LUND UNIVERSITY

Pharmacological treatment of osteopenia induced by gastrectomy or ovariectomy in young female rats

Andersson, Niklas U.; Surve, Vikas; Lehto-Axtelius, Daisy; Andersson, Kjell; Ryberg, Birgitta; Ohlsson, Claes; Håkanson, Rolf

Published in:
Acta Orthopaedica Scandinavica

DOI:
10.1080/00016470412331294465

2004

Link to publication

Citation for published version (APA):
Andersson, N. U., Surve, V., Lehto-Axtelius, D., Andersson, K., Ryberg, B., Ohlsson, C., & Håkanson, R. (2004). Pharmacological treatment of osteopenia induced by gastrectomy or ovariectomy in young female rats. *Acta Orthopaedica Scandinavica*, *75*(2), 201-209. https://doi.org/10.1080/00016470412331294465

Total number of authors:
7

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Pharmacological treatment of osteopenia induced by gastrectomy or ovariectomy in young female rats

Niklas Andersson*[1, 2], Vikas V Surve*[1], Daisy Lehto-Axtelius[3], Kjell Andersson[4], Birgitta Ryberg[4], Claes Ohlsson[2] and Rolf Håkanson[1]

[1]Department of Pharmacology, Institute of Physiological Sciences, Lund University, BMC F-13, SE-221 84 Lund, [2]Division of Endocrinology, Department of Internal Medicine, Sahlgrenska Academy at Gothenburg University, Bruna Stråket 16, SE-413 45 Gothenburg, [3]Centre for Oral Health Sciences, Department of Radiology, Malmö University, Malmö, [4]AstraZeneca R&D, Mölndal, Sweden
Correspondence RH: rolf.hakanson@farm.lu.se
Submitted 02-11-29. Accepted 03-07-08
* Equal first authors

Background Both gastrectomy (GX) and ovariectomy (OVX) induce osteopenia in man and experimental animals. The present study addresses the question — can alendronate, estrogen or parathyroid hormone (PTH) be used to treat established GX- or OVX -evoked osteopenia?

Methods Rats were GX-, OVX- or SHAM-operated 8 weeks before starting the treatment with drugs. Each group was then treated for 8 weeks with 50 μg/kg/day alendronate, 10 μg/kg/day estrogen or 75 μg/kg/day PTH(1-84); n = 8 rats/group. Peripheral Quantitative Computed Tomography (pQCT) was used to measure trabecular bone mineral density (BMD) and various cortical bone parameters.

Results At killing, 16 weeks after surgery, GX and OVX rats had a greatly reduced trabecular BMD in the metaphysis of the distal femur (GX –44% and OVX –55%). Alendronate increased the trabecular BMD by 44% in GX rats and by 64% in OVX rats, while PTH increased it by 51% and 115%, respectively. However, estrogen increased the trabecular BMD in GX rats (35%), but not in OVX rats (15%, not significant). Cortical bone parameters were adversely (but moderately) affected by GX, but not by OVX or by treatment with the three drugs.

Interpretation Alendronate, estrogen and PTH restored the trabecular bone loss in rats with an established GX-evoked osteopenia. In contrast, alendronate and PTH, but not estrogen, restored the trabecular bone loss after OVX. Hence, the mechanism underlying GX-evoked bone loss differs from that underlying OVX-evoked bone loss. The ability of alendronate, estrogen and PTH to reverse the GX-evoked osteopenia in the rat may be of clinical interest when dealing with bone loss in humans after GX.

In experimental animals, osteopenia can be induced by various methods, including dietary calcium restriction, gastrectomy (GX), immobilization, and ovariectomy (OVX). The OVX rat is a commonly used model for testing ways to prevent and treat postmenopausal osteoporosis. Estrogen deficiency increases bone turnover with excess bone resorption, and leads to a net loss of trabecular bone (Bagi et al. 1997). In cortical bone, an increase in the rate of bone turnover results in endocortical resorption. However, this is compensated for by accelerated periosteal growth and the net cortical bone loss is small or nonexistent (Turner et al. 1987).

Gastrectomy (GX)—i.e., resection of the glandular part of the stomach—leads to osteopenia in the rat (Klinge et al. 1995, Lehto-Axtelius et al. 1998). In humans, GX causes osteopenia; the underlying mechanisms are poorly understood (Tovey et al. 1991, Heiskanen et al. 2001). The GX-evoked osteopenia in rats differs from that induced by OVX (Lehto-Axtelius et al. 1998, Surve et al. 2001a, b), suggesting that the underlying mechanisms are different. GX and OVX affect mainly trabecular bone. Moreover, GX (but not OVX) induces a spectacular loss of bone in the calvaria.

