Drug-induced prevention of gastrectomy- and ovariectomy-induced osteopaenia in the young female rat

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(N Andersson and V V Surve contributed equally to this work)

Abstract

Both ovariectomy (Ovx) and gastrectomy (Gx) induce osteopaenia in rats and humans. While the effect of Ovx has been ascribed to oestrogen deficiency, the underlying mechanism behind Gx is poorly understood. Alendronate, oestrogen and parathyroid hormone (PTH) are known to prevent the osteopaenia induced by Ovx in rats. The purpose of the present study was to determine whether alendronate, oestrogen or PTH could also prevent Gx-evoked osteopaenia. Rats were Ovx-, Gx-, or were sham-operated (Sham) and were then treated with alendronate, oestrogen or PTH for eight weeks. At sacrifice, serum PTH was una...ed trabecular BMD in the metaphyseal area of the distal femur (Ovx, $-37 \pm 7\%$; Gx, $-32 \pm 7\%$). The cortical BMC of the femur was only slightly reduced. Alendronate prevented trabecular bone loss after both Ovx and Gx, while oestrogen and PTH prevented trabecular bone loss after Ovx but not after Gx.

In conclusion, the bisphosphonate alendronate prevented both Ovx- and Gx-induced trabecular bone loss. In contrast, PTH and oestrogen prevented Ovx-induced but not Gx-induced trabecular bone loss, suggesting that the mechanism behind the trabecular bone loss in Ovx rats differs from that in Gx rats. The results support the notion that the mechanism of action for the bone-sparing effect of these drugs differs. The ability of alendronate, and probably also other bisphosphonates, to prevent Gx-evoked osteopaenia in the rat might be of potential clinical interest when dealing with post-Gx osteopaenia in humans.

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Introduction

The ovariectomized (Ovx) rat is an established animal model for testing ways to prevent and treat postmenopausal osteoporosis (Wronska et al. 1985, Yamazaki & Yamaguchii 1989, Kall 1991, Thompson et al. 1995). Oestrogen deficiency results in increased bone turnover with excess bone resorption and significant loss of trabecular bone (Bagi et al. 1997). In cortical bone, an increased bone turnover rate results in endocortical resorption. However, this is compensated by accelerated periosteal growth and the net cortical bone loss is small or non-existent (Turner et al. 1987, Bain et al. 1993).

as being the major causes of Gx-evoked bone loss. Gx-evoked osteopaenia differs in some respects from that induced by Ovx (Surve et al. 2001a,b), suggesting that the underlying mechanisms are different. Both Ovx and Gx affect trabecular bone and at times also cortical bone. In addition, however, Gx induces a quite spectacular effect in the calvaria of the rat (Klinge et al. 1995, Lehto-Axtelius et al. 1998, Mühlbauer et al. 1998, Surve et al. 2001a,b).

Osteoporotic patients are currently treated with oestrogen or bisphosphonates. Oestrogen replacement is the most common treatment of post–menopausal osteoporosis (Cosman & Lindsay 1999). Bisphosphonates, such as alendronate, reduce fracture risk because they increase bone mass by reducing bone turnover (Ravn et al. 1996, Cummings et al. 1998, Fisher et al. 1999). Parathyroid hormone (PTH) is an anabolic hormone, which has attracted attention recently as a potential remedy for osteoporosis. Animal studies (Mitlak et al. 1996, Sato et al. 1997) as well as clinical studies (Rosen & Rackoff 2001) have shown that PTH, when administered intermittently, exerts an anabolic effect on bone. The anabolic action of PTH is most evident in trabecular bone while cortical bone mass is either unaffected or only moderately increased. However, if PTH is administered continuously it has a catabolic effect on bone (Dempster et al. 1993, 1995).

In the present study, we assessed the ability of alendronate, oestrogen, and PTH to prevent osteopaenia in the rat following Gx or Ovx. The purpose of the study was to explore whether the osteopaenia induced by Gx and Ovx responded similarly to preventive treatment with any of these drugs. Bones were removed and examined after 8 weeks of preventive drug treatment.

Materials and Methods

Animals

Ninety-six female, three-month-old Sprague-Dawley rats were obtained from M&B, Skensved, Denmark. The rats were allowed an acclimatisation period of seven days prior to the start of the study. The animals were maintained in groups of three in Macrolon cages, on a 12 h light/12 h darkness cycle with access to standard rat food pellets (1-0% calcium and 0-7% phosphorus, Lactamin, Vadstena, Sweden) and tap water available ad libitum. Body weights were determined at the beginning of the study and weekly thereafter. In order to prevent anaemia, Gx rats were injected with 400 µg/kg vitamin B12 (Betolvex, Dumex, Copenhagen, Denmark) and 20 mg/kg iron(Fe3+)sorbitol (Jectofer, AstraZeneca, Södertälje, Sweden) by the intramuscular route once every second week (beginning the first week after surgery). The experiments had been approved by the local animal welfare committee before the study started.

