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ORIGINAL CONTRIBUTION

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Effect of ovariectomy on bone histology and plasma parameters of bone metabolism in nulliparous and multiparous sows

Einfluß der Ovariektomie auf Knochenhistologie und Plasmaparameter des Knochenstoffwechsels bei nulliparen und multiparen Sauen

Summary To investigate the suitability of the pig as animal model for postmenopausal osteoporosis, effects of ovariectomy (OVX) on bone metabolism and histology were studied in two groups of sows (9 months, nulliparous or 35 months, multiparous). A standard diet of about 1.5 % calcium (Ca) was fed till sacrifice at either 12 or 20 months post OVX when mineral content and histology were studied in representative bone specimens of proximal tibia, iliac crest and lumbar vertebrae. At 4, 8, 12, and 18 months post OVX, total and bone-specific alkaline phosphatase (AP_t, AP_b) calcidiol, calcitriol and parathyroid hormone (PTH) were measured in plasma.

In young sows OVX did not significantly affect plasma variables except for calcitriol, which was higher at 4 months post OVX. No significant differences between OVX or control animals were observed in the variables of bone chemical and histological analyses, neither 12 nor 20 months post OVX.

In multiparous sows OVX significantly increased PTH plasma concentrations at 8 months post OVX and plasma calcitriol, AP_t and AP_b at 12 months post OVX. All effects were moderate and transient. OVX did not significantly affect the variables of bone chemical and histological analyses neither 12 nor 20 months post OVX.

Although undoubtedly the clinical-chemical changes observed were not accompanied by any histomorphometric signs of osteopenia/osteoporosis, it must be left to future experiments as to whether this resulted from the ample calcium supply provided. This possibility is supported by recent observations showing that porcine osteopenia could be induced by OVX in animals maintained on only 0.75 % dietary calcium but not on higher (0.9 %) Ca regimens (33).

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Zusammenfassung In der vorliegenden Untersuchung sollte die Eignung des ovariektomierten Schweins als Modell für die postmenopausale Osteoporose beschrieben werden. Hierzu wurde der Einfluß der Ovariektomie (OVX) auf Plasmaparameter des Knochenstoffwechsels und auf knochenhistologische Parameter bei zwei Gruppen von Sauen (9 Monate alt, Nullipara oder 35 Monate alt, Multipara) untersucht. Eine Standarddiät für Schweine mit einem Calciumgehalt von 1,5 % wurde verabreicht. Gesamt- und knochenspezifische alkalische Phosphatase (AP_t, AP_b) Calcidiol, Calcitriol und Parathormon (PTH) wurden im Plasma 4, 8, 12 und 18 Monate nach OVX bestimmt. Der Mineralgehalt sowie histologische Parameter wurden in repräsentativen Knochenproben 12 Monate nach OVX und am Ende des Versuchs 18 Monate nach OVX gemessen.

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Bei jungen Sauen führte die Ovariektomie zu keinen signifikanten Veränderungen der Plasmaparameter mit Ausnahme höherer Konzentrationen des Calcitriols 4 Monate nach OVX. Es wurden weder 12 noch 20 Monate nach OVX signifikante Unterschiede zwischen OVX- und Kontrolltieren in den Parametern der chemischen oder histologischen Knochenanalyse beobachtet.

Bei multiparen Sauen waren die Plasmakonzentrationen des PTH gegenüber den Kontrolltieren 8 Monate nach OVX signifikant erhöht. Die Konzentrationen des Calcitriols sowie die Aktivität der AP_t und AP_b lagen 12 Monate nach OVX signifikant über den Werten der intakten Tiere. Alle Effekte waren moderat. Weder 12 Monate noch 20 Monate nach OVX kam es zu signifikanten Veränderungen in den Parametern der chemischen oder histologischen Knochenanalyse.

Obwohl die beobachteten Veränderungen in den klinisch-chemischen Plasmaparametern zweifellos keine histomorphometrischen Anzeichen einer Osteopenie/Osteoporose zur Folge hatten, bleibt es zukünftigen Untersuchungen überlassen nachzuprüfen, ob das Ausbleiben des Osteopenie ein Ergebnis der in diesem Experiment hohen Calciumzufuhr war. Eine solche Möglichkeit sollte aufgrund der Beobachtung in Betracht gezogen werden, daß Osteopenie beim Schwein nach OVX eintritt, wenn eine Diät mit geringerer Calciumzufuhr (0,75 % im Vergleich zu 0,9 %) verabreicht wurde (33).

