Effects of estrogen and growth hormone on skeleton in the ovariectomized rat with hypophysectomy

JAMES K. YEH,¹ ² MENG-MENG CHEN,¹ AND JOHN F. ALOIA¹ ²

Department of Medicine, Winthrop-University Hospital, Mineola, New York 11501; and The Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794

Yeh, James K., Meng-Meng Chen, and John F. Aloia. Effects of estrogen and growth hormone on skeleton in the ovariectomized rat with hypophysectomy. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E734–E742, 1997.—To investigate whether growth hormone (GH) and 17β-estradiol (E2) replacement can prevent osteopenia induced by pituitary and ovarian hormone deficiency [by hypophysectomy and ovariectomy (HX+OV)], we administered relatively low doses of GH (2.3 IU·kg⁻¹·day⁻¹) and E2 (100 µg·kg⁻¹·wk⁻¹) in experiment 1 and relatively high doses of GH (13.5 IU·kg⁻¹·day⁻¹) and E2 (3,500 µg·kg⁻¹·wk⁻¹) in experiment 2 to 2-mo-old HX+OV Sprague-Dawley rats for 6 wk. Our data show that the HX+OV of rats results in diminished periosteal bone formation, longitudinal bone growth, and decreased cancellous bone volume. Administration of either the low or high dose of GH to these rats increased their systemic growth, serum levels of osteocalcin, and cortical bone formation. Either low or high doses of GH or E2 alone only partially prevent cancellous bone loss. However, the combined treatment of GH plus E2 resulted in an additive increase in the cancellous bone mass. We conclude that the additive effect of GH plus E2 on cancellous bone is attributed to the suppressive effect of E2 on bone resorption and the anabolic effect of GH on bone formation.

PITUITARY AND OVARIAN hormones play an important role in the regulation of bone formation and bone resorption. Ovarian hormone deficiency after menopause or ovariectomy (OV) results in a high bone turnover rate and an increase in bone loss (7, 29). Administration of estrogen to postmenopausal women or OV animals has been shown to suppress bone resorption and prevent bone loss (12, 15, 28). Hypophyseal hormone deficiency by hypophysectomy (HX) in rats results in a decrease in periosteal bone formation and a loss in cancellous bone (4, 10, 22, 31). Administration of growth hormone (GH) to HX rats increases cortical and cancellous bone formation (6, 10, 21, 22).

Both HX and OV result in estrogen deficiency, but the mechanisms through which bone is lost differ. Bone loss after HX is due to a decrease in bone turnover with less bone formation than bone resorption (4, 14, 18, 22, 31), whereas bone loss induced by OV is due to a high bone turnover rate with more bone resorption than bone formation (28, 29). Recently, our laboratory observed that, when an OV rat was HX (HO), the effect OV has on bone turnover rate was abolished (5, 32). These findings suggest that the factor causing high bone turnover by OV would be diminished without pituitary hormones present. Furthermore, the antiresorptive effect of estrogens or raloxifene in the OV rat has also been found to be diminished through HX (3, 33). It is not known which pituitary hormone(s) can reactivate the OV-induced high bone turnover or restore the antiresorptive effect of estrogen. Uncovering the pituitary-derived factor(s) that interacts with estrogens in the maintenance of bone is pivotal to the success of finding a means to prevent osteoporosis.

Although several pituitary hormones are needed for bone growth, GH is the only one that has been shown to stimulate longitudinal bone growth in a dose-dependent manner (16). In addition to its growth-promoting effect, GH has important regulatory functions in bone formation and osteogenesis (10, 11, 13, 18, 20, 24). The anabolic effect of GH on skeletal bone growth and bone formation has been demonstrated in both human and animal models (1, 6, 19, 21, 26, 27). This evidence prompted us to investigate whether or not the OV-induced high bone turnover rate and/or the antiresorptive effect of estrogens that has been inhibited by HX would resurface if GH were reintroduced to HO rats.

In this study we chose to examine the effects of GH, with or without 17β-estradiol (E2), on bone turnover and bone mass in HO rats. The specific aim of this proposal was to study the influence of GH, E2, and GH+E2 on bone density, bone mass, and dynamic histomorphometry of cancellous and cortical bone in these animals. We investigated 1) whether or not GH administration to HO rats could unmask OV-induced high bone turnover and bone loss or exert an anabolic effect on bone as occurs in ovarian-intact rats, 2) whether or not the antiestrogenic effect of estrogens in OV rats is dependent on GH or whether other hormones are also required, and 3) whether or not GH and E2 in a combined intervention can prevent bone loss and also increase bone mass in the HO rats. Analysis of the results of experiment 1 revealed that the doses of E2 and GH used were unable to fully prevent cancellous bone loss in HO rats. To be certain that any failure to fully prevent cancellous bone loss was not due to an insufficient dose of hormone, we performed experiment 2 using relatively high doses of E2 and GH.