Copyright © Taylor & Francis *2004. ISSN 0001–6470. Printed in Sweden – all rights reserved.*

Antiresorptive agents, such as bisphosphonates and estrogen, are used clinically to treat postmenopausal osteoporosis. Bisphosphonates, such as alendronate, lower the risk of fracture because they increase bone mass by reducing bone turnover (Cummings et al. 1998). Parathyroid hormone (PTH) has recently attracted attention as a potential remedy for osteoporosis. Studies have shown that PTH, when given intermittently, has an anabolic effect on bone. This effect was evident in trabecular bone while cortical bone was unaffected or increased only moderately (Dempster et al. 1993).

We have previously evaluated the effectiveness of alendronate, estrogen and PTH in preventing the development of OVX- or GX-evoked osteopenia in the rat (Andersson et al. 2002). We found that alendronate prevented trabecular bone loss after GX and OVX, while estrogen and PTH prevented trabecular bone loss after OVX, but not after GX. In the present study, we assessed the effectiveness of alendronate, estrogen, and PTH in reversing an established GX- or OVX-induced osteopenia in the rat. Selected bones were removed and examined by peripheral Quantitative Computed Tomography (pQCT) after 8 weeks of treatment (16 weeks after surgery).

Experiments

Animals

96 female, 3-month old Sprague-Dawley rats were obtained from M&B, Skensved, Denmark. The rats were allowed an acclimatization period of 7 days before the study was started. The animals were kept in groups of 3 in Macrolon cages, on a 12-hour light/dark cycle with access to standard rat food pellets (1.0% calcium and 0.7% phosphorus, Lactamin, Vadstena, Sweden) and tap water ad libitum. Body weights were determined at the beginning of the study and weekly thereafter. To prevent anemia, GX rats were given intramuscular injections of 400 μg/kg vitamin B12 (Betolvex, Dumex, Copenhagen, Denmark) and 20 mg/kg of iron(III)sorbitol (Jectofer, AstraZeneca, Södertälje, Sweden) once every other week (beginning the first week after surgery). The local animal welfare committee had approved the experiments before the study started.

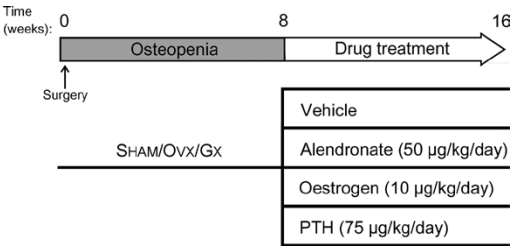

Figure 1. Experimental design: Rats were subjected to SHAM operation, ovariectomy (OVX), or gastrectomy (GX). Eight weeks after surgery, the three groups were divided into four treatment groups (n=8 rats in each group).

Drugs

The drugs were administered subcutaneously once daily in the doses indicated: alendronate (50 μg/kg/day), estrogen (10 μg/kg/day) and PTH (75 μg/kg/day). Alendronate hydrochloride was synthesized at AstraZeneca R&D, Mölndal, Sweden, estradiol-3-benzoate was obtained from Boehringer Ingelheim, Ingelheim am Rhein, Germany. Human PTH(1-84) was obtained from Allelix Biopharmaceuticals, Mississauga, Ontario, Canada. The alendronate dose corresponds to an effective daily dose of 10 μg compound phosphorus per kg. 10 mM citrate buffered saline, pH 5.5, was used as vehicle for PTH, saline as vehicle for alendronate, and sesame oil (Apoteksbolaget, Gothenburg, Sweden) for estrogen. The doses chosen are effective for preventing OVX-induced osteopenia (Wronski et al. 1988, Seedor et al. 1991, Shen et al. 1995).

Experimental design

The rats were randomly assigned to 1 of 3 groups and then subjected to various types of surgery (Figure 1): gastrectomy (GX, n = 32), ovariectomy (OVX, n = 32), and sham operation (SHAM, n = 32). They were subdivided into 4 treatment groups (n=8 in each)—i.e., SHAM + vehicle, SHAM + alendronate, SHAM + estrogen , SHAM + PTH, OVX + vehicle, OVX + alendronate, OVX + estrogen, OVX + PTH, GX + vehicle, GX + alendronate, GX + estrogen, and GX + PTH. The treatment was started 8 weeks after surgery and lasted for 8 weeks.

The rats were killed by exsanguination (cardiac puncture) under isoflurane anesthesia (Forene, Abbott Laboratories, Abbot Park, IL, USA). Blood was drawn and serum stored at –20 ºC pending

analysis (gastrin, osteocalcin and PTH). Uteri were removed, and wet weights 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 ºC pending determination of bone density. The femurs and tibiae were fixed in 4% buffered formaldehyde for 4 days at 4 ºC, washed thoroughly thereafter and stored in 70% ethanol at 4 ºC.

