Repeated *in vivo* determinations of bone mineral density during parathyroid hormone treatment in ovariectomized mice

N Andersson^{1,2}, M K Lindberg³, C Ohlsson³, K Andersson¹
and B Ryberg¹

¹AstraZeneca R&D, Mölndal, Sweden

²Institute of Physiological Sciences, Department of Pharmacology, Lund University, Lund, Sweden

³Department of Internal Medicine, Sahlgrenska University Hospital, Göteborg, Sweden

(Requests for offprints should be addressed to Niklas Andersson, AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden;

Email: niklas.andersson@astrazeneca.com)

Abstract

The recent development of different genetically modified mice with potentially interesting bone phenotypes has increased the demand for effective non-invasive methods to evaluate effects on bone of mice during growth and development, and for drug evaluation. In the present study, the skeleton was analyzed by repeated *in vivo* scans using dual energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT). Ovariectomized (ovx) mice treated with parathyroid hormone (PTH) were used as an animal model to evaluate these two techniques at different times after the onset of treatment. Female mice (6 weeks of age) were allocated randomly to four groups: (1) sham-operated+vehicle; (2) ovx+vehicle; (3) sham-operated+PTH(1–84) 150 µg/kg per day; (4) ovx+PTH. Six weeks after ovariectomy the drug treatment began and was continued for 8 weeks. The total body bone mineral content (BMC) and total body areal bone mineral density (BMD) were measured by DXA. Ovariectomy reduced total body BMC and total body areal BMD by $6.2 \pm 1.7\%$ and $2.6 \pm 0.9\%$

respectively. No effect of PTH on total body BMC was seen during the treatment period. The trabecular volumetric BMD was measured by pQCT. Ovariectomy reduced the trabecular volumetric BMD by $52 \pm 6.7\%$. The pQCT technique detected a clear effect on trabecular volumetric BMD after 2 weeks of PTH treatment (ovx $94 \pm 29\%$ and sham-operated $46 \pm 10\%$ more than vehicle-treated). The cortical bone was measured in a mid-diaphyseal pQCT scan of the tibia. Ovariectomy reduced the cortical BMC by $9 \pm 2\%$. PTH treatment for 8 weeks increased cortical BMC in ovx mice.

In conclusion, the pQCT technique is more sensitive than the DXA technique in the detection of bone loss after ovariectomy and increased bone mass after PTH treatment in mice. Notably, the pQCT, but not the DXA, technique detected a dramatic effect as early as after 2 weeks of PTH treatment. Dynamic pQCT measurements will be useful for monitoring skeletal changes during growth and development, and for drug evaluation in mice.

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Introduction

The recent development of different genetically modified mice with potentially interesting bone phenotypes has increased the demand for effective non-invasive methods to evaluate influences on the bones of mice during growth and development, and for drug evaluation. Dual-energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) are widely used non-invasive methods for determining bone mineral parameters in animals and humans. DXA can be used to estimate bone mineral content (BMC) and areal bone mineral density (BMD) of the entire body and of individual bones. However, the areal BMD measured with DXA should not be mistaken for the true volumetric BMD obtained with

pQCT. The pQCT can differentiate between cortical and trabecular bone and, in addition to density, it can measure parameters such as cortical area and cortical thickness (Rosen *et al.* 1995, Sandstedt *et al.* 1996, Windahl *et al.* 1999).

Parathyroid hormone (PTH) is an anabolic agent that has attracted much attention as a potential candidate in the treatment of osteoporosis. Clinical trials have shown PTH, when administered intermittently, to have an anabolic effect on bone (Reeve *et al.* 1976, 1980). Intermittent PTH treatment is also effective in preventing ovariectomy-induced bone loss in rats (Dempster *et al.* 1993, 1995, Mitlak *et al.* 1996, Sato *et al.* 1997). Ovariectomy in the rat is an established animal model for studies on the prevention and treatment of osteoporosis. Estrogen

deficiency results in an increase in bone turnover, with excess bone resorption, and significant loss of trabecular bone. In cortical bone, an increased bone turnover rate results in endosteal resorption; however, because this is compensated by periosteal growth, the net cortical bone loss is small (Shen *et al.* 1995, Yamauchi *et al.* 1995, Li *et al.* 1996, Bagi *et al.* 1997). The ovariectomy model is better established in rats than it is in mice (Turner *et al.* 1987, Broulik 1991, Edwards *et al.* 1992, Bain *et al.* 1993, Okada *et al.* 1998, Windahl *et al.* 1999), in which the effect of ovariectomy appears to be highly strain-dependent (Zeng *et al.* 1998). There is little information available on anabolic effects of PTH in mice (Rihani-Bisharat *et al.* 1998, Zeng *et al.* 1998, Stanislaus *et al.* 2000).

