Androstenedione treatment reduces loss of cancellous bone volume in ovariectomised rats in a dose-responsive manner and the effect is not mediated by oestrogen

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Abstract

We have tested the hypothesis that androstenedione (administered as 21-day, slow-release pellets) is converted to active sex steroids and reduces bone turnover in the ovariectomised rat model. We found that ovariectomy resulted in a minor but significant reduction in plasma concentrations of androstenedione and testosterone and a more significant reduction in oestrone (E₁) and oestradiol (E₂). This was associated with the expected substantial loss of metaphyseal cancellous bone volume. Androstenedione (1·5–100 mg) pellets increased the plasma concentrations of androstenedione and testosterone above those in the ovariectomised (ovx) rats in a dose-responsive manner, whereas E₂ plasma concentrations were increased to a minor but significant degree above those in the ovx animals. Androstenedione reduced loss of cancellous bone volume in a dose-dependent fashion by reducing bone turnover. The 1·5, 5 and 100 mg androstenedione-induced effect on bone turnover was not abrogated by simultaneous treatment with Arimidex, an aromatase inhibitor. This implies that the skeletal-protective effect of androstenedione was not oestrogen-mediated.


Introduction

There are several lines of evidence to support the hypothesis that peripheral conversion of sex steroid plays an important part in maintaining the skeleton. These include the findings (1) that there is greater bone loss in rats that have been subjected to both adrenalectomy and ovariectomy than in rats that have undergone ovariectomy only (Durbridge et al. 1990), (2) that postmenopausal women with primary adrenal failure and reduced concentrations of adrenal precursors including androstenedione have a lower bone mineral density (BMD) than normal postmenopausal women (Devogelaer et al. 1987), (3) that plasma concentrations of androstenedione and oestrone (E₁) correlate positively with BMD in postmenopausal women (Marshall et al. 1977, Cauley et al. 1986) and (4) that reduced concentrations of these hormones have been shown to be associated with an increased risk of fracture (Marshall et al. 1977, Cauley et al. 1986). Finally, it has been shown that treatment with dehydroepiandrosterone protects against cancellous bone loss in ovariectomised rats (Turner et al. 1990). However, that study did not show if the protective effect was the result of conversion to androgens or oestrogens.

Androstenedione is a sex steroid hormone precursor that is secreted by the ovaries and adrenals. It is converted to testosterone and E₁ and the latter, although relatively biologically inactive, can be further converted to 17β-oestradiol (E₂). In this study, we investigated further the influence of peripheral conversion of androstenedione on the skeleton in the ovariectomised (ovx) rat, using static and dynamic bone histomorphometry. The experiments were performed in sexually mature animals that had reached a plateau in their growth phase (Kalu 1991); the characteristics of bone loss in these animals are largely similar to those of the aged rat model (Kalu et al. 1989). Because our interest lies essentially in the role of sex steroids secreted from ovarian stroma in the regulation of bone metabolism, we tested the effect of androstenedione, rather than adrenal precursor hormones.

Material and Methods

Animal experimentation and histomorphometry

Female Sprague–Dawley rats (13 weeks old) were purchased from Harlan Olac Ltd (Bicester, Oxon, UK) and housed at 21 °C with a 12 h light : 12 h darkness cycle and fed rat chow (Lillico, Betchworth, Surrey, UK) and water. They were pair-fed. Androstenedione and placebo slow-release tablets were purchased from Innovative Research of America (Toledo, Ohio, USA) and inserted s.c. on the
day of the ovariectomy. Animals were subjected to ovariectomy or a sham operation on day 1. Surgery was performed with the animal under halothane anaesthesia using a dorsal approach. The experiment was completed on day 21, when the animals were killed by exsanguination using cardiac puncture under anaesthesia, followed by cervical dislocation. Plasma samples were stored at −70°C until required for use. The aromatase inhibitor, Arimidex (kindly provided by Dr Vose, Zeneca Pharmaceuticals, Macclesfield, UK), was administered orally, daily at a dose of 0·1 mg/kg.

