
<td>4.93 ± 0.10</td>
<td>5.05 ± 0.09</td>
<td>4.87 ± 0.08</td>
<td>4.96 ± 0.08</td>
<td>0.452</td>
</tr>
<tr>
<td>Ct.Ar (%)</td>
<td>81.14 ± 0.37</td>
<td>77.92 ± 0.85ab</td>
<td>78.11 ± 0.81a</td>
<td>70.08 ± 0.73ab</td>
<td>71.55 ± 0.73a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ps-MS/BS (%)</td>
<td>27.82 ± 2.84</td>
<td>13.50 ± 1.33ab</td>
<td>16.93 ± 1.26a</td>
<td>9.21 ± 1.14ab</td>
<td>13.03 ± 1.14a</td>
<td>0.002</td>
</tr>
<tr>
<td>Ps-MAR (μm/day)</td>
<td>1.11 ± 0.06</td>
<td>0.74 ± 0.11a</td>
<td>0.82 ± 0.11a</td>
<td>0.48 ± 0.10ab</td>
<td>0.55 ± 0.10a</td>
<td>0.016</td>
</tr>
<tr>
<td>Ps-BFR/BS (μm³/μm²/day)</td>
<td>27.01 ± 4.16</td>
<td>10.14 ± 2.07ab</td>
<td>14.20 ± 1.95a</td>
<td>4.77 ± 1.76ab</td>
<td>8.43 ± 1.76a</td>
<td>0.006</td>
</tr>
<tr>
<td>Ec-MS/BS (%)</td>
<td>16.39 ± 2.34</td>
<td>32.89 ± 3.34ab</td>
<td>39.32 ± 3.34a</td>
<td>44.41 ± 2.99ab</td>
<td>51.42 ± 2.85a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ec-MAR (μm/day)</td>
<td>0.90 ± 0.10</td>
<td>1.20 ± 0.05ab</td>
<td>1.17 ± 0.05a</td>
<td>1.52 ± 0.04ab</td>
<td>1.46 ± 0.04a</td>
<td>0.001</td>
</tr>
<tr>
<td>Ec-BFR/BS (μm³/μm²/day)</td>
<td>14.23 ± 2.90</td>
<td>39.91 ± 5.61a</td>
<td>42.92 ± 5.29a</td>
<td>69.19 ± 4.78a</td>
<td>75.34 ± 4.78a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ec-ES/BS (%)</td>
<td>5.56 ± 0.63</td>
<td>6.97 ± 1.26a</td>
<td>5.03 ± 1.19ab</td>
<td>12.72 ± 1.07ab</td>
<td>9.09 ± 1.07ab</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± SEM, n = 10

T.Ar total area, Ct.Ar percent cortical bone area (cortical area/total area), Ps-MS/BS percent periosteal mineralized surface/bone surface, Ps-MAR periosteal mineral apposition rate, Ps-BFR/BS periosteal bone-formation rate, Ec-MS/BS percent endocortical mineralized surface/bone surface, Ec-MAR endocortical mineral apposition rate, Ec-BFR/BS endocortical bone-formation rate, Ec-ES/BS endocortical eroded surface/bone surface

* Significantly different from the B group (B vs. SH-C; B vs. ORX-C) based on t-test, P < 0.05. Different letters (a and b for GTP effect) are significantly different by two-way ANOVA and Fisher’s LSD test (P < 0.05)

Bone Quality of Femur

Relative to the B group, both SH-C and ORX-C groups decreased bone strength of femur in aged male rats, as assessed by three-point bending test (Fig. 4). Castration reduced femoral bone strength, while GTP supplementation enhanced it. There was no interaction between ORX and GTP supplementation in bone strength levels.

Liver Enzymatic Activities

Compared to the B group, the ORX-C group suppressed liver GPX activity (Fig. 5). Castration resulted in a significant decrease in GPX in rats. GTP supplementation led to an increase in the activity of GPX in both SH and ORX rats. There was no interaction between ORX and GTP supplementation in GPX activity of rats.
In the present study, models of sham- and ORX-operated aged male rats were successfully utilized to investigate the effect of GTP supplementation in drinking water in mitigating aging-induced and aging-plus-androgen-deficiency-induced deterioration of bone microarchitecture and strength. Compared to the 15-month-old B group, the SH-C and ORX-C groups without GTP supplementation for 16 weeks had lower values for femur BMD (240, 228, and 196 mg/cm² for the B, SH-C, and ORX-C groups, respectively). We found that aging plus androgen deficiency produced a greater detrimental impact on BMD than aging alone. On the other hand, we also observed that, relative to the B group, aging in the SH-C group led to lowered cortical bone volume via a reduction in periosteal bone-formation rate and an increase in endocortical bone-formation rate (Table 2). Similar to the observation of BMD, aging plus androgen deficiency in the ORX-C group also produced a greater detrimental effect on microarchitecture (at both trabecular and cortical bones) and bone strength than aging alone in the SH-C group, which is consistent with the results reported by others [18–20].

