Plasma nitrate+nitrite levels are regulated by ovarian steroids but do not correlate with trabecular bone mineral density in rats

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Abstract

Nitric oxide (NO) is a mediator of bone metabolism and its production is under the control of gender hormones in several cell types or tissues. Changes in endogenous NO production, measured as plasma nitrate+nitrite levels, may therefore contribute to ovariectomy (OVX)-induced bone loss. We studied plasma nitrate+nitrite levels and trabecular bone mineral density (TBMD) 4 weeks after sham-operation or OVX in rats receiving various hormonal treatments. OVX decreased plasma nitrate+nitrite levels significantly and this was accompanied by a significant decrease in TBMD. Treatment with oral ethinyl oestradiol (EE) and subcutaneous 17β-oestradiol dose-dependently prevented the decrease in plasma nitrate+nitrite levels after OVX, but treatment with oral 17β-oestradiol did not. Oestrogen treatment, 17β-oestradiol (s.c. or orally) or EE (orally), prevented the OVX-induced decrease in TBMD. Treatment of sham-operated rats with the anti-oestrogen ICI164,384 induced a significant decrease in TBMD that corresponded to 54% of the decrease observed after OVX, but did not affect plasma nitrate+nitrite levels. Treatment of ovariectomized rats with Org 2058, a pure progestagen, did not prevent bone loss, but prevented the decrease in plasma nitrate+nitrite levels dose-dependently. Treatment with tibolone, a synthetic steroid with combined weak oestrogenic, progestagenic, and androgenic properties, or with progestagen in combination with EE completely prevented bone loss after OVX. These treatments, however, only partly prevented the OVX-induced decrease in plasma nitrate+nitrite levels. In conclusion, OVX decreased both TBMD and plasma nitrate+nitrite levels. Although plasma nitrate+nitrite levels were under the control of both oestrogen and progesterone, TBMD was affected by oestrogen only. Decreased systemic production of NO is, therefore, not involved in OVX-induced bone loss in rats.


Introduction

Loss of ovarian function is associated with bone loss as a consequence of an imbalance between bone resorption and formation in favour of the first. This can be prevented by oestrogen replacement. Progesterone may also be involved, as some progestagens have been shown to reduce postmenopausal bone loss (reviewed by Prior 1990). The mechanisms by which these ovarian steroids may exert their protective effect on bone are not well understood.

may have a down-regulating effect on NO₃⁺NO₂ plasma levels in the presence of oestrogen (Rosselli et al. 1994, 1995, Cicinelli et al. 1996, Kawano et al. 1996, Imthurn et al. 1997).

In the present study, we hypothesized that loss of ovarian function causes a decrease in NO production, which in turn contributes to bone loss. To investigate this, sham-operated or ovariectomized rats were treated with various hormonal therapies and trabecular bone mineral density (TBMD) and NO₃⁺NO₂ plasma levels were determined 4 weeks after surgery.

Materials and Methods

Animals and materials

Mature 3-month-old unmated female Wistar rats (strain Hsd/Cpd:Wu), weighing approximately 225 g, were obtained from Harlan, the Central Institute for Breeding of Laboratory Animals, Zeist, The Netherlands. The animals were housed under standard conditions and kept with a ratio of 14 h light:10 h darkness in an air conditioned room (21 °C ± 2 °C). During the experiment the animals were housed individually, had free access to tap water and were pair-fed (16 g pelleted food per day (Hope Farms, Linschoten, The Netherlands)). All protocols were approved by the Animal Ethics Committee. Animals were divided into groups according to a randomized block design using body weight as the selection parameter. Rats were subjected to either OVX (n=140) or sham-operation (n=72) under general anaesthesia. From the day of operation onwards, the ovariectomized rats received various hormonal treatments (twice daily), consisting of vehicle (0·5% w/v gelatine and 5% w/v mannitol; n=54), ethinyl oestradiol (EE) (orally; n=6), 17β-oestradiol (orally; n=4–10, s.c.; n=8), (16α)-16-ethyl-21-hydroxy-19-norpregn-4-ene-3,20-dion (s.c.; Org 2058; n=6); a pure progestagen which exerts its effect via the progesterone receptor only, progestagen (s.c.) and EE (orally; n=6), or tibolone (orally; Org OD14: (7α,17α)-17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one; n=12) (de Visser et al. 1984). Sham-operated rats received control solution (n=60), the anti-oestrogenICI164,384 (s.c.; (7α,17β)-N-butyl-3,17-dihydroxy-N-methylene-1,3,5(10)-trienes-7-undecanamide; n=6) (Wakeling & Bowler 1987 in arachis oil, or the anti-progestagen RU 486 (orally, 11β-(4-(dimethyl-amino)phenyl)-17β-hydroxy-17α-(1-propynyl)-ESTRA-4,9-dien-3-one; n=6) (Baulieu 1989). All preparations were made by the Department of Pharmaceutics, N.V. Organon, Oss, The Netherlands and were of analytical grade. After 4 weeks treatment and overnight fasting, blood was collected from the abdominal aorta under general anaesthesia. Heparin plasma samples were centrifuged (3000 g) for 10 min at 4 °C, divided into small samples, and stored at −20 °C. The right femora were dissected out for TBMD measurement at autopsy.

