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Original Article

Hylonome

Lean, fat and bone masses are influenced by orchidectomy in the rat. A densitometric X-ray absorptiometric study

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Abstract

In man, hypogonadism is a risk factor for osteoporosis. Orchidectomy (ORX) in the rat leads to an imbalance between resorption and formation resulting in bone loss. We have measured whole body weight, lean and fat mass, whole bone mass (BMC) in the ORX rat model by dual X-ray densitometry (DXA). Forty-eight male Wistar rats (18-19 weeks old) were studied at 2, 4, 8 and 16 weeks. In each group, 6 rats were ORX and 6 sham-operated were used as control. DXA was performed on the whole body and isolated tibia. The whole body weight of the ORX animals became significantly decreased only at 16 weeks. Whole body BMC was reduced from 8 weeks in the ORX group. The most striking result was a net decrease in lean mass that reached -15.7% at 16 weeks. On the other hand, fat mass remained unchanged during the time series in the ORX animals.

Keywords: Fat Mass, Lean Mass, Densitometry, DXA, Orchidectomy, Animal Model, Osteoporosis

Introduction

Osteoporosis has been defined as a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk[1]. Sex hormones are recognized as important factors in the maintenance of bone mass and architecture. An increased bone remodeling rate (leading to a marked reduction of skeletal mass) is observed in women after menopause or surgical castration. Reid et al.[2,3] reported that bone mineral density was determined by fat body mass. Other studies demonstrate that both fat mass and lean body mass have important effects on bone mass, depending on the bone mass parameter used, the skeletal site measured and menopausal status[4-6]. Recently, the importance of leptin has been stressed and helps to explain the protective effect of obesity on bone mass in humans[7]. In men, the decline of the gonadal function with age is slower than in women. However risk factors are usually observed in men to induce osteoporosis (smoking, alcohol abuse, previous gastric surgery, reduced physical activity and glucocorticoid therapy). Hypogonadism is also recognized as a risk factor for osteoporosis in men[8].

In men, the prevalence of osteoporosis has often been underestimated. Recent epidemiological studies have shown that about 30% of hip fractures and 20% of vertebral fractures occurred in men. A recent American study estimated that the lifetime risk of hip fracture was about 6% and the risk of vertebral fracture was about 5% in 50 year-old white men[9]. The decrease in testosterone in men is related to modification of the body composition (fat, lean and body mass)[10-12]. These changes are similar to those that occur in hypogonadal men (natural or acquired) and are highly correlated with androgen levels[13,14].

The mechanisms governing the fat mass, lean mass and their relationships to the decrease in bone mass with age and sex are still unclear[15]. The studies of Horber et al. suggest that the impact of age on the fuel metabolism and body composition is different in men and women[16]. Appropriate animal models are needed to elucidate further the pathogenesis of bone loss induced by androgen deficiency. The orchidectomized rat (ORX) has been proposed to simulate male osteoporosis due to hypogonadism[17-19]. Dual energy X-ray absorptiometry (DXA) is recognized as the most valuable non-invasive method. It is currently used in clinical practice to measure bone mass (bone mineral content (BMC) and density (BMD)). DXA has also been proposed as an accurate method in nutritional studies to

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measure lean and fat mass[20]. The aim of the present study was to investigate the time-related changes in lean, fat, and bone mass induced by ORX.

Materials and methods

Animals

An homogenous cohort of forty-eight male Wistar rats (Charles River, Cléon, France), weighing 466 ± 36 g (18-19 weeks old) were acclimated for two weeks old to the local vivarium conditions (24°C and a 12h / 12h light dark cycle). These were adult and mature Wistar rats according to the growth provided by the supplier. Rats were given a standard laboratory food (UAR, Villemoison sur Orge, France) and water *ad libitum*.

Twenty-four rats were sham operated and served as controls. Twenty-four rats were orchidectomized by scrotal incisions under halothane anesthesia and constituted the ORX group. After ligature of the testicular arteries, the castration was performed and the incisions were closed with three clips. All rats were weighed weekly and sacrificed with chloroform at 2, 4, 8 or 16 weeks after the beginning of study. At sacrifice, whole body DXA measurements were performed. Thereafter, the tibias were dissected, cleaned of surrounding tissues and fixed in an ethanol-based fluid for 24 hours at +4 °C.

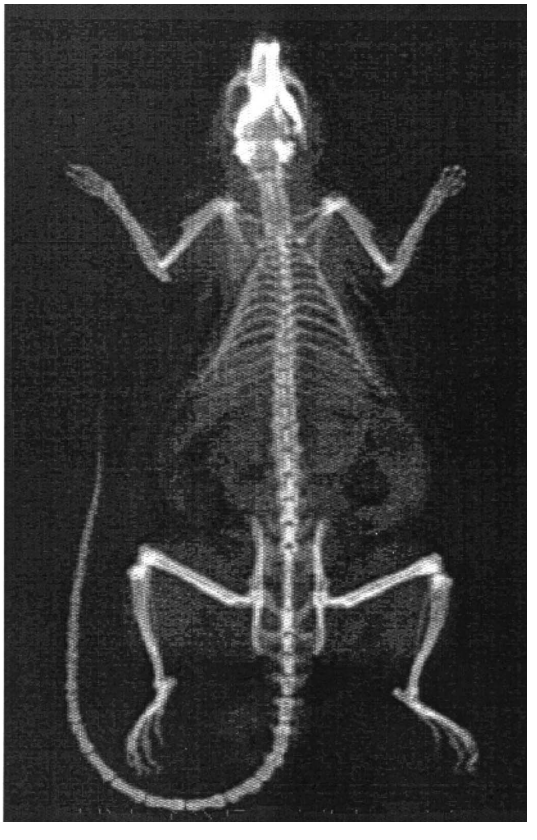

Figure 1. Whole body image of a Wistar rat analyzed with the Hologic QDR 2000.