Key words Ovariectomy – pig – bone histology – calcitriol – parathyroid hormone – alkaline phosphatase

Schlüsselwörter Ovariektomie – Schwein – Knochenhistologie – Calcitriol – Parathormon – alkalische Phosphatase

Abbreviation index AP_t = total alkaline phosphatase · AP_b = bone specific alkaline phosphatase *BV/TV* = bone volume/tissue volume · *CV* = coefficient of variation · *OVX* = ovariectomy *PTH* = parathyroid hormone *RIA* = radioimmunoassay *TBPf* = trabecular bone pattern factor · $25(OH)D_3$ = calcidiol $1,25\ (OH)_2D_3$ = calcitriol

Introduction

Osteoporosis is a multifactorial disease of the skeleton with considerable prevalence in Western society and increasing relevance for developing countries. Postmenopausal osteoporosis – a specific form of this disease – has been related to estrogen deficiency (39). Symptoms of osteoporosis develop over a long time period. Controlled long-term experiments in humans to elucidate the etiology or possibilities for prevention and therapy of osteoporosis are difficult and often impossible to perform. Therefore one has to rely on suitable animal models.

Ovariectomy has been established as a method to simulate the hormonal status of postmenopausal women. In rats, ovariectomy resulted in changes of bone mass and bone structure comparable to postmenopausal women (49, 12). Still, there are basic differences in bone metabolism between both species. It has been accepted for a long time that in rats there is no epiphyseal closure although in a more recent study this has been questioned (15). Furthermore, in the rat, in contrast to humans, bone modeling prevails; almost no intracortical remodeling has been observed (13, 14). Whereas remodeling is characterized by a coupling of osteoblast and osteoclast activities, modeling implies the independence of osteoblast and osteoclast activities (14).

Other species-specific differences, e.g. no reduction of circulating osteocalcin following Vitamin K antagonist administration in rats (36) in contrast to humans (47) demonstrate some limitations of the rat as animal model in the field of bone metabolism.

With respect to bone metabolism pigs are closer to humans than rats are. Miniature pigs have been used as models to investigate bone mineralization in the neonatal state (40) and remodeling has been described (48, 26). Moreover, the general anatomy and physiology of the pig is very similar to humans (6) and in many respects, especially in nutritional physiology, the pig or miniature pig is regarded as a very suitable animal model with regard to humans.

This experiment was performed to investigate the suitability of the ovariectomized pig as a model for postmenopausal osteoporosis in women. Both gilts (young, nulliparous) and old multiparous sows were ovariectomized and compared with intact control animals. Clinical-chemical parameters relevant to bone metabolism, and chemical composition and histological structure of bone were analyzed. In analogy to the human postmenopausal state bone loss and a rise in AP_b were to be expected.

Material and methods

Animals and sampling

Two different groups of pigs (German Landrace) were used: group one consisting of 14 young sows with an average age of 9 months, group two consisting of 14 old sows with an average age of 35 months. The young sows were fertile but had never been pregnant (nulliparous). The number of pregnancies of old sows (multiparous) varied between 3 and 7. The mean body weights at slaughter were 230 kg and 300 kg for young and old sows, respectively (Table 1).

Table 1 Basic data of sows

	Nulliparous sows		Multiparous sows	
	OVX[1]	C[2]	OVX	C
Age[3] (months)	9 ± 0	9 ± 0	30.4 ± 2.3	39.7 ± 3.8
Litters (n)	–	–	3.8 ± 0.4*	6.1 ± 0.7
Body weight[4] (kg)	226 ± 20	232 ± 15	294 ± 12	318 ± 12
Uterus weight (g)	104 ± 9***	974 ± 105	221 ± 27***	1 404 ± 172

Mean ± SEM, n = 7; [1] ovariectomized; [2] control group; [3] at timepoint of ovariectomy; [4] at slaughter;
* $p \leq 0.05$; *** $p \leq 0.001$ significantly different from control group

Estrogen deficiency was induced by bilateral ovariectomy (OVX) in 7 animals per group at time zero. Permission according to the national law for animal welfare was obtained from the authorities. Four animals per group were sacrified 12 months, and the remaining 20 animals 20 months after OVX. Blood samples were taken after an overnight fast and always at the same time 4, 8, 12, and 18 months after OVX by puncturing the vena cava cranialis to follow the time-course of parameters related to bone- or calcium metabolism such as calcium (Ca), phosphate, total alkaline phosphatase (AP_t), bone-specific alkaline phosphatase (AP_b), parathyroid hormone (PTH), calcidiol ($25(OH)D_3$), and calcitriol ($1,25(OH)_2D_3$).

