

# Progestagens Androgenic Action on the Bone of Male Castrated Mice

**Broulík P. D.<sup>1</sup>, Broulíková K.<sup>1</sup>, Nečas E.<sup>2</sup>**

<sup>1</sup>Third Medical Department of the First Faculty of Medicine, Charles University in Prague, and General Teaching Hospital, Czech Republic;

<sup>2</sup>Institute of Pathological Physiology of the First Faculty of Medicine, Charles University in Prague, Czech Republic

Received November 28, 2006, Accepted December 20, 2006.

**Key words:** Progestagens – Androgenic effect – Bone density

*This work was supported by the research project 305/04/1528 granted by the GA ČR, and by projects MSM 0021620806 and LC06044 granted by MSM ČR.*

**Mailing Address:** Professor Petr Broulík, MD., DSc., Third Medical Department of the First Faculty of Medicine and General Teaching Hospital, U Nemocnice 1, 128 08 Prague 2, Czech Republic, Phone: +420 224 912 954, e-mail: pbrou@lf1.cuni.cz

**Abstract:** It has been suggested that some progestagens could have an androgenic stimulatory effect on bone formation. The androgenic effects of progestagens were tested *in vivo* in the absence of androgens and estrogens in the castrated male mice, species extraordinary responsive to the withdrawal or administration of androgens. Three progestagens (norethisterone, uterogestan and medroxyprogesterone acetate) were compared as to their androgenic activity. Tissues especially sensitive to androgens, the seminal vesicles and kidney of the mice fell significantly after castration and all three progestagens did not affect their weights. The present results confirm the well known fact that castration leads to osteopenia in mice. Uterogestan micronized progesterone and MPA have no effect on the bone density or mineral content of the tibia of tested mice. Only in the case of NETA we observed slight statistically significant ( $p < 0.05$ ) increase in bone density. Progestagens do not appear to have the androgenic effect on the skeleton and NETA has been suggested as one of the exception. Our results indicate that only NETA at the dose which is used in hormonal therapy for prevention of osteoporosis has a slight protective effect against bone mineral loss in castrated mice.

---

## Introduction

In contrast to the abundance of work on the effects of estrogens on bone metabolism, there is little evidence of direct effects of progesterone on bone [1]. It has been suggested that some progestagens could have a stimulatory effect on bone formation. Clinical studies suggest that the effects of progesterone treatment on postmenopausal women are similar to those of estrogen, but combined estrogen and progesterone treatment has effects that differ from those of treatment with either steroid alone [2]. Progesterone receptors have been identified in human and murine osteoblast cells, so progesterone could exert direct effects on bone through its own receptor [3]. It has been suggested that some progestagens could have androgenic effect on bone. Progestagens may mimic, inhibit or potentiate the action of androgens. The androgenic actions vary with the steroid tested.

The androgenic effect of progestagens was tested *in vivo* under standardized condition in the absence of androgens and estrogens. A model of osteoporosis in the castrated mice has been used to evaluate the effects of three progestagens. Three progestagens (norethisterone – NETA, utrogestan, medroxyprogesterone acetate – MPA) were compared as to their androgenic activity. In order to ascertain the androgenic activity of progestagens the drugs were administered to the castrated male mice.

It seemed therefore reasonable to see whether in this species the androgenic effect of progestagens could be demonstrated not only in typical androgen dependent organ as seminal vesicles but also in kidney, which are especially sensitive in mice to renotrophic effect of androgens and in bone as it is well established that androgens exert a remarkable effect on the bone homeostasis.

