J Bone Miner Metab (2000) 18:258–263

Journal of
Bone and
Mineral
Metabolism

Original articles

The effects of nandrolone decanoate on bone mass and metabolism in ovariectomized rats with osteopenia

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Abstract The effects of nandrolone decanoate (ND) treatment on bone mass and metabolism were studied in ovariectomized (OVX) rats with osteopenia. The 6-month-old rats were divided into Sham (n = 12) and OVX (n = 24). The OVX rats were allowed to lose bone for 6 weeks. At 6 weeks post ovariectomy, the OVX rats were divided into two groups: (1) OVX + Vehicle and (2) OVX + ND. The effects of ND on bone mineral density (BMD), bone mineral content (BMC), and bone metabolism were studied by dual-energy X-ray absorptiometry (DXA) and biochemical markers including urinary pyridinoline (Pyr), deoxypyridinoline (Dpyr), and serum osteocalcin. After 24 weeks of treatment, histomorphometry of the right tibiae and the wet weight of the gastrocnemius and soleus skeletal muscles were also examined. Ovariectomy resulted in a significant increase in biochemical markers and a significant decrease in spine BMD (0.221 ± 0.016 g/cm^2 in OVX group *vs* 0.239 ± 0.008 g/cm^2 in Sham group) and BMC (0.550 ± 0.055 g in OVX group *vs* 0.605 ± 0.042 g in Sham group) at 6 weeks post ovariectomy. Spine BMD (0.227 ± 0.017 g/cm^2), femoral BMD (0.263 ± 0.012 g/cm^2), and bone density of femur (1.035 ± 0.036 g/cm^3) in the OVX + ND group were significantly greater than those in the OVX + Vehicle group (0.204 ± 0.013 g/cm^2 for spine BMD, 0.243 ± 0.009 g/cm^2 for femoral BMD, 0.938 ± 0.06 g/cm^3 for bone density of femur) after 24 weeks of treatment. ND treatment decreased urinary Pyr and Dpyr significantly in OVX rats. Histomorphometric findings indicated that ND-treated rats had greater cancellous bone volume, greater trabecular number, greater trabecular thickness, and less trabecular separation than vehicle-treated OVX rats. OVX rats had greater wet weight of the gastrocnemius and soleus muscles than rats treated with ND. The data suggest that the effect of ND on bone mass is not influenced by the condition of the muscles in OVX rats. Our findings indicate that ND blocks further bone loss by inhibition of bone resorption in OVX rats with osteopenia.

Key words Anabolic steroid · Biochemical markers · Bone mineral density · Osteocalcin · Pyridinium cross-links

Introduction

Nandrolone decanoate (ND) is a refined anabolic steroid that has been used for many years in the treatment of osteoporosis in Europe, Australia, and Japan [1–3]. Although the effectiveness of ND in the treatment of established osteoporosis has been documented, the mechanism of ND remained unknown.

Although ND treatment has been reported to increase midradius bone mineral density (BMD) by 5%–7% in the first year of treatment [2,3], one study showed that most of this increase was an artifact caused by alterations in the content of fat and muscle [4]. The recent study also suggested that a higher muscle mass induced by ND could partly explain the higher BMD in the group treated with hormonal replacement therapy with addition of ND [5]. Their results implied that at least part of the positive effect of anabolic steroids on bone mass might be mediated through increased muscle mass.

On the other hand, in vitro studies indicated that anabolic steroids may affect bone directly. Anabolic steroids are derivatives of the male hormone testosterone. In vitro studies demonstrated that androgen receptors are present at low densities in osteoblast-like cells [6]. Osteoblast-like cells may be able to aromatize androgens into estrogens [7]. Androgens are shown to stimulate human and murine osteoblastic cell proliferation in vitro and to induce expression of the osteoblast line differentiation marker alkaline phosphatase, presumably through an androgen receptor-mediated mechanism [8].