MATERIALS AND METHODS

Animal Preparation

Experiment 1. Fifty-six female Sprague-Dawley rats were purchased from Hilltop Animal Care (Pittsburgh, PA). They were randomly divided into six groups containing basal control, age-matched control (Con), HO, HO+E2, HO+GH, and HO+GH+E2. The HO and OV surgeries were done at Hilltop Animal Care when the rats were 2 mo of age. Upon arrival (3 days postoperative) and until the end of the
human GH (rhGH) was 13.5 IU·kg$^2$ and one animal in the HO·GH group and one animal in the HO group and one animal in the HO·E2 group was subcutaneously injected twice a week at a dosage of 100 µg·kg$^{-2}$·wk$^{-1}$ in olive oil. Ovine GH was subcutaneously injected at a dosage of 2.3 IU·kg$^{-2}$·day$^{-1}$. Ovine GH (lot no. AFP-9220A) was kindly provided by the National Institute of Diabetes and Digestive and Kidney Diseases, The Center for Population Research of the National Human and Pituitary Program. All animals were housed singly in cages (25 × 20 × 18 cm$^3$) under local vivarium conditions (temperature 23.8°C and 12:12-h on-off light cycle). Eight rats were killed at 2 mo of age as baseline controls, and the rest of the rats were killed 6 wk after surgery. Animals were maintained in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals, and animal protocols were approved by the Laboratory Animal Care Committee of Winthrop-University Hospital. The body weight of the rats was monitored weekly, and their serum levels of insulin-like growth factor I (IGF-I) were measured at death to assess the completeness of HX. Serum levels of estradiol and progesterone and uterine weight were also measured to assess the completeness of OV. One rat in the HO·GH group was excluded from this study due to a cessation in weight gain. In this case, only a small increase in serum IGF-I was observed and antibodies against the ovine GH were detected (30).

Experiment 2. Fifty-six female Sprague-Dawley rats were used. Animal age, grouping, and experimental periods were the same as in experiment 1, except the dose of recombinant human GH (rhGH) was 13.5 IU·kg$^{-2}$·day$^{-1}$ and the dose of E$^2$ was 3,500 µg·kg$^{-2}$·wk$^{-1}$. The rhGH was supplied by Genentech, South San Francisco, CA. One animal in the HO·GH group and one animal in the HO·GH + E$^2$ group were excluded from the study due to a cessation in body weight gain, a reduction of IGF-I serum levels, and detection of antibodies against rhGH.

Preparation of Specimens

All rats were labeled with 15 mg/kg of demeclocycline intraperitoneally (Sigma Chemical, St. Louis, MO) and 8 mg/kg of calcein subcutaneously (Sigma Chemical) at 10 and 3 days, respectively, before being killed. Rats were killed under CO$_2$ anesthesia, and blood specimens were collected from the choroidal artery and jugular vein for serum chemistries. The gastrocnemius muscle and uterus were isolated for measurement of wet weight. The right tibia was removed and processed for methylmethacrylate embedding without decalcification (4, 31). In experiment 2, the left tibia was also removed and decalcified for 5 wk at 4°C in 10% EDTA containing 7.0% sucrose (pH 7.2). After a thorough wash in phosphate-buffered saline solution, the decalcified bones were dehydrated in graded alcohol and embedded in 2-hydroxyethyl methacrylate (J B4-Plus embedding kit, Polysciences, Warrington, PA). Polymerization was carried out at 4°C in a cold room overnight. Polymerized blocks were kept in a desiccator at 4°C until sectioning. Sections of 4-µm thickness were cut with a microscope (2040 Reichert-Jung) and then mounted on glass slides. Tartrate-resistant acid phosphatase (TRAP) was localized using a commercially available kit with α-naphthyl phosphate disodium salt as substrate (Sigma Chemical) (31).

Histomorphometric Analysis

The proximal sections of each right tibia and the tibial shaft were cut using an Isomet saw (Buehler, Lake Bluff, IL) and stained by Villanueva Osteochrome Bone Stain (Polysciences) for 5 days. The specimens were destained and dehydrated with sequential changes (70, 95, and 100%) of ethanol solution and xylene and then embedded in methyl methacrylate (Eastman Organic Chemicals, Rochester, NY). Proximal tibiae were frontal cut longitudinally and the tibial shaft was cut cross-sectionally on the tibia and femoral junction. A histosaw that utilizes a diamond wire (Delaware Diamond Knives, Wilmington, DE) was used to cut the specimen to a thickness of 40 µm, and the specimen was then placed under a coverslip with Eukitt (Calibrated Instruments, Hawthorne, NY).