**Experiment I** The animals were divided into 11 groups of six each. They weighed an average of 220 g (range 207–233 g). Group 1 was subjected to a sham operation. Group 2 had a bilateral ovariectomy. Groups 3, 5, 7, 8, 9 and 10 were subjected to bilateral ovariectomy and had androstenedione pellets (1·5, 5, 15, 35, 75 and 100 mg respectively) inserted s.c. in the back of the neck. Groups 4, 6 and 11 had bilateral ovariectomy, and placebo pellets of 1·5, 5 and 100 mg were inserted respectively.

**Experiment II** The animals were divided into nine groups of six animals each. They weighed an average of 219 g (range 203–233 g). Group 1 was subjected to a sham operation. Group 2 had a bilateral ovariectomy. Group 3 had a bilateral ovariectomy and was treated with Arimidex. Groups 4, 5 and 6 had bilateral ovariectomy and were treated with 1·5 mg androstenedione pellets, 1·5 mg pellets and Arimidex, and a 1·5 mg placebo pellet respectively. Groups 7, 8 and 9 were treated the same as groups 4, 5 and 6, except that a 5 mg pellet was used.

**Experiment III** The animals (average weight 228 g, range 193–239 g) were divided into six groups of six. Group 1 was subjected to a sham operation. Group 2 had a bilateral ovariectomy. Group 3 and 4 had a bilateral ovariectomy and were treated with 100 mg androstenedione and placebo pellets respectively. Group 5 had a bilateral ovariectomy and were treated with 100 mg androstenedione pellets and Arimidex. Group 6 had a bilateral ovariectomy and were treated with Arimidex.

Calcine (30 mg/kg; Sigma Chemicals, Poole, Dorset, UK) and tetracycline hydrochloride (25 mg/kg; Lederle Laboratory, Gosport, Hants, UK) were injected i.p. on day 7 and day 14 of the experiment respectively.

The uteri were removed and weighed and ovariectomy was confirmed by the absence of ovarian tissue. The tibiae were cleaned of soft tissue, fixed in 70% alcohol for 24 h, dehydrated through graded alcohols and embedded without decalcification in London Resin (London Resin Co. Ltd, Basingstoke, Hants, UK). Longitudinal sections of the proximal metaphysis were cut using a Reichart–Jung microtome (Leica Ltd, Milton Keynes, UK); 5 μm sections were stained with toluidine blue and 12 μm unstained sections were used for fluorescent microscopy. Bone histomorphometry was performed using transmitted and epifluorescent microscopy linked to a computer-assisted image analyser (Seescan Ltd, Cambridge, UK). Bone volume and surface parameters were measured by tracing the relevant features with a cursor on the video screen. Bone volume/cancellous volume (BV/TV) measurements were performed at ×40 magnification and the surface parameters were measured at ×400 magnification. All sections were analysed without knowledge of the group from which the section came.

BV/TV at the proximal metaphyseal cancellous bone was measured on two non-consecutive sections. A standard area of 2 mm² (at least 2 mm from the growth plate, to exclude the primary spongiosa) was measured. Trabeculae number and thickness were calculated as previously described (Parfitt et al. 1983). Static parameters were measured in the same way as that described for BV/TV and included osteoblast surface (Ob.S/BS), osteoclast surface (Oc.S/BS) and osteoclast number (No.c./BS). Longitudinal growth rate (LGR) was derived by measuring the distance between the tetracycline and the calcine fluorescent bands that parallel the growth plate at four equally placed sites per section and dividing by the time interval between the two injections. The rate of formation of bone (BFR/BS; tissue concentration, total surface referent) was calculated from the product of the percentage of the trabecular bone surface with a double fluorochrome label (dLS/BS) and the mineral apposition rate; the former was obtained by measuring the percentage of the trabecular bone surface, covered by two fluorochrome labels and the latter by dividing the interlabel distance by the time interval between the injections of the labels in the corresponding area. Appositional rate values were not corrected for the obliquity of the plane of section of cancellous bone.

**RIA for plasma hormone concentrations**

Plasma concentrations of androstenedione, E₁ and testosterone (Diagnostics Systems Laboratories, Webster, TX, USA) and E₂ (Inc Star, Wokingham, Berks, UK) were measured by RIA as instructed in the manufacturer’s guidelines. The RIAs had intra-assay coefficients of variation less than 5%, and interassay coefficients of variation less than 6%. Each assay was validated by spiking plasma samples with known amounts of the four samples being tested.