At slaughter, the uterus was removed and weighed to provide a measure of estrogen production. Representative bone specimens were taken for chemical and histological analyses: *Iliac crest* 1 cm below the spina ileaca anterior superior, a transiliacal specimen of 15–20 mm x 5–10 mm; *proximal tibia* about 5 cm distal from the eminentia intercondylaria, a specimen of 5–15 mm medial, and first and second *lumbar vertebra*, sagittal, a specimen of 5–10 mm central.

Diet

The animals were fed 2.5–3 kg of a standard diet for breeding sows, based on barley and soy, containing 1.5 % calcium and 0.9 % phosphorous. The composition was as follows (g/kg): barley: 500; oats: 60; wheat: 150; alfalfa green flour: 60; protein concentrate: 100; soy concentrate: 80; dry yeast: 20; mineral and vitamin premixture: 30. Free access to tap water, containing 20.2 mg calcium/L, was given. Water intake was about 8 L/d, as observed in sows in similar housing conditions.

Analytical methods

Plasma calcium was analyzed directly by atomic absorption spectroscopy (Perkin Elmer 1100) using an air/acetylene-flame at 2 300 °C after dilution with a 0.5 % lanthanium chloride solution. Phosphate was estimated in EDTA plasma as inorganic phosphorous with an automatic analyzer (Cobas Bio, Hoffmann La Roche, Basel, Switzerland) using a test kit (Boehringer-Mannheim, Germany). PTH was determined in EDTA plasma using a radioimmunoassay (RIA) (Amersham Buchler, Braunschweig, Germany). The kit contained an antiserum of guinea pigs directed against the C-terminal end, and a goat-anti-guinea pig-globulin as precipitation reagent. Values for sensitivity, intra- and inter-assay variability were 15.4 pg/L, 6.5 % coefficient of variation (CV) and 7.2 % CV, respectively. Calcidiol was measured in EDTA plasma by RIA (Immundiagnostik, Bensheim, Germany) after acetonitrile precipitation. Values for sensitivity, intra- and inter-assay variability were 2.5 nmol/L, 9.8 % CV and 14 % CV, respectively. Calcitriol was measured in EDTA plasma by RIA (Immundiagnostik, Bensheim, Germany). For this process the plasma was cleaned by Extrelut extraction. Subsequently, a separation from other vitamin-D metabolites was achieved by HPLC (41). Values for intra- and inter-assay variability were 12 % CV and 17 % CV, respectively. AP_t and AP_b were determined in heparinized plasma either directly or as the difference after lectin precipitation, respectively. The kit (Boehringer-Mannheim, Germany) was based on a kinetic color reaction. Values for intra- and inter-assay variability were 1.55 % CV and 2.5 % CV, respectively.

The *chemical composition of bone* was analyzed in representative samples of iliac crest, tibia and 1st lumbar vertebra. Bone specimens were dried for 4 h at 105 °C to determine dry matter, and consequently ashed in a muffle furnace at 450 °C overnight. The ash was dissolved in 20 % (v/v) hydrochloric acid. Ca was analyzed by atomic absorption spectroscopy using an air/acetylene-flame at 2 300 °C (Perkin Elmer 1100) after dilution 1:1 000 with 0.5 % lanthanium chloride solution. Inorganic phosphorous was estimated with an automatic analyzer (Cobas Bio, Hoffmann La Roche, Basel, Switzerland) using a test kit (Boehringer-Mannheim, Germany).

For *histological analyses*, defined bone specimens of the 2nd lumbar vertebra, the iliac crest, and the proximal tibia were cut with a diamond saw, fixed in buffered formalin (4 %) and embedded in epon. The embedded material was cut with a microtome to 5 µm-thick sections.