### Material and Method

Adult male strain H mice (Velaz Prague) weighting approximately 30 g were used for the experiments. They were fed on a standard laboratory diet containing 23 % protein, 1.2 % calcium and 0.6 % phosphorus with water ad libitum, and were kept in an indirectly illuminated room with controlled temperature at  $24 \pm 2$  C. After acclimatization to the new environment one group of mice was sham operated (control) and four groups were orchidectomized. All orchidectomized mice were equally distributed across four treatment groups: 1. castrated, 2. castrated fed by uterogestan, 3. castrated fed by NETA and 4. castrated fed by medroxyprogesterone acetate. The drugs were incorporated into the animals' diet. Each animal received 5.0 g of food per day and treatment was continued for 3 months. Because pharmacokinetic data are not available for the administration of gestagens in mice, we used the dose proportional to that given to humans for clinical treatment of osteoporosis. Medroxyprogesterone acetate (Provera Pharmacia NV Puurs Belgic) 5mg/kg/day Progesterone micronisatum Utrogestan (Laboratories Besius Paris) 200 mg/kg/day and Norethisterone acetate (Slovakofarma) 1 mg/kg/day were mixed into the diet for three months. The animals were weighted before and after the experiment and their food consumption was measured daily in order to standardise the administration of the drug in the dose shown above. When the animals were killed, blood was withdrawn from the heart and the kidneys, seminal vesicles and tibias and femurs were removed, cleaned and weighed on a torsion balance. Each left tibia was removed cleaned of muscle and tendon. The left tibias were collected, stripped of soft tissues, and stored in glass vials at  $-20^{\circ}$  C. For determination of the bone density, left tibias were placed in unstoppered glass vials filled with deionised water. Vials were placed into a desiccator that was connected to a vacuum for 60 min so that trapped air diffused out of the bone. All bones were weighed before being immersed in deionised water previously equilibrated to room temperature. Bone density was calculated by Archimedes principle as described previously [4]. Briefly according to Archimedes principle a buoyant force on submerged object in a fluid equals the density of the fluid multiplied by the volume of the fluid that was displaced. The latter is the ratio of buoyant force to fluid density. The density of the object is then obtained as the mass divided by the volume that is displaced. However it is important to note that the buoyant force does not depend on the weight or shape of the submerged object but on the weight of the displaced fluid. Although assessing bone density using Archimedes principle is time consuming it measures true rather than apparent density.

The bones were then dried to constant weight and then incinerated for 24 hours at  $600^{\circ}$  C to white ash which was weighed. The ash weight was expressed per millilitre of volume of unashed tibia. Bone ashes were then dissolved in 3 mol/l hydrochloric acid before the determination of calcium and phosphorus content.

Calcium was measured by the method of Gitelman [5] and phosphorus according to Kraml [6].

Testosterone in blood plasma was determined by conventional radioimmunoassays (CIBA – Corning Diagnostic Corp.).

For the bone morphology we used the method presented by [7, 8]. Also left femurs were removed and cleaned of tissue for X-ray. Standardized roentgenographs of femur were made using Philips mammo diagnost 3000 X-ray machine at controlled exposures of 26 kV at 5.5 mA. Morphometric measurements were performed directly on the X rays after magnification by fine calliper. On the roentgenographs at 40 % of the total length starting from the distal end the external, inner bone diameter and cortical width were measured after magnification with fine calliper.

Kidneys and seminal vesicles were cleaned and weighed and the weight was expressed in relative values (mg/100 g body weight).

Differences between groups were determined statistically by analysis of variance followed by Duncan's [9] multiple range test.

## Results

The effects of progestagens administration on all groups of castrated animals are summarized in Table 1. At the end of the study the body weight of mice administered with progestagens were not significantly lower than sham operated mice. Surgical castration showed no significant effect on the body weight. Administration of progestagens did not significantly affect low plasma testosterone which was produced by castration. In mice receiving exogenous progestagens the

**Table 1 – Effect of castration, norethisterone, utrogestan and medroxyprogesterone acetate on seminal vesicle weight, serum testosterone and bone mineral content of the tibia in intact mice (means  $\pm$  SD)  $p < 0.05$  v. s intact animals**