Experimental design

The rats were randomly assigned to one of three groups and then subjected to various types of surgery (Fig. 1): gastrectomy (Gx, n=32), ovariectomy (Ovx, n=32), and sham-operation (Sham, n=32). The three groups were subdivided into four treatment groups (8 rats in each). Drug treatment started the day after surgery. The drugs were administered on a daily basis for eight weeks. The treatment groups were: Sham+vehicle (sesame oil), subcutaneous injection (s.c. inj.), Sham+50 µg/kg/day
alendronate (corresponding to an effective daily dose of 10 µg compound phosphorus per kg; alendronate hydrochloride was synthesised at AstraZeneca R&D, Mölndal, Sweden), s.c. inj., Sham +10 µg/kg/day oestrogen (oestradiol-3-benzoate, Boehringer Ingelheim, Germany), s.c. inj., Sham +75 µg/kg/day PTH (human recombinant PTH(1–84), Allelix Biopharmaceuticals, Mississauga, ON, Canada), s.c. inj., Ovx+vehicle, Ovx+alendronate, Ovx+oestrogen, Ovx+PTH, Gx+vehicle, Gx+alendronate, Gx+oestrogen, and Gx+PTH. Citrate buffered saline (10 mM, pH 5·5) was used as vehicle for PTH. Saline was used as vehicle for alendronate. The doses chosen have been shown to be effective in preventing Ovx-induced osteopaenia (Wronski et al. 1988, Seedorff et al. 1991, Shen et al. 1995).

The rats were killed under isoflurane (Forene, Abbot, Abbot Park, IL, USA) anaesthesia by exsanguination (cardiac puncture). Blood was drawn and serum was stored at −20 °C until analysis (for gastrin and osteocalcin). Uteri were removed, and wet weights were recorded. Femurs, tibiae, and the fifth lumbar vertebrae (L5) were dissected out and cleaned of soft tissue. Each L5 was wrapped in saline-soaked gauze and stored at −20 °C until determination of bone density. The left femurs and tibiae were fixed in 10% formalin for four days at 4 °C, washed thoroughly thereafter and stored in 70% ethanol at 4 °C.

**Surgery**

Before surgery, the rats were anaesthetised with an intraperitoneal injection of ketamine hydrochloride (Ketalar, Parke-Davis, Barcelona, Spain) and xylazine (Rompun, Bayer, Leverkusen, Germany) at doses of 90 and 4·5 mg/kg respectively. Gx was performed by resection of the glandular part of the stomach and anastomosing the rumen to the duodenum (Lehto-Axtelius et al. 1998). Successful Gx was verified by the demonstration of reduced concentrations of gastrin in serum. Ovx and sham operations were performed using an abdominal approach (mid-line abdominal incision). Successful Ovx was verified by demonstrating reduced uterine weight (data not shown). There was no mortality in the Ovx or Sham groups. In the Gx group the mortality was about 10%.

**Measurement of serum gastrin, osteocalcin and PTH**

Determination of serum gastrin was performed as previously described (Stadil & Refeld 1973). Rat gastrin-17 was used as standard. The concentration of gastrin in serum was expressed as pmol equivalents of rat gastrin-17 per litre. Serum osteocalcin was measured using a commercially available enzyme-linked immunosorbent assay (Rat-MID Osteocalcin ELISA, Osteometer BioTech, Herlev, Denmark). The concentration of osteocalcin was expressed as ng equivalents of rat osteocalcin per ml. Serum PTH was measured using a commercially available immunoradiometric assay (Rat PTH IRMA, Immunotopics Inc., San Clemente, CA, USA). The concentration of PTH in serum was expressed as pg equivalents of rat PTH(1–34) per ml.

**Measurement of bone mineral density**

The volume of the vertebral body of L5 (with the two epiphysial ends, the posterior pedicle arch, and the spinous process removed, height ~4 mm) was determined by Archimedes’ principle. The vertebrae were subsequently incinerated at 600 °C for 12 h; the resulting ash was weighed after cooling in a desiccator. The ash weight divided by the volume gave the bone mineral density (BMD, mg/cm³).