**Statistics**

The results were analysed using Fisher’s least significant difference method for multiple comparisons in a one-way analysis of variance and all results are expressed as mean ± s.e.m. Significance was considered when P<0·05. Statview 4·0 (Abacus Concepts, Cupertino, CA, USA) was used to analyse the results.
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Results

The sham-operated animals gained 22·2 ± 3·3 g, 37·5 ± 6·7 g and 25·3 ± 3·8 g body weight over the 21-day period in experiments I, II and III respectively. Despite pair feeding, all ovx rats gained significant body weight compared with sham-operated animals. The ovx animals gained 56·8 ± 5·9 g in experiment I, 53·4 ± 4·6 g in experiment II and 56·1 ± 4·7 g in experiment III. In the three experiments, ovariectomy was confirmed by the absence of ovarian tissue, and by the significantly reduced plasma concentrations of E2.

Experiment I

Ovariectomy caused a significant reduction in plasma concentrations of androstenedione, testosterone (Fig. 1), E1 (sham-operated 37·90 ± 1·36 pg/ml, ovx 23·19 ± 0·85 pg/ml; P<0·0001) and E2 (sham-operated 42·59 ± 1·26 pg/ml, ovx 10·22 ± 0·20 pg/ml; P<0·0001). Insertion of androstenedione pellets increased the plasma concentrations of androstenedione and testosterone in a dose-responsive manner (Fig. 1). Interestingly, all sizes of androstenedione pellet resulted in a similar increase in plasma concentrations of E1 (1·5 mg pellet
50±50 ± 3·30 pg/ml; 5 mg pellet 47·86 ± 1·52 pg/ml; 15 mg pellet 56·06 ± 4·05 pg/ml; 35 mg pellet 47·07 ± 2·18 pg/ml; 75 mg pellet 49·90 ± 2·90 pg/ml; 100 mg pellet 43·01 ± 3·19 pg/ml). These values were significantly greater than those in the sham-operated group (P<0·0001). Androstenedione treatment of the ovx rats also increased, to a minor but significant extent, the plasma concentrations of E2 to values greater than those in ovx, untreated rats (P<0·01). As with plasma concentrations of E1, this did not increase in a dose–response manner, but reached a maximum in response to 1·5 mg androstenedione pellets (12·77 ± 0·2 pg/ml). No effect on hormone concentrations was seen in placebo-treated animals (Fig. 1).

The uterine weight increased in a dose-responsive manner when the rats were treated with increasing amounts of androstenedione (Table 1). The LGR was increased in ovx rats compared with sham-operated animals. The 1·5 and 5 mg androstenedione pellets resulted in a reduction in LGR; 5 mg androstenedione returned the LGR to that in the sham-operated group, but this effect was lost in a dose-dependent manner with administration of larger pellets (Table 1).

Ovariectomy resulted in approximately 50% loss of metaphyseal cancellous bone compared with sham-operated animals (Table 1). Insertion of androstenedione pellets resulted in a dose-responsive increase in BV/TV% compared with the placebo-treated ovx animals (Table 1). This increased BV/TV% was largely the result of an increase in the thickness of the metaphyseal trabeculae, rather than in their number (Table 1). The changes in cancellous bone volume in the animals treated with 1·5 and 5 mg androstenedione pellets were small and only just reached statistical significance (Table 1). However, the data in experiment II showed similar results, and when the data for these variables from the two experiments were pooled, so that the number of animals per variable was increased from six to 12, the results became statistically highly significant (P<0·0001; ovx compared with ovx and 1·5 and 5 mg androstenedione).

Osteoclast numbers and the percentage of osteoclasts that covered the trabecular bone surface were increased in the ovx animals compared with the sham-operated group (Fig. 2). Androstenedione pellets of all sizes caused a reduction in these parameters and the 100 mg androstenedione pellet returned the osteoclast parameters to values found in the sham-operated group (Fig. 2).

The predicted increase in BFR was present in the ovx rats compared with the sham-operated animals. All sizes of androstenedione pellets caused reduction in this BFR. Such a finding is explained by the reduction in both mineral apposition rate and dLS/BS% in response to androstenedione; however, the latter was more significantly affected (Fig. 3). The androstenedione pellets up to 15 mg caused a dose-responsive reduction in BFR parameters, but larger pellets reversed this effect (Fig. 3).