In vitro studies have suggested that anabolic steroids may affect bone by an androgen receptor-mediated

Offprint requests to: M. Takahashi
Received: August 25, 1999 / Accepted: January 28, 2000

mechanism; however, very little is known about how anabolic steroids affect bone in vivo. Recently, the effect of ND on bone in ovariectomized (OVX) rats was reported when ND was injected at the same time as ovariectomy [9]. However, the rats were young; the effect of ND on bone in adult OVX rats with osteopenia has not been published.

In this study, the longitudinal and long-term effects of ND treatment on bone metabolism and bone mass were studied in adult OVX rats with osteopenia, using dual- energy X-ray absorptiometry (DXA), biochemical markers, and histomorphometry.

Materials and methods

Groups and protocol

The experiment was conducted in 6-month-old female Wistar rats. Thirty-six rats were randomly divided into two groups: one group (n = 12) was sham operated (Sham) and the other group underwent operation (OVX) (n = 24). Bilateral ovariectomies were performed from a dorsal approach in 24 animals under general anesthesia. The OVX rats were allowed to lose bone for 6 weeks. At 6 weeks post ovariectomy, the OVX rats were further divided into two groups: one group (n = 12) received the solvent vehicle (OVX + Vehicle) and the other group (n = 12) was injected with ND (OVX + ND). The ND treatment began 6 weeks post ovariectomy. ND (Deca Durabolin; Nippon Organon, Tokyo, Japan) was injected subcutaneously once a week for 24 weeks at 1.0 mg/kg body weight; the rats in the OVX + Vehicle group were injected subcutaneously once a week with 0.2 ml sesame oil.

Treatments lasted 24 weeks. The animals were maintained on normal rat chow and housed with a 12-h light:dark cycle. Tap water and rat chow were supplied ad libitum. The spine bone mineral density (BMD) and bone mineral content (BMC) were measured before ovariectomy and at 6, 12, 18, and 24 weeks after ovariectomy. Urine samples were collected for 24 h in tubes containing 1 ml of 1 N HCl before ovariectomy and at 6, 12, 18, and 24 weeks after ovariectomy. The animals were killed at 30 weeks after the commencement of the experiment and the right femora were excised for determination of BMD and BMC. The right tibiae were excised for histomorphometric examination.

Biochemical markers

Serum osteocalcin was measured by radioimmunoassay (RIA). The RIA protocol was essentially that recommended by the supplier of the reagents (Biomedical Technologies, Stoughton, MA, USA). Urinary pyridinoline (Pyr) and deoxypyridinoline (Dpyr) were measured by automated solid-phase extraction and reversed-phase high performance liquid chromatography [10]. The values of Pyr and Dpyr in urine samples were expressed per millimole of urinary creatinine. Before hydrolysis, urinary creatinine content was determined enzymatically with an aliquot of each urine sample using a clinical chemistry analyzer (CL–20; Shimadzu, Tokyo, Japan). The coefficients of variation of Pyr and Dpyr for several measurements performed on the same day on the same urine sample were 2.9% and 4.4%, respectively.

Bone mineral measurements

BMD and BMC of lumbar vertebrae were measured by DXA using a Hologic QDR-1000 plus bone densitometer (Hologic, Waltham, MA, USA) adapted for measurement in small animals. A regional high-resolution software program (line spacing, 0.0254 cm; resolution, 0.0127 cm) was used with a 1-mm-diameter X-ray collimator that was simply inserted over the original collimator. The lumbar spine was scanned at the levels of the vertebrae L1–L6. The lumbar spine scan was performed in the supine position. The coefficients of variation of BMD and BMC for several measurements performed at different weeks on the same frozen rats were 0.91% and 1.06%, respectively. Excised femora were measured as described previously [11].

Measurement of bone density

After densitometry, the volumes of femora were measured according to Archimedes' principle. To measure dry and ash weight of femora, the right femora were dried at 120°C for 12 h and thereafter ashed in a muffled furnace at 800°C for 12 h. Bone density was calculated by dividing dry weight by volume.