	Intact n-8	Castrated n-8	Castrated + Uterogestan n-8	Castrated + NETA n-8	Castrated + MPA n-8
Initial body weight (g)	35.2 $\pm$ 1.0	35.0 $\pm$ 1.0	36.0 $\pm$ 1	35.0 $\pm$ 2	35.0 $\pm$ 1
Final body weight (g)	38.0 $\pm$ 2	38.6 $\pm$ 2.5	43.0 $\pm$ 3	40.7 $\pm$ 3	41.2 $\pm$ 3
Density of tibia(g/ml)	1.48 $\pm$ 0.03	1.38 $\pm$ 0.03*	1.37 $\pm$ 0.01*	1.41 $\pm$ 0.02*	1.36 $\pm$ 0.03*
Ash content (g/ml)	0.78 $\pm$ 0.02	0.64 $\pm$ 0.02*	0.63 $\pm$ 0.02*	0.69 $\pm$ 0.01*	0.62 $\pm$ 0.03*
Bone calcium (mg/ml)	300 $\pm$ 9	241 $\pm$ 6.0*	239 $\pm$ 7.0*	250 $\pm$ 7.0*	240 $\pm$ 6.0*
Bone phosphate (mg/ml)	132 $\pm$ 4.0	105 $\pm$ 3.9*	104 $\pm$ 3.0*	110 $\pm$ 4.0*	104 $\pm$ 5.0*
S. vesicl. (mg/100g b.wt)	433 $\pm$ 17.6	62 $\pm$ 2.8*	60 $\pm$ 3.0 *	66 $\pm$ 3.2*	61 $\pm$ 2.9*
Pl. calcium (mmol/l)	2.2 $\pm$ 0.09	2.1 $\pm$ 0.07	2.2 $\pm$ 0.9	2.3 $\pm$ 0.08	2.2 $\pm$ 0.07
Pl. phosphate (mmol/l)	4.2 $\pm$ 0.12	4.2 $\pm$ 0.10	4.1 $\pm$ 0.10	4.2 $\pm$ 0.2	4.0 $\pm$ 0.3
Pl. testosterone ( $\mu$ mol/l)	23.0 $\pm$ 3.2	0.6 $\pm$ 0.2 *	0.5 $\pm$ 0.2 *	0.5 $\pm$ 0.3*	0.6 $\pm$ 0.2*
Kidney (mg/100g.b.wt)	1873 $\pm$ 112	1161 $\pm$ 59*	1118 $\pm$ 40*	1172 $\pm$ 34*	1107 $\pm$ 43*

extremely low weight of seminal vesicles was not increased compared with that in castrated untreated animals.

Kidney weight fell significantly after castration ( $p < 0.01$ ) and MPA, NETA and uterogestan did not significantly affect relative kidney weight in the castrated animals.

Mean tibial density, ash weight, mineral content of the tibia of castrated mice was significantly decreased ( $1.38 \pm 0.03$ ) compared with that in the control group ( $1.48 \pm 0.03$ ) ( $p < 0.01$ ). MPA and uterogestan did not have any positive effect on bone density, ash weight and mineral content. Only in the case of NETA has been observed slight but statistically significant increase in bone density ( $1.40 \pm 0.02$ ), ash weight and mineral content in comparison with castrated group ( $1.38 \pm 0.03$ ), ( $p < 0.05$ ).

Femoral length and outer diameter were not significantly different between the groups 3 months after orchidectomy. At this time however orchidectomized mice had significantly thinner cortical widths ( $0.53 \pm 0.02$  v.s  $0.27 \pm 0.02$ ). The decrease in cortical widths was not prevented by administration of progestagens.

## Discussion

All progestagens exert progestational activity via binding to a specific intracellular nuclear progesterone receptor [10]. It should be noted that natural progesterone and some of the synthetic progestagens show a weak binding affinity for other intracellular receptors for steroid hormones such as the glucocorticoid or the androgen receptor [11].

Studies indicate that progestagens may mimic, inhibit or potentiate the action of androgens. This effects have been termed the androgenic, antiandrogenic and synandrogenic actions of progestagens [12, 13]. These synandrogenic and antiandrogenic actions, like the androgenic actions of progestins are dependent not only upon steroid structure but upon the responsiveness of individual tissues,

**Table 2 – Variables of morphometric measurements on femur in individual groups of animals (mean  $\pm$  SE)  $p < 0.01$  vs. intact animals**

	Intact n-8	Castrated n-8	Castrated + Uterogestan n-8	Castrated + NETA n-8	Castrated + MPA n-8
Femur length (mm $\pm$ SE)	17.5 $\pm$ 0.2	17.2 $\pm$ 0.3	17.3 $\pm$ 0.3	17.4 $\pm$ 0.3	17.4 $\pm$ 0.2
Outer diameter (mm $\pm$ SE)	2.14 $\pm$ 0.07	2.11 $\pm$ 0.09	2.12 $\pm$ 0.08	2.12 $\pm$ 0.09	2.13 $\pm$ 0.09
Inner diameter (mm $\pm$ SE)	1.62 $\pm$ 0.11	1.84 $\pm$ 0.13*	1.85 $\pm$ 0.12*	1.81 $\pm$ 0.10*	1.86 $\pm$ 0.11*
Cortical width (mm $\pm$ SE)	0.53 $\pm$ 0.02	0.27 $\pm$ 0.02*	0.27 $\pm$ 0.03*	1.31 $\pm$ 0.10*	0.27 $\pm$ 0.10*

within a specific animal and between species. All progestagens which either mimic or modify androgen action compete with testosterone for binding sites on the cytoplasmic androgen receptor from kidney and the male reproductive tract. When gestagens are assayed in the adult rat progesterone and its C 21 derivatives have only minimal effects on the male reproductive tract, whereas compounds structurally related to 19-nortestosterone are more active [14]. The mechanism by which progestins act on androgen-sensitive end organs could involve a process which is either independent of or similar to that of testosterone.