Experiments II and III
To determine if the androstenedione-induced effect was mediated by oestrogen, Arimidex was administered to the
ovx animals in the presence and absence of 1·5, 5 and 100 mg androstenedione. BV/TV% in the ovx rats in both experiments was reduced by approximately 50% (Tables 2 and 3). Treatment with Arimidex did not reduce the androstenedione-induced increase in BV/TV% (Table 2 and 3). As in experiment I, the increase in cancellous bone volume in the androstenedione-treated rats was the result of reduced bone turnover. The sham-operated, ovx, ovx + 1·5 mg, ovx + 5 mg and ovx + 100 mg androstenedione-treated rats in experiments II and III were found to have values for Oc.S/BS%, NOc./BS (n/mm), BFR (10^{-2} \, \text{mm}^3 \, \text{mm}^{-2} \, \text{day}^{-1}), \text{MAR} (\mu m/day), \text{dLS/BS}%, \text{dLS/BS}%, \text{Ob.S/BS}% and Ob.S/BS% within the same ranges as those seen in experiment I. Arimidex treatment in the presence of 1·5, 5 and 100 mg androstenedione pellets did not alter the values of these parameters significantly. Neither placebo pellets nor Arimidex alone were found to have an effect.

Ovariectomy resulted in a significant reduction in plasma androstenedione and testosterone in experiment II (Tables 4 and 5). The plasma concentrations of androstenedione and testosterone in the ovx-androstenedione-treated

Figure 3 Effect of androstenedione (adione) and placebo (P) pellets on histomorphometric indices of cancellous bone formation after ovariectomy. Values are mean ± S.E.M.; where the S.E.M. is not shown, it was too small to be seen. BFR, Bone formation rate; MAR, mineral apposition rate; dLS/BS%, percentage of cancellous bone surface with a double fluorochrome label; Ob.S/BS%, percentage of bone surface covered with osteoblasts; sham, sham-operated; ovx, ovariectomised. BFR: *P<0·0001 compared with all treatment groups except P groups, **P<0·03 compared with all groups. MAR: *P<0·0001 compared with all groups except P groups, **P<0·001 compared with all androstenedione- and P-treatment groups except 5 mg androstenedione-treated rats. dLS/BS%: *P<0·01 compared with all groups except P-treated groups, **P<0·005 compared with all treatment groups. Ob.S/BS%: *P<0·0001 compared with all groups except P-treated groups.

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rats were similar to those in the ovx–androstenedione–Arimidex–treated animals, with the exception that testosterone was increased in the 1.5 mg androstenedione–Arimidex–ovx rats compared with the 1.5 mg androstenedione–ovx rats without Arimidex (Table 4). This is probably the result of a greater proportion of the androstenedione being converted to testosterone, as it was prevented from being converted to E₁. The reason why similar differences did not occur in the animals treated with 5 and 100 mg androstenedione may be that their greater plasma concentrations of testosterone limited conversion to testosterone in the presence of Arimidex. Table 5 shows that ovariectomy in experiment III resulted in reduced plasma concentrations of androstenedione and testosterone, and that 100 mg androstenedione increased androstenedione and testosterone to supraphysiological concentrations. As in experiment II, Arimidex had no effect on these values. In experiment II, plasma concentrations of E₁ and E₂ were also significantly reduced in the ovx animals (E₁, sham-operated 38.28 ± 10.83 pg/ml, ovx 14.79 ± 10.47 pg/ml, ovx + 1.5A 23.83 ± 11.24 pg/ml, ovx + 1.5A + Ar 17.66 ± 20.10 pg/ml). E₂ and E₃ plasma concentrations were increased in the ovx animals in response to 1.5 mg androstenedione compared