The aim of the present study was to compare the usefulness of the two non-invasive radiological techniques, pQCT and DXA, in the detection of early treatment effects on cortical and trabecular bone in mice. Ovariectomized (ovx) mice treated with PTH were used as an animal model to evaluate these two techniques at different time points after the onset of treatment.

Materials and Methods

Animals

Six-week-old, C57BL/6 female mice (Møllegaard's Breeding Center, Skensved, Denmark) were maintained on a 12-h light:12-h darkness cycle at 22 °C with food and water available *ad libitum*. The mice were divided into two groups: bilateral ovariectomies or sham operations were performed. Six weeks after surgery, the mice were divided into four treatment groups: (1) sham-operated+vehicle; (2) ovariectomized (ovx)+vehicle; (3) sham-operated+PTH(1–84) 150 µg/kg per day; (4) ovx+PTH ($n=7-10$ mice/group). Body weights were determined at the start of drug treatment and every week thereafter. All animal procedures were reviewed and approved by the local animal welfare committee before the study started.

Drug treatment

Human recombinant PTH(1–84) was obtained from Allelix Biopharmaceuticals, Mississauga, Canada. All animals received daily subcutaneous (s.c.) injections (5 ml/kg) of PTH (150 µg/kg) or the vehicle (10 mmol/l citrate-buffered saline, pH 5.5) for 8 weeks. The mice were anesthetized with a mixture of ketamine 75 mg/kg (Ketalar, Parke-Davis, Barcelona, Spain) and medetomidine 1 mg/kg (Domitor vet., Orion, Espoo, Finland; intraperitoneal injection) during surgery and during all *in vivo* X-ray measurements. After X-ray measurements, an α_2 -antagonist (atipamezole, Antisedan vet., Orion), 1 mg/kg (s.c. injection), was administered to promote recovery and reduce sleeping time.

Tissue collection

After the cessation of treatment, the mice were anesthetized, subjected to cardiac puncture, and killed by cervical dislocation. The uterus was removed from each animal and weighed to confirm successful ovariectomy. Femora, tibiae and lumbar vertebrae L5 were removed, cleaned of soft tissue, fixed in 10% formalin, and stored in 70% ethanol at 4 °C.

Dual-energy X-ray absorptiometry

BMC and areal BMD were measured using the Norland pDEXA Sabre (Norland, Fort Atkinson, WI, USA). The software Sabre Research (v3.6) was used (Windahl *et al.* 1999). The machine was calibrated daily with a phantom provided by the manufacturer.

Total body BMC and total body areal BMD were measured *in vivo* 6 weeks after ovariectomy (at the start of drug treatment) and after 2, 4 and 8 weeks of drug treatment. Medium-resolution scans, with line spacing set at 0.05 cm, were used. Three mice could be analyzed simultaneously in the same scan, therefore a mouse that was killed at the beginning of the experiment was included in each scan as an internal standard in order to avoid between-scan variations. The interassay coefficients of variation (CV) for the DXA measurements were less than 5%.

The femur, tibia and vertebrae L5 were measured *ex vivo* on excised bones under a 15 mm layer of 70% ethanol. All excised bones were measured simultaneously in a single scan. High-resolution scans were used (line spacing 0.02 cm or 0.01 cm).

Peripheral quantitative computed tomography

Tomographic measurements were made using the Stratec pQCT XCT Research M (Norland) specifically modified for use on small bone specimens (software version 5.4B; resolution 70 µm) (Rosen *et al.* 1995). The *in vivo* measurements were made on the tibia, for technical reasons.

In vivo measurements of the left tibia were made 6 weeks after surgery and after 2, 4 and 8 weeks of drug treatment. A metaphyseal pQCT scan was used to determine trabecular bone. The metaphyseal scan was positioned at 1% of the total length of the tibia distal to the growth plate. The trabecular bone region/compartments was defined as the density of the inner 45% of the scanned bone area. A mid-diaphyseal pQCT scan was made of the same tibia to determine the cortical BMD, the cortical cross-sectional area, the cortical thickness, the periosteal circumference, the endosteal circumference, the moment of resistance, and the cross-sectional moment of inertia.