**Peripheral quantitative computed tomography (pQCT)**

Computerised tomography was performed with the Stratec pQCT XCT Research M (Norland Corp., Fort Atkinson, WI, USA) specifically modified for use on small bone specimens (software version 5·4B; operating at 70 µm resolution) (Rosen et al. 1995, Windahl et al. 1999, Andersson et al. 2001). The machine was calibrated with a standard of hydroxyapatite embedded in acrylic plastic. pQCT was used to analyse cross-sections of the distal metaphysis and mid-diaphysis of the left femur. During the measurements the excised femurs were placed in a test tube filled with 70% ethanol. Metaphyseal scans were performed to measure the trabecular BMD (mg/cm³). The scout view of the pQCT system was used to locate the growth plate. The metaphyseal scan line was positioned 2·5 mm proximal to the distal growth plate. This area of the femoral metaphysis is rich in trabecular bone. The trabecular bone region was defined as the inner 45% of the scanned bone area. Cortical BMD (mg/mm³) was determined in a 0·1 mm cross-section of the mid-diaphysis. This scan was also used to determine geometrical parameters such as the cortical thickness (mm), the cortical cross sectional area (mm²), the periosteal circumference (mm), the endocortical circumference (mm) and the cortical bone mineral content (BMC, mg/mm). The inter-assay coefficient of variation for the pQCT measurements was less than 2%. The lengths of the femur and tibiae were also determined at the same time.

**Statistical analysis**

The results are presented as means ± s.e.m. The effects of drugs or the effects of surgery were analysed by one-way analysis of variance (ANOVA); P<0·05 was considered statistically significant. Whenever statistically significant differences were found between the various experimental
groups by ANOVA, individual differences were assessed by post hoc analysis (Dunnett’s test).

**Results**

**Body weights and skeletal dimensions of bones**

Ovx rats gained weight more rapidly than Gx or sham-operated rats (Table 1). Oestrogen treatment reduced the body weight gain of Ovx rats but not that of Gx and Sham rats. Neither PTH nor alendronate treatment affected the final body weights in any of the groups.

The lengths of the tibia and femur were not adversely affected by surgery. The length of the tibia was reduced by alendronate treatment in Sham and Ovx rats and was slightly reduced by oestrogen in Ovx rats. No effects on the lengths of the tibia or femur were noted in the other groups (Table 2).

**Table 1** Effects of the three drugs on the body weight gain. Results are means ± S.E.M. (*n* = 7–8 rats per group)

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (g)</th>
<th>Alendronate (g)</th>
<th>Oestrogen (g)</th>
<th>PTH (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starting</td>
<td>225 ± 5</td>
<td>229 ± 5</td>
<td>226 ± 4</td>
<td>226 ± 4</td>
</tr>
<tr>
<td>Final</td>
<td>294 ± 6</td>
<td>288 ± 10</td>
<td>296 ± 7</td>
<td>291 ± 8</td>
</tr>
<tr>
<td>Change in weight (%)</td>
<td>31 ± 2</td>
<td>26 ± 2</td>
<td>31 ± 4</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>Ovx</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starting</td>
<td>213 ± 5</td>
<td>213 ± 5</td>
<td>209 ± 6</td>
<td>211 ± 6</td>
</tr>
<tr>
<td>Final</td>
<td>347 ± 12 #</td>
<td>336 ± 16 #</td>
<td>274 ± 6 *</td>
<td>364 ± 16 #</td>
</tr>
<tr>
<td>Change in weight (%)</td>
<td>63 ± 2 #</td>
<td>58 ± 4 #</td>
<td>31 ± 4 *</td>
<td>73 ± 5 #</td>
</tr>
<tr>
<td>Gx</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starting</td>
<td>234 ± 2</td>
<td>231 ± 5</td>
<td>231 ± 3</td>
<td>231 ± 4</td>
</tr>
<tr>
<td>Final</td>
<td>287 ± 7</td>
<td>276 ± 15</td>
<td>273 ± 8</td>
<td>288 ± 5</td>
</tr>
<tr>
<td>Change in weight (%)</td>
<td>23 ± 3</td>
<td>19 ± 1</td>
<td>18 ± 4</td>
<td>25 ± 2</td>
</tr>
</tbody>
</table>

Statistical significance was assessed for the differences between the sham-operated rats and each of the two surgery groups or between the vehicle-treated groups and each of the three drug-treated groups.  
*P < 0.05 versus sham; **P < 0.05 versus vehicle (ANOVA).

**Table 2** Effects of the three drugs on length (mm) of tibia and femur in Gx, Ovx and Sham rats. Results are means ± S.E.M. (*n* = 7–8 rats per group)

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Alendronate</th>
<th>Oestrogen</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibia</td>
<td>39·7 ± 0·3</td>
<td>38·3 ± 0·4 *</td>
<td>39·5 ± 0·4</td>
<td>39·1 ± 0·3</td>
</tr>
<tr>
<td>Femur</td>
<td>35·0 ± 0·2</td>
<td>34·5 ± 0·2</td>
<td>34·7 ± 0·2</td>
<td>34·9 ± 0·1</td>
</tr>
<tr>
<td>Ovx</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibia</td>
<td>39·9 ± 0·3</td>
<td>38·4 ± 0·3 *</td>
<td>38·8 ± 0·2 *</td>
<td>39·7 ± 0·4</td>
</tr>
<tr>
<td>Femur</td>
<td>35·6 ± 0·4</td>
<td>34·8 ± 0·4</td>
<td>34·4 ± 0·3</td>
<td>35·7 ± 0·5</td>
</tr>
<tr>
<td>Gx</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibia</td>
<td>39·1 ± 0·2</td>
<td>38·2 ± 0·4</td>
<td>38·8 ± 0·4</td>
<td>39·7 ± 0·2</td>
</tr>
<tr>
<td>Femur</td>
<td>35·4 ± 0·3</td>
<td>34·7 ± 0·2</td>
<td>34·7 ± 0·3</td>
<td>35·5 ± 0·3</td>
</tr>
</tbody>
</table>