Histomorphometric parameters of cortical bone of the tibial shaft and cancellous bone of the proximal tibia were measured with a digitizing morphometry system and the nomenclature standard (17), as described previously (4, 31). Briefly, the measured parameters included total tissue area, cortical area, marrow area, growth plate-labeled width, trabecular bone area and parameters, single- and double-labeled perimeters, interlabeled widths, and eroded perimeters. These parameters were then used to calculate cortical area per tissue area, cancellous bone volume per total volume, longitudinal growth rate, total labeled surface per bone surface, mineral apposition rate, bone formation rate on the periosteum, cancellous bone, and the eroded surface per trabecular surface. In experiment 2, osteoclasts, as identified by TRAP-positive staining, were counted in the region from the distal end of the growth plate to 0.2 mm from the growth plate.

Lumbar Vertebral Volume, Dry Weight, and Length

The L$_2$ vertebra of each animal in experiments 1 and 2 was dissected free of soft tissue. The weight of the bone in air and in deionized water was measured using a Sartorius balance with a thin wire to which the bone was attached. The difference between the two weights is the volume of the bone. Each bone was then extracted with ethanol and chloroform to remove water and fat for dry weight and bone length measurements (4).

Chemical Analysis

Serum levels of IGF-I were measured with a commercial radioimmunoassay kit after acid-ethanol precipitation (Nichols Institute, San Juan Capistrano, CA) (30). Serum osteocalcin was measured by radioimmunoassay using goat anti-rat osteocalcin and donkey anti-goat immunoglobulin G (IgG) as antibodies. The antibody against rat osteocalcin and the antibody against goat IgG were from Biomed Technologies (Stoughton, MA). Serum estradiol and progesterone were measured with commercial kits from Diagnostic Products (Los Angeles, CA).

Statistical Analysis

All data are presented as the means ± SD. Because the variance of each measurement was proportional to the mean, data were converted to logarithms for statistical analysis. Two-factor analysis of variance (ANOVA) was used to examine the individual effect and interaction between treatments (25). The multiple comparisons between groups were done by Fisher’s protected least significant difference test. Statistical analyses were performed using the StatView II program on a Macintosh computer. A significance level of $P < 0.05$ was used for all comparisons.
Table 1. Body weight, gastrocnemius muscle, uterine weights, and L4 vertebral wet weight, volume, density, and dry weight of experimental animals

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Wt, g</th>
<th>Gastrocnemius Muscle Wt, g</th>
<th>Uterine Wt, mg</th>
<th>L4 Vertebral Wet Wt, mg</th>
<th>L4 Vertebral Volume, mm³</th>
<th>L4 Vertebral Density, mg/mm³</th>
<th>L4 Vertebral Dry Wt, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>8</td>
<td>211 ± 9</td>
<td>1.44 ± 0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>8</td>
<td>267 ± 23</td>
<td>1.85 ± 0.15</td>
<td></td>
<td>502 ± 117</td>
<td>317 ± 24</td>
<td>225 ± 23</td>
<td>1.43 ± 0.013</td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
<td>185 ± 11</td>
<td>1.22 ± 0.10</td>
<td></td>
<td>147 ± 34</td>
<td>214 ± 21</td>
<td>161 ± 16</td>
<td>1.33 ± 0.027</td>
</tr>
<tr>
<td>HO + E2</td>
<td>10</td>
<td>169 ± 7</td>
<td>1.10 ± 0.07</td>
<td></td>
<td>398 ± 38</td>
<td>218 ± 16</td>
<td>162 ± 12</td>
<td>1.35 ± 0.029</td>
</tr>
<tr>
<td>HO + GH</td>
<td>9</td>
<td>254 ± 15</td>
<td>1.67 ± 0.18</td>
<td></td>
<td>181 ± 24</td>
<td>229 ± 23</td>
<td>168 ± 18</td>
<td>1.36 ± 0.036</td>
</tr>
<tr>
<td>HO + GH + E2</td>
<td>10</td>
<td>237 ± 17</td>
<td>1.63 ± 0.13</td>
<td></td>
<td>550 ± 58</td>
<td>232 ± 19</td>
<td>167 ± 14</td>
<td>1.39 ± 0.035</td>
</tr>
</tbody>
</table>