Ex vivo measurements were performed on excised bones in order to enable us to correlate effects on the femur *ex vivo* with the effects on the tibia *in vivo*. Metaphyseal

Table 1 Body weight, uterine weight and lengths of the femur and tibia in ovariectomized (ovx) and sham-operated (Sham) mice after 8 weeks of intermittent PTH treatment

	Sham Vehicle	Ovx Vehicle	Sham PTH	Ovx PTH
Body weight (g)	23.6 ± 0.6	22.9 ± 0.5	24.1 ± 0.6	23.8 ± 0.7
Uterine weight (mg)	74 ± 7	16 ± 2*	98 ± 13	18 ± 1*
Femur length (mm)	15.5 ± 0.1	15.6 ± 0.3	16.1 ± 0.1†	15.6 ± 0.3
Tibia length (mm)	18.1 ± 0.2	18.5 ± 0.3	18.2 ± 0.3	17.7 ± 0.2

Values are mean ± S.E.M., $n=7-10$ mice per group. *Significantly different from sham-operated ($P<0.05$); †significantly different from vehicle-treated ($P<0.05$) (ANOVA).

Figure 1 Effects of ovariectomy. Total body areal BMD (A), total body BMC (B), trabecular volumetric BMD (C) and cortical BMC (D) in sham-operated (SHAM) and ovx mice 6 weeks after surgery. Means ± S.E.M., $n=17$. *Significantly different from sham-operated mice ($P<0.05$, Student's *t*-test).

pQCT scans of the left femora were used to measure trabecular BMD. The metaphyseal scan was positioned at 3% of the total length of the femur proximal to the distal growth plate. The trabecular bone region was defined as the density of the inner 45% of the scanned bone area. Mid-diaphyseal pQCT scans of the left femora were used to determine the cortical BMD, the cortical cross-sectional area, the cortical thickness, the periosteal circumference, the endosteal circumference, the moment of resistance, and the cross-sectional moment of inertia. The inter-assay CVs for the pQCT measurements were less than 2%.

It should be emphasized that DXA gives the areal BMD, whereas the pQCT gives the real/volumetric BMD. Thus DXA gives the mineral content per area (mg/cm^2), not per volume (mg/ml). Therefore, a factor regulating the outer dimensions of a bone will affect the areal BMD (DXA) but not the volumetric BMD (pQCT).

Statistical analysis

Results are presented as means ± S.E.M., $n=7-10$. For analysis of the effects of ovariectomy before the start of drug treatment, the mice were pooled into two groups ($n=17$ each). Student's *t*-test was used to evaluate the effects of ovariectomy and the effect of drug treatment. $P<0.05$ was considered statistically significant. Data from excised bones were analyzed by one-way analysis of variance (ANOVA). Whenever statistically significant differences ($P<0.05$) were found between the experimental groups by ANOVA, individual differences were assessed by *post hoc* analysis (Tukey's test).

Results

Neither ovariectomy nor PTH treatment had any effects on body weight or the length of the tibia (Table 1). The

Figure 2 Effects of PTH treatment in ovx mice. The mice were ovariectomized 6 weeks before the start of treatment. Total body areal BMD (A), total body BMC (B), trabecular volumetric BMD (C) and cortical BMC (D) were measured by DXA (A, B) and pQCT (C, D). Means $\pm$ S.E.M., $n=7-10$. *Significantly different from vehicle-treated ($P<0.05$, Student's *t*-test).

length of the femur was increased by $4 \pm 1\%$ by PTH treatment in sham-operated mice, but no effect of PTH was found in ovx mice. Uterine weights were reduced in all ovx animals, confirming successful ovariectomy.

In vivo measurements of BMD were made at the start of PTH administration (6 weeks after ovariectomy) and 2, 4 and 8 weeks after the start of PTH treatment. Both DXA and pQCT scans were performed on each occasion.

Total body areal BMD and total body BMC were measured by DXA. At the start of treatment, 6 weeks after ovariectomy, total body areal BMD (Fig. 1A) and total body BMC (Fig. 1B) were decreased by $2.6 \pm 0.9\%$ and $6.2 \pm 1.7\%$ respectively, compared with the values for sham-operated mice. PTH treatment for 4 weeks increased total body areal BMD ($7.7 \pm 1.4\%$) compared with pretreatment values in ovx mice. However, after 8 weeks of treatment there was no statistically significant effect of PTH (Fig. 2A). PTH had no statistically significant effect on total body BMC in ovx mice (Fig. 2B). In sham-operated mice, PTH treatment had no significant effects on either total body areal BMD or total body BMC (Fig. 3A, B).