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effect of 1·5–5 mg androstenedione (A) pellets and Arimidex (Ar) on uterine weight, longitudinal growth rate (LGR) and cancellous bone volume (BV/TV%) of ovariectomised (ovx) rats. Values are means ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine weight (mg)</td>
<td>LGR (µm/day)</td>
</tr>
<tr>
<td>sham</td>
<td>473.23 ± 28.58**</td>
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<td>ovx+veh</td>
<td>101.84 ± 4.68</td>
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<tr>
<td>ovx+Ar</td>
<td>96.67 ± 3.96</td>
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<td>ovx+1·5A</td>
<td>116.16 ± 5.04*</td>
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<tr>
<td>ovx+1·5A + Ar</td>
<td>105.51 ± 3.36</td>
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<tr>
<td>ovx+1·5P</td>
<td>103.89 ± 2.16</td>
</tr>
<tr>
<td>ovx+5A</td>
<td>122.83 ± 5.03*</td>
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<tr>
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<td>ovx+5P</td>
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<th>Table 3</th>
<th>Effect of 100 mg androstenedione (A) pellets and Arimidex (Ar) on uterine weight, longitudinal growth rate (LGR) and cancellous bone volume (BV/TV%) of ovariectomised rats. Values are means ± S.E.M.</th>
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</thead>
<tbody>
<tr>
<td>Uterine weight (mg)</td>
<td>LGR (µm/day)</td>
</tr>
<tr>
<td>sham</td>
<td>640.13 ± 38.38</td>
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<tr>
<td>ovx+veh</td>
<td>196.00 ± 27.22</td>
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<tr>
<td>ovx+100A</td>
<td>549.38 ± 17.66**</td>
</tr>
<tr>
<td>ovx+100P</td>
<td>190.38 ± 14.79</td>
</tr>
<tr>
<td>ovx+100A + Ar</td>
<td>326.00 ± 20.83*</td>
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<tr>
<td>ovx+Ar</td>
<td>173.38 ± 14.03</td>
</tr>
</tbody>
</table>

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<tr>
<th>Table 4</th>
<th>Plasma concentrations of androstenedione (A) and testosterone (T) in sham-operated (sham), ovariectomised (ovx) and ovx rats treated with A (1·5 or 5 mg) ± Arimidex (Ar). Values are means ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>ovx + veh</td>
</tr>
<tr>
<td>A (pg/ml)</td>
<td>173.0 ± 13.0*</td>
</tr>
<tr>
<td>T (pg/ml)</td>
<td>2180 ± 80*</td>
</tr>
</tbody>
</table>

A: *P<0·0001 compared with ovx+veh, ovx+Ar. T: *P<0·05, **P<0·0001 compared with ovx, ovx+veh.
with the ovx rats (E₁, 48·03 ± 2·02 pg/ml, P<0·0001; E₂, 13·60 ± 0·68 pg/ml, P<0·01) and 5 mg androstenedione pellets (E₁, 49·53 ± 0·69 pg/ml, P<0·0001; E₂, 13·09 ± 0·49 pg/ml, P<0·01), and these increases were returned to ovx values in the presence of Arimidex. Oestrogen concentrations were also reduced as a consequence of ovariectomy in experiment III (E₁, sham-operated 37·12 ± 1·54, ovx 20·18 ± 0·98 pg/ml; E₂, sham-operated 41·11 ± 2·51, 10·09 ± 0·29 pg/ml; P<0·0001). Oestrogen plasma concentrations in the ovx rats were also increased in response to 100 mg androstenedione compared with ovx rats (E₁, 43·02 ± 3·19 pg/ml, P<0·0001; E₂, ovx 13·19 ± 0·29 pg/ml, P<0·01). The androstenedione-induced values in response to E₁ and E₂ were returned to ovx values in the presence of Arimidex.

Tables 1, 2 and 3 show the effect of androstenedione and Arimidex on uterine weights. Treatment with both Arimidex and androstenedione 100 mg resulted in a significant reduction in uterine weights to values mid-way between those of the sham-operated and ovx rats (Table 3), demonstrating that the uterine hypertrophy induced by androstenedione treatment was caused only in part by an oestrogen–mediated effect, but that high concentrations of testosterone, as reported previously, also contributed to this finding (Schmidt & Katzenellenbogen 1979). Treatment with Arimidex and androstenedione 1·5 and 5 mg also reduced the uterine weights compared with androstenedione alone; however, in this case they were returned to ovx values (Table 2), indicating that low plasma concentrations mediated this effect, and suggesting that low plasma concentrations of androgens had little effect on the uterus.