Bone histomorphometry

The excised proximal tibiae were stained by Villanueva bone stain (Maruto, Tokyo, Japan) without demineralization, and then were processed, embedded, and sectioned longitudinally using a Reichert-Jung Supercut microtome (Reichert-Jung, NuBloch, Germany). From the center of the proximal tibiae, 10-mm nonconsecutive sections were obtained. Bone parameters were quantified within a 2 × 2 mm area of trabecular bone tissue that was standardized at distances greater than 1 mm from the growth plate–metaphyseal junction to exclude the primary spongiosa. Five fields from each section, situated equidistant from the cortex and 1 mm distal to the lowest point of the growth plate, were measured. The measured parameters included total tissue area,

trabecular bone area, and perimeter. These data were used to calculate the percentage of cancellous bone volume, trabecular thickness, number, and separation according to the formula described by Parfitt et al. [12].

Statistical analysis

The significance of difference between the two groups was evaluated with an unpaired two-tailed Student's *t* test, using StatView II on a Macintosh computer. Comparison among three groups was done by one-way analysis of variance (ANOVA). Values of P less than 0.05 were considered significant.

Results

Establishment of osteopenia model

Ovariectomy resulted in significant decreases in spine BMD and BMC and significant increases in urinary Pyr, Dpyr, and serum osteocalcin at 6 weeks after operation (Table 1).

Effects of ND on body weight, spine BMD, femoral BMD, and bone density of femur

Both OVX + Vehicle and OVX + ND rats gained body weight significantly during the experiment. There was no significant change in percent change of body weight between OVX + ND and OVX + Vehicle except a significant decrease in OVX + ND at 12 weeks post ovariectomy (data not shown). Figure 1 shows the effects of ND on spine BMD, femoral BMD, and bone density of the femur in OVX rats. There were significant differences in spine BMD, femoral BMD, and bone density of femur between the Sham and OVX + Vehicle groups. There were also significant differences in spine BMD, femoral BMD, and bone density of femur between the OVX + ND and OVX + Vehicle groups.

Longitudinal effects of ND on biochemical markers

ND did not result in significant change in urinary excretion of creatinine (data not shown). Urinary Pyr and Dpyr decreased 39% and 41%, respectively, at 12 weeks post ovariectomy in the OVX rats treated with ND. There was a significant difference in urinary excretion of Pyr and Dpyr between the OVX + ND group and the OVX + Vehicle group. There was no significant differ-

Table 1. Effects of ovariectomy on body weights, spine BMD and BMC, and biochemical markers in rats

	Sham (n = 12)	OVX (n = 24)
Body weight (g)	277.27 ± 14.38	298.94 ± 25.60**
Spine BMD (g/cm^2)	0.2389 ± 0.008	0.2213 ± 0.013***
Spine BMC (g)	0.6052 ± 0.042	0.5501 ± 0.055**
Serum osteocalcin (ng/ml)	95.83 ± 29.86	127.36 ± 18.72***
Urinary Pyr (nmol/mmol creatinine)	38.77 ± 13.59	73.73 ± 30.59***
Urinary Dpyr (nmol/mmol creatinine)	16.09 ± 8.39	39.74 ± 18.39***

Data are means ± SD
BMD, bone mineral density; BMC, bone mineral content; Pyr, pyridinoline; Dpyr, deoxypyridinoline
** $P < 0.01$; *** $P < 0.001$ vs Sham group