Trabecular BMD was measured by pQCT in the metaphysis of the tibia. The trabecular bone compartment

was defined as the inner 45% of the scanned bone area. Six weeks after ovariectomy (i.e. at the start of PTH treatment), the trabecular volumetric BMD was decreased by $52 \pm 6.7\%$ compared with that in sham-operated controls (Fig. 1C). Two weeks of PTH treatment increased the trabecular volumetric BMD compared with pretreatment values both in ovx and in sham-operated mice, by $94 \pm 29\%$ and $46 \pm 10\%$ respectively (Figs 2C and 3C). In fact, the ovariectomy-evoked trabecular osteopenia was reversed after 4 weeks of PTH treatment (trabecular volumetric BMD in sham-operated vehicle-treated mice and ovx PTH-treated mice was 130 ± 10 mg/ml and 120 ± 12 mg/ml, respectively).

The cortical BMC was determined in a mid-diaphyseal pQCT scan. Six weeks after ovariectomy, the cortical BMC was reduced by $9.2 \pm 1.9\%$ compared with that in the sham-operated animals (Fig. 1D). In ovx mice, PTH showed a statistically significant effect after 8 weeks of treatment (Fig. 2D). In contrast, no effect of PTH treatment was seen on cortical BMC in sham-operated mice (Fig. 3D).

Ex vivo DXA measurements were made on the excised femur, tibia and the fifth lumbar vertebra (L5). OvX mice had lower BMC in the femur and the L5 than

Figure 3 Effects of PTH treatment in sham-operated mice. The mice were sham-operated 6 weeks before the start of treatment. Total body areal BMD (A), total body BMC (B), trabecular volumetric BMD (C) and cortical BMC (D) were measured by DXA (A, B) and pQCT (C, D). Means $\pm$ S.E.M., $n=8-9$. *Significantly different from vehicle-treated ($P<0.05$, Student's t -test).

sham-operated mice (femur $-19 \pm 2.1\%$ and L5 $-20 \pm 7.6\%$ compared sham-operated; Fig. 4). PTH treatment increased the BMC in the femur and in the L5 of ovx mice ($20 \pm 2.7\%$ and $24 \pm 7.6\%$ respectively). PTH treatment also increased the BMC in the femur of sham-operated mice ($10 \pm 1.7\%$). Neither ovariectomy nor PTH treatment had any statistically significant effect on BMC of the intact tibia (Fig. 4).

High-resolution (line spacing 0.01 cm) DXA scans were also performed on one representative femur and the L5 from one animal in each group. The scans confirmed the pronounced effects of both ovariectomy and PTH treatment (Fig. 5).

Discussion

It is well known that ovariectomy results in a reduced BMC mainly because of a decrease in trabecular bone, whereas PTH treatment reverses the trabecular bone loss. Most studies on this subject have been performed using traditional methods including ash weight (BMC) and histomorphometry. These techniques are well established and useful for the determination of bone mass and bone

quality in killed animals. However, they are labour-intensive and not possible to perform longitudinally without killing a relatively large number of mice at each time point. DXA and pQCT might be useful tools with which to determine the phenotype of new genetically modified mice and for estimating treatment effects during drug treatment. We have previously demonstrated that DXA determinations of BMC in mice correlate well with BMC as measured by ash weight (Sandstedt *et al.* 1996) and that trabecular volumetric BMD estimated using pQCT correlate well with bone volume/total volume as measured by histomorphometry (Windahl *et al.* 2001).