Surgery

The rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (Ketalar, Park-Davis, Barcelona, Spain) and xylazine (Rompun, Bayer, Leverkusen, Germany) in doses of 90 and 4.5 mg/kg, respectively. GX was performed by resecting the glandular part of the stomach and anastomosing the rumen to the duodenum, as described elsewhere (Lehto-Axtelius et al. 1998). Successful GX was verified by the demonstration of a low serum gastrin concentration. OVX or SHAM-operations were performed, using an abdominal approach (midline abdominal incision). Successful OVX was verified by a reduction in uterine weight (data not shown). None of the animals in the OVX or SHAM groups died. In the GX group, 3 rats died.

Measurements of serum gastrin, osteocalcin and parathyroid hormone

Determination of serum gastrin was performed as previously described (Stadil and Rehfeld 1973). Rat gastrin-17 was used as a standard. The concentration of gastrin in serum was expressed as pmol equivalents of rat gastrin-17 per L. Serum osteocalcin was determined, 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, 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 epiphyseal ends, the posterior pedicle arch and the spinous process removed, height of ~4 mm) was determined by Archimedes' principle. The vertebrae were then incinerated at 600 ºC for 12h; the resulting ash was weighed after cooling in a desiccator. The ash weight, divided by the volume, gave the bone mineral density (BMD, mg/cm^3).

Peripheral quantitative computed tomography (pQCT)

pQCT was performed with the Stratec peripheral quantitative computed tomograph (pQCT) XCT Research M (Norland Corp., Fort Atkinson, WI) specifically modified for use on small bone specimens (software version 5.4B; operating at 70 μm resolution) (Rosen et al. 1995, Andersson et al. 2002). The machine was calibrated with a standard of hydroxyapatite embedded in acrylic plastic. pQCT was used to analyze cross-sections of the distal metaphysis and middiaphysis of the left femur. During the measurements, the excised femurs were placed in a test tube filled with 70% ethanol. Metaphyseal scans were taken to measure the trabecular BMD (mg/cm^3). 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. Hence, no cortical bone was included in the trabecular BMD measurement (Andersson et al. 2002). The middiaphysis of the rat femur contains cortical bone alone. Cortical BMD (mg/mm^3) was determined in a 0.1 mm cross-section of the middiaphysis. This scan was also used to determine geometrical parameters, such as the cortical thickness (mm), the cortical cross-sectional area (mm^2), the periosteal circumference (mm), the endocortical circumference (mm) and the cortical bone mineral content (BMC, mg/mm). The inter-assay CV for the pQCT measurements was less than 2%. The lengths of the femur and tibiae were determined at the same time.

Statistics

The results are presented as means (SEM). Effects of drugs or surgery were analyzed by one-way analysis of variance (ANOVA). $P < 0.05$ was con-

Table 1. Effects of the three drugs on gain in body weight

	Vehicle	Alendronate	Estrogen	PTH
SHAM-operated rats				
Weight at start, g	231 (4)	233 (5)	237 (5)	230 (6)
Weight at end, g	323 (6)	322 (11)	338 (4)	327 (9)
Change in weight, %	40 (2)	38 (3)	43 (1)	42 (3)
OVX rats				
Weight at start, g	239 (7)	239 (7)	225 (3)	232 (5)
Weight at end, g	394 (13) [a]	381 (8) [a]	346 (11) [b]	379 (12) [a]
Change in weight, %	65 (3) [a]	59 (2) [a]	53 (3) [b]	63 (3) [a]
GX rats				
Weight at start, g	236 (6)	242 (8)	246 (9)	239 (9)
Weight at end, g	312 (8)	321 (9)	329 (7)	307 (10)
Change in weight, %	32 (3)	33 (3)	34 (2)	28 (3)

Mean (SEM) (n = 7–8 rats per group). Statistical significance was assessed by comparing the differences between the vehicle-treated groups and each of the three drug-treated groups or between the SHAM-operated rats and each of the two groups subjected to surgery.
[a] $p < 0.05$ versus SHAM (ANOVA).
[b] $p < 0.05$ versus vehicle

sidered statistically significant. Whenever statistically significant differences were found between the experimental groups, individual differences versus control 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). Estrogen treatment reduced the body weight of OVX rats, but not of GX and SHAM rats. Neither PTH nor alendronate affected the final body weights in any of the groups.

The length of the femur increased more in OVX rats than in SHAM-operated ones (OVX 37.8 (0.3) mm, SHAM 36.6 (0.2) mm, $p = 0.04$). No other effects of surgery on the length of the femur or tibia were seen in the groups (data not shown). Indeed, all femur and tibia lengths were unchanged in all groups regardless of treatment.