Plasma nitrate+nitrite analysis

Plasma samples were deproteinized with 3% (final concentration) ZnSO₄ for 15 min and centrifuged at 30 000 g for 15 min. NO₃ in supernatants was reduced to NO₂ with oxidized cadmium in 0·56% NH₄Cl and 0·15% Borax-buffer (Sigma, Zwijndrecht, The Netherlands) for 30 min and vortexed every 5 min. Cadmium was oxidized by two treatments with 2% HCl for 30 min under continuous shaking. Before oxidized cadmium was added to plasma supernatants, pH was neutralized by repeated washing with distilled water. NO₂ levels in the reduced plasma supernatants were determined with Griess reagent consisting of 0·5% sulfanilamide, 0·05% naphthylethelene-diamine-dihydrochloride, and 2·5% H₃PO₄. Equal volumes (50 µl) of reduced samples and Griess reagent were mixed and incubated in a 96-well plate for 15 min at room temperature under continuous shaking. NO₂ concentration, proportional to OD₅₅₀, was determined using a microtiterplate reader (Thermomax, Molecular Devices, Menlo Park, CA, USA). Results are not differentiated into NO₃ and NO₂ but expressed as NO₃⁺NO₂ levels, as very little or no NO₂ is present in plasma of humans (Green et al. 1982) and rats (own observation). The percentage of NO₃ that was reduced to NO₂ was extrapolated from comparing standard curves of serial dilutions of NaNO₃ and NaNO₂ which had undergone the same procedure as the plasma samples. NO₃⁺NO₂ concentrations of the plasma samples were estimated from the NaNO₂ standard curve and corrected for the percentage NO₃ reduction to NO₂. The mean percentage NO₃ reduction to NO₂ was 72·7% ± 2·2 (range 64–79). The mean correlation coefficients of the NaNO₃ and NaNO₂ standard curves were 0·998 ± 0·001 (range 0·992–1·000) and 0·997 ± 0·001 (range 0·990–0·999) respectively.

Trabecular bone mineral density

TBMD (mg/cc) was measured directly in the distal metaphyseal part of the right femur by peripheral quantitative computed tomography (pQCT) adapted for measurements in small animals (Stratec XCT–960A, Stratec, Birkenfeld, Germany). A 360 ° X-ray scan, which had a standard thickness of 1 mm was taken. The scan had a resolution of 0·148 × 0·148 mm and was taken at 5·5 mm from the distal end of the femur, where TBMD of the metaphysyeal part was measured. Intra- and interassay variation for the measurements were 3%. The XCT–960A was calibrated with a standard of hydroxyapatite embedded in acrylic plastic.