In the present study, 6-week-old female mice underwent ovariectomy or were sham-operated. The mice were then treated with PTH intermittently for 8 weeks, and the bone mineral was followed longitudinally *in vivo* by repeated measurements using both DXA and pQCT. The dose of PTH used in the present study (PTH(1-84) 150 $\mu\text{g/kg}$ per day) was chosen because it has been shown to reverse the effect of ovariectomy in rats (Andersson *et al.* 1998). In the present study, we used 150 $\mu\text{g/kg}$ per day PTH(1-84), whereas most previous studies used PTH(1-34). The equimolar amount of PTH(1-34) would be 66 $\mu\text{g/kg}$ per day. *In vivo* studies directly comparing the

Figure 4 Bone mineral content (BMC) measured *ex vivo* in the femur (A), tibia (B) and vertebra L5 (C) after 8 weeks of PTH treatment in ovx and sham-operated (SHAM) mice. BMC was measured by DXA; all bones that were compared were present in a single scan. Means $\pm$ S.E.M., $n=7-10$. ^aSignificantly ($P<0.05$) different from sham-operated; ^bsignificantly ($P<0.05$) different from vehicle-treated (ANOVA).

effects of human PTH(1–34) and human PTH(1–84) found that the two peptides at equimolar doses increased

the bone mass to a similar extent in rats (Mosekilde *et al.* 1991, Ejerstedt *et al.* 1993, Kimmel *et al.* 1993). Little information is yet available as to the *in vivo* effect of PTH in mice (Rihani-Bisharat *et al.* 1998, Zeng *et al.* 1998). However, the two peptides appear to be equipotent in mice also (Stanislaus *et al.* 2000).

Ovariectomy in the rat is associated with an increase in body weight (Wronski *et al.* 1987). Existing data on the effect of ovariectomy on the body weight in mice are inconsistent: one study has shown that ovariectomy results in an increased body weight (Bain *et al.* 1993), whereas other reports have failed to do so (Edwards *et al.* 1992, Sandstedt *et al.* 1996, Yoshitake *et al.* 1999). No significant effect on body weight was seen in the present study. Successful ovariectomy was confirmed by a significant decrease in uterine weights. In the present study we used young (but sexually mature) mice, whereas older mice were used in previous studies. This difference may, at least partly, explain the difference with respect to body weight gain after ovariectomy.

Total body BMC and total body areal BMD were measured by DXA at regular intervals throughout the study. Ovariectomy resulted both in reduced total body BMC and reduced total body areal BMD. In ovx mice, DXA revealed statistically significant PTH-evoked treatment effects on total body areal BMD only after 4 weeks, whereas no effect was seen after 8 weeks of treatment. No other treatment-evoked effects on total body areal BMD or on total body BMC were detected by DXA in ovx and sham-operated mice *in vivo*. *Ex vivo*, the excised bones were measured by DXA. These measurements revealed effects of PTH treatment on BMC in the femur and vertebrae L5 of both ovx and sham-operated mice. In a DXA scan, the areal BMD is a presentation of the bone mineral content (BMC) per bone area. As the cortical bone represents about 80% of the bone mass in humans (Ma *et al.* 1999), the amount of cortical bone in a mouse is likely to be more than 95%. Trabecular and cortical bone can not be separated by DXA. Therefore, most of the total body areal BMD and total body BMC, as measured by DXA, is accounted for by cortical bone. Thus the relatively low trabecular bone content in mice may explain the small effect of ovariectomy on total body areal BMD and total body BMC. Detection of treatment effects of an anabolic agent such as PTH, which mainly acts on trabecular bone, will therefore be difficult using the DXA technique in mice. If the DXA scan is performed on excised bones that contain a relatively larger amount of trabecular bone, such as a vertebra, the treatment effects will become more pronounced. As discussed above, some of the effects of PTH treatment in the present study were detected by DXA on the excised bones, but not by the *in vivo* measurements. The inter-scan variations between different DXA scans of mice *in vivo* are another problem. In the *in vivo* measurements, an internal standard was included in each scan to reduce this variation. This

Table 2 Trabecular volumetric BMD and cortical bone parameters measured in the femur by pQCT. Measurements were performed *ex vivo* after 8 weeks of intermittent PTH treatment

	Sham Vehicle	Ovx Vehicle	Sham PTH	Ovx PTH
Trabecular BMD (mg/ml)	87 ± 13	42 ± 12	171 ± 18†	84 ± 16*
Cortical BMC (mg/mm)	1.02 ± 0.02	0.81 ± 0.01*	1.03 ± 0.03	0.93 ± 0.03*†
Cortical BMD (mg/ml)	1116 ± 7	1152 ± 7*	1121 ± 9	1085 ± 5*†
Cortical area (mm ²)	0.86 ± 0.02	0.72 ± 0.01*	0.87 ± 0.02	0.80 ± 0.02†
Periosteal circumference (mm)	5.001 ± 0.042	4.761 ± 0.036*	4.963 ± 0.043	4.821 ± 0.080
Endosteal circumference (mm)	3.774 ± 0.046	3.686 ± 0.033	3.701 ± 0.044	3.632 ± 0.076
Cortical thickness (mm)	0.195 ± 0.004	0.171 ± 0.001*	0.200 ± 0.005	0.189 ± 0.003†
Moment of resistance (mm ³)	0.389 ± 0.015	0.282 ± 0.010*	0.384 ± 0.017	0.326 ± 0.023†
Moment of inertia (mm ⁴)	0.396 ± 0.013	0.307 ± 0.007*	0.398 ± 0.014	0.335 ± 0.014*