The current investigation showed that GTP supplementation in drinking water preserves bone mass and microarchitectural changes at both cancellous and cortical bone compartments in SH and ORX aged male rats, as shown in the SH + GTP and ORX + GTP groups, respectively.
Such results support our stated hypothesis that long-term administration of GTP mitigates aging- and aging-plus-androgen-deficiency-induced bone loss and bone microstructure deterioration as determined by BMD, histomorphometric, and μ-CT analyses. The results of GTP mitigating bone loss and sustaining bone microstructure are similar to findings in sham and ovariectomized aged female rats supplemented with GTP in the drinking water [9, 10].

In the GTP-supplemented rats, both SH + GTP and ORX + GTP groups had significantly greater serum OC concentrations (a bone-formation biomarker) and bone-formation rate (as measured at trabecular bone of proximal tibia and at endocortical bone of tibial shaft) relative to non-GTP-supplemented rats. In addition, the inhibitory effect of GTP supplementation on serum TRAP concentrations (a bone-resorption biomarker) seemed to be more significant in ORX-treated rats than in SH-treated rats. Based on the histomorphometric findings, both SH + GTP and ORX + GTP groups had less bone-resorption activity than non-GTP-supplemented rats. The abilities of GTP to increase indices of bone formation and to suppress indices of bone resorption in both groups have been demonstrated in previous animal studies [10, 21] and further supported by cellular studies, in terms of GTP’s beneficial influence on osteoblastic and osteoclastic activity, as reviewed by Shen et al. [22]. For example, in vivo studies, EGCG, an abundant GTP, increased osteoblastogenesis via stimulating the mRNA expression of osteogenic genes [23], protein expression of alkaline phosphatase activity [24], and finally enhanced mineralization [25]. On the other hand, EGCG has also been found to suppress osteoclastogenesis via enhancing osteoclastic apoptosis, mediated by Fenton action [26] or caspase activation [27].

It was noted that the bioavailability of EGCG or catechins, however, is relatively low due to a short half-life by nature. The distribution of catechin concentration is dependent on tissue sites. Watkins’ laboratory reported in a mouse study [28] that tea catechins can be accumulated in long bones (e.g., femur and tibia) with continuous tea consumption as short as 16 days. In their study, 15-week-old mice were treated with the AIN-93G basal diet supplemented with green tea extract (30 g/kg diet) for 16 days. After 16 days, HPLC-ECD analysis detected major tea catechins in bone content (μmol/g bone weight) in the order of EGC, EC, EGCG, and ECG and totals were 7.3 ± 1.8, 2.2 ± 0.9, 76.4 ± 21.9, 8.6 ± 3.2, and 94.4 ± 26.3, respectively. On the other hand, in the present study, aged rats with 400 g average body weight consumed approximately 35 mL drinking water containing 0.05% (wt/vol) GTP daily for 16 weeks. Although we did not measure bone catechins in our study, based on the observations of Watkins’ team [28], we speculated that the route of water consumption in the present study would also generate significant amounts of catechins accumulated in the long bones of rats, resulting in an impact on bone remodeling.

In this study, we compared the efficacy of GTP at decreasing bone loss between intact-treated (SH + GTP) and ORX-treated (ORX + GTP) aged male rats. In general, the benefits of GTP were more pronounced in the SH group than in the ORX group. For instance, GTP supplementation increased by 17.5% the trabecular bone volume of the tibia in the SH + GTP group over that in the SH-C group, while it only increased by 12.3% in the ORX + GTP group over that in the ORX-C group. Nevertheless, such a protective action of GTP in both intact and androgen-deficient aged male rats may be due to GTP’s antioxidant capacity as shown in elevated activities of liver GPX in both the SH + GTP and ORX + GTP groups (Fig. 5), which agrees with our previous study using estrogen-deficient aged female rats also supplemented with GTP [9, 10]. Moreover, in the present study, GTP was assessed as a dietary supplement option for palliating bone loss and deterioration of bone microstructure due to aging or aging plus androgen deficiency. Our results demonstrated that GTP supplementation has potential benefits on BMD and microstructure in aged male rats. These potential benefits of GTP may be mediated in part through enhanced bone formation and suppressed bone erosion, leading to a larger net bone volume and modulated trabecular and endocortical bone compartments [10].

Conclusions

In the present study, we examined the effects of GTP on skeletal remodeling during male aging, which were hypothesized to augment bone preservation in 15-month-old SH and ORX rats. Our data have demonstrated that drinking water rich in GTP mitigated aging-induced bone loss in gonad-intact aged female rats and aging-plus-androgen-deficiency-induced bone loss in ORX aged male rats during skeletal remodeling. Additionally, GTP supplementation to aged male rats for 16 weeks had a positive effect on preserving both cancellous and cortical bone compartment via increasing bone formation and suppressing bone erosion. Such a bone-protective role of GTP may contribute to an increase of antioxidant capacity.

Despite the differences between gonad-intact (or ORX) aged male rats and elderly men in terms of the impact of GTP supplementation on bone mass, microarchitecture, and strength, we believe that our study presents a first step toward assessing the effects of green tea consumption on skeletal remodeling in elderly men. Future studies may focus on translating data from animal observations to investigate the possible therapeutic efficacy of GTPs in...
elderly men with low bone mass along with related mechanisms.

Acknowledgement This study was supported by Winthrop-University Hospital and Texas Tech University Health Sciences Centers.

References