Statistics

Statistical analysis was performed by one-way ANOVA for multiple comparison followed by Fisher’s PLSD (least significant difference test) test. Values are expressed as
Table 1  Plasma oestradiol levels and uterine weights of sham-operated and ovariectomized rats receiving various hormonal treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>17β-oestradiol plasma levels (µg/ml)</th>
<th>Uterine weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM and vehicle</td>
<td>–</td>
<td>18.3 ± 7.2</td>
<td>531.1 ± 21.1</td>
</tr>
<tr>
<td>OVX and vehicle</td>
<td>–</td>
<td>6.0 ± 0.7</td>
<td>133.5 ± 11.5*</td>
</tr>
<tr>
<td>OVX and EE (µg/rat per day, orally)</td>
<td>2 × 2.5</td>
<td>ND</td>
<td>380.2 ± 53.7†</td>
</tr>
<tr>
<td></td>
<td>2 × 10</td>
<td>ND</td>
<td>443.7 ± 30.0*†</td>
</tr>
<tr>
<td></td>
<td>2 × 40</td>
<td>ND</td>
<td>450.8 ± 22.6†</td>
</tr>
<tr>
<td>OVX and 17β-oestradiol (µg/rat per day, orally)</td>
<td>2 × 2</td>
<td>10.0 ± 2.7</td>
<td>205.9 ± 19.5*</td>
</tr>
<tr>
<td></td>
<td>2 × 125</td>
<td>11.8 ± 2.4</td>
<td>396.9 ± 28.0*†</td>
</tr>
<tr>
<td></td>
<td>2 × 250</td>
<td>18.3 ± 0.6</td>
<td>378.2 ± 21.9*†</td>
</tr>
<tr>
<td></td>
<td>2 × 500</td>
<td>37.1 ± 3.3†</td>
<td>468.4 ± 28.5*†</td>
</tr>
<tr>
<td>OVX and 17β-oestradiol (µg/rat per day, s.c.)</td>
<td>2 × 2</td>
<td>244 ± 102.0*</td>
<td>173.3 ± 73.4*</td>
</tr>
<tr>
<td>SHAM and ICI164,384 (mg/rat per day, s.c.)</td>
<td>2 × 10</td>
<td>ND</td>
<td>150.0 ± 68.4*</td>
</tr>
<tr>
<td>OVX and progestagen (Org 2058) (µg/rat per day, s.c.)</td>
<td>2 × 2.5</td>
<td>ND</td>
<td>161.0 ± 12.2*</td>
</tr>
<tr>
<td></td>
<td>2 × 40</td>
<td>ND</td>
<td>155.4 ± 12.5*</td>
</tr>
<tr>
<td>SHAM and RU 486 (mg/rat per day, orally)</td>
<td>2 × 2</td>
<td>29.5 ± 11.5</td>
<td>530.3 ± 65.6</td>
</tr>
<tr>
<td>OVX and progestagen and EE (µg/rat per day, s.c. and orally respectively)</td>
<td>2 × 40 and</td>
<td>ND</td>
<td>404.2 ± 12.7†</td>
</tr>
<tr>
<td></td>
<td>2 × 40</td>
<td>ND</td>
<td>514.7 ± 25.1†</td>
</tr>
<tr>
<td>OVX and tibolone (µg/rat per day, orally)</td>
<td>2 × 500</td>
<td>7.1 ± 2.4</td>
<td>379.9 ± 11.0†</td>
</tr>
</tbody>
</table>

*Significant versus SHAM (P<0.05); †significant versus OVX (P<0.05). ICI164,384, anti-oestrogen; RU 486, anti-progesterone; ND, not determined.

Results

In all experiments, OVX induced statistically significant decreases in uterine weight (Table 1), NO3+NO2 plasma levels (Figs 1A, 2A, 3A and 4A) and TBMD (Figs 1B, 2B, 3B and 4B). Four weeks after surgery, the mean plasma NO3+NO2 levels of the sham-operated and ovariectomized groups were 33.6 ± 23.1 (range 23.1–24.3) respectively. The mean plasma oestradiol levels of rats treated with control solution, but had no effect on the plasma levels of NO3+NO2 (Fig. 2A). As expected, plasma oestrogen levels of rats treated with ICI164,384 increased by about 13-fold (Table 1).

A discrepancy between plasma NO3+NO2 levels and TBMD was also found after progestagen treatment. Progestagen (2 × 40 µg/rat/day, s.c.) given to ovariectomized rats treated with control solution, but had no effect on the plasma levels of NO3+NO2 (Fig. 2A). As expected, plasma oestrogen levels of rats treated with ICI164,384 increased by about 13-fold (Table 1).