Values are mean ± S.E.M., *n* = 7–9 mice per group. *Significantly different from sham-operated (*P* < 0.05); †significantly different from vehicle-treated (*P* < 0.05) (ANOVA).

limited the number of mice that could be scanned at the same time. In the *ex vivo* measurements, however, all bones were scanned at the same time and inter-scan variation was avoided.

The pQCT technique measures the true volumetric BMD, and it may be used to measure trabecular and cortical bone separately. Trabecular volumetric BMD can be measured in the central part of the metaphysis of the distal femur and proximal tibia. Ovariectomy resulted in a markedly decreased trabecular volumetric BMD, confirm-

ing the findings of a recent study in which the same technique was used in mice (Windahl *et al.* 1999).

Notably, the pQCT – but not the DXA – technique detected a pronounced effect of PTH on trabecular bone as soon as after 2 weeks of treatment. After the animal was killed, the femur was excised and the trabecular volumetric BMD was measured in the metaphysis of the distal femur. The trabecular volumetric BMD in the excised femurs was very similar to that obtained *in vivo* in the metaphysis of the proximal tibia, suggesting that the trabecular bone in the metaphyseal region of the proximal tibia reflects the trabecular bone at other locations also.

The effects of ovariectomy and PTH treatment on cortical bone were studied by pQCT in the diaphysis of the tibia and femur. Estrogen-deficient rats have been reported to have decreased cortical BMC (Yamazaki & Yamaguchi 1989), whereas PTH has been shown to exert anabolic effects on cortical bone (Dempster *et al.* 1993). Mid-diaphyseal pQCT scans on excised bones revealed that the cortical BMC, the cortical cross-sectional area and the cortical thickness were reduced after ovariectomy in both the femur (Table 2) and the tibia. The effects of ovariectomy were counteracted by 8 weeks of PTH treatment. This means that the effect of PTH on cortical bone of mice was similar to that reported in the rat (Wronski & Yen 1994). In contrast, no effect on cortical bone of PTH treatment was seen in sham-operated mice. Thus PTH treatment resulted in a pronounced and fast increase in the trabecular volumetric BMD in both ovx and sham-operated mice. In contrast, a statistically significant effect of PTH on cortical bone was only detected in ovx mice. It was, however, both delayed and less pronounced.

Both DXA and pQCT are equally time-consuming and labour-intensive. One disadvantage with the DXA equipment used in the present study was a relatively large between-scan variation in the *in vivo* measurements (see

Figure 5 High-resolution DXA scan of the femur (top) and vertebra L5 (bottom) from one representative animal in each of the drug treatment groups. The relative areal BMD is indicated: H=high areal BMD; L=low areal BMD.

above). The advantage of DXA compared with pQCT is the possibility of measuring the dimensions and the BMC both in the total body and in individual bones. One important disadvantage of using DXA for determination of bone mass is that it provides a picture of an attenuated X-ray passing through the body; accordingly, the picture obtained is two-dimensional (areal BMD, g/cm²). DXA therefore only recognizes changes in width and length, but does not account for changes in the third dimension. The major advantage of pQCT over DXA is the ability to examine a specific bone compartment. Thus cortical bone and trabecular bone can be analyzed separately. Furthermore, the between-scan variations are small in the pQCT measurements.

In conclusion, the pQCT technique is more sensitive than the DXA technique in the detection of bone loss after ovariectomy and increased bone mass after PTH treatment in mice. PTH treatment resulted in a pronounced and fast increase in trabecular volumetric BMD, whereas a less pronounced and later effect was seen on the cortical bone. Notably, the pQCT – but not the DXA – technique detected a pronounced effect as early as after 2 weeks of PTH treatment. Therefore dynamic *in vivo* measurements, using pQCT, are useful for the monitoring of skeletal changes in mice.

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