Bone mineral density

The BMD (ash weight/volume) of L5 was determined. OVX and GX reduced the BMD by 16 (5)% ($p < 0.001$) and 19 (4)% ($p = 0.001$), respectively (Figure 2). PTH increased the BMD of L5 in OVX and GX rats by 16 (3)% ($p < 0.001$) and 16 (2)% ($p = 0.002$), respectively, as compared to vehicle. Neither alendronate nor estrogen affected the BMD in any group.

L5 contains both trabecular and cortical bone. In order to determine whether surgery and drug treatment affected mainly trabecular or cortical bone, the trabecular BMD in the metaphysis and the cortical bone parameters in the middiaphysis of the femur were measured using pQCT.

16 weeks after surgery, OVX and GX rats showed a significant reduction in trabecular BMD in the metaphysis of the distal femur (OVX –55 (9)% ($p < 0.001$) and GX –44 (10)% ($p = 0.001$), respectively) (Figure 2). Alendronate increased the trabecular BMD in OVX and GX rats by 64 (7)% ($p = 0.007$) and 44 (9)% ($p = 0.006$), respectively. Estrogen increased the trabecular BMD in GX rats (35 (6)%, $p < 0.03$), but not in OVX rats (15 (9)%, $p = 0.8$). PTH treatment increased the trabecular BMD in OVX and GX rats by 115 (8)% ($p < 0.001$) and 51 (7)% ($p = 0.002$), respectively. The trabecular BMD of PTH-treated OVX was similar to that of SHAM-operated controls.

Unlike trabecular bone, cortical bone responded poorly to GX, OVX and treatment with drugs (Table 2). The cortical bone mineral content (BMC) was reduced in GX rats (GX –9.4 (3.1)%,

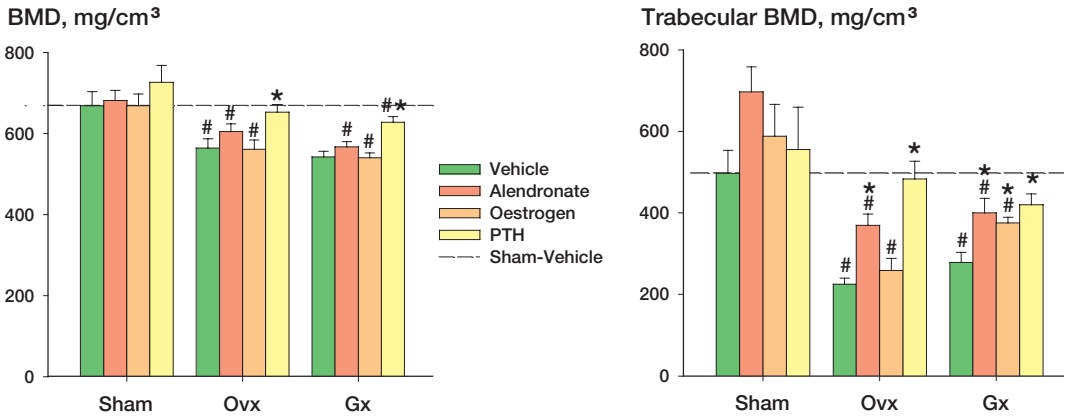

Figure 2. Bone mineral density (BMD) of the fifth lumbar vertebral body (ash weight/volume) and trabecular BMD in the metaphysis of the femur (measured by pQCT). The trabecular bone region was defined as the inner 45% of the bone area scanned. Means ± SEM (n = 7–8 rats in each group). Vehicle-treated rats were compared with the various drug-treated groups and the SHAM-operated rats were compared with the two surgery groups. * $p < 0.05$ versus vehicle; # $p < 0.05$ versus SHAM (ANOVA).

Table 2 Effects of the three drugs on cortical bone parameters measured in the middiaphysis of the femur by pQCT