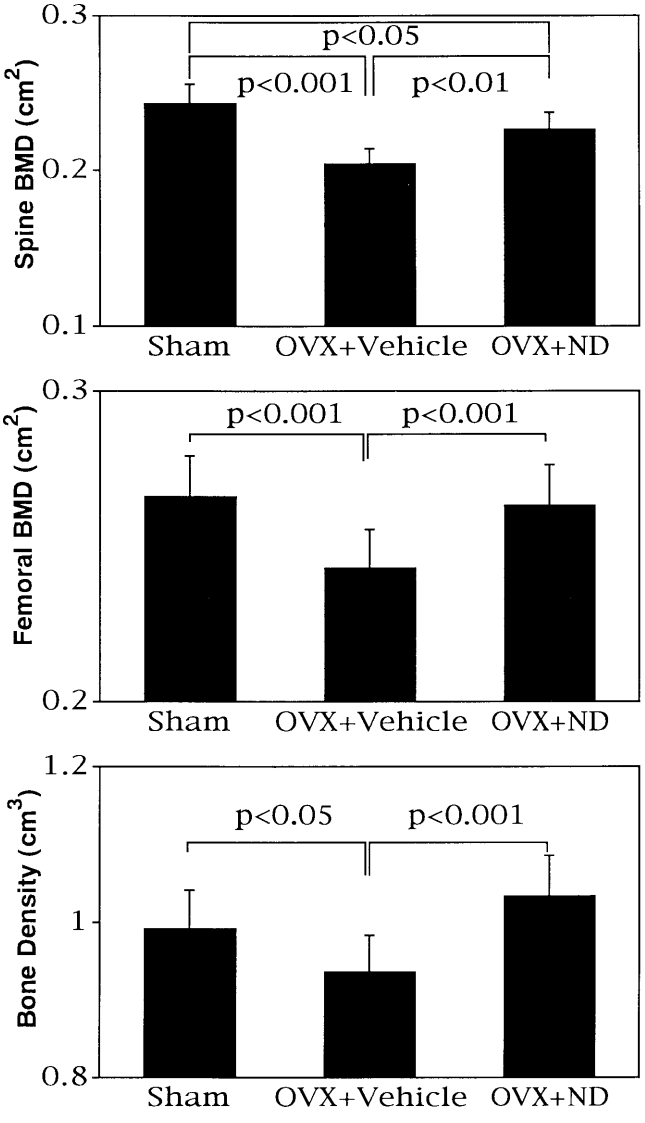

Fig. 1. Effects of nandrolone decanoate (*ND*) on spine bone mineral density (*BMD*), femoral BMD, and bone density of femur at 30 weeks post ovariectomy in ovariectomized (*OVX*) rats. Data are given as mean ± SD

ence in serum osteocalcin between the OVX + ND group and the OVX + Vehicle group (Fig. 2).

Long-term effects of ND on cancellous bone volume and trabecular architecture of secondary spongiosa in the proximal tibia

In the OVX + Vehicle rats, cancellous bone volume was significantly decreased when compared with both Sham and OVX + ND rats at 30 weeks post ovariectomy. The decrease of cancellous bone volume accompanied a poor trabecular architecture as reflected by significantly decreased trabecular thickness and number and increased trabecular separation compared with both Sham and OVX + ND rats. ND treatment increased cancellous bone volume significantly; the increase of cancellous bone volume was accompanied by an improved trabecular architecture as reflected by trabecular thickness, number, and separation (Fig. 3).

Effect of ND on wet weight of muscle

At 30 weeks post ovariectomy, OVX rats had greater wet weight of gastrocnemius and soleus than rats treated with ND (Table 2). There was no significance in wet weight of gastrocnemius and soleus between the Sham and the OVX + ND groups.

Discussion

This is the first report about the effects of ND on bone in OVX rats with osteopenia. Our findings indicate that ND blocks further bone loss by inhibiting bone resorption, as indicated by densitometry, histomorphometry, and urinary pyridinium cross-links in OVX rats with osteopenia. The effect of ND on bone mass is not influenced by the condition of the muscles in OVX rats.

Recently, a study in which ND was injected at the same time as ovariectomy in the rat suggested that ND prevented bone loss caused by ovariectomy [9]. However, the effect of ND on bone in OVX rats with osteopenia has not been published. In our study, ND-treated rats had greater spine BMD and femoral BMD measured in vivo and in vitro, respectively. Histomorphometric findings also indicated that ND-treated rats had greater cancellous bone volume, greater trabecular number, greater trabecular thickness, and less trabecular separation than vehicle-treated OVX rats.

