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# Bone and muscle protective potential of the prostate-sparing synthetic androgen 7α-methyl-19-nortestosterone: Evidence from the aged orchidectomized male rat model

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**Abstract**

This study reports the preclinical evaluation of the bone and muscle protective potential of the synthetic androgen 7α-methyl-19-nortestosterone (MENT™), as assessed in the aged orchidectomized rat model. Aged (13-month-old) orchidectomized Wistar rats were treated with different doses of MENT (4, 12 or 36 μg/day) subcutaneously for 16 weeks via mini-osmotic pumps. Analysis of the effects of androgen deficiency versus MENT replacement was performed using quantitative computed tomography (pQCT), dual energy X-ray absorptiometry (DEXA) and biochemical markers of bone turnover. At the end of the study period, prostate weight in orchidectomized rats treated with low- (4 μg/day) or mid-dose (12 μg/day) MENT remained significantly lower compared to the sham-operated animals (−47% and −25%, respectively). High-dose MENT (36 μg/day), on the other hand, induced prostate hypertrophy (+21% versus sham). Low-, mid- and high-dose MENT were found to be effective in suppressing the acceleration of bone remodeling following orchidectomy, as assessed by osteocalcin and deoxypyridinoline. In addition, low-, mid- and high-dose were able to prevent the orchidectomy-induced bone loss, as evaluated by DEXA at the femur and total-body and by pQCT at the femur. Compared to sham-operated animals, the low- and mid-dose MENT groups showed no decline in lean body mass and no muscle atrophy (as measured by *m. quadriceps* weight) at 16 weeks, whereas high-dose MENT was associated with a significant decline in lean body mass (−8.5% versus sham) and quadriceps weight (−10.6%). We conclude that, in the aged orchidectomized rat model, low- and mid-doses of the synthetic androgen MENT have bone and muscle protective effects and do not induce prostate hypertrophy. The bone protective action of high-dose MENT, however, occurs at the expense of muscle wasting and prostate hypertrophy. Our findings support the need for human studies to explore the potential of MENT as an option for androgen replacement in aging men.


*Keywords:* Androgens; Orchidectomy; Bone; Lean body mass; Fat mass

## Introduction

Sex steroids play a major role in the maintenance of bone mass in both women and men [1]. Aging, however, results in the steady decline of sex steroids with concomitant decline in bone mass [2,3]. Testosterone deprivation in men induces a degree of bone loss which is similar to the bone loss observed in early menopause or following surgical ovariectomy [4,5]. Within 1 year after castration, lumbar spine bone mineral density (BMD) has declined by about 5–10%, followed by a more gradual but continuing decrease in axial BMD [4–7]. Significant bone loss, albeit to a lesser extent, is also observed at appendicular skeletal sites, including the hip. In addition to the decline in BMD, aging men lose

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muscle tissue and gain fat mass [7–9]. Androgen deficiency is likely to contribute to the age-associated decline in lean body mass and muscle strength which, in turn, may lead to loss of bone quality and increased susceptibility for falls, two major determinants of the risk for fracture [10].

There is an ever-increasing interest in androgen supplementation in aging men. Stimulated by the clinical potential of selective estrogen receptor modulators (SERMs) in postmenopausal women, the search for androgenic SARMs is currently being pursued [11,12]. Ideally, a SARM for use in aging men should support libido and protect both muscle and bone mass without having adverse effects on the prostate and other sex accessory glands [13], but no SARMs have been clinically approved yet. The data obtained so far have not clearly defined the balance between risks and benefits of androgen replacement therapy in these men [14–16]. In clinical trials, testosterone supplementation induces minimal increase in the prostate volume with modest increase in levels of prostate specific antigen (PSA). Some of the other potential undesirable effects of androgen replacement, such as increase in erythropoiesis, sleep apnea, gynecomastia and suppression of spermatogenesis, are also observed in men and should be examined during treatment [14].