The seminal vesicles and kidney of the mice can be considered as a target organ responding very sensitively to the alteration of the androgen homeostasis. Some human study indicated that progestagens may also be effective as an estrogen in prevention of postmenopausal bone loss [15]. The mouse bone tissue is also a known androgen responsive tissue [16]. It has been suggested that some progestagens could have a stimulating effect on bone formation. Therefore it was of interest to investigate the interactions of progestins on these organs.

The present results confirm the well known fact that castration leads to osteopenia in experimental animals. An extraordinary responsiveness to the withdrawal or administration of androgens can be demonstrated in mice. The bones of our castrated mice were characterized by reduction of ash weight, bone density and calcium and phosphorus content. Utrogestan and MPA have no effect on the bone density or mineral content of the femur tested mice and only in the case of NETA we observed slight increase in bone density.

The mechanism of the skeletal effects of norethisterone acetate is not known but it has been speculated that norethisterone acetate may enhance bone formation without decreasing bone resorption [17]. Progestagens do not appear to have any androgenic effect on the skeleton and norethisterone acetate has been suggested as one of the exceptions.

These results indicate that only norethisterone acetate at the dose which is used in hormonal replacement therapy for prevention of osteoporosis has a slight protective effect against bone mineral loss in castrated mice [18].

There is controversy concerning the effects of progestins on bone. In the work of [19] norethindrone acetate for 9 weeks does not have a potent short-term anabolic effect on bone, but does have effects that are likely to be mediated through the estrogen and androgen receptors.

In some studies a small additional beneficial effect of the progestagens was documented [20] but more often no significant differences were seen between estrogens alone or plus progestagens [21, 22].

Micronized progesterone has similar protective effects on the uterus and fewer effects on the lipid profile than other preparations, but its effects on bone are unknown. The work of [23] did not prove an effect of micronized progesterone on bone turnover. These data suggest that effects on bone demonstrated using other progestogens preparations might be due to androgenic or estrogenic effects.

Progestin medroxyprogesterone acetate (MPA) is one of the most commonly prescribed progestins for hormone replacement therapy and in gynaecologic practice. However it appears that MPA with significant glucocorticoid activity even may decrease bone density [24].

Few progestagens alone have been used in HRT without estrogens [21]. The conclusion from these papers is that use of progestins may prevent just only a small part of the bone losses seen without the use of steroids.

Sex steroids are important not only for the maintenance of the female skeleton but also for the male skeleton. The relative contribution of androgens versus estrogens in the regulation of the male skeleton is unclear [25].

Estrogen remains the primary bone active agent in hormone therapy while progestagens have significantly less activity. The selection of the appropriate progestagens in hormone therapy should be based on criteria other than bone activity. Progestagens do not have a potent short term anabolic effect on bone.