**Experiment 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Wt, g</th>
<th>Gastrocnemius Muscle Wt, g</th>
<th>Uterine Wt, mg</th>
<th>L4 Vertebral Wet Wt, mg</th>
<th>L4 Vertebral Volume, mm³</th>
<th>L4 Vertebral Density, mg/mm³</th>
<th>L4 Vertebral Dry Wt, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>10</td>
<td>205 ± 11</td>
<td>1.40 ± 0.04</td>
<td></td>
<td>491 ± 131</td>
<td>249 ± 18</td>
<td>184 ± 14</td>
<td>1.356 ± 0.017</td>
</tr>
<tr>
<td>Con</td>
<td>10</td>
<td>265 ± 13</td>
<td>1.80 ± 0.09</td>
<td></td>
<td>544 ± 89</td>
<td>313 ± 31</td>
<td>221 ± 23</td>
<td>1.421 ± 0.020</td>
</tr>
<tr>
<td>HO</td>
<td>9</td>
<td>191 ± 12</td>
<td>1.29 ± 0.08</td>
<td></td>
<td>162 ± 55</td>
<td>262 ± 19</td>
<td>194 ± 15</td>
<td>1.350 ± 0.022</td>
</tr>
<tr>
<td>HO + E2</td>
<td>9</td>
<td>167 ± 5</td>
<td>1.14 ± 0.08</td>
<td></td>
<td>513 ± 124</td>
<td>249 ± 20</td>
<td>183 ± 16</td>
<td>1.366 ± 0.025</td>
</tr>
<tr>
<td>HO + GH</td>
<td>8</td>
<td>301 ± 13</td>
<td>1.99 ± 0.11</td>
<td></td>
<td>203 ± 57</td>
<td>298 ± 22</td>
<td>216 ± 15</td>
<td>1.379 ± 0.014</td>
</tr>
<tr>
<td>HO + GH + E2</td>
<td>8</td>
<td>276 ± 12</td>
<td>1.95 ± 0.11</td>
<td></td>
<td>652 ± 80</td>
<td>304 ± 24</td>
<td>216 ± 19</td>
<td>1.410 ± 0.019</td>
</tr>
</tbody>
</table>

**Experiment 2**

**RESULTS**

Systemic Effects of E2 and GH

The systemic effects of E2 and GH observed in experiment 1 are similar to those observed in experiment 2 (Table 1). Table 1 illustrates that the body weight and gastrocnemius muscle weight of the Con group increased significantly by the end of the 6-wk period, whereas the body weight and the gastrocnemius muscle weight of the HO groups decreased in comparison with the baseline. Administration of low- or high-dose E2 resulted in a further loss in these weights in the HO rats. Administration of low- or high-dose GH to the HO rats, on the other hand, increased both body weight and gastrocnemius muscle weight. The average body weight of the GH + E2 combination treatment group was less than that of the GH treatment group in both experiments.

Atrophy of the uterus and a significant decrease in estradiol and progesterone serum levels of the HO groups were used to assess completeness of OV. Cessation of body weight gain and a decrease in serum levels of IGF-I to nearly 5% of the intact levels were indicative of complete HX in the HO rats. Administration of the low dose of E2 alone in HO rats partially prevented and the high dose of E2 fully prevented the atrophy of the uterus. The combination of E2 and GH administration to the HO rats resulted in a further increase in the uterine weight in both experiments. However, administration of GH alone in the HO rats did not result in a significant increase in their uterine weight. The synergistic interaction of E2 and GH treatment on the uterine weight of the HO rats was significant (P < 0.0001).

Serum levels of progesterone in the HO rats were not affected by either E2 or GH administration, whereas serum levels of estradiol were increased by E2 administration (Table 2). HO resulted in a decrease in serum levels of osteocalcin compared with the Con rats, and this decrease was not affected by the administration of either low- or high-dose E2 alone. Administration of GH, however, increased serum levels of osteocalcin in the HO rats. Osteocalcin serum levels were also increased by a combined treatment of GH and E2, but to a lesser degree than observed in the GH alone treatment group. The serum levels measured in experiment 2 gave similar results with the exception of estradiol, which showed an increase in proportion to the increased dose of E2.