Statistical significance was assessed for the differences between the vehicle-treated groups and each of the three drug-treated groups.  
*P < 0.05 (ANOVA).

BMD

The BMD (ash weight/volume) of L5 was determined. Ovx and Gx reduced the BMD by 15 ± 4% (*P* < 0.05) and 22 ± 3% (*P* < 0.05) respectively (Fig. 2A). Compared with vehicle treatment, alendronate raised the BMD of L5 in both Ovx (34 ± 5%, *P* < 0.05) and Gx (21 ± 3%, *P* < 0.05) rats, but not in sham-operated rats. No effects of oestrogen were seen in any of the groups. PTH significantly increased the BMD in the L5 of Ovx and sham-operated, but not of Gx rats (*P* < 0.05).

L5 contains both trabecular and cortical bone. In order to determine whether the main effects of surgery and of the preventive drug treatments were on trabecular or cortical bone, the trabecular BMD in the metaphysis and the cortical bone in the mid-diaphysis of the femur were measured using pQCT. Eight weeks after surgery both Ovx and Gx rats had a significantly reduced trabecular BMD in the distal femur (Ovx, −37 ± 7%, *P* < 0.05;
Gx, –49 ± 7%, \( P<0.05 \) (Fig. 2B). Alendronate modified the loss of trabecular BMD in all three surgery groups, while PTH and oestrogen prevented the trabecular osteopaenia induced by Ovx but not that induced by Gx.

In contrast to trabecular bone, cortical bone responded poorly to both Gx and Ovx as well as to the preventive drug treatment (Table 3). However, the cortical thickness was reduced – albeit moderately – in all Gx groups compared with Sham, while Ovx seemed to be without effect on cortical bone. Oestrogen-treated Ovx rats showed a reduced periosteal and endocortical circumference compared with oestrogen-treated sham-operated rats.

**Serum gastrin, osteocalcin and PTH**

The serum concentration of gastrin was low in all Gx groups at sacrifice while it was normal in all other groups (Gx, 23 ± 1 pmol/l; Ovx, 74 ± 4 pmol/l; Sham, 72 ± 4 pmol/l \( P<0.05 \) ANOVA)). The serum concentration of osteocalcin, a marker of bone formation/turnover, was increased in Ovx rats but not in Gx rats (Fig. 3). Alendronate and oestrogen treatment suppressed the serum osteocalcin concentration in the Ovx rats to Sham levels. The serum concentration of PTH at sacrifice was unchanged in all groups (Gx, 75 ± 13 pg/ml; Ovx, 64 ± 8 pg/ml; Sham, 58 ± 11 pg/ml (not significant, ANOVA)).

**Discussion**

Ovx and Gx cause osteopaenia in both rats and humans. Although both Ovx-evoked and Gx-evoked osteopaenia reflect decreased formation and accelerated degradation and turnover of bone, the pathogenetic mechanisms behind the bone loss seem to differ (Surve et al. 2001a,b). Gx reduces the percentage of bone in the calvaria and lowers the trabecular and cortical bone content. Ovx affects trabecular bone (and only affects cortical bone to a minor extent) but not calvarial bone (Surve et al. 2001b). Today, bisphosphonates and oestrogen replacement are used clinically to treat osteoporosis and soon PTH will be available as an additional treatment option. In this study we examine whether alendronate, oestrogen or PTH will
prevent the osteopenia induced by Ovx or Gx in rats. This is the first study in which these drugs have been used in an attempt to prevent Gx-evoked osteopenia. Our results confirm that alendronate and oestrogen are able to prevent the trabecular bone loss induced by Ovx (Wronska et al. 1988, Seedorf et al. 1991). In addition, we show that PTH at a dose previously shown to be effective in the treatment of an established Ovx-evoked osteopenia (Shen et al. 1995) also prevented Ovx-induced bone loss. In contrast, Gx-induced bone loss could be prevented by alendronate but not by PTH or oestrogen. Thus, the evidence suggests that the mechanism behind the Ovx-evoked osteopenia in rats differs from that induced by Gx.