Ovariectomy resulted in a higher turnover of bone as indicated by urinary Pyr, Dpyr, and serum osteocalcin (see Table 1) in this study. Our results concerning biochemical markers in OVX rats are consistent with earlier studies as to biochemical markers and histomor-

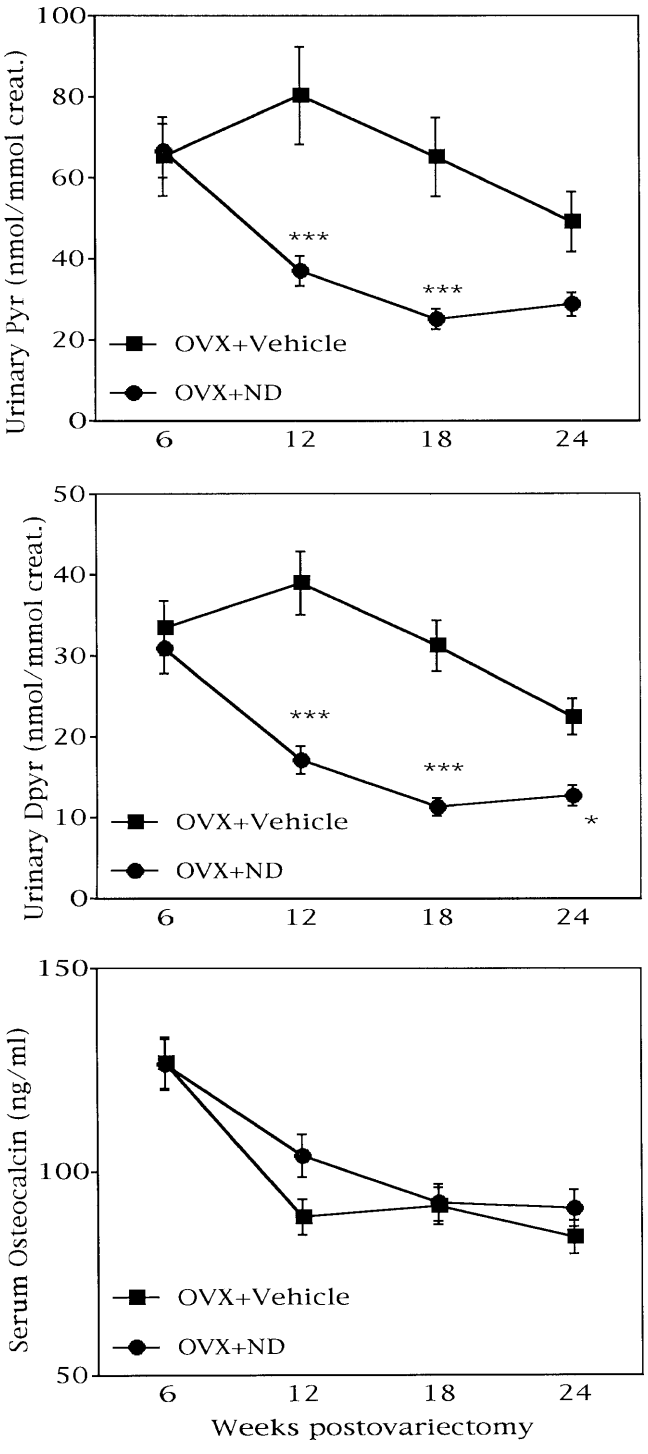

Fig. 2. Effects of ND on urinary excretion of pyridinoline (*Pyr*), deoxypyridinoline (*Dpyr*), and serum osteocalcin in OVX rats. OVX + Vehicle group ($n = 12$); OVX + ND group ($n = 12$). Data are given as mean ± SE. $^{*}P < 0.05$; $^{***}P < 0.001$ vs OVX + Vehicle

Table 2. Effects of ND on wet weight of musculi gastrocnemius and soleus in OVX rats