Treatment of ovariectomized rats with EE prevented the decrease in plasma NO3+NO2 levels induced by OVX (Fig. 2A). In contrast, administration of 17β-oestradiol subcutaneously (2 × 2.5 µg/rat/day) prevented the decrease in uterine weight (Table 1) and TBMD (Fig. 2B) in addition to the decrease in plasma NO3+NO2 levels in the ovariectomized rats (Fig. 2A).

The anti-oestrogen ICI164,384 (2 × 2 mg/rat/day, s.c.) given to sham-operated rats reduced uterine weights (Table 1) and TBMD (Fig. 2B) compared with sham-operated rats treated with control solution, but had no effect on the plasma levels of NO3+NO2 (Fig. 2A). As expected, plasma oestrogen levels of rats treated with ICI164,384 increased by about 13-fold (Table 1).

The anti-oestrogen ICI164,384 (2 × 2 mg/rat/day, s.c.) given to sham-operated rats reduced uterine weights (Table 1) and TBMD (Fig. 2B) compared with sham-operated rats treated with control solution, but had no effect on the plasma levels of NO3+NO2 (Fig. 2A). As expected, plasma oestrogen levels of rats treated with ICI164,384 increased by about 13-fold (Table 1).

A discrepancy between plasma NO3+NO2 levels and TBMD was also found after progestagen treatment. Progestagen (2 × 40 µg/rat/day, s.c.) given to ovariectomized rats treated the decrease in plasma NO3+NO2 levels in a dose-dependent manner (Fig. 3A), but had no effect on TBMD which remained low (Fig. 3B). As expected, progestagen treatment did not prevent the decrease in uterine weight after OVX (Table 1). Treatment of sham-operated rats with the anti-progestagen RU 486 (2 × 2 mg/rat/day, orally) had no effect on uterine weight (Table 1) and TBMD (Fig. 3B), but tended to decrease plasma NO3+NO2 levels (Fig. 3A).

Concurrent administration of effective doses of progestagen (2 × 40 µg/rat/day, s.c.) and EE (2 × 40 µg/rat/day, orally) partly prevented the decrease in plasma NO3+NO2 levels after OVX (Fig. 4A). This treatment, however, did not prevent the decrease in plasma NO3+NO2 levels induced by OVX (Fig. 2A). In contrast, administration of 17β-oestradiol subcutaneously (2 × 2.5 µg/rat/day) prevented the decrease in uterine weight (Table 1) and TBMD (Fig. 2B) in addition to the decrease in plasma NO3+NO2 levels in the ovariectomized rats (Fig. 2A).
however, prevented the decrease in uterine weight (Table 1) and TBMD (Fig. 4B) in ovariectomized rats. Treatment of ovariectomized rats with tibolone (2\#500 µg/rat/day, orally), a synthetic steroid with combined weak oestrogenic, progestagenic, and androgenic properties, prevented partly the OVX-induced decrease in plasma NO$_3$+NO$_2$ levels (Fig. 4A) and totally the decrease in TBMD (Fig. 4B). Tibolone had a partial effect on uterine weight after OVX (Table 1).

The effects of the various hormonal treatments on both plasma NO$_3$+NO$_2$ levels and TBMD are summarized in Table 2.

![Graph A](image1.png)  
**Figure 1** Plasma NO$_3$+NO$_2$ levels and TBMD of sham-operated and ovariectomized rats receiving EE. Effect of EE treatment (µg/rat/day, orally) on (A) plasma NO$_3$+NO$_2$ levels and (B) TBMD 4 weeks after OVX (n=6). *Significant versus SHAM (P<0.05); #Significant versus OVX (P<0.05). SHAM, sham-operation.

![Graph B](image2.png)  
**Figure 2** Plasma NO$_3$+NO$_2$ levels and TBMD of sham-operated and ovariectomized rats receiving 17β-oestradiol or anti-oestrogen. Effect of 17β-oestradiol (orally or s.c., µg/rat/day) and anti-oestrogen (s.c, mg/rat/day) treatment on (A) plasma NO$_3$+NO$_2$ levels and (B) TBMD 4 weeks after surgery. SHAM, n=24; OVX, n=20; OVX and 17β-E2 (2 × 32), n=5; OVX and 17β-E2 (2 × 125), n=5; OVX and 17β-E2 (2 × 250), n=4; OVX and 17β-E2 (2 × 500) n=10; OVX and 17β-E2 (s.c, 2 × 2.5), n=8; SHAM and anti-oestrogen (ICI164,384, 2 × 2), n=6. *Significant versus SHAM (P<0.05); #Significant versus OVX (P<0.05). 17β-E2, 17β-oestradiol; SHAM, sham-operation; Data from four experiments.