Both Ovx and Gx were found to induce a significant decrease in the BMD of L5. The vertebral body is rich in trabecular bone but contains also significant amounts of cortical bone. In order to determine whether the main effects of Ovx and Gx were on trabecular or cortical bone we measured the trabecular BMD in the metaphysis of the distal femur using pQCT. Cortical bone was measured in the mid-diaphysis of the same femur. The trabecular BMD was reduced following Ovx and Gx while the cortical BMC was unaffected, indicating that Ovx as well as Gx primarily affect trabecular bone. However, in the Gx group a reduced cortical thickness was observed. This was not the case in the Ovx group. These results are in accordance with those that have been reported previously (Wronska et al. 1985, 1988, Surve et al. 2001a,b).

Ovx was associated with an increased body weight, which was prevented by treatment with oestrogen, confirming the results of several earlier studies (Yamazaki & Yamaguchi 1989, Kalu 1991, Kalu et al. 1991). The effects of oestrogen on body weight and uterine weights (data not shown) demonstrate that a functional dose of oestrogen was given. This dose of oestrogen reversed the Ovx-induced trabecular bone loss but did not prevent the Gx-induced osteopenia. This finding indicates that the trabecular bone loss following Gx is initiated and driven by a mechanism other than that causing Ovx-evoked osteopenia.

Alendronate prevented both Ovx-induced and Gx-induced trabecular bone loss while no effect was seen on cortical bone. The exact mechanism of action for alendronate, as well as other bisphosphonates, is not well understood. The antiresorptive effect of bisphosphonates impairs the final steps in bone remodelling, directly interfering with the activated osteoclasts. Once internalised within the osteoclasts, it is thought to interfere with the mevalonate-to-cholesterol pathway (Bergstrom et al. 2000). The reduced length of the tibia observed in this study has been described previously with a similar dose of alendronate (Seedorf et al. 2002). The fact that alendronate prevented the Gx-evoked trabecular bone loss supports the view that calcium malabsorption is not responsible for the bone loss (Lehto-Axelius et al. 2002, Surve et al. 2002).

Intermittent injections of PTH result in an anabolic effect on bone in both Sham and Ovx rats (Dempster et al. 1993, 1995). Although most studies of the in vivo effects of PTH have been performed on Ovx rats with established osteopenia, PTH has been given at different dosages to

Table 3 Effects of drug treatment on cortical bone parameters measured in the mid-diaphysis of the femur by pQCT. Results are means ± S.E.M. (n = 7–8 rats per group)

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Alendronate</th>
<th>Oestrogen</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortical BMC (mg/mm)</td>
<td>7.53 ± 0.15</td>
<td>7.55 ± 0.20</td>
<td>7.53 ± 0.15</td>
<td>7.83 ± 0.20</td>
</tr>
<tr>
<td>Cortical BMD (mg/mm³)</td>
<td>1.356 ± 0.004</td>
<td>1.364 ± 0.003</td>
<td>1.348 ± 0.002</td>
<td>1.358 ± 0.007</td>
</tr>
<tr>
<td>Cortical area (mm²)</td>
<td>5.56 ± 0.12</td>
<td>5.54 ± 0.15</td>
<td>5.59 ± 0.11</td>
<td>5.77 ± 0.14</td>
</tr>
<tr>
<td>Cortical thickness (mm)</td>
<td>0.62 ± 0.01</td>
<td>0.62 ± 0.01</td>
<td>0.60 ± 0.01</td>
<td>0.65 ± 0.01</td>
</tr>
<tr>
<td>Periosteal circumference (mm)</td>
<td>10.96 ± 0.13</td>
<td>10.85 ± 0.18</td>
<td>11.19 ± 0.11</td>
<td>10.87 ± 0.09</td>
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<tr>
<td>Endocortical circumference (mm)</td>
<td>7.10 ± 0.11</td>
<td>6.94 ± 0.20</td>
<td>7.41 ± 0.10</td>
<td>6.77 ± 0.09</td>
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<tr>
<td>Ovx rats</td>
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<tr>
<td>Cortical BMC (mg/mm)</td>
<td>7.41 ± 0.13</td>
<td>7.87 ± 0.33</td>
<td>7.11 ± 0.16</td>
<td>7.81 ± 0.27</td>
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<td>Cortical BMD (mg/mm³)</td>
<td>1.353 ± 0.004</td>
<td>1.351 ± 0.006</td>
<td>1.355 ± 0.004</td>
<td>1.332 ± 0.005*#</td>
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<td>Cortical area (mm²)</td>
<td>5.48 ± 0.09</td>
<td>5.83 ± 0.26</td>
<td>5.25 ± 0.11</td>
<td>5.86 ± 0.19</td>
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<td>Cortical thickness (mm)</td>
<td>0.60 ± 0.01</td>
<td>0.62 ± 0.01</td>
<td>0.60 ± 0.01</td>
<td>0.65 ± 0.01*</td>
</tr>
<tr>
<td>Periosteal circumference (mm)</td>
<td>10.99 ± 0.14</td>
<td>11.33 ± 0.31</td>
<td>10.62 ± 0.13#</td>
<td>11.08 ± 0.21</td>
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<tr>
<td>Endocortical circumference (mm)</td>
<td>7.20 ± 0.18</td>
<td>7.45 ± 0.26</td>
<td>6.85 ± 0.14#</td>
<td>7.02 ± 0.20</td>
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<td>Gx rats</td>
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<tr>
<td>Cortical BMC (mg/mm)</td>
<td>7.24 ± 0.10</td>
<td>7.23 ± 0.07</td>
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<td>7.18 ± 0.14</td>
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<td>Cortical BMD (mg/mm³)</td>
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<td>1.340 ± 0.005</td>
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<td>5.38 ± 0.06</td>
<td>5.04 ± 0.09#</td>
<td>5.36 ± 0.12</td>
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<tr>
<td>Cortical thickness (mm)</td>
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<td>Periosteal circumference (mm)</td>
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<td>11.17 ± 0.10</td>
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<td>11.05 ± 0.15</td>
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<td>Endocortical circumference (mm)</td>
<td>7.34 ± 0.20</td>
<td>7.56 ± 0.12</td>
<td>7.13 ± 0.23</td>
<td>7.39 ± 0.16#</td>
</tr>
</tbody>
</table>