These were stained with a modification of van Kossa technique which results in a black staining of mineralized tissue and a red staining of osteoid. The method was chosen because it allows an automatic image analysis by means of a video-based automatic image analysis system (IBAS II; Kontron, München, Germany) after a 10-fold magnification. Bone volume/tissue volume (BV/TV) and connectivity of trabeculae, i.e., trabecular bone pattern factor (TBPf) were analyzed. For calculating TBPf the spongiosa was assessed for bone area (A) and bone perimeter (P). The TBPf is the ratio of two measurements, before (1) and after (2) dilatation: TBPf (mm^{-1}) = (P1-P2)/(A1-A2). This value can be positive, if mainly convex forms prevail, i.e., isolated trabeculae are predominating. A negative value indicates mainly concave forms, i.e., a good connectivity between trabeculae. The method has been described in detail (19).

Statistics

The statistical evaluation was based on analysis of variance using the program "Statgraphics" (Vers. 5.0). Age, ovariectomy and the interaction of both were taken into account as fixed effects. The significance of differences was tested using an unpaired, two-tailed *t*-test (for AP, a one-tailed *t*-test was used). Since the time point of necropsy (12 months or 20 months) had no effect on bone parameters, analysis of variance for chemical and histological bone parameters (BV/TV and TBPf) was done on pooled samples of both time points. Means and standard error of the mean (SEM) are given throughout.

Results

In Table 1 some basic parameters of the experimental groups are given. Significant differences between the ovariectomized and the intact sows were observed in uterus weight, indicating a complete OVX.

In Figs. 1 and 2 the time-courses of clinical plasma parameters of multiparous old sows are given. Ca and phosphate plasma levels are nonspecific parameters regarding primary osteoporosis. Usually they remain constant unless a secondary form of osteoporosis occurs. In the present experiment OVX did not affect plasma calcium and phosphate plasma concentrations in old sows. Calcidiol tended to be elevated following OVX. However, plasma calcitriol and alkaline phosphatase (AP_b and AP_t) were significantly increased at 12 months post OVX, and PTH at 8 months post OVX.

In nulliparous sows OVX did not affect plasma parameters, except for calcitriol, which was significantly higher 4 months post OVX, and PTH which tended to be higher most of the time compared to control animals (Figs. 3 and 4). Compared to multiparous sows the age-dependent decrease in AP observed in growing minipigs

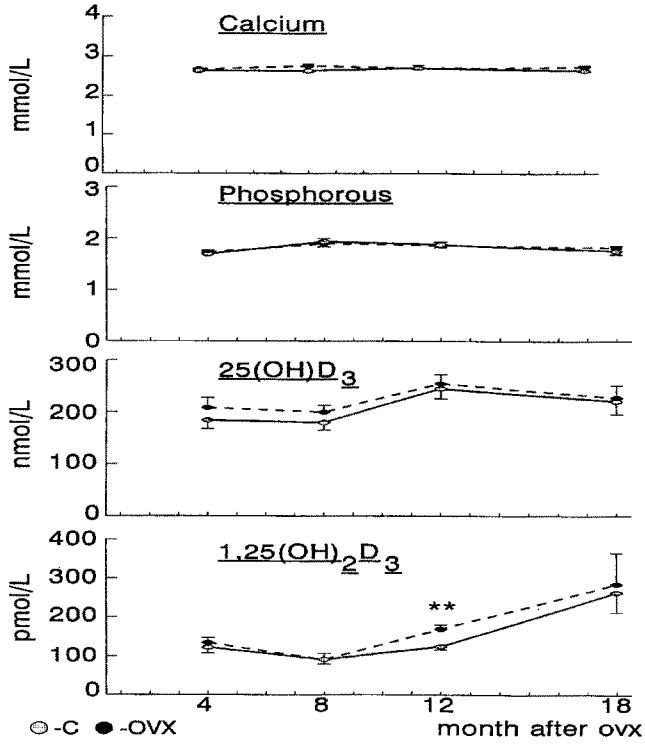

Fig. 1 Time-course of calcium, phosphorous, calcidiol, and calcitriol in plasma of ovariectomized multiparous sows. Mean and standard error of the mean, n = 7, resp. 5 at month 18; **: $p < 0.01$.