**Discussion**

Previous studies in premenopausal women have shown that plasma NO$_3$+NO$_2$ levels increase during follicular...
development, peak at mid cycle and are correlated with plasma 17β-oestradiol levels (Rosselli et al. 1994, Cicinelli et al. 1996, Kawano et al. 1996). In addition, peak expiratory NO concentrations were found to be significantly higher at mid cycle than during menstruation (Kharitonov et al. 1994). Postmenopausal women, on the other hand, had lower plasma NO₃+NO₂ levels; 20.2 ± 2.4 µM compared with 38 ± 3.0 µM in premenopausal women (Rosselli et al. 1995). The latter differences are very similar to those found in the present study between ovariectomized and sham-operated rats (23.1 ± 0.8 µM versus 33.6 ± 1.8 µM). Taken together,
these results strongly suggest that NO$_3$+NO$_2$ plasma levels are regulated by ovarian steroids. In addition, in all experiments OVX was associated with parallel decreases in plasma NO$_3$+NO$_2$ levels and in TBMD.

Plasma NO$_3$+NO$_2$ levels have been found to reflect endogenous NO production in humans receiving a low NO$_3$+NO$_2$ diet (Hibbs et al. 1992). In rats, lipopolysaccharide (LPS) treatment induced an increase in NO$_3$+NO$_2$ plasma levels that was prevented by l-NAME, suggesting that NO$_3$+NO$_2$ plasma levels reflect endogenous NO production (Shultz & Raij 1992). The short half-life of approximately 5 h of NO$_3$ in plasma (Wagner et al. 1983) and the excretion of dietary NO$_3$ in the urine within 18 h of intake (Wasserman 1978), allowed us to determine the plasma NO$_3$+NO$_2$ levels after an overnight fasting period in rats receiving a regular diet. Indeed, the plasma NO$_3$+NO$_2$ levels (33·6 ± 1·8 µM) in sham-operated rats in the present study are in agreement with plasma concentrations (38 µM) in virgin rats and TBMD in rats receiving various hormonal treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NO$_3$+NO$_2$ levels</th>
<th>TBMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVX and vehicle</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>OVX and EE</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>OVX and 17β-oestradiol (oral)</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>OVX and 17β-oestradiol (s.c.)</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>SHAM and anti-oestrogen</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>OVX and progestagen</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>SHAM-operation and anti-progesterone</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>OVX and progestagen and EE</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>OVX and tibolone</td>
<td>←</td>
<td>←</td>
</tr>
</tbody>
</table>

Compared to SHAM: no change (●); partially decreased (↓); decreased (↓↓).

Treatment with oral EE or subcutaneous 17β-oestradiol increased plasma NO$_3$+NO$_2$ levels following OVX in rats — a result that is similar to the increase in plasma NO$_3$+NO$_2$ levels reported after treatment of postmenopausal women with transdermal 17β-oestradiol (Rosselli et al. 1995) or premenopausal women with a non-specific oestrogen preparation (Ramsay et al. 1995). Both oral EE or subcutaneous 17β-oestradiol also prevented the decrease in TBMD following OVX in rats. However, treatment with oral 17β-oestradiol up to 2 × 500 µg/rat/day, while exerting all expected oestrogenic effects on uterine weight and TBMD, had no effect on plasma NO$_3$+NO$_2$ levels which remained at the OVX level. This may be due to differences in the activities of metabolites of 17β-oestradiol generated after passage of the liver on uterus and bone and on cells responsible for endogenous NO production. EE is relatively slowly transformed into inactive metabolites in the liver (Kuhl 1990) and may, therefore, be active on endogenous NO production after oral administration.