A synthetic androgen, 7α-methyl-19-nortestosterone (MENT), is being developed for androgen replacement therapy and male contraception. Compared to testosterone, MENT is almost 10-fold more potent on the muscle while its androgenic potency on the sex accessory glands is 4- to 5-fold higher [17]. Unlike testosterone, MENT does not undergo 5α-reduction. Thus, for maintaining most androgenic functions including anabolic action, the dose of MENT required is estimated to be only half the dose required to maintain prostate weights in castrated rats [17]. This confers an additional advantage over the high potency so that the replacement dose in men will be able to maintain most androgenic functions without over-stimulating the prostate. This prostate-sparing effect of MENT has also been demonstrated in monkeys [18]. In addition, it has been demonstrated that MENT is a substrate for aromatase enzyme and can be converted to an estrogenic compound with significant binding for estrogen receptors (ER) [19]. MENT, based on its less stimulatory effects on the prostate versus muscle and favorable safety profile, might potentially be an interesting androgen for hormone replacement therapy. However, the effects of MENT on bone maintenance and replacement have not been studied.

Previous studies from our group have provided evidence that the aged male rat model allows preclinical evaluation of sex steroids on bone and muscle tissue [20–24]. In this animal model, orchidectomy induces a significant loss in spine and hip BMD, along with a decline in lean body mass and muscle atrophy. Loss of bone and lean body mass is prevented by testosterone, even at low concentrations [24]. Dihydrotestosterone (DHT), on the other hand, appears to have less bone-sparing capacity than testosterone [20]. In fact, in the context of this model, only supra-physiological doses of DHT are able to prevent orchidectomy-induced (trabecular) bone loss, and this bone-sparing action occurs at the expense of hypertrophy of the prostate and seminal vesicles [20]. These unwanted side effects may limit the clinical potential of DHT, particularly in aging men. MENT, due to its less stimulatory effects on the sex accessory glands, might potentially become as an option for androgen replacement therapy for aging men. The effects of MENT on bone, muscle and prostate in the aged male orchiectomized rat model were assessed in this study.

## Materials and methods

### *Animals*

Male 13-month-old Wistar rats (600–650 g) were obtained from Bioservices (Schaijk, The Netherlands) and housed under standard conditions: a 12-h light/dark cycle, in an air-conditioned room, and a standard diet (1% calcium, 0.76% phosphate) (Hope Farms, Woerden, The Netherlands) and free access to tap water.

Sixty rats were randomly divided into six groups. A baseline group was killed at the start of the experiment. The other rats were either sham-operated (Sham) or orchidectomized (ORX) using Nembutal (ip, 60 mg/kg) anaesthesia (Sanofi Pharmaceuticals, Inc., Sante Animal, Brussels, Belgium). ORX rats were treated with either vehicle (1,2-propandiol, Merck) or with different doses of 7α-methyl-19-nortestosterone (MENT) (4, 12 or 36 μg/day), through subcutaneously implanted mini-osmotic pumps (Alzet, model 2004, Durect Cooperation, Cupertino, CA). Rats were treated for an experimental period of 16 weeks and pumps were replaced every 4 weeks. MENT was kindly provided by the Population Council (New York, USA).

Body weight and food intake were determined weekly during the experimental period. At 8 and 16 weeks of treatment, rats were put in metabolic cages to collect 24-h urine for measurement of collagen cross-links. Serum was collected by tail bleeding at 8 weeks of treatment. After 16 weeks, the rats were anaesthetized with Nembutal and sacrificed by exsanguination via the abdominal aortic artery. Serum was collected and stored at −20°C until assay. The effects of ORX and ORX + treatment were verified by measurement of ventral prostate, seminal vesicles and levator ani wet weight immediately after sacrifice. *M. quadriceps* weight was assessed as a marker of treatment effects on muscle. All experimental procedures were conducted after obtaining formal approval from the ethical committee of the Katholieke Universiteit Leuven.

### *Assays*

Serum osteocalcin was measured by an in-house radioimmunoassay (RIA) [25]. After acid-ethanol extraction serum IGF-I concentrations were measured by an in-house