Table 2. The serum levels of IGF-I, estradiol, progesterone, and osteocalcin of animals in experiment 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Serum IGF-I, ng/ml</th>
<th>Serum Estradiol, ng/ml</th>
<th>Serum Progesterone, ng/ml</th>
<th>Serum Osteocalcin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>8</td>
<td>1,213 ± 188</td>
<td>17.4 ± 7.3</td>
<td>7.4 ± 3.5</td>
<td>43.6 ± 4.5</td>
</tr>
<tr>
<td>Con</td>
<td>8</td>
<td>1,175 ± 205</td>
<td>22.6 ± 4.5</td>
<td>9.6 ± 2.2</td>
<td>37.8 ± 3.6</td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
<td>167 ± 12</td>
<td>5.4 ± 0.9</td>
<td>0.15 ± 0.07</td>
<td>30.2 ± 3.1</td>
</tr>
<tr>
<td>HO + E2</td>
<td>10</td>
<td>74 ± 14</td>
<td>26.6 ± 3.5</td>
<td>0.23 ± 0.07</td>
<td>28.8 ± 4.4</td>
</tr>
<tr>
<td>HO + GH</td>
<td>9</td>
<td>1,156 ± 325</td>
<td>4.8 ± 1.2</td>
<td>0.19 ± 0.08</td>
<td>53.1 ± 6.2</td>
</tr>
<tr>
<td>HO + GH + E2</td>
<td>10</td>
<td>1,056 ± 214</td>
<td>27.7 ± 2.8</td>
<td>0.21 ± 0.11</td>
<td>46.1 ± 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SD; GH and E2 values same as described for Table 1, experiment 1. *Vs. Basal group; †vs. Con group; ‡vs. HO group; §vs. HO + E2 group; ¶vs. HO + GH group. All differences (P < 0.05) were tested by multiple comparison with ANOVA when data were transformed to logarithm.
Effects of E2 and GH on L4 Vertebral Growth

Whereas bone volume and dry weight of the L4 vertebra increased with age in the Con group in comparison with the respective baseline, bone volume and dry weight of the L4 vertebra of the HO animal remained the same (Table 1). Administration of either the low or high dose of E2 to the HO rats had no significant effect on lumbar volume, dry weight, or length, but the low and high doses of GH resulted in an increase in these parameters. Unlike with the body weights, combined administration of GH and E2 resulted in an additional increase in the dry weight and density of the L4 vertebra.

Effects of HO on Cortical and Cancellous Bone

Table 3 illustrates the static and dynamic difference of cortical and cancellous bone among the baseline, age-matched Con, and HO rats without hormonal treatment. Whereas the cortical area and cancellous bone volume increased with age in the Con rats, the tibial longitudinal growth rate and periosteal and cancellous bone formation rate of the Con rats decreased in comparison with the baseline. HO resulted in a cessation of the gain in cortical area and a decrease in the periosteal bone formation rate. Conversely, HO decreased cancellous bone volume without a significant decrease in the cancellous bone formation rate. These data were similar in both experiments. The eroded surface of cancellous bone did not differ between the Con and HO groups (data from experiment 2 are consistent with those of experiment 1 but are not shown). However, the number of osteoclasts was lower in the HO than in the Con groups (osteoclast number was not measured in experiment 1).

Effects of E2 and GH on Cortical Bone of Tibial Shaft

Administration of either the low or high dose of E2 to the HO rats resulted in no significant effect on the cancellous bone formation rate, eroded surface per bone surface area, and the number of osteoclasts found in the metaphysis (Fig. 4, C and F). The eroded surface and osteoclast number are both used as indicators of bone resorption. Administration of the low or high dose of GH increased the longitudinal growth rate to an undetectable range in the HO rats in both experiments, and E2 administration to the HO rats did not result in a significant change in this activity (Fig. 1, E and F). When administration of E2 was combined with GH, the periosteal bone formation rate was increased to levels observed in the Con rats, and the suppressive effect of E2 on bone formation rate was not significant in either experiment.

Effects of E2 and GH on Cancellous Bone

Administration of either E2 or GH alone to the HO rats at the different doses increased bone volume, but both levels were still lower than those observed in the respective Con groups (Fig. 2, A and B). Administration of the low or high dose of GH increased the longitudinal growth rate to levels observed in the Con group (Fig. 4 and Table 3). ANOVA shows that the effect of E2 and GH on increasing cancellous bone volume in the HO rats is additive (synergistic interaction was not significant, P > 0.05).

The longitudinal growth rate of the tibia of the HO rats was decreased to an undetectable range, and neither dose of E2 had a significant effect on this growth rate (Fig. 4, A and B). Administration of the low or high dose of GH increased the longitudinal growth rate to levels observed in the Con group (Fig. 4 and Table 3). When in combination with GH, E2 expressed a suppressive effect on the longitudinal growth rate. This effect was significant when the low doses of GH and E2 were used in combination. Administration of either the low or high dose of E2 alone to the HO rats did not affect the cancellous bone formation rate or the eroded surface, but it decreased the number of osteoclasts found in the metaphysis (Fig. 4, C and F). The eroded surface and osteoclast number are both used as indicators of bone resorption. Administration of the low or high dose of GH resulted in an increase in the cancellous bone formation rate, eroded surface per bone surface area, and the number of osteoclasts per tissue area. Also, when E2 and GH were combined in treatment, the GH-induced high bone formation rate was suppressed.