Treatment with the anti-oestrogen ICI164,384 diminished TBMD to about half the decrease seen after OVX and decreased uterine weight to a level similar to that found in ovariectomized rats. Anti-oestrogen treatment, however, had no effect on plasma NO$_3$+NO$_2$ levels. These results also revealed a dissociation between circulating NO$_3$+NO$_2$ plasma levels and TBMD. While there was a clear relation between the changes in oestrogen plasma levels and TBMD, the present data suggest that another ovarian steroid besides oestrogen may be involved in the regulation of plasma NO$_3$+NO$_2$ levels. This may be progesterone, as progestagen treatment prevented the OVX-induced decrease in NO$_3$+NO$_2$ dose-dependently. In humans, progesterone serum levels did not correlate with NO$_3$+NO$_2$ plasma levels (Rosselli et al. 1994), but most of the progesterone plasma concentrations in that study were within a narrow range (1–3 nM). In later studies by the same group, treatment of postmenopausal women with both oestrogen and progesterone induced a slight elevation in plasma NO$_3$+NO$_2$ levels, while oestrogen alone increased these levels significantly (Rosselli et al. 1995, Inthurn et al. 1997). Plasma NO$_3$+NO$_2$ levels are lower in the secretory phase of the menstrual cycle than at the mid cycle and it has been suggested that this may be due to increased progesterone production during the secretory phase (Cicinelli et al. 1996, Kawano et al. 1996). These observations correspond well with the finding in the present study that concurrent administration of oestrogen and progestagen to ovariectomized rats partly prevented the decrease in plasma NO$_3$+NO$_2$ levels. In addition, tibolone, a synthetic steroid with weak oestrogenic, androgenic, and progestational activity (Tax et al. 1987, van der

**Table 2** Effects of various hormonal treatments on NO$_3$+NO$_2$ plasma levels and TBMD in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NO$_3$+NO$_2$ plasma levels</th>
<th>TBMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVX and vehicle</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>OVX and EE</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>OVX and 17β-oestradiol (oral)</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>OVX and 17β-oestradiol (s.c.)</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>SHAM and anti-oestrogen</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>OVX and progestagen</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>SHAM-operation and anti-progesterone</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>OVX and progestagen and EE</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>OVX and tibolone</td>
<td>←</td>
<td>←</td>
</tr>
</tbody>
</table>

Compared to SHAM: no change (●); partially decreased (↓); decreased (↓↓).
Vies 1987), slightly elevated plasma NO$_3$+NO$_2$ levels in ovariectomized rats but did not prevent the OVX-induced decrease. Taken together, these results suggest that the combination of oestrogen and progesterone decreases plasma NO$_3$+NO$_2$ levels.

The effect of progesterone on bone is still controversial. Although short term positive effects of progesterone on bone have been reported in both humans (Abdalla et al. 1985, consensus report by Whitehead & Lobo 1988, reviewed by Prior 1990) and rodents (Barbagallo et al. 1989, Barengolts et al. 1990), other studies failed to demonstrate a positive effect of progesterone (Isserow et al. 1995). While progesterone has been reported to abolish the protective effect of oestrogen against bone mineral loss after OVX in rats (Barbagallo et al. 1989), no negative effect of progestagen on the bone sparing effect of oestrogen was observed in the present study. The available data in humans indicate that the addition of progestagen to oestrogen therapy does not influence the skeletal response (consensus report by Whitehead & Lobo 1988). Treatment with tibolone completely prevented bone loss after OVX in the present study. This protective effect of tibolone against bone loss has also been reported in postmenopausal women (Lindsay et al. 1980, Fogelman et al. 1981, Rymer et al. 1994, Lyritis et al. 1995, Bjarnason et al. 1996).

In conclusion, OVX decreased both TBMD and plasma NO$_3$+NO$_2$ levels in rats. These two parameters, however, did not correlate with each other as they were differently affected by various hormonal treatments. While plasma NO$_3$+NO$_2$ levels were regulated by both oestrogen and progesterone, TBMD was affected by changes in oestrogen levels only. Decreased systemic NO production, therefore, is not involved in bone loss after OVX. It is possible, however, that a reduction in local NO production within the bone microenvironment may be involved.

Acknowledgement
The authors thank H D Petronilia, M Scheepers, C Spanjers, and A Weekers for their technical assistance.

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Hormonal regulation of TBMD and NO production in rats