## References

1. FARISH E., BARNES J. F., O DONOGHUE E.: The role of megestrol acetate as an alternative to conventional hormone replacement therapy. *Climacteric* 3: 125–134, 2000.
2. CHESNUT CH., BELL N. H., CLARK G. S., DRINKWATER B. L., ENGLISH S. C., JOHNSTON C. C., NOTELOVITZ M., ROSEN C., CAIN D. F., FLESSLAND K. A., MALLINAK N. S. J.: Hormone replacement therapy in postmenopausal women, urinary N-telopeptide of type I collagen monitors therapeutic effects and predicts response of bone mineral density. *Am. J. Med* 102: 29–37, 1997.
3. GUNNET J. W., GRANGER K., CRYAN E., DEMAREST K. T.: Characterization of the progestin receptors in the human TE85 and murine MC3T3-E1 osteoblast-like cell lines. *J. Endocrinol.* 163: 139–147, 1999.
4. KALU D. N., HARDIN R. R., CECKERHAM R.: Evaluation of the pathogenesis of skeletal changes in ovariectomized rats. *Endocrinology* 115: 507–511, 1994.
5. GITELMAN H.: An improved automated procedure for the determination of calcium in biological specimens. *Anal. Biochem.* 18: 521–531, 1967.
6. KRAML J.: A semiautomated determination of phospholipids. *Clin. Chim. Acta* 13: 442–448, 1966.
7. VANDERSCHUEREN D. E., VAUTTERCK A. M. H., SUIKER W. J., WISSER L. P. C., SCHOT R., BOUILLON R.: Bone and mineral metabolism in aged male rats. Short and long term effects of androgen deficiency. *Endocrinology* 130: 2906–2916, 1992.
8. BEALL P. T., MISRA L. K., YOUNG R. L., SPJUT H. J., EBAVANS H. J., LE BLAME A.: Clomiphene protects against osteoporosis in the mature ovariectomized rat. *Calcif. Tissue Int.* 36: 123–125, 1984.
9. DUNCAN D.: Multiple range and multiple F test. *Biometrics* 11: 1–4, 1955.
10. GRAHAM J. D., CLARKE C. L.: Physiological action of progesterone in target tissues. *Endocr. Rev.* 18: 502–519, 1997.
11. DARNEY P. D.: The androgenicity of progestins. *Am. J. Med.* 98: 104S–110S, 1995.
12. GUPTA C. H., BULLOCK L. P., BARDIN C. W.: Further Studies on the androgenic, antiandrogenic and synandrogenic actions of progestins. *Endocrinology* 120: 736–744, 1978.
13. BROWN T. R., BULLOCK L., BARDIN C. W.: In vitro and in vivo binding of progestins to the androgen receptor of mouse kidney: correlation with biological activities. *Endocrinology* 105: 1281–1287, 1979.

14. BULLOCK L. P., BARTHE P. L., MOWSZOWICZ I., ORTH D. N., BARDIN C. W.: The effect of progestins on submaxillary gland epidermal growth factor: Demonstration of androgenic, synandrogenic and antiandrogenic actions. *Endocrinology* 97: 189–195, 1975.
15. CHRISTIANSEN C., RIIS B. J.: 17 beta estradiol and continuous norethisterone a unique treatment for established osteoporosis in elderly women. *J. Clin. Endocrinol. Metab.* 71: 836–841, 1990.
16. BROULIK P. D., STARKA L.: Effect of antiandrogens casodex and epitestosterone on bone composition in mice. *Bone* 20: 473–475, 1997.
17. BELAISCH J., DELCHAMBRE J.: Progestagens and bones *Contracept. Fertil. Sex.* 21: 853–860, 1993.
18. DELMAS P. D., CONFAVREUX E., GARNERO P., FARDELLONE P., VERNEJOU M. C., CORMIER C., ARCE J. C.: A combination of low doses of 17 beta estradiol and norethisterone acetate prevents bone loss and normalizes bone turnover in postmenopausal women. *Osteoporosis Int.* 11: 177–187, 2000.
19. ONOBRAKPEYA O. A., FALL P. M., WILLARD A., CHAKRAVARTHI P., HANSEN A., RAISZ L. G.: Effect of norethidronate acetate on hormone levels and markers of bone turnover in estrogen-treated postmenopausal women. *Endocr. Res.* 27: 473–480, 2001.
20. RIIS B. J., LEHRMANN H. J., CHRISTIANSEN C.: Norethisterone acetate in combination with estrogen effects on the skeleton and other organs. *Am. J. Obstet. Gynecol.* 187: 1101–1116, 2002.
21. THIJSSEN J. H. H.: Overview on the effects of progestins on bone. *Maturitas* 46: S77–S87, 2003.
22. LEPESCHEUX L., SECCHI J., GAILLARD-KELLY M., MILLER P.: Effects of 17 beta estradiol and trimesthene alone, and in combination on the bone and uterus of ovariectomized rats. *Gynecol. Endocrinol.* 15: 312–320, 2001.
23. IKRAM Z., DULIPSINGH L., PRESTWOOD K. M.: Lack of effect of short-term micronized progesterone on bone turnover in postmenopausal women. *J. Womens Health Gen. Based Med.* 8: 973–979, 1999.
24. ISHIDA Y., ISHIDA Y., HEERSCHKE J. N.: Pharmacologic doses of medroxyprogesterone may cause bone loss through glucocorticoid activity: a hypothesis. *Osteoporosis Int.* 13: 601–605, 2002.
25. BROULIK P. D., HALUZIK M., NEDVIDKOVA J.: *Effect of exogenous estrogen on male rats bone. Sborník lékařský* 100: 219–222, 1999.