	Sham (n = 12)	OVX + Vehicle (n = 12)	OVX + ND (n = 12)
Soleus and gastrocnemius (g)	1.73 ± 0.11***	2.00 ± 0.18	1.80 ± 0.08**
Soleus (g)	0.40 ± 0.03*	0.48 ± 0.07	0.42 ± 0.08
Gastrocnemius (g)	1.33 ± 0.11**	1.52 ± 0.15	1.38 ± 0.09*

Data are means ± SD
ND, nandrolone decanoate; OVX, ovariectomized
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs OVX + Vehicle

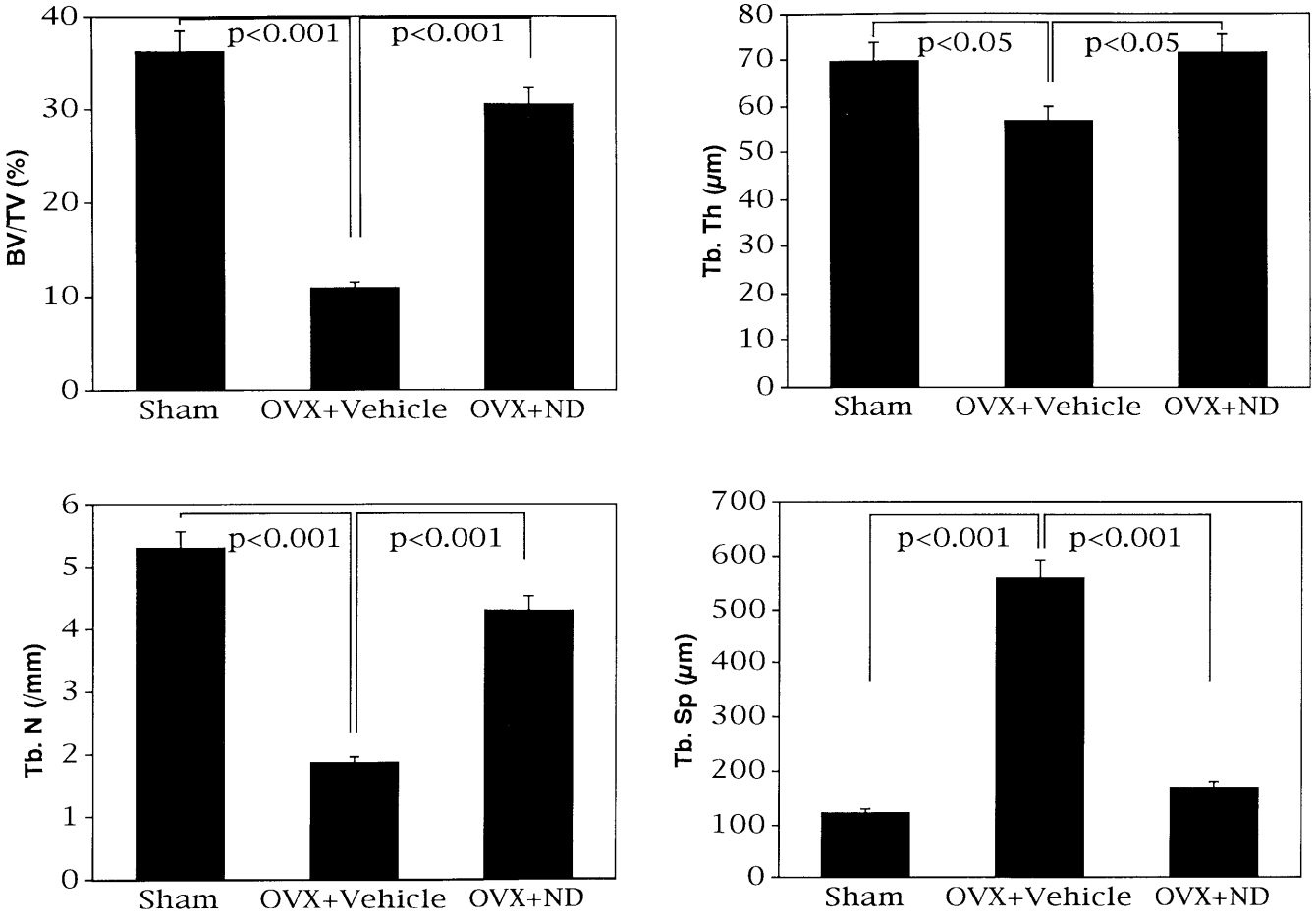

Fig. 3. Effects of ND on cancellous bone volume (BV/TV), trabecular thickness (*Tb.Th*), trabecular number (*Tb.N*), and trabecular separation (*Tb.Sp*) of the proximal tibia at 30 weeks post ovariectomy in OVX rats. Data are given as mean ± SE

phometry [13–15]. ND treatment inhibited bone resorption in OVX rats as indicated by the changes in Pyr and Dpyr. Other studies have shown significant reduction of hydroxyproline after ND treatment [2,16]. Urinary calcium/creatinine was found to be reduced after 6 months of treatment with ND in rats [9]. Our results with urinary Pyr and Dpyr confirmed the earlier studies. Although a tendency to increased values of osteocalcin was present in the OVX + ND group, we could not find a significant difference in serum osteocalcin between the OVX + ND and OVX + Vehicle groups (see Fig. 2). The decreases in urinary Pyr and Dpyr could explain the effects of ND in preventing bone loss after ovariectomy. However, the changes in osteocalcin could not explain the further increase in BMD by stimulating bone formation.

Recent studies have suggested that aromatization of androgens into estrogens may be an important metabolic pathway for skeletal maintenance and may explain the protective effects of androgen on skeletal maintenance [17]. However, ND is a nonaromatizable androgen, so ND could not be aromatized into estrogen. Nonaromatizable androgens probably induce proliferation and differentiation of osteoblasts [8]. A direct effect of androgens on osteoclasts has not yet been proven. Androgens may inhibit bone resorption by inhibition of the recruitment of osteoclast precursors from bone marrow, by decreased secretion of interleukin 6 or prostaglandin E_2, or