The increases in BV/TV%, LGR, parameters of bone resorption and bone formation in response to 1·5, 5 and 100 mg androstenedione identified in experiment I were confirmed in experiments II and III (Tables 2 and 3). Arimidex failed to abrogate the effect of androstenedione treatment on bone turnover. However, Arimidex blocked the reduction in LGR induced by 1·5 and 5 mg androstenedione (Table 2).

**Discussion**

In this study we have demonstrated that insertion of increasing sizes of pellet of androstenedione in ovx rats caused a dose–dependent increase in plasma concentrations of androstenedione and testosterone that were paralleled by an increase in cancellous bone volume. This, together with the finding that the reduction in osteoclast parameters and indices of bone formation in response to high and low concentrations of androstenedione were not abrogated by Arimidex, although the plasma concentrations of E₁ and E₂ were returned to ovx values, demonstrates that the skeletal-protective effect was not mediated by oestrogens. Taken together, these results imply that the effect was mediated by androgens. It is not clear from this study whether the effects are mediated directly by androstenedione or by testosterone. Further investigation of this question is currently difficult, as specific inhibitors for the various forms of 17β-hydroxysteroid dehydrogenase (Andersson 1995), the enzyme which catalyses the interconversion of these hormones, are not available. The use of an anti-androgen such as Casodex or Flutamide would be unhelpful, as the effect of both hormones would be antagonised. The site of the skeletal response to testosterone has not been demonstrated and requires further investigation; androgen receptors have been identified on osteoblasts (Colvard et al. 1989), but there are also reports that they are present on osteoclasts (Ousler et al. 1993). The reduction in osteoclast parameters that occurred in a dose-responsive manner with the increasing concentrations of androstenedione and testosterone indicates that the protection against the loss of cancellous bone can largely be accounted for by attenuation of the increased catabolic effect seen as a consequence of ovariectomy. It might be expected that the continuous downward trend of the bone resorption parameters, in response to increasing plasma concentrations of testosterone, would be paralleled by a similar reduction in the indices of bone formation, as has been seen in similar experiments in which the ovx rats were treated with E₂. However, the dLS/BS% and the mineral apposition rate in the androstenedione-treated animals paralleled the reduction in bone resorption parameters only in response to 1·5, 5 and 15 mg pellets; use of larger pellets resulted in reversal of this trend, and the 75 mg androstenedione pellet was found to have the same effect on BFR as the 1·5 mg pellet. The reason for this finding is not clear, but in view of the increasing BFR, it suggests that androgens are exerting an anabolic effect on the skeleton – a finding that has been reported previously in the rat (Tobias et al. 1994, Gallagher et al. 1996, Lea

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**Table 5 Plasma concentrations of androstenedione (A) and testosterone (T) in sham-operated (sham), ovariectomised (ovx) and ovx rats treated with A (100 mg) ± Arimidex (Ar).** Values are means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>ovx + veh</th>
<th>ovx + A</th>
<th>ovx + 100P</th>
<th>ovx + 100A + Ar</th>
<th>ovx + AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (ng/ml)</td>
<td>0·185 ± 0·035**</td>
<td>0·05 ± 0·01</td>
<td>13·92 ± 1·474*</td>
<td>0·049 ± 0·011</td>
<td>15·11 ± 0·796*</td>
<td>0·056 ± 0·002</td>
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<tr>
<td>T (ng/ml)</td>
<td>0·193 ± 0·023**</td>
<td>0·044 ± 0·001</td>
<td>15·03 ± 1·474*</td>
<td>0·038 ± 0·011</td>
<td>16·07 ± 0·796*</td>
<td>0·021 ± 0·002</td>
</tr>
</tbody>
</table>

A: *P<0·0001, **P<0·01 compared with ovx + veh, ovx + Ar. T: *P<0·0001, **P<0·01 compared with ovx + veh, ovx + Ar.
et al. 1996). However, this is difficult to reconcile, as the cancellous bone volume and the trabecular bone thickness did not exceed those of the sham-operated animals. Hence a definitive explanation cannot be presented without further experiments. Nevertheless, the answer may lie in the fact that androgens are reported to be anabolic in oestrogen-replete rats (Goulding & Gold 1993, Gallagher et al. 1996, Lea et al. 1996) and oestrogen–deficient rats (Tobias et al. 1994), whereas they are not anti-catabolic in oestrogen–replete rats (Goulding & Gold 1993, Gallagher et al. 1996, Lea et al. 1996). This suggests that androstenedione treatment would exert its anabolic effect on the skeleton of the ovx rat immediately it was administered, whereas there may be a lag period before androgens exert their anti-catabolic effect on the same animals. This may explain why the cancellous bone volume does not exceed that of the sham-operated animals in an experiment of this duration.