Table 3. Bone histomorphometry in proximal tibia and tibial shaft of Basal, Con, and HO rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Longitudinal Growth Rate, µm/day</th>
<th>Tissue Area, mm²</th>
<th>Cortical Area, %</th>
<th>Periosteal Bone Formation Rate, µm³·µm⁻²·day⁻¹</th>
<th>Cancellous Bone Volume %</th>
<th>Cancellous Bone Formation Rate, µm³·µm⁻²·day⁻¹</th>
<th>Cancellous Eroded Surface, %</th>
<th>Cancellous Osteoclast No. no./mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>8</td>
<td>149.1 ± 9.3</td>
<td>3.52 ± 0.11</td>
<td>81.4 ± 1.9</td>
<td>224.7 ± 62.0</td>
<td>16.9 ± 3.8</td>
<td>36.0 ± 7.6</td>
<td>3.1 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>8</td>
<td>42.6 ± 8.5a</td>
<td>4.16 ± 0.20a</td>
<td>83.9 ± 1.4a</td>
<td>91.2 ± 23.5a</td>
<td>25.1 ± 2.5a</td>
<td>19.6 ± 4.8a</td>
<td>2.9 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>HO</td>
<td>10</td>
<td>0.0 ± 0.0b,c</td>
<td>3.64 ± 0.32b</td>
<td>81.3 ± 1.7b</td>
<td>3.2 ± 3.9b</td>
<td>2.9 ± 1.9b</td>
<td>21.8 ± 10.1b</td>
<td>3.2 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>10</td>
<td>150.8 ± 8.8</td>
<td>3.46 ± 0.16</td>
<td>80.3 ± 3.3</td>
<td>266.8 ± 54.1</td>
<td>17.2 ± 3.0</td>
<td>37.0 ± 3.0</td>
<td>137 ± 9</td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>10</td>
<td>42.3 ± 4.6a</td>
<td>3.88 ± 0.19a</td>
<td>84.4 ± 1.6a</td>
<td>79.7 ± 10.9a</td>
<td>22.6 ± 4.0a</td>
<td>24.5 ± 4.8a</td>
<td>98 ± 6a</td>
<td></td>
</tr>
<tr>
<td>HO</td>
<td>9</td>
<td>0.0 ± 0.0b,c</td>
<td>3.54 ± 0.08b</td>
<td>82.4 ± 1.5b</td>
<td>0.9 ± 2.1b</td>
<td>5.9 ± 2.5b</td>
<td>21.0 ± 7.8</td>
<td>44 ± 13b</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 vs. Basal group; **P < 0.05 vs. Con group. All differences were tested by multiple comparison with ANOVA when data were transformed to logarithm.
by E2, and this suppression was significant with the high-dose treatment. Figure 4, C and D, shows that the bone formation rate did not differ between the HO+GH+E2 and HO+GH groups in experiment 1 but was lower in the HO+GH+E2 than in the HO+GH group in experiment 2. Administration of E2 also suppressed the GH-induced increase in bone-eroded surface in experiment 1 (Fig. 4E). The suppressive effect of E2 on the GH-induced increase in osteoclast number, as measured in experiment 2, was not significant (Fig. 4F). ANOVA analysis shows that the suppressive effect of E2 and the stimulatory effect of GH on trabecular eroded surface in experiment 1 and on metaphyseal osteoclast number in experiment 2 are both significant (P < 0.05). In both experiments, the stimulatory effect of GH on bone formation is significant. Again, the interaction between E2 and GH is not significant (P > 0.05).

**DISCUSSION**

Cancellous bone loss through HX in young rats is believed to be due not only to the imbalance and suppression of bone formation over bone resorption in the trabeculae but also to a cessation of new bone formation from the growth plate (4, 22, 31). Conversely, OV-induced cancellous bone loss is due to increased bone turnover rate with more resorption than formation (5, 29, 32). The present finding consists with our previous report that HX of OV rats abolished the OV-induced high bone turnover rate (5, 32), yet cancellous bone volume in HO rats still decreased, and the rate of this decrease was equal to or greater than that observed in the age-matched OV rats (5). Although the measured bone formation parameters and the eroded surface per trabecular bone surface in the HO rats did not differ from those of the age-matched control rats, the loss of cancellous bone in these rats indicates that bone resorption must be higher than formation.