by increased sensitivity of marrow cells or osteoblasts for bone resorption-stimulating factors such as parathyroid hormone (PTH) [8]. A recent study suggested that male sex steroids, acting through the androgen-specific receptor, inhibit the expression of the interleukin 6 gene [18]. Therefore, ND may inhibit bone resorption by the androgen-specific receptor in OVX rats.

The studies of anabolic steroids in human suggested that at least part of the positive effect of anabolic steroids on bone mass might be mediated through increased muscle mass. Hassager et al. found that ND treatment changed the soft tissue composition in osteoporotic postmenopausal women toward a leaner and more muscular body [4]. A recent study also suggested that a higher muscle mass induced by ND could partly explain the higher BMD in the group treated with hormonal replacement therapy with the addition of ND [5]. However, in this study ND treatment did not increase muscle weight, because OVX + Vehicle rats had greater wet weight of gastrocnemius and soleus than the OVX rats treated with ND. The first reason for the difference between the expected increase in muscle weight after ND treatment and our results could be the effect of ovariectomy on body weight, as it is well known that ovariectomy increases body weight in rats. The increases in body weight caused by ovariectomy may be responsible for the increase in the wet weight of muscles in OVX rats. The second reason could be that the data on muscle weight were cross-sectional. The present study suggests that the effect of ND on bone mass is not influenced by the muscles in OVX rats.

In conclusion, our findings indicate that ND blocks further bone loss in OVX rats with osteopenia. The effect of ND on bone mass is not influenced by muscles in OVX rats.

Acknowledgments. The authors thank Dr. Hideyuki Murata for suggestion of the effect on muscles. The authors express their gratitude to NV Organon, So, The Netherlands, and Nippon Organon K.K., Tokyo, Japan, for providing nandrolone decanoate (Deca-Durabolin).

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