	Vehicle	Alendronate	Estrogen	PTH
SHAM-operated rats				
Cortical BMC, mg/mm	8.80 (0.23)	9.10 (0.28)	8.78 (0.19)	8.92 (0.20)
Cortical BMD, mg/mm^3	1.39 (5)	1.40 (6)	1.39 (5)	1.40 (7)
Cortical area, mm^2	6.34 (0.15)	6.52 (0.20)	6.32 (0.12)	6.40 (0.16)
Cortical thickness, mm	0.68 (0.01)	0.68 (0.01)	0.66 (0.01)	0.70 (0.01)
Periosteal circumference, mm	11.5 (0.15)	11.7 (0.27)	11.7 (0.15)	11.3 (0.17)
Endocortical circumference, mm	7.22 (0.12)	7.47 (0.27)	7.53 (0.18)	6.89 (0.15)
OVX rats				
Cortical BMC, mg/mm	9.26 (0.10)	8.95 (0.23)	8.73 (0.31)	9.22 (0.25)
Cortical BMD, mg/mm^3	1.38 (5)	1.40 (3) [a]	1.39 (3)	1.40 (5) [a]
Cortical area, mm^2	6.70 (0.07)	6.39 (0.17)	6.27 (0.22)	6.57 (0.18)
Cortical thickness, mm	0.69 (0.01)	0.69 (0.01)	0.66 (0.01)	0.72 (0.01)
Periosteal circumference, mm	11.9 (0.10)	11.4 (0.16)	11.6 (0.25)	11.4 (0.17)
Endocortical circumference, mm	7.53 (0.15)	7.07 (0.13)	7.48 (0.22)	6.93 (0.13) [a]
GX rats				
Cortical BMC, mg/mm	7.97 (0.29) [b]	8.34 (0.20)	8.09 (0.17)	8.01 (0.25) [b]
Cortical BMD, mg/mm^3	1.40 (3)	1.40 (5)	1.38 (3)	1.39 (4)
Cortical area, mm^2	5.70 (0.20) [b]	5.98 (0.12)	5.85 (0.11)	5.78 (0.19)
Cortical thickness, mm	0.64 (0.02)	0.66 (0.01)	0.63 (0.01)	0.65 (0.01) [b]
Periosteal circumference, mm	10.9 (0.13) [b]	11.1 (0.12)	11.3 (0.10)	11.0 (0.29)
Endocortical circumference, mm	6.85 (0.04)	6.98 (0.14)	7.31 (0.10)	6.90 (0.29)

Mean (SEM) (n = 7–8 rats in each group). Statistical significance was assessed for the differences between the vehicle-treated groups and each of the three drug-treated groups or between the SHAM-operated rats and each of the two surgery groups.
[a] $p < 0.05$ versus vehicle
[b] $p < 0.05$ versus SHAM (ANOVA).

p = 0.03), but not in OVX rats (OVX +5.3 (1.9)%, p = 0.3). The GX-evoked reduction in cortical BMC was due mainly to a reduction in the cortical area because of a smaller periosteal circumference. Treatment with the three drugs did not affect the cortical bone parameters in the GX and SHAM groups (not shown). In OVX rats, PTH treatment significantly reduced the endocortical circumfer-

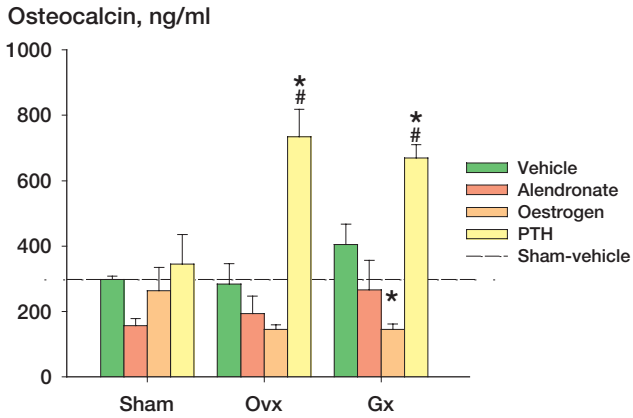

Figure 3. Effects of alendronate, estrogen or PTH on the serum osteocalcin concentration at sacrifice. Means ± SEM (n = 7–8 rats in each group). Vehicle-treated rats were compared with the various drug-treated groups and the SHAM-operated rats were compared with the two surgery groups. * p<0.05 versus vehicle; # p<0.05 versus SHAM (ANOVA).

ence. In OVX rats, the cortical BMD increased slightly after alendronate and PTH treatment. No other effects on the various cortical bone parameters were noted in the OVX rats.

Serum gastrin, osteocalcin and parathyroid hormone

On sacrifice, as expected, the serum concentration of gastrin was reduced in GX rats (32 (7) pmol/L, $p < 0.001$), as compared to OVX (109 (11) pmol/L) and SHAM rats (155 (19) pmol/L). Osteocalcin, a marker of bone formation/turnover, increased after PTH treatment in OVX (159 (17)%, $p = 0.001$) and GX (65 (11)%, $p = 0.007$) rats (Figure 3). Estrogen reduced the serum osteocalcin levels in GX rats (–64 (39)%, $p = 0.007$), but not in OVX ones (–49 (30)%, $p = 0.2$). The serum concentration of endogenous PTH was unchanged in GX (61 (8) pg/mL, $p = 0.7$) and OVX rats (47 (6) pg/mL, $p = 0.9$), as compared to SHAM rats (53 (5) pg/mL).