In the current study, all animal groups were fed ad libitum. Although GH administration causes an increase in food intake (including calcium), which can contribute to the increase in body weight, muscle weight, and bone gain, this increase in food consumption cannot fully account for the increase in bone gain of the GH-treated groups. The specific effect of GH on local bone growth has been established by the unilateral injection of GH into one hindlimb of the HX rat (11,
respectively, in experiment 2. Interactions between GH and E₂ in both experiment 1 and experiment 2 were tested by 1-way ANOVA. Increase in cancellous bone of HO+E₂, HO+GH, and HO+GH+E₂ was 2.8, 3.5, and 7.4%, respectively, compared with HO in experiment 1. Increase was 2.2, 4.0, and 8.6%, respectively, in experiment 2. Interactions between GH and E₂ in both experiments were P < 0.05 by 2-way ANOVA.

Fig. 2. Cancellous bone volume of groups in experiment 1 (A) and experiment 2 (B). Values are means ± SD. GH and E₂ values same as described in legend of Fig. 1. *P < 0.05 vs. HO group; †P < 0.05 vs. HO+E₂ group; ‡P < 0.05 vs. HO+GH group. All differences were tested by 1-way ANOVA. Increase in cancellous bone of HO+E₂, HO+GH, and HO+GH+E₂ was 2.8, 3.5, and 7.4%, respectively, compared with HO in experiment 1. Increase was 2.2, 4.0, and 8.6%, respectively, in experiment 2. Interactions between GH and E₂ in both experiments were P < 0.05 by 2-way ANOVA.

20). The GH-treated limb showed a greater gain in bone mass than its counterpart. Furthermore, in a pair feeding study, the distinctive effect of GH on body size and total body bone mass has also been demonstrated (27). Wright et al. (27) controlled for body weight and still concluded that the GH-treated group experienced greater bone gain than the untreated group.

GH replacement in HX rats is known to activate tibial longitudinal growth and increases new bone formation from the growth plate (6, 11, 13, 16). It also increases cancellous bone formation and resorption activity (1, 6, 14). One of the aims of the current study was to determine whether GH administration to HO rats would unmask the OV-induced high bone turnover and bone loss or if it would exert an anabolic effect on the skeleton. In HO rats, GH administration restores body weight, serum levels of IGF-I, osteocalcin (a serum marker of bone formation activity) of the high-dose GH-treated rats reached the level of the age-matched control rats after 6 wk of treatment. The systemic growth (body and muscle weights and bone growth) was even greater in the GH-treated group than in the age-matched Con group. These findings indicate that the dose of GH used is equal to or above the physiological range and the inability to prevent cancellous bone loss was not due to an insufficient dose of GH. Whether or not the sensitivity of cancellous bone to exogenous GH differs from that of cortical bone is not known. Andreassen et al. (1) have reported that aged rats treated with rhGH at a dosage of 8.1 IU·kg⁻¹·day⁻¹ for 80 days experienced a significant anabolic effect of GH in their cortical bone without any significant changes in the quantity of their cancellous bone.

Wright et al. (27) used dwarf rats as a model of isolated GH deficiency and demonstrated that GH replacement completely restored cancellous bone volume to the level found in the pair-fed control rats. This discrepancy between the effect of GH on preventing cancellous bone loss induced by GH deficiency alone vs. the effect on cancellous bone loss induced by complete pituitary hormone deficiency (HX) suggests that pituitary hormones in addition to GH are critical to the conservation of cancellous bone. GH is an important participant in the regulation of skeletal mass, but it apparently does not act alone. An interaction among several hormones, including GH, is necessary to maintain normal bone growth and development. This phenomenon has also been observed in human studies. Investigators have attempted to use GH replacement therapy to prevent osteopenia in hypopituitary patients (19, 26). However, long-term treatment of hypopituitary patients with GH does not appreciably improve the osteopenia associated with this condition, despite an increased bone formation and bone resorption rate. Data obtained in these studies support the hypothesis that pituitary-related hormones in addition to GH are required in the prevention of HX-induced osteopenia.

Lack of hypophyseal hormones alone cannot explain the phenomenon that osteoblastic activity is suppressed on the periosteal surface of cortical bone while continuing on the trabecular surface of cancellous bone of the same tissue. Frost and Jee (8) propose that cortical bone is somewhat overloaded during growth, significantly higher than that of the HO group. Therefore, the increased bone formation and resorption rate of cancellous bone observed in the HO+GH rats is due to the anabolic effect of GH and not to the effect of OV. From these observations we further conclude that the bone-resorbing effect of OV is not GH dependent.