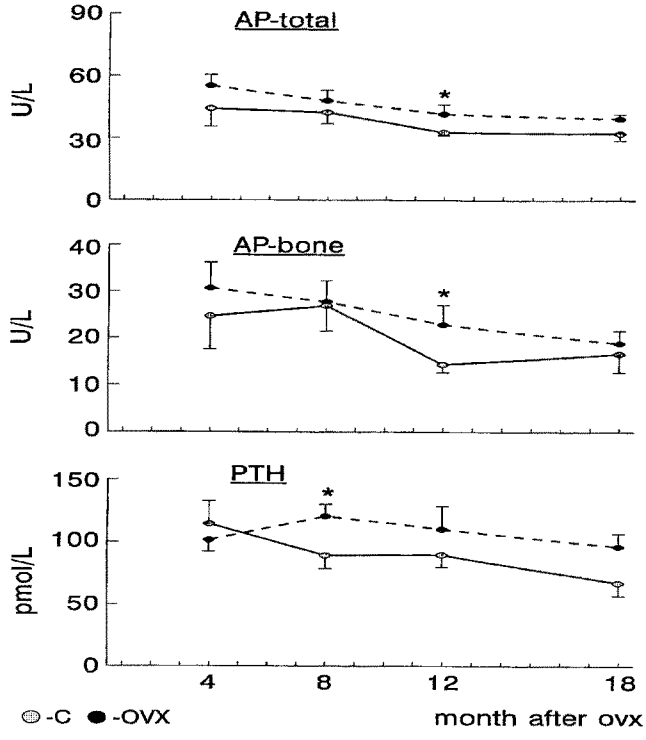

Fig. 2 Time-course of total alkaline phosphatase, bone specific alkaline phosphatase, and parathyroid hormone in plasma of ovariectomized multiparous sows. Mean and standard error of the mean; n = 7, resp. 5 at month 18; *: $p < 0.05$.

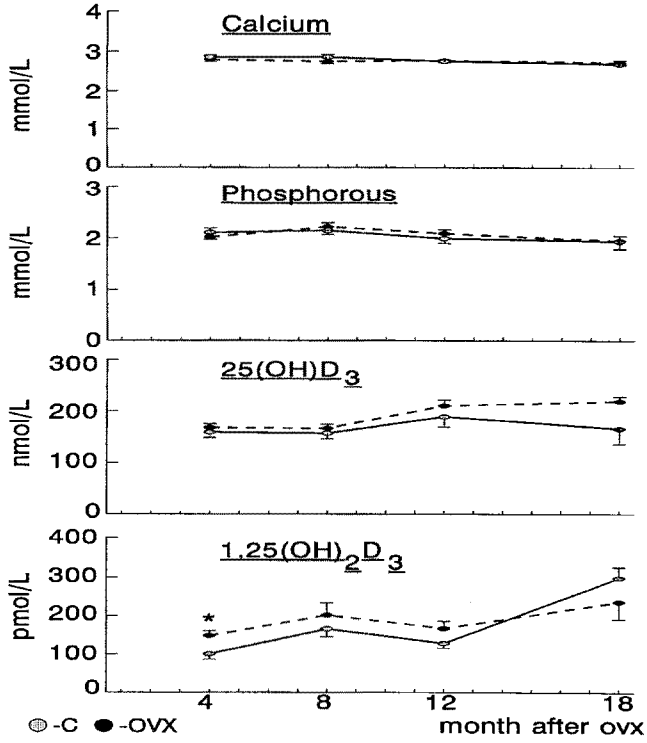

Fig. 3 Time-course of calcium, phosphorous, calcidiol, and calcitriol in plasma of ovariectomized young nulliparous sows. Mean and standard error of the mean; n = 7, resp. 5 at month 18;
*: $p < 0.05$.