Discussion

It is well known that OVX causes an increase in bone turnover and an imbalance between resorption and formation of bone that may result in a reduced bone mass in man and experimental animals. GX also accelerates bone turnover—i.e., increases resorption and reduces formation. However, the pathogenetic mechanisms underlying GX- and OVX- evoked osteopenia seem to differ since GX (unlike OVX) greatly reduces the percentage of bone in the calvaria (Klinge et al. 1995, Lehto-Axtelius et al. 1998, Surve et al. 2001a, b). Bisphosphonates and estrogen replacement are used clinically to treat osteoporosis and PTH will probably soon be available as an additional treatment. In an earlier study, we showed that OVX-induced osteopenia could be prevented by treatment with alendronate, estrogen or PTH. However, GX-induced bone loss can be prevented by giving alendronate, but not PTH or estrogen (Andersson et al. 2002). Here we studied whether alendronate, estrogen or PTH is an effective treatment of established osteopenia induced by OVX or GX in rats. Our results with OVX rats confirm previous reports that PTH restores trabecular bone, while estrogen does not (Abe et al. 1993, Shen et al. 1995). This is the first study in which alendronate, estrogen and PTH have been used in an attempt to reverse GX-evoked osteopenia. Unlike our previous preventive study, all three drugs restored trabecular bone lost after GX at least to a certain degree. Thus, available evidence suggests that the mechanism underlying the OVX-evoked osteopenia differs from that underlying osteopenia induced by GX.

OVX and GX reduced the BMD of L5. The vertebral body is rich in trabecular bone, but it also contains cortical bone. To determine whether the effects of OVX and GX were mainly on trabecular or cortical bone, we measured trabecular BMD in the metaphysis of the distal femur, using pQCT. Cortical bone was measured in the middiaphysis of the same femur. Both OVX and GX had a negative effect on the trabecular bone. However, in the GX group, but not in the OVX group, cortical BMC was reduced as well. These findings accord with those of others (Wronski et al. 1985, Surve et al. 2001a, b).

OVX was associated with an increase in body weight. This was reversed by treatment with estrogen, which confirms the results of several previous studies (Wronski et al. 1988, Abe et al. 1993, Shen et al. 2000). The effects of estrogen on body weight and uterine weight (data not shown) indicate that the dose given was effective. As in other studies (Abe et al. 1993, Shen et al. 2000) estrogen failed to restore the bone lost after OVX. It is commonly believed that estrogen maintains bone mass by an antiresorptive mechanism. After OVX, bone resorption therefore increases and estrogen replacement reverses it. However, in the GX group, estrogen increased the trabecular BMD. Although estrogen had no significant effect in the SHAM-operated rats in this study, we have reported earlier that the same daily dose of estrogen increased the trabecular bone mass in SHAM-operated rats (Andersson et al. 2002). Moreover, higher daily doses of estrogen have been shown to increase the trabecular bone mass in normal, intact rats (Wronski et al. 1988).

Alendronate increased the trabecular BMD in OVX and GX rats. These findings accord with those of others in which an established OVX-evoked osteopenia was treated with alendronate and other bisphosphonates (Wronski et al. 1993, Iwaniec et al. 2001). However this is the first study in which alendronate has been used to treat an established GX-induced osteopenia.

PTH increased the trabecular bone mass of OVX rats more effectively than alendronate or estrogen. This is in agreement with previous observations in trabecular bone (Wronski et al. 1993). In the GX group, PTH increased the trabecular BMD to the same extent as alendronate or estrogen. It is noteworthy that PTH prevented the OVX-induced but not the GX-induced bone loss, while being effective in the treatment of OVX- and GX-evoked osteopenia (Andersson et al. 2002).

Biochemical markers of bone turnover, such as osteocalcin, are widely used clinically to monitor the metabolic activity of bone. At the end of the study (16 weeks after surgery and 8 weeks after starting the drug treatment), we found no statistically significant difference between the serum osteocalcin concentrations in OVX, GX and SHAM-operated rats. Some authors have reported reduced serum osteocalcin levels after treatment with alendronate and estrogen in rats (Shen et al. 1995, Frolik et al. 1996). In this study, alendronate had no effect on serum osteocalcin. However, estrogen reduced the serum osteocalcin concentration in GX rats, but not in SHAM-operated or OVX rats. On the other hand, PTH increased the serum osteocalcin concentration in GX (65 (11)%, $p = 0.007$) and OVX rats (159 (17)%, $p = 0.001$), but not in SHAM-operated rats (16 (15)%, $p = 0.91$). PTH has previously been reported to increase serum osteocalcin levels in OVX rats (Shen et al. 1995).