Dual energy X-ray absorptiometry

Whole body and tibia DXA measurements were done on a Hologic QDR 2000 (Hologic inc., Waltham, MA) functioning in the pencil beam mode with the small animal software (release V5-71 and V4-74 respectively). Before each series of measurements, a tissue calibration scan was performed with the Hologic phantom. Each rat was placed on a Plexiglas platform in a prone position. Several parameters were obtained: BMC, BMD and whole body weight (comprising the BMC + lean body mass + fat mass) (Fig. 1). BMC and BMD of the left tibia were measured using a proximal height resolution option with 0.0267 cm line spacing. Tibias were placed in a plastic dish containing water at a constant depth for all measurements and placed on the Plexiglas platform.

Statistical analysis

Statistical study was performed using the Systat statistical software release 8.0.1 (SPSS inc. Chicago, IL). All data were expressed as mean ± SEM. At each studied time, differences between the SHAM and ORX groups were analyzed using a Mann-Whitney U-test. Differences were considered as significant when $p<0.05$.

Results

The whole body weight of the ORX animals became significantly decreased only at 16 w ($p = 0.01$) (Fig. 2). The body weight of the animals was well correlated between DXA and the weighing scale ($r = 0.993$; $p<0.000001$). BMC of the whole body was decreased in the ORX groups at all times but only reached significance at 8 weeks ($p<0.05$). Bone loss became more marked at 16 w with a -12.7% decrease ($p<0.001$) (Fig. 3). When BMD values were considered, the decrease was only significant at 16 w ($p<0.01$). BMC of the tibia became significantly lower at 8 weeks in the ORX group ($p<0.01$) (Fig. 4). As of the second

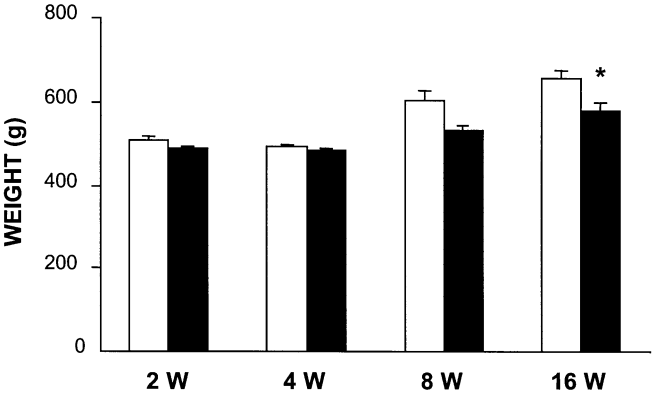

Figure 2. Time evolution of whole body weight measured by DXA in SHAM rats: hollow bar and ORX rats: solid bar. * significant difference: $p<0.01$.

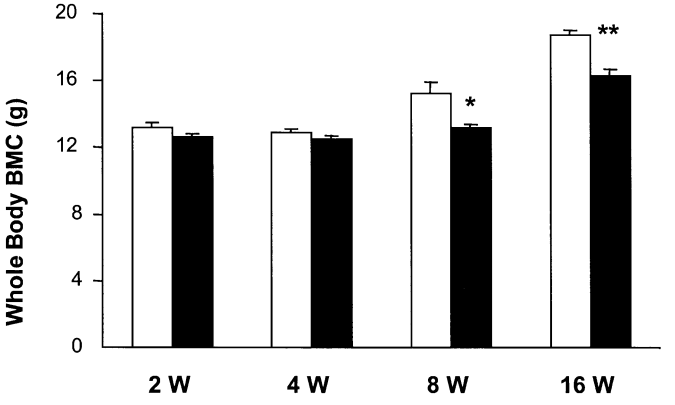

Figure 3. Time evolution of whole body BMC measured by DXA in SHAM rats: hollow bar and ORX rats: solid bar. * significant difference: $p<0.05$, ** significant difference: $p<0.001$.

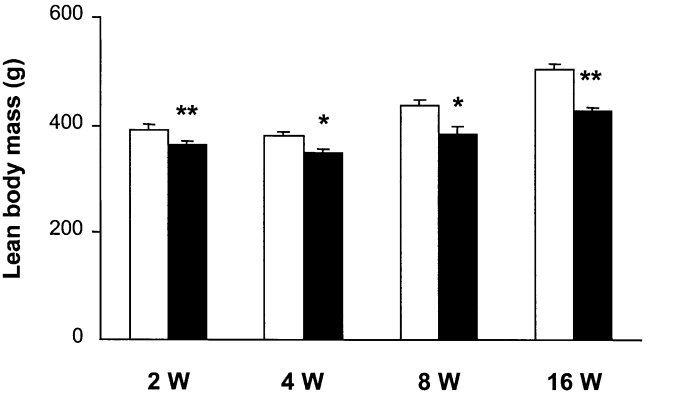

Figure 5. Time evolution of lean body mass measured by DXA in SHAM rats: hollow bar and ORX rats: solid bar. * significant difference: $p<0.05$, ** significant difference: $p<0.01$.

week, a significant reduction of the lean body mass was noted in the ORX group (-7.4% $p<0.01$ at 2 w). The difference was accentuated during the following weeks and a -15.4% was obtained at 16 w in the ORX group ($p<0.01$ at 16 w) (Fig. 5). On the contrary, no significant change in fat mass was observed during all the duration of the study