Statistical significance was assessed for the differences between the sham-operated rats and each of the two surgery groups (P<0.05) or between the vehicle-treated groups and each of the three drug-treated groups (P<0.05) (ANOVA).
intact or sham-operated rats (4–7 weeks old) for relatively short periods of time (6–24 days) (Hock et al. 1988, Gunness-Hey & Hock 1989, Hock & Gera 1992, Uzawa et al. 1995, Frolik et al. 1999). These studies have shown an increase in the trabecular bone volume (measured by histomorphometry) of 15–100%. In a fairly recent study by Frolik et al. (1999), 4-week-old male rats were treated with PTH(1–34) (equivalent of 100 µg/kg/day PTH(1–84)) for 4 weeks. As a result, the BMD in the metaphysis of the proximal tibia was increased by 52%. In the present study we treated 3-month-old female rats with PTH for 8 weeks. The increase in trabecular BMD in the metaphysis of the distal femur following PTH treatment of sham-operated rats was not statistically significant. Interestingly, PTH prevented the Ovx-evoked but not the Gx-evoked bone loss. The results of the present study confirm earlier observations that intermittent PTH treatment prevents Ovx-induced trabecular bone loss (Dempster et al. 1993, 1995). However, as seen for oestrogen, the anabolic/protective effect of PTH seen in the Ovx rats was not apparent in the Gx rats, supporting the notion that the mechanism behind Gx-induced osteopaenia differs from that behind Ovx-induced osteopaenia. 

Biochemical markers of bone turnover, such as serum osteocalcin, are widely used clinically to monitor the metabolic activity of the bone (Woigt & Seibel 2001). Our study showed elevated serum osteocalcin concentrations 8 weeks after Ovx, and this elevation was suppressed by alendronate or oestrogen. These findings are consistent with previous results (Shen et al. 1995, Frolik et al. 1996). Although previous studies (Shen et al. 1993, 1995, Meng et al. 1996) have reported increased osteocalcin levels following PTH treatment in Ovx rats, this study failed to show statistical significance. However, PTH prevented the Ovx-induced bone loss. On the other hand, PTH raised the serum osteocalcin concentration in the Gx rats, suggesting an increased rate of bone formation (Woigt & Seibel 2001). However, this was not associated with an altered bone mass. Again, the mechanism behind Gx-induced osteopaenia seems to differ from that behind Ovx-induced osteopaenia.

There is a frequently voiced suspicion that nutritional deficiencies cause or at least contribute to the Gx-evoked osteopaenia. This suspicion is triggered by earlier observations that the normal body weight gain is slowed down by Gx (see e.g. Klinge et al. 1995). However, it is unlikely that either the Gx-induced or the Ovx-induced osteopaenia is a result of generalised nutritional deficiency, as in this study neither the Gx group nor the Ovx group were growth retarded (see also Surve et al. 2001a,b). A specific deficiency of calcium is also unlikely because previous reports have described a lack of effect of calcium supplementation (Persson et al. 1993, Klinge et al. 1995, Lehto-Axtelius et al. 1998, 2002) and because the serum PTH concentration was unaffected by surgery (this study). Vitamin D deficiency is known to cause impaired absorption and osteomalacia (Imawari et al. 1980). This is unlikely to be the cause of the Gx-evoked osteopaenia since the serum concentration of 1,25-dihydroxyvitamin D$_3$ is raised following Gx (Axelson et al. 1991, Rümenapf et al. 1998). Elevated levels of 1,25-dihydroxyvitamin D$_3$ could be caused by secondary hyperparathyroidism. However, there was no evidence of hyperparathyroidism in this study confirming several observations made earlier (Mühlbauer et al. 1998, Rümenapf et al. 1998, Wojtyczka et al. 1998). It may be argued that removal of the acid-producing part of the stomach may cause acidosis with consequences for calcium/bone metabolism. However, complete inhibition of gastric acid secretion by proton pump inhibitors does not cause osteopaenia (Persson et al. 1993). Hence, the results support the view that the stomach is important for bone metabolism through mechanisms that are unrelated to dietary deficiencies or lack of gastric acid.