It has frequently been stated that nutritional deficiencies contribute to the GX-evoked osteopenia. This view is in line with the finding that normal body weight gain slowed down after GX (Klinge et al. 1995). The findings in the present study do not support this view. Indeed, it seems unlikely that either GX-induced or OVX-induced osteopenia results from generalized nutritional deficiency, as neither the GX rats nor the OVX rats were growth retarded (see Surve et al. 2001a, b, Andersson et al. 2002). Moreover, a specific deficiency of calcium is unlikely since it has been shown earlier that calcium supplements have no effect (Persson et al. 1993, Klinge et al. 1995). Vitamin D deficiency is known to cause impaired calcium absorption and osteomalacia. However, vitamin D deficiency seems to be an unlikely cause of the GX-evoked osteopenia because the serum concentrations of 1,25-dihydroxyvitamin D3 increased after GX (Axelson et al. 1991, Rümenapf et al. 1997, 1998). These increases may reflect secondary hyperparathyroidism, but the serum PTH concentration was not affected by GX. This accords with the results of previous studies (Andersson et al. 2002). It may be argued that removal of the acid-producing part of the stomach may cause acidosis and affect calcium/bone metabolism. However, complete inhibition of gastric acid secretion by proton pump inhibitors does not cause osteopenia (Persson et al. 1993). The findings support the view that the stomach is important for bone metabolism by mechanisms that are unrelated to dietary deficiencies or lack of gastric acid (see Surve et al. 2002).

In conclusion, alendronate and PTH, but not estrogen, restored trabecular bone loss after OVX, while all three drugs restored trabecular bone loss after GX. This suggests that the mechanisms

underlying GX-evoked and OVX-evoked trabecular bone differ. The ability of PTH, estrogen and alendronate (and probably other bisphosphonates as well) to reverse GX- evoked osteopenia may be of clinical interest when dealing with post-GX osteopenia in humans.

The authors thank Ms. Susanne Arvidsson, Ms. Agneta Karlsson, Ms. Dorota Kakol-Palm, Ms. Maud Petterson and Ms. Anna Themner-Persson for expert technical assistance. We also thank the SWEGENE Centre for Bio-Imaging (CBI), Gothenburg University, for technical support regarding image analysis. The study was supported by AstraZeneca, the Swedish Medical Research Council (K2001-72X-04250-28A & 04x-1007), the Swedish Foundation for Strategic Research, the Lundberg Foundation, the Swedish Medical Society, the Novo Nordisk Foundation, the Torsten and Ragnar Söderberg Foundation, the Emil and Vera Cornell Foundation, the Petrus and Augusta Hedlund Foundation, the Swedish Association Against Rheumatic Disease, the A. Påhlsson Foundation, the Crafoord Foundation and the Medical Faculty, Lund University.

This study constitutes a scientific collaboration between the Department of Pharmacology, Institute of Physiological Sciences, Lund University, the Division of Endocrinology, Department of Internal Medicine, Sahlgrenska Academy at Gothenburg University, and AstraZeneca R&D, Mölndal. AstraZeneca provided unconditional financial support for this study.

Abe T, Chow J W, Lean J M, Chambers T J. Estrogen does not restore bone lost after ovariectomy in the rat. J Bone Miner Res 1993; 8 (7): 831-8.

Andersson N, Surve V V, Lehto-Axtelius D, Ohlsson C, Håkanson R, Andersson K, Ryberg B. Drug-induced prevention of gastrectomy- and ovariectomy-induced osteopenia in the young female rat. J Endocrinol 2002; 175 (3): 695-703.

Axelson J, Persson P, Gagnemo-Persson R, Håkanson R. Importance of the stomach in maintaining calcium homoeostasis in the rat. Gut 1991; 32 (11): 1298-302.

Bagi C M, Ammann P, Rizzoli R, Miller S C. Effect of estrogen deficiency on cancellous and cortical bone structure and strength of the femoral neck in rats. Calcif Tissue Int 1997; 61 (4): 336-44.

Cummings S R, Black D M, Thompson D E, Applegate W B, Barrett-Connor E, Musliner T A, Palermo L, Prineas R, Rubin S M, Scott J C, Vogt T, Wallace R, Yates A J, LaCroix AZ. Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: Results from the fracture intervention trial. JAMA 1998; 280 (24): 2077-82.

Dempster D W, Cosman F, Parisien M, Shen V, Lindsay R. Anabolic actions of parathyroid hormone on bone. Endocr Rev 1993; 14 (6): 690-709.

Frolik C A, Bryant H U, Black E C, Magee D E, Chandrasekhar S. Time-dependent changes in biochemical bone markers and serum cholesterol in ovariectomized rats: Effects of raloxifene HCl, tamoxifen, estrogen, and alendronate. Bone 1996; 18 (6): 621-7.

Heiskanen J T, Kroger H, Paakkonen M, Parviainen M T, Lamberg-Allardt C, Alhava E. Bone mineral metabolism after total gastrectomy. Bone 2001; 28 (1): 123-7.