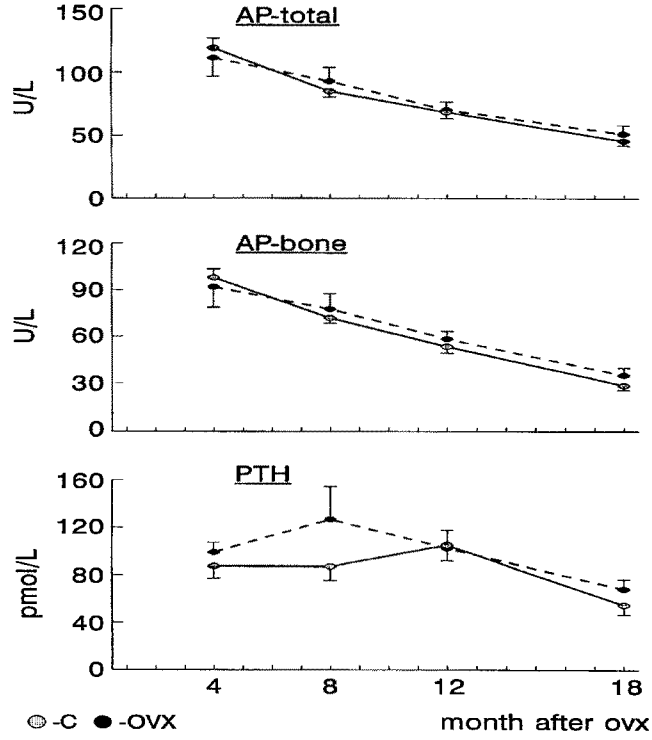

Fig. 4 Time-course of total alkaline phosphatase, bone specific alkaline phosphatase, and parathyroid hormone in plasma of ovariectomized young nulliparous sows. Mean and standard error of the mean; n = 7, resp. 5 at month 18.

(30) was more marked in these younger nulliparous animals following time according to their higher growing rate.

Multiparous sows tended to have less dry matter in the iliac crest and tibia following OVX as compared to intact animals. In total, however, OVX did not affect the parameters of bone density: Dry matter, ash, calcium and phosphorous as % of wet weight were not significantly different in iliac crest, tibia or lumbar vertebra compared to control animals. This was true for old multiparous (Table 2) and for young nulliparous sows (Table 3).

The results of the chemical analyses were supported by histological analyses. A normal architecture of spongiosa and trabecular connectivity was observed at the proximal tibia and ileac crest (not shown), and the 2nd lumbar vertebra (Fig. 5).

Signs for increased osteoclastic resorption were not observed and there was no increase in osteoid. Differences in structure of the 2nd lumbar vertebra between intact and OVX nulliparous and multiparous sows were not observed: The spongiosa in specimens of OVX animals showed a normal architecture (TBPf) and bone vol-

Table 2 Parameters of bone mass and bone density of iliac crest, proximal tibia and 1st lumbar vertebra of ovariectomized multiparous sows

	Ileac crest		Tibia		1st Lumbar vertebra	
Parameter	OVX[1)]	C[2)]	OVX	C	OVX	C
Dry matter (%)	77.6 ± 1.4	81.6 ± 1.0	88.6 ± 0.8	90.0 ± 0.7	77.2 ± 1.1	74.1 ± 1.0
Ash (% ww[3)])	37.0 ± 1.2	37.7 ± 2.2	43.5 ± 1.8	41.9 ± 0.8	42.8 ± 0.4	43.5 ± 1.6
Ca (mg/g ww)	137.4 ± 3.8	136.1 ± 8.3	152.2 ± 7.8	152.0 ± 3.1	146.7 ± 3.7	151.0 ± 3.5
P (mg/g ww)	57.5 ± 1.9	58.2 ± 3.8	69.6 ± 2.3	65.6 ± 1.2	68.1 ± 1.2	69.2 ± 1.8

Mean ± SEM; n = 7; [1)] ovariectomized; [2)] control group; [3)] wet weight

Table 3 Parameters of bone mass and bone density of ileac crest, proximal tibia and 1st lumbar vertebra of ovariectomized nulliparous sows

	Ileac crest		Tibia		1st Lumbar vertebra	
Parameter	OVX[1]	C[2]	OVX	C	OVX	C
Dry matter (%)	74.8 ± 1.8	71.1 ± 1.0	90.7 ± 2.6	90.1 ± 2.1	72.1 ± 0.5	73.7 ± 1.8
Ash (% ww[3])	32.6 ± 2.1	31.8 ± 1.2	44.3 ± 3.5	42.9 ± 1.8	40.4 ± 0.5	42.0 ± 1.2
Ca (mg/g ww)	120.6 ± 7.6	116.2 ± 4.3	163.8 ± 14.2	160.3 ± 7.4	144.4 ± 2.5	144.8 ± 3.1
P (mg/g ww)	51.8 ± 3.4	50.0 ± 2.1	71.6 ± 5.2	78.0 ± 4.7	65.1 ± 1.0	67.4 ± 1.5

Mean ± SEM; n = 7; [1] ovariectomized; [2] controlgroup; [3] wet weight

ume (BV/TV) (Table 4). This was true 12 and 20 months post OVX. Signs of degeneration were visible in multiparous (M) vs nulliparous sows as an effect of age and/or multiple lactations.