In conclusion, PTH and oestrogen prevented Ovx-induced but not Gx-induced trabecular bone loss, suggesting that the mechanism behind the trabecular bone loss in Gx rats differs from that in Ovx rats. In contrast, the bisphosphonate alendronate prevented both Gx- and Ovx-induced trabecular bone loss. The preventive effect of alendronate but not of oestrogen or PTH on Gx-induced bone loss supports the notion that the mechanism of action for the bone-sparing effect of these substances differs. The ability of alendronate, and probably also other bisphosphonates, to prevent Gx-evoked osteopaenia in the rat, might be of potential clinical interest when dealing with post-Gx osteopaenia in humans.

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References

during parathyroid hormone treatment in ovariectomized mice.
Journal of Endocrinology 170 529–537.

Importance of the stomach in maintaining calcium homeostasis in the rat.
Gut 32 1298–1302.

Bagi CM, Ammann R, Rizzoli R & Miller SC 1997 Effect of estrogen
deficiency on cancellous and cortical bone structure and strength of

Bain SD, Jensen E, Celino DL, Bailey MC, Lantry MM & Edwards
MW 1993 High-dose gestagens modulate bone resorption and
formation and enhance estrogen-induced endosteal bone formation
in the ovariectomized mouse. Journal of Bone and Mineral Research 8
219–230.

Bergstrom JD, Bostedor RG, Masarachia PJ, Reszka AA & Rodan GA
2000 Alendronate is a specific, nanomolar inhibitor of farnesyl
diphosphate synthase. Archives of Biochemistry and Biophysics 373
231–241.

Cosman F & Lindsay R 1999 Selective estrogen receptor modulators:
clinical spectrum. Endocrine Reviews 20 418–434.

Cummings SR, Black DM, Thompson DE, Applegate WB,
Barrett-Connor E, Mulsiner TA, Palermo L, Prinzes R, Rubin
Effect of alendronate on risk of fracture in women with low bone
density but without vertebral fractures: results from the Fracture
Intervention Trial. Journal of the American Medical Association 280
2077–2082.

Dempster DW, Cosman F, Parisen M, Shen V & Lindsay R 1993
Anabolic actions of parathyroid hormone on bone. Endocrine Reviews
14 690–709.

Dempster DW, Cosman F, Parisen M, Shen V & Lindsay R 1995
Anabolic actions of parathyroid hormone on bone: update 1995. In
Endocrine Reviews Monograph, vol. 4, pp 247–250. Ed. A

Fisher JE, Rogers MJ, Halasy JM, Luckman SP, Hughes DE,
Masarachia PJ, Wesołowski G, Russel RG, Rodan GA & Reszka
AA 1999 Alendronate mechanism of action: geranylgeranol,
an intermediate in the mevalone pathway, prevents inhibition of
osteoclast formation, bone resorption, and kinase activation in vitro.
PNAS 96 133–138.

Frelik CA, Bryant HU, Black EC, Magee DE & Chandrasekhar S
1996 Time–dependent changes in biochemical bone markers and
serum cholesterol in ovariectomized rats: effects of raloxifene
HCl, tamoxifen, estrogen, and alendronate. Bone 18 621–627.

Frelik CA, Cain RL, Sato M, Harvey AK, Chandrasekhar S, Black
EC, Tashjian AH Jr & Hock JM 1999 Comparison of recombinant
human PTH(1–34) (LY333334) with a C-terminally substituted
activity and in vivo pharmacological effects in rats. Journal of Bone

Gepp H, Koch M, Schwille PO, Erben RG, Rümengap G, Schmiedl
A & Fries W 2000 Vagus-sparing gastric fundectomy in the rat:
development of osteopaenia, relationship to urinary phosphate and
net acid secretion, serum gastrin and vitamin D. Research in
Experimental Medicine 200 1–16.

Gunness-Hey M & Hock JM 1989 Loss of the anabolic effect
of parathyroid hormone on bone after discontinuation of hormone in

Heiskanen JT, Kröger H, Pääkkönen MT, Parviainen MT,
Lambert-Allardt C & Alhava E 2001 Bone mineral density after

Hock J M & Gera I 1992 Effects of continuous and intermittent
administration and inhibition of resorption on the anabolic response
of bone to parathyroid hormone. Journal of Bone and Mineral Research
7 65–72.