The data in these experiments demonstrate that bone resorption parameters were reduced in all androstenedione-treated groups, even when the androstenedione and testosterone plasma concentrations were slightly less than those in sham-operated animals (that is, in response to the 1·5 mg androstenedione pellet). The reductions in the osteoclast parameters were small in the animals treated with the 1·5 and 5 mg pellets, but they were significant; nevertheless, it is necessary to be cautious about interpretation of such minor changes. However, it appears that the anti-catabolic effect that we have found in the ovx rats treated with the 1·5 and 5 mg androstenedione pellets was real, as the data were obtained blind and, importantly, were reproduced in two separate experiments. Furthermore, these results are also supported by the findings that the same reduction in osteoclast parameters occurred in animals treated with Arimidex and androstenedione, and that these results correlated with reduced indices of bone formation compared with those in ovx rats. Arimidex alone did not alter the plasma hormone concentrations in the ovx rats, suggesting that the adrenal as a source of these steroids is not significant, and that the adrenals do not have an important role in maintaining the skeleton in the ovx rat. This is at odds with previous data (Durbridge et al. 1990), and the difference might be accounted for by the different duration of the two studies.

Androstenedione treatment exerted a bimodal effect on the LGR in ovx rats. This is probably accounted for by the opposing influences of oestrogen and testosterone. The reduction in the LGR in the rats treated with 1·5 and 5 mg androstenedione was reversed by Arimidex, demonstrating that this effect was oestrogen-mediated. In contrast, Arimidex had no effect on LGR in the presence of the 100 mg androstenedione pellets, possibly because the oestrogenic effect was masked by the very high plasma concentrations of androgen. Because the E_2 concentrations were so low in the androstenedione-treated animals, it is unlikely that circulating E_2 accounts for this effect at the growth plate, and this raises the possibility that peripheral synthesis of E_2 occurs at this site. The finding that the body weights of the androstenedione-treated rats were not reduced also provides supportive evidence that the low concentrations of circulating androstenedione are insignificant in terms of exerting a demonstrable physiological effect.

Although testosterone has been shown to reduce osteoclast numbers in orchidectomised rats (Wakely et al. 1991), the effect of androgens on osteoclasts in female rats is less clear. The reduction of osteoclast parameters in these experiments is particularly interesting, in view of previous reports that showed no demonstrable effect on osteoclast parameters in oestrogen–replete female rats when anti-androgens, either Casodex (Lea et al. 1996) or Flutamide (Goulding & Gold 1993, Gallagher et al. 1996), were administered. On the basis of our current results, it appears that an androgen-mediated effect on osteoclasts, at least at physiological plasma concentrations, may only become detectable in the absence of oestrogen. Therefore osteoclasts in the oestrogen–deficient ovx rats may only have become sensitive to the effect of androgens some days after ovariection. If this were the case, we would predict that low concentrations of androgens such as that induced by the 1·5 mg pellets would have a more significant effect over a longer period of time. This argument is based on the knowledge that parameters of bone resorption increase for approximately 100 days after ovariection (Wronska et al. 1988a). Therefore, if the osteoclast parameters are stabilised 21 days after ovariection in androstenedione-treated rats, bone turnover should also stabilise and further bone loss should not occur. This is obviously an important issue, because the greatest effect of androstenedione in our study was brought about by supraphysiological concentrations of androgens, and this may increase the risk of cardiovascular disease and outweigh the benefits to the skeleton.

As postmenopausal osteoporosis occurs against a background of increased osteoclastic bone resorption (Steiniche et al. 1989), which is the same process whereby oestrogen-deficient rats become osteopenic (Wronska et al. 1988a, b), the results of our study have important implications in terms of androgen treatment for osteoporosis in postmenopausal women. Furthermore, endogenous androgens secreted by ovarian stroma may be important in determining the rate of bone turnover in postmenopausal women and hence be a predictor of the risk of the disease.

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**References**


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