Table 4 Bone volume (BV/TV)[1] and bone structure (TBPf)[2] of 2nd lumbar vertebra in sows following ovariectomy

	BV/TV	TBPf
	(%)	(mm^{-1})
N[3]/OVX[4]	31.88 ± 2.28	-1.44 ± 0.22
N/C[5]	35.46 ± 1.92	-1.54 ± 0.19
M[6]/OVX	30.55 ± 1.92	-1.33 ± 0.19
M/C	32.65 ± 1.92	-1.38 ± 0.19

Mean ± pooled SEM, n = 7 except group N/OVX where n = 5; [1] bone volume/tissue volume; [2] trabecular bone pattern factor, for details see "methods"; [3] nulliparous; [4] ovariectomized; [5] control; [6] multiparous. There were no significant differences between groups

Discussion

In the present experiment we investigated whether the ovariectomized sow is a suitable model for postmenopausal osteoporosis in women. The criteria selected were clinical-chemical plasma parameters and the chemical and histological analyses of representative bone specimens. A decrease in bone mass and – structure was to be expected accompanied with a rise in AP_b in case that a high turnover osteoporosis had occurred.

We observed that OVX had no significant effect on clinical-chemical plasma parameters in *nulliparous* sows, with the exception of elevated calcitriol concentrations 4 months after surgery. Moreover, chemical and histological analyses of bone did not reveal significant differences between OVX and control sows. Consequently, nulliparous sows appear to be less suitable as animal model than multiparous sows for human postmenopausal osteoporosis. At the moment we have no explanation for the lower sensitivity of nulliparous sows to OVX compared with multiparous sows. In this connection it is of note that Spencer has described a lactational osteoporosis

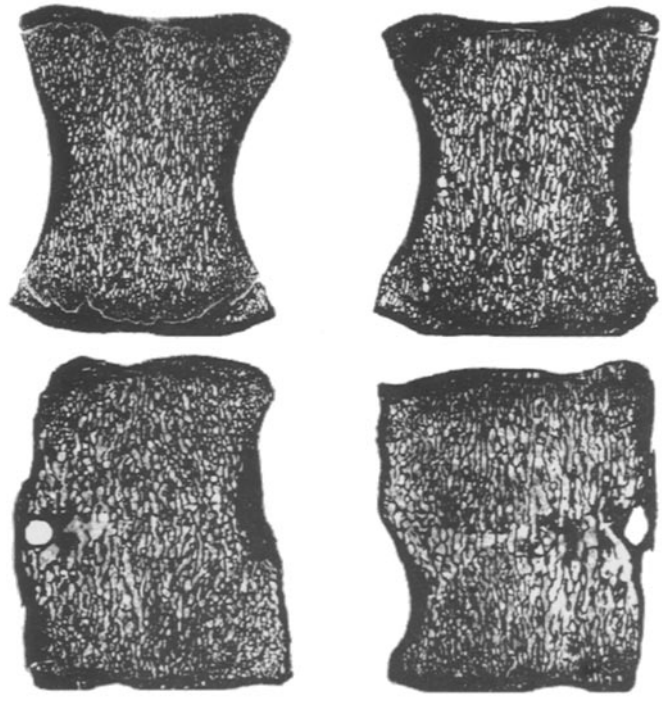

Fig. 5 Representative surface stained block grindings of undecalcified 2nd lumbar vertebra: Nulliparous sows (top) and multiparous sows (bottom); control (left), ovariectomized (right).

in pigs (44). In women, a correlation between the number of pregnancies or total time of breastfeeding and bone density is controversial (20, 22, 31).

In *multiparous* sows we observed a significant rise in plasma concentrations of PTH and calcitriol and in the activity of AP_t and AP_b as a consequence of OVX.

PTH stimulates the hydroxylation of calcidiol to calcitriol in the kidney. Furthermore, PTH stimulates osteoclast activity if it is administered in physiological doses (23) and therefore is regarded as indirect parameter of increased bone resorption, too (1). Elevated PTH concentrations have been observed by some authors in postmenopausal osteoporotic patients (46), and elderly people with hip fracture compared to healthy age-matched con-

trols (4), but not by others (17). In our experiment, PTH concentrations of multiparous sows were significantly increased 8 months after OVX and tended to remain higher for the rest of the experiment (Fig. 2). The chronological order of these changes could imply that OVX primarily stimulates PTH secretion in multiparous sows and as a consequence raises calcitriol concentration.