GH administered at 2.3 IU·kg⁻¹·day⁻¹ in experiment 1 partially restored both the cortical bone mass and cancellous bone volume of the tibia of the HO rats. The higher dosage of 13.5 IU·kg⁻¹·day⁻¹ used in the second experiment fully restored the growth and mass of cortical bone but was not able to fully restore the cancellous bone volume of the HO rats. The serum levels of IGF-I and osteocalcin (a serum marker of bone formation activity) of the high-dose GH-treated rats reached the level of the age-matched control rats after 6 wk of treatment. The systemic growth (body and muscle weights and bone growth) was even greater in the GH-treated group than in the age-matched Con group. These findings indicate that the dose of GH used is equal to or above the physiological range and the inability to prevent cancellous bone loss was not due to an insufficient dose of GH. Whether or not the sensitivity of cancellous bone to exogenous GH differs from that of cortical bone is not known. Andreassen et al. (1) have reported that aged rats treated with rhGH at a dosage of 8.1 IU·kg⁻¹·day⁻¹ for 80 days experienced a significant anabolic effect of GH in their cortical bone without any significant changes in the quantity of their cancellous bone.

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but the metaphysial spongiosa is somewhat underloaded for its usual mechanical usage. Therefore, cortical bone mass will continue to increase for adaptation to overload. In the metaphysial spongiosa, cancellous bone mass will continue to decrease for adaptation to underload. Hypophysectomized rats are not only deficient in hypophysial hormones, they also take in less food and are more physically inactive in their cages than the control or GH-treated groups. Their muscle growth has also stopped, and essentially their muscles have become weaker. HX in OV rats is a condition similar to partial immobilization and underloading. Thus mechanical disuse in HO could cause an increase in bone loss of the metaphysial spongiosa and a decrease in radial growth (periosteal bone formation rate) in cortical bone. Due to the mechanical disuse and hypophysial hormone-deficiency, GH’s effects on preventing cancellous bone loss in HO rats could be diminished. Therefore, the difference between cancellous and cortical bone in their mechanical loading properties could also contribute to the difference in response to exogenous GH administration.

It has also been demonstrated that the daily injection of 35 or 70 µg·kg⁻¹·wk⁻¹ E₂ is sufficient to prevent cancellous bone loss in OV rats (12, 28). In this study, however, either 100 µg·rat⁻¹·wk⁻¹ or 3,500 µg·kg⁻¹·wk⁻¹ of E₂ were administered to HO rats, and either dosage resulted in only a partial prevention of cancellous bone loss. The bone volume of both the low- and high-dose E₂ treatment groups was approximately one-third that of the control rats. Thus the antosteopenic effect of estrogen in OV rats is diminished by HX (33). The mechanism through which HX causes a reduction of the preventive effect of E₂ on cancellous bone loss in the HO rat is not known. Perhaps the factors that cause high bone turnover in OV and the factors that cause suppression by E₂ are pituitary hormone dependent.

Because the antosteopenic effect of E₂ on preventing cancellous bone loss in OV rats proved to be diminished through HX, we wanted to examine whether or not GH replacement alone could restore the antosteopenic effect of estrogen in HO rats or whether other hormones are required. When GH and E₂ were combined in treatment, the level of cancellous bone volume was higher than with either treatment alone. However, the combined intervention was still only able to prevent cancellous bone loss to the level equivalent to less than one-half of the intact control group. Therefore, we conclude that the antosteopenic effect of E₂ is not GH dependent. The result of ANOVA reveals that there is no synergistic interaction between GH and E₂ and supports the suggestion that the increase in cancellous bone is the result of an additive and independent effect of the two hormones. This conclusion is supported by histomorphometric results; GH increased cancellous
bone formation and bone resorption, whereas E₂ depressed the bone-eroded surface without significantly affecting the bone formation rate. Thus the increase in cancellous bone volume through the combined intervention is attributed to the suppressive effect of E₂ on bone resorption and the anabolic effect of GH on bone formation.

In conclusion, pituitary deficiency abolishes estrogen deficiency-induced high bone turnover and diminishes the cancellous bone conservation effect of estrogens. The causative mechanism is not known, but mechanical usage and certain endocrine and local growth factor regulation in addition to hypophysial hormones all may be involved (2). GH administration to HO rats results in an anabolic effect on systemic growth, skeletal bone formation, and bone growth but does not result in an unmasking of the OV-induced high bone turnover and bone loss. When GH and E₂ were combined in treatment, the additive effect observed in the prevention of cancellous bone loss is attributed to the suppression of E₂ on bone resorption and the anabolic effect of GH on bone formation.

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Address for reprint requests: J. K. Yeh, Metabolism Laboratory, Dept. of Medicine, Winthrop-Univ. Hospital, Mineola, NY 11501.

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