Iwaniec U T, Samnegard E, Cullen D M, Kimmel D B. Maintenance of cancellous bone in ovariectomized, human parathyroid hormone [hPTH(1-84)]-treated rats by estrogen, risedronate, or reduced hPTH. Bone 2001; 29 (4): 352-60.

Klinge B, Lehto-Axtelius D, Åkerman M, Håkanson R. Structure of calvaria after gastrectomy. An experimental study in the rat. Scand J Gastroenterol 1995; 30 (10): 952-7.

Lehto-Axtelius D, Stenström M, Johnell O. Osteopenia after gastrectomy, fundectomy or antrectomy: An experimental study in the rat. Regul Pept 1998; 78 (1-3): 41-50.

Persson P, Gagnemo-Persson R, Chen D, Axelson J, Nylander A G, Johnell O, Håkanson R. Gastrectomy causes bone loss in the rat: Is lack of gastric acid responsible? Scand J Gastroenterol 1993; 28 (4): 301-6.

Rosen H N, Tollin S, Balena R, Middlebrooks V L, Beamer W G, Donohue L R, Rosen C, Turner A, Holick M, Greenspan S L. Differentiating between orchiectomized rats and controls using measurements of trabecular bone density: A comparison among DXA, histomorphometry, and peripheral quantitative computerized tomography. Calcif Tissue Int 1995; 57 (1): 35-9.

Rümenapf G, Schwille P O, Erben R G, Schreiber M, Fries W, Schmiedl A, Hohenberger W. Osteopenia following total gastrectomy in the rat-State of mineral metabolism and bone histomorphometry. Eur Surg Res 1997; 29 (3): 209-21.

Rümenapf G, Schwille PO, Erben R G, Schreiber M, Berge B, Fries W, Schmiedl A, Koroma S, Hohenberger W. Gastric fundectomy in the rat: Effects on mineral and bone metabolism, with emphasis on the gastrin-calcitonin-parathyroid hormone-vitamin d axis. Calcif Tissue Int 1998; 63 (5): 433-41.

Seedor J G, Quartuccio H A, Thompson D D. The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. J Bone Miner Res 1991; 6 (4): 339-46.

Shen V, Birchman R, Xu R, Otter M, Wu D, Lindsay R, Dempster D W. Effects of reciprocal treatment with estrogen and estrogen plus parathyroid hormone on bone structure and strength in ovariectomized rats. J Clin Invest 1995; 96 (5): 2331-8.

Shen V, Birchman R, Wu D D, Lindsay R. Skeletal effects of parathyroid hormone infusion in ovariectomized rats with or without estrogen repletion. J Bone Miner Res 2000; 15 (4): 740-6.

Stadil F, Rehfeld J F. Determination of gastrin in serum. An evaluation of the reliability of a radioimmunoassay. Scand J Gastroenterol 1973; 8 (2): 101-12.

Surve V V, Andersson N, Alatalo S, Lehto-Axtelius D, Halleen J, Väänänen K, Håkanson R. Does combined gastrectomy and ovariectomy induce greater osteopenia in young female rats than gastrectomy alone? Calcif Tissue Int 2001a; 69 (5): 274-80.

Surve V V, Andersson N, Lehto-Axtelius D, Håkanson R. Comparison of osteopenia after gastrectomy, ovariectomy and prednisolone treatment in the young female rat. Acta Orthop Scand 2001b; 72 (5): 525-32.

Surve V V, Höglund P, Håkanson R. Gastrectomized rats respond with exaggerated hypercalcemia to oral and intravenous calcium loads because of impaired ability of bone to take up Ca2+. Scand J Gastroenterol 2002; 37 (5): 523-30.

Tovey F I, Hall M L, Ell P J, Hobsley M. Postgastrectomy osteoporosis. Br J Surg 1991; 78 (11): 1335-7.

Turner R T, Vandersteenhoven J J, Bell N H. The effects of ovariectomy and 17 beta-estradiol on cortical bone histomorphometry in growing rats. J Bone Miner Res 1987; 2 (2): 115-22.

Wronski T J, Lowry P L, Walsh C C, Ignaszewski L A. Skeletal alterations in ovariectomized rats. Calcif Tissue Int 1985; 37 (3): 324-8.

Wronski T J, Cintron M, Doherty A L, Dann L M. Estrogen treatment prevents osteopenia and depresses bone turnover in ovariectomized rats. Endocrinology 1988; 123 (2): 681-6.

Wronski T J, Yen C F, Qi H, Dann L M. Parathyroid hormone is more effective than estrogen or bisphosphonates for restoration of lost bone mass in ovariectomized rats. Endocrinology 1993; 132 (2): 823-31.