Calcitriol acts at different sites of calcium- or bone metabolism. Apart from stimulating renal calcium reabsorption and synthesis of calcium-binding protein in the intestinal mucosa, which means increasing calcium absorption (21), calcitriol also stimulates osteoclast activity (38, 28). In our experiment, multiparous old sows had significantly higher plasma concentrations of the biologically active metabolite $1,25(OH)_2D_3$, 12 months after OVX. The rise in calcitriol might also be related to estrogen reduction by OVX, since there is recent evidence that the calcitriol receptor of different organs is estrogen-regulated (10). In osteoporotic women conflicting results have been reported (16, 21).

It has been shown that patients with osteoporosis often suffer from a low vitamin-D status (2, 35). This could then result in a reduced calcium absorption rate contributing to osteoporosis. In our experiment there were no significant differences in plasma calcidiol between groups, indicating that no differences in vitamin D status caused the metabolic differences we observed following OVX.

Bone-specific alkaline phosphatase is a measure of osteoblast activity and positively correlated with the activity of these bone building cells. According to Delmas (9), increased activity of AP is an indicator of increased bone turnover. Total AP activity was elevated in old multiparous sows at all time points, significantly however only 12 months after OVX as compared to intact animals (Fig. 2). Bone-specific AP almost paralleled total AP. This finding agrees with AP activities in postmenopausal osteoporotic (37) or ovariectomized women (27, 43), but is in contrast to plasma AP concentrations of dogs following ovariectomy (29). The rise 12 months post OVX in the present study was of comparable magnitude as in women 12 months post OVX (45).

It is conceivable that the sum of the above changes in plasma concentrations of clinical-chemical parameters reflects an increase in bone turnover as a result of OVX. However, under these experimental conditions no significant changes either in bone mass or in histomorphometric parameters occurred. It is interesting to note that in one study reporting on premenopausal women following surgical ovariectomy, no significant decrease in bone mass was observed although plasma parameters of bone metabolism indicated a rise in bone turnover (43). No information on habitual or supplemented calcium intake of the patients was given.

At present, we are unable to answer our initial question with respect to the suitability of the ovariectomized pig as a model for postmenopausal osteoporosis in women. With regard to the chemical and histomorphometric bone analyses the pig may not appear to be a suitable model, since there was no osteopenia/osteoporosis following ovariectomy in our experiment, in contrast to primates (32), dogs (8), rats (49, 42) or even sheep (18).

However, the most probable explanation for our findings, namely a significant change in plasma parameters of bone metabolism indicating a rise in bone turnover but without symptoms of osteopenia, e.g. without decrease in bone mass or -structure may be the calcium content of the diet. The high intake of calcium, resulting from feeding a pig diet with 1.5 % Ca (this corresponds to 8 g Ca/70 kg body weight x day) may have prevented the demineralization of bone. The role of dietary calcium in preventing or postponing bone demineralization is controversial (34, 25). Differences in methodical approach (7) and difficulties assessing true calcium intake over a long period of time in investigations with humans (3) may contribute to these different data. In rats bone mineralization of ovariectomized animals on a high calcium diet (1.1 %) was not different from the intact control group on a diet containing 0.35 % calcium (5). We could confirm this positive correlation between calcium intake and bone development in growing and ovariectomized rats (24).

A recent study with ovariectomized minipigs (33) has shown that osteopenia also developed in this species, and that osteopenia was more pronounced in animals kept on a diet containing 0.75 % as compared to 0.9 % Ca in the diet. The observation that PTH did not rise following OVX (33) is in good agreement with our findings in nulliparous sows.

The calcium dependency of manifestation of osteoporosis as reported by Mosekilde et al. (33) can also be deduced from clinical observations: An intervention study in postmenopausal women has shown that a high calcium supply (1 or 2 g/d) reduced demineralization of bone. This dietary effect was more marked in late than in early postmenopausal women (11) indicating a higher susceptibility to dietary intervention at times not immediately following natural or artificial estrogen deficiency. In contrast to our experiment, all pigs were nulliparous and the time post OVX was only 6 months (33). Therefore a compensatory mineralization at longer times post OVX (i.e. 12–20 months) could have occurred in our study. In conclusion, the increased concentrations of plasma AP_b, PTH, and calcitriol leads us to hypothesize that ovariectomy initiated a rise in bone turnover in pigs. In this respect multiparous sows appear to be more sensitive than nulliparous animals. However, osteopenia may have been prevented by a high intake of calcium over a prolonged period of time.

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