Hock JM, Gera I, Fonseca J & Raisz LG 1988 Human parathyroid
hormone(1–34) increases bone mass in ovariectomized and

Imawari M, Kozawa K, Akanuma Y, Koizumi S, Itakura H & Kosaka
K 1980 Serum 25-hydroxy-vitamin D and vitamin D-binding
protein levels and mineral metabolism after partial and total

Kalu DN 1991 The ovariectomized rat model of postmenopausal bone

Hollis BW 1991 A comparative study of the actions of tamoxifen,
estrogen and progesterone in the ovariectomized rat. Bone and
Mineral 15 109–123.

Klinge B, Lehto-Axelius D, Åkerman M & Håkanson R 1995
Structure of calvaria after gastrectomy. An experimental study in

Lehto-Axelius D, Stenström M & Johnell O 1998 Osteopaenia after
gastrectomy, fundectomy or antrectomy: an experimental study in
the rat. Regulatory Peptides 78 41–50.

Lehto-Axelius D, Surve VV, Johnell O & Håkanson R 2002 Effects
of estrogen deficiency and calcium supplementation on
gastrectomy-induced osteopaenia in the young male rat.

Melton III LJ, Crouson CS, Khoda S & O’Fallon WM 1999 Fracture
risk after surgery for peptic ulcer disease: a population-based cohort

Meng XW, Liang XG, Birchman R, Wu DD, Dempster DW,
Lindsay R & Shen V 1996 Temporal expression of the anabolic
action of PTH in cancellous bone of ovariectomized rats. Journal of

Mittal BH, Burdette Millerer P, Schoenfeld D & Neer RM 1996
Sequential effects of chronic human PTH (1–84) treatment of
estrogen-deficiency osteopaenia in the rat. Journal of Bone and

Mulhauer RC, Schenk RK, Chen D, Lehto-Axelius D & Håkanson
Calcified Tissue International 62 323–326.

Persson P, Gagnemo-Persson R, Chen D, Axelson J, Nylander AG,
Johnell O & Håkanson R 1993 Gastrectomy causes bone loss in the
rat: is lack of gastric acid responsible? Scandinavian Journal of
Gastroenterology 28 301–306.

Ravn P, Clemmensen B, Riis BJ & Christiansen C 1996 The effect on
bone mass and bone markers of different doses of ibandronate – a
new bisphosphonate for prevention and treatment of
postmenopausal osteoporosis – a 1-year, randomized, double-blind,

Rosen CJ & Rackoff PJ 2001 Emerging anabolic treatments for
osteoporosis. Rheumatic Disease Clinics of North America 27

Rosen HN, Tollin S, Balena R, Middlebrooks VL, Beamer WG,
Donohue LR, Rosen C, Turner A, Holick M & Greenspan SL
1995 Differentiating between orchidectomized rats and controls
using measurements of trabecular bone density: a comparison among
DXA, histomorphometry, and peripheral quantitative computerized

Rümengap G, Schwille PO, Erben RG, Schreiber M, Fries W,
Schmiedl A & Hohenberger W 1997 Osteopaenia following total
gastrectomy in the rat – state of mineral metabolism and bone
histomorphometry. European Surgical Research 29 209–221.

Rümengap G, Schwille PO, Erben RG, Bergé B, Fries W, Schmiedl
A, Koroma S & Hohenberger W 1998 Gastric fundectomy in the
rat: effects on mineral bone metabolism, with emphasis on the
gastrin(1–16)–calcinin–parathyroid hormone–vitamin D axis. Calcified
Tissue International 63 433–441.

Sato M, Zen GQ & Turner CH 1997 Biosynthetic human
parathyroid hormone (1–34) effects on bone quality in aged

Seedor JG, Quartuccio HA & Thompson DD 1991 The
bisphosphonate alendronate (MK-217) inhibits bone loss due to
339–346.

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www.endocrinology.org

Downloaded from Bioscientifica.com at 12/02/2018 08:48:32PM via free access
Shen V, Dempster DW, Birchman R, Xu R & Lindsay R 1993 Loss of cancellous bone mass and connectivity in ovariectomized rats can be restored by combined treatment with parathyroid hormone and estradiol. *Journal of Clinical Investigation* 91 2479–2487.


Surve VV, Höglund P & Häkanson R 2002 Gastrectomized rats respond with exaggerated hypercalcemia to oral and intravenous calcium loads because of impaired ability of bone to take up Ca²⁺. *Scandinavian Journal of Gastroenterology* 37 523–530.

Thompson DD, Simmons HA, Pirie CM & Ke HZ 1995 FDA Guidelines and animal models for osteoporosis. *Bone* 17 125S–133S.


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