

## Short- and long-term effects of calcium and exercise on bone mineral density in ovariectomized rats

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At the level of prevention of bone mineral loss produced by ovariectomy, the aim of the present study was to determine the effect produced by supplementation of Ca in the diet and a moderate exercise programme (treadmill), simultaneously or separately, in ovariectomized rats, an experimental model of postmenopausal bone loss. Female Wistar rats (*n* 110, 15 weeks old) were divided into five groups: (1) OVX, rats ovariectomized at 15 weeks of age, fed a standard diet; (2) SHAM, rats sham operated at 15 weeks of age, fed a standard diet; (3) OVX–EX, ovariectomized rats, fed a standard diet and performing the established exercise programme; (4) OVX–Ca, ovariectomized rats fed a diet supplemented with Ca; (5) OVX–EXCa, ovariectomized rats with the exercise programme and diet supplemented with Ca. The different treatments were initiated 1 week after ovariectomy and were continued for 13 weeks for subgroup 1 and 28 weeks for subgroup 2, to look at the interaction of age and time passed from ovariectomy on the treatments. Bone mineral density (BMD) was determined, at the end of the study, in the lumbar spine (L2, L3 and L4) and in the left femur using a densitometer. Bone turnover was also estimated at the end of the study, measuring the serum formation marker total alkaline phosphatase (AP) and the resorption marker serum tartrate-resistant acid phosphatase (TRAP). As expected, OVX rats showed a significant decrease ( $P < 0.05$ ) in BMD, more pronounced in subgroup 2, and a significant increase in AP and TRAP with regard to their respective SHAM group. The simultaneous treatment with Ca and exercise produced the best effects on lumbar and femoral BMD of ovariectomized rats, partially avoiding bone loss produced by ovariectomy, although it was not able to fully maintain BMD levels of intact animals. This combined treatment produced a significant increase in AP, both in subgroups 1 and 2, and a decrease in TRAP in subgroup 1, with regard to OVX group. The exercise treatment alone was able to produce an increase in BMD with regard to OVX group only in subgroup 1 of rats (younger animals and less time from ovariectomy), but not in subgroup 2. In agreement with this, there was an increase of AP in both subgroups, lower than that observed in animals submitted to exercise plus Ca supplement, and a decrease of TRAP in subgroup 1, without significant changes in this marker in the older rats. Ca treatment did not produce any significant effect on BMD in OVX rats in both subgroups of animals, showing a decrease of AP and TRAP levels in the younger animals with no significant variations in markers of bone remodelling in the older female rats compared with their respective OVX group.

### Osteoporosis: Calcium: Exercise: Bone mass: Bone markers: Rat

Bone is a dynamic tissue in a continuous process of formation and resorption, activities performed by the osteoblasts and the osteoclasts respectively. The increase in bone mass or, on the contrary, bone loss, depends on the balance between these two processes (Canalis, 1996). Bone turnover is regulated by systemic hormones and by

local factors. Oestrogens are included among these systemic hormones. Their action on bone remodelling seems to be mainly due to an inhibitory effect on osteoclast activity, as a result of both direct and indirect effects on these cells (Spelsberg *et al.* 1999).

After menopause, the depletion of oestrogens results in an

**Abbreviations:** AP, alkaline phosphatase; BMD, bone mineral density; EX, exercise programme; OVX, ovariectomized; SHAM, sham-operated; TRAP, tartrate-resistant acid phosphatase.

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increase in bone turnover with the rate of osteoclastic resorption exceeding the rate of osteoblastic formation, a fact that results in a loss of bone mass (Dempster & Lindsay, 1993). A rise in urinary Ca at menopause is another factor that contributes to the development of osteopenia (Nordin *et al.* 1991). These authors suggest that oestrogens promote tubular reabsorption of Ca and that the rise in bone resorption at the menopause could be accounted for, at least in part, by the effect of oestrogen deficiency on the kidney.

On the other hand, it is known that mechanical stress produced by physical activity increases bone mass (Smith & Raab, 1986). This effect seems to be mediated by the release of insulin-like growth factors by osteoblasts, which promote the proliferation of these cells (Zaman *et al.* 1997). On the contrary, immobilization has an effect on bone modelling and remodelling, through an increased activation of remodelling loci and a decrease of the osteoblast activity (Minaire, 1989).

There is controversy about the effectiveness that Ca or exercise have separately in the prevention of bone loss after menopause (Heaney, 1989; Wang & Zhang, 1998), although the negative effect that both low Ca intake or immobilization have on bone mass is well known (Cheng *et al.* 1991; Peterson *et al.* 1995).

There are some studies that have examined the combined effects of exercise and Ca supplementation, reflecting the existing controversy as to whether the combined treatment significantly improves the independent action of exercise or Ca (Mazess & Barden, 1991; Lau *et al.* 1992). This controversy is based on the variability among the different studies, due to the difficulty in performing well-controlled trials on this type of habits in human subjects. In this respect, experimental models present the advantage of a better control of the different variables, making quite reproducible studies possible. Thus, the ovariectomized (OVX) rat is a suitable experimental model for post-menopausal bone loss that faithfully reproduces the changes observed in human subjects and has the added benefit that the effects are detectable only a few months after intervention (Kalu, 1991; Díaz-Curiel & Gala, 1994).

At the level of prevention of bone mineral loss produced by ovariectomy, the aim of the present work was to determine the effect produced by a supplement of Ca in the diet and a moderate exercise programme (treadmill), together or separately, in OVX rats. Bone mineral density (BMD) techniques, and the determination of biochemical markers of bone formation (alkaline phosphatase, AP) and resorption (tartrate-resistant acid phosphatase, TRAP) have been used. These measurements were performed at two time-points after ovariectomy to examine the shorter-term and the long-term effects of the treatments on bone.

## Materials and methods

### *Experimental animals and treatment*

Female Wistar rats (*n* 110, 15 weeks old), weighing 252 (SD 30) g were used. Animals were kept under constant living conditions (22°C, 12 h light–dark cycle) and were allowed free access to food and drinking water. Five groups of twenty-two rats were established: group OVX, rats

ovariectomized at 15 weeks of age, fed with a standard diet; group SHAM, rats sham-operated at 15 weeks of age, fed with a standard diet; group OVX–EX, ovariectomized rats fed with a standard diet and performing the established exercise programme; group OVX–Ca, ovariectomized rats, fed with a diet supplemented with Ca; group OVX–EXCa, ovariectomized rats, performing the established exercise programme and fed with a diet supplemented with Ca.

In order to make the ovariectomy, a median laparotomy was performed to identify the right and left *cornu uteri* and their corresponding ovaries. After suture of the vascular plexus with fine linen thread, both ovaries were removed, followed by closure of the abdominal cavity.

Ten animals from each group were killed at 28 weeks of age (13 weeks after the beginning of the experiment, subgroup 1) and the other twelve animals were sacrificed at 43 weeks of age (28 weeks after the beginning of the experiment, subgroup 2). These two different subgroups were done in order to look at the influence of age and time since ovariectomy (menopause).

The different treatments were initiated 1 week after ovariectomy or sham operation and were continued until the end of the experiment (after 13 weeks for subgroup 1 and after 28 weeks for subgroup 2). The two different diets were supplied by PANLAB, S.A.<sup>®</sup> (Barcelona, Spain). Table 1 shows the composition of both diets in pellet form. They were identical in all their components except for their Ca concentration (6.6 g/kg standard diet and 15 g/kg Ca-supplemented diet) and were supplied to the rats *ad libitum*. The Ca content of the diet was confirmed through the determination of Ca content in weighed ashes. The exercise regimen consisted of daily training on a flat-bed treadmill. Rats ran 1080 m/d at 8 m/min, at intervals of 15 min exercise and 30 min of rest for 5 d/week. The compliance of the animals to the exercise regimen was total, because in the rolling band they could not be immobile. The run performed by the rats was equivalent to 4 km/d in man, taking into account comparisons of body weight, height and length from hip to heel.

The experimental animals were killed, after treatment for 12 weeks (subgroup 1) or 27 weeks (subgroup 2), by exanguination under diethyl ether anaesthesia. Once the blood was collected, animals were frozen at –20°C and

**Table 1.** Analytical composition of standard diet (g/kg)\*†

Protein	176.2
Fat	25.0
Cellulose	40.5
Total ash	43.8
Starch	443.3
Sodium	1.4
Retinyl acetate	0.0225
Ergocalciferol	0.000375
α-Tocopherol acetate	0.15
Lysine	8.5
Methionine	2.9
Calcium	6.6
Phosphorus	4.9
Moisture	105.4

\* Supplied by PANLAB<sup>®</sup> S.A. (Barcelona, Spain).

† Calcium-enriched diet had the same composition, except calcium content 15 g/kg.

transferred to a refrigerator 1 d before BMD determination. All procedures were carried out according to European Community Standards on the Care and Use of Laboratory Animals.

#### Serum assays

Serum obtained by cardiac puncture on the day of death was immediately frozen ( $-20^{\circ}\text{C}$ ) as aliquots.

Serum AP, creatinine and Ca levels were determined with use of an autoanalyser (Dade Dimension; Dade Behring, Newark, DE, USA), which uses the *p*-nitrophenyl phosphate kinetic, alkaline picrate and *O*-cresolphthalein complexone methods. Intra-assay and inter-assay CV of these methods were <1.2 and 4.7%, 1.4 and 1.9%, and 1.3 and 1.4% respectively.

TRAP values were determined with use of 4-nitrophenyl phosphate as substrate (1 mmol/l) in acetate buffer (50 mmol sodium acetate/l, 10 mmol sodium tartrate/l, pH 4.8). Samples were incubated at  $37^{\circ}\text{C}$  for 60 min. The reaction was stopped by the addition of 3 ml 1 M-NaOH. The absorbance of the samples was measured at 410 nm. Intra-assay and inter-assay CV were <3 and 6% respectively.

#### Bone mineral density

BMD was determined *in situ* in the lumbar spine (L2, L3 and L4) and in the left femur (after extraction) with use of a Hologic QDR 1000 (S/N 277; Hologic, las Rozas, Madrid, Spain) densitometer with a special program for short bones (Gala *et al.* 1998). Intra-assay and inter-assay CV were <0.53 and 1.2% respectively.

Lumbar bone mineral density was expressed as the mean of the values obtained for the L2, L3 and L4 vertebrae.

#### Statistical analysis

The results of the experiments were expressed as the mean values and standard deviations of the different variables. A comparison of the results of the different groups was performed with use of the unpaired Student's *t* test. A *P* value <5% ( $P<0.05$ ) was accepted as denoting a significant difference.

#### Results

Serum values of Ca and creatinine (99 (SD 6.5) mg/l, and 6.8 (SD 0.5) mg/l respectively) were all within the normal range.

Table 2 shows femoral BMD values for the different groups and the percentage of BMD loss with regard to the SHAM group. As expected, OVX rats showed a significant decrease in BMD (21.4% in subgroup 1 and 28.0% in subgroup 2) with regard to their corresponding SHAM group. In the subgroup 1 (13 weeks after ovariectomy and 12 weeks with the treatment), the treatment exclusively with Ca (group OVX-Ca) did not prevent the decrease in BMD (19.7%) produced by ovariectomy. However, both exercise alone or exercise plus Ca supplement produced a significant increase in femoral BMD compared with the OVX group without any treatment (femoral loss 12.4 or 10.3% respectively *v.* 21.4%), although femoral BMD was still significantly lower than that of the SHAM group. In the subgroup 2 (28 weeks after ovariectomy and 27 with the corresponding treatment), only the combined action of exercise and Ca, but not exercise or Ca, produced a significant increase in femoral BMD compared with the OVX group (bone loss 20.8 *v.* 28.0%).

Table 3 shows lumbar BMD in the different groups. The two subgroups of OVX rats showed a significant decrease in BMD with regard to their corresponding SHAM subgroup

**Table 2.** Femoral bone mineral density (fBMD) of 28-week-old rats, 13 weeks after ovariectomy (subgroup 1) and of 43-week-old rats 28 weeks after ovariectomy (subgroup 2)\*†  
(Mean values and standard deviations for ten rats in subgroup 1 and twelve in subgroup 2)

	fBMD (g/cm <sup>2</sup> per kg body weight)		Statistical significance of effect <i>v.</i> OVX‡	Mean BMD loss <i>v.</i> SHAM (%)
	Mean	SD		
<b>Subgroup 1</b>				
SHAM	0.959	0.066	$P<0.01$	–
OVX	0.754	0.089	–	21.4
OVX-EX	0.840	0.050	$P<0.05$	12.4
OVX-Ca	0.770	0.050	NS	19.7
OVX-EXCa	0.864	0.080	$P<0.01$	10.3
<b>Subgroup 2</b>				
SHAM	0.978	0.078	$P<0.01$	–
OVX	0.704	0.072	–	28.0
OVX-EX	0.742	0.061	NS	24.2
OVX-Ca	0.722	0.039	NS	26.2
OVX-EXCa	0.774	0.068	$P<0.05$	20.8

OVX, rats ovariectomized at 15 weeks of age, fed a standard diet; SHAM, rats sham-operated at 15 weeks of age, fed a standard diet; OVX-EX, ovariectomized rats fed a standard diet and performing the established exercise programme; OVX-Ca, ovariectomized rats fed a diet supplemented with calcium.

\* For details of diets and procedures, see Table 1 and p. 522.

† Treatments were initiated 1 week after ovariectomy.

‡ Student's *t* test.

**Table 3.** Lumbar bone mineral density (LBMD) of 28-week-old rats, 13 weeks after ovariectomy (subgroup 1) and of 43-week-old rats 28 weeks after ovariectomy (subgroup 2)\*†  
(Mean values and standard deviations for ten rats in subgroup 1 and twelve in subgroup 2)

	LBMD (g/cm <sup>2</sup> per kg body weight)		Statistical significance of effect v. OVX‡	Mean BMD loss v. SHAM (%)
	Mean	SD		
<b>Subgroup 1</b>				
SHAM	0.799	0.049	<i>P</i> <0.01	–
OVX	0.643	0.100	–	19.5
OVX–EX	0.701	0.050	<i>P</i> <0.05	12.4
OVX–Ca	0.614	0.050	NS	23.6
OVX–EXCa	0.731	0.080	<i>P</i> <0.01	8.6
<b>Subgroup 2</b>				
SHAM	0.818	0.078	<i>P</i> <0.01	–
OVX	0.570	0.056	–	30.3
OVX–EX	0.580	0.048	NS	29.1
OVX–Ca	0.576	0.063	NS	29.6
OVX–EXCa	0.663	0.067	<i>P</i> <0.05	18.9

OVX, rats ovariectomized at 15 weeks of age, fed a standard diet; SHAM, rats sham-operated at 15 weeks of age, fed a standard diet; OVX–EX, ovariectomized rats fed a standard diet and performing the established exercise programme; OVX–Ca, ovariectomized rats fed a diet supplemented with calcium.

\* For details of diets and procedures, see Table 1 and p. 522.

† Treatments were initiated 1 week after ovariectomy.

‡ Student's *t* test.

(19.5 and 30.3% respectively). In subgroup 1, exercise combined with Ca supplementation produced values of lumbar BMD between the OVX and the SHAM group (BMD loss 8.6%). The exercise treatment, group OVX–EX, also decreased bone loss due to ovariectomy (12.4 v. 19.5%). However, the treatment exclusively with Ca (group OVX–Ca) was not able to produce a significant increase in BMD compared with the OVX group. In a similar way to changes observed in femoral BMD, in subgroup 2, Ca

supplement (group OVX–Ca) or exercise (group OVX–EX) did not produce any significant change in the decrease in lumbar BMD due to ovariectomy (lumbar BMD loss of 29.6 and 29.1% respectively). However, the combined Ca plus exercise treatment produced values of BMD in femur between those of the OVX and the SHAM group (lumbar BMD loss 18.9%).

Table 4 shows serum TRAP levels in the different groups studied. The ovariectomy produced an increase in TRAP levels in subgroups 1 and 2 when compared with their respective SHAM groups. In subgroup 1 (younger rats and a shorter period of time after ovariectomy), the treatment with Ca, exercise or Ca plus exercise avoided the increase in TRAP levels produced by ovariectomy, showing OVX–Ca, OVX–EX and OVX–EXCa group levels of TRAP similar to those of the SHAM group. However, in subgroup 2, none of these treatments avoided the increase in TRAP levels produced by ovariectomy.

Table 5 shows AP levels in the serum of the studied groups. As was expected, ovariectomy produced a significant increase in AP levels in both subgroups of rats with regard to SHAM animals. The exercise alone (OVX–EX), or combined with Ca (OVX–EXCa), produced an increase in AP with regard to the OVX group. However, Ca supplementation does not produce significant changes in AP levels with regard to the OVX group without any treatment.

## Discussion

Previous studies have shown that the OVX rat is a suitable experimental model for postmenopausal osteoporosis that faithfully reproduces the changes observed in women. At present, many studies evaluating the effects produced by different treatments for postmenopausal osteoporosis in human subjects have employed rodent models (Kalu, 1991;

**Table 4.** Tartrate resistant acid phosphatase (TRAP) of 28-week-old rats, 13 weeks after ovariectomy (subgroup 1) and of 43-week-old rats 28 weeks after ovariectomy (subgroup 2)\*†

(Mean values and standard deviations for ten rats in subgroup 1 and twelve in subgroup 2)

	TRAP (lu/l)		Statistical significance of effect v. OVX‡
	Mean	SD	
<b>Subgroup 1</b>			
SHAM	21.6	4.6	<i>P</i> <0.05
OVX	26.6	6.0	–
OVX–EX	20.7	6.1	<i>P</i> <0.05
OVX–Ca	18.3	5.6	<i>P</i> <0.05
OVX–EXCa	20.2	6.5	<i>P</i> <0.05
<b>Subgroup 2</b>			
SHAM	11.5	2.1	<i>P</i> <0.01
OVX	19.2	5.8	–
OVX–EX	21.9	5.2	NS
OVX–Ca	18.2	5.5	NS
OVX–EXCa	17.5	3.2	NS

OVX, rats ovariectomized at 15 weeks of age, fed a standard diet, SHAM, rats sham-operated at 15 weeks of age, fed a standard diet; OVX–EX, ovariectomized rats fed a standard diet and performing the established exercise programme; OVX–Ca, ovariectomized rats fed a diet supplemented with calcium.

\* For details of diets and procedures, see Table 1 and p. 522.

† Treatments were initiated 1 week after ovariectomy.

‡ Student's *t* test.

Wronsky *et al.* 1991; Frost & Jee, 1992; Díaz-Curiel *et al.* 1998).

The current study shows that, in 13 weeks, ovariectomy induced the loss of BMD by 21.4% in the femur and by 19.5% in the lumbar vertebrae in rats ovariectomized at 15 weeks of age. BMD losses were 28.0 and 30.3% respectively compared to an age-matched sham group, 28 weeks after ovariectomy. In a previous study, Sato *et al.* (1994) reported that because significance could not be consistently shown for bone mineral content or area between SHAM and OVX rats for either vertebra or femora, BMD appears to be the better variable to quantitate bone changes in the rat.

In order to study the possible variations in bone remodelling which could explain BMD variations, we measured AP and TRAP, biochemical markers for bone formation and resorption respectively. Total AP in serum exists in various isozyme forms, the most common from skeletal, liver, intestine and placenta sources. Bone AP is localized in the plasma membrane of osteoblasts and released into the circulation during bone mineralization process. Due to the fact that liver and bone AP isoenzymes are quantitatively the most important in serum, in absence of liver disease, total AP can be considered, in spite of its lack of specificity, an indirect index of osteoblast activity and it is commonly used in diagnosing and monitoring bone formation rate (Moss, 1982).

TRAP is a lysosomal hydrolase, which has been shown to be released from the osteoclasts during bone resorption (Zaidi *et al.* 1989). Acid phosphatase is present in bone, spleen, prostate, erythrocytes and platelets. In serum, only bone and erythrocytes isoenzymes of acid phosphatase are insensitive to tartrate. Therefore, in absence of haemolysis, the activity of this isoenzyme, TRAP, can be used as an index of osteoclast activity, i.e. bone resorption (Ly *et al.* 1973).

The increase observed in AP and TRAP in OVX rats compared with their respective SHAM group, both 13 or 28 weeks after ovariectomy, reflects increased bone turnover which resulted in bone loss and the decline in BMD. Other studies reported higher levels of serum osteocalcin and total AP, and a higher urinary excretion of deoxypyridinoline: creatinine, a marker of bone resorption, 3 months after surgery in OVX rats, compared with the SHAM group (Kippo *et al.* 1998). This pattern is quite similar to that observed in postmenopausal osteoporotic women, in which a general increase of biochemical markers of bone turnover is found with a predominance of the resorptive process, leading to BMD loss (Garnero *et al.* 1996).

With regard to the effect produced by exercise, OVX rats of subgroup 1 undergoing the exercise programme (OVX-EX) presented a significant increase in femoral and lumbar BMD with regard to their corresponding OVX group. However, in subgroup 2, female rats which had oestrogen depletion for a longer period of time, values of lumbar and femoral BMD do not differ significantly between OVX and OVX-EX rats. These results agree with the hypothesis sustained by several authors that exercise produces a positive effect in bone mass in young (Newhall *et al.* 1991; Yeh *et al.* 1993b; Mosekilde *et al.* 1994) but not in adult (Raab *et al.* 1990; Yeh *et al.* 1993a, 1994; Chen *et al.* 1994) animals.

**Table 5.** Serum alkaline phosphatase (AP) of 28-week-old rats, 13 weeks after ovariectomy (subgroup 1) and of 43-week-old rats 28 weeks after ovariectomy (subgroup 2)\*†

(Mean values and standard deviations for ten rats in subgroup 1 and twelve in subgroup 2)

	AP (Iu/l)		Statistical significance of effect v. OVX‡
	Mean	SD	
Subgroup 1			
SHAM	125	48	$P < 0.05$
OVX	157	41	–
OVX-EX	179	51	$P < 0.05$
OVX-Ca	132	16	NS
OVX-EXCa	195	31	$P < 0.01$
Subgroup 2			
SHAM	77	19	$P < 0.05$
OVX	112	32	–
OVX-EX	171	60	$P < 0.01$
OVX-Ca	109	50	NS
OVX-EXCa	183	9	$P < 0.01$

OVX, rats ovariectomized at 15 weeks of age, fed a standard diet; SHAM, rats sham-operated at 15 weeks of age, fed a standard diet; OVX-EX, ovariectomized rats fed a standard diet and performing the established exercise programme, OVX-Ca, ovariectomized rats fed a diet supplemented with calcium.

\* For details of diets and procedures, see Table 1 and p. 522.

† Treatments were initiated 1 week after ovariectomy.

‡ Student's *t* test.

The variations in the levels of TRAP and AP produced by the exercise can explain the results observed in BMD. Exercise in the young rats whose menopause was more recent (subgroup 1) reduced losses in femoral and lumbar BMD and this was reflected in lower TRAP and higher AP levels, compared with the OVX group. However, in the older animals (subgroup 2), although AP levels were higher than those of the OVX group, TRAP levels were not reduced, and this could be the reason why these animals cannot improve their low BMD levels due to ovariectomy.

With regard to the benefits produced by exercise on BMD in the younger group of rats, our results agree with previous works (Iwamoto *et al.* 1998a,b) which justify the benefits produced by exercise on BMD because exercise decreases bone resorption and increases bone formation.

According to the finding of an increase in bone formation markers produced by exercise, it has been found, in *in vitro* experiments, that a mechanical strain produces an increase in cellular proliferation (Damien *et al.* 1998).

Ca supplementation does not produce any significant increase either in femoral or in lumbar BMD in the two subgroups of OVX rats. In the group of younger OVX rats, mean values of AP decreased, although not significantly with regard to OVX rats, and values of TRAP decreased significantly with regard to OVX rats. However, the decrease in TRAP was not followed by an increase in BMD values. In this respect, it is commonly accepted that Ca slows the remodelling and in this way it is important to take into account that this ion could slow remodelling-related loss (Heaney, 1991). In subgroup 2, OVX rats after a longer period of ovariectomy, Ca treatment did not produce significant changes either in AP or in TRAP with regard to non-treated OVX rats, and in consequence, BMD of OVX-Ca group was similar to that of OVX group.

As has been discussed, in our present study, Ca supplementation alone did not produce any increase in cortical or in trabecular bone in ovariectomized rats. However, in a study by Iwamoto *et al.* (1998a), the supplement of Ca to OVX rats produced a significant increase in lumbar (trabecular bone), but not in femoral BMD. Our work differs from that of Iwamoto *et al.* (1998a) in that these authors produced the osteopenia in the rats by ovariectomy and a Ca-deficient diet. This could be the reason why animals in the experiment performed by Iwamoto *et al.* presented an increase in BMD when they were fed with a normal-Ca diet. However, in our present study, all the groups of rats were fed with a standard content of Ca in the diet, although some groups received a Ca supplement. Other authors (Riis *et al.* 1987; Nordin & Morris, 1989; Dawson-Hughes *et al.* 1991; Fujita *et al.* 1993) found that Ca supplementation exerts beneficial effects especially on cortical bone. On the other hand, in a study recently performed on adolescent girls and young women in Europe, Kardinaal *et al.* (1999) observed that Ca itself has no significant effect on BMD.

In conclusion, the results of the present study suggest that the exercise treatment in OVX rats can partially restore lumbar and femoral loss in the early menopause, but not when more time has passed from the loss of ovary function. Ca treatment is not able to produce any significant improvement on bone loss due to ovariectomy. However, the combined action of Ca and exercise produces the best effects on BMD, partially avoiding bone loss produced by ovariectomy both in younger and older rats. We think that these findings could be extrapolated to human postmenopausal osteoporosis prevention.

Our results agree with several previous reports performed in human subjects. In an interesting study including 1075 women and 690 men aged 69 (SD 6.7) years, Nguyen *et al.* (2000) concluded that adequate dietary Ca intake and maintaining a physically active lifestyle in late decades of life could potentially translate into a reduction in the risk of osteoporosis and hence improve the quality and perhaps quantity of life in the elderly population. At this point, it is interesting to comment on the results published by Specker (1996) in a meta-analysis about the possible interaction between Ca intake and bone response to physical activity in adult subjects. According to the results of this analysis, physical activity has beneficial effects on BMD at high Ca intakes, with no effect at mean Ca intakes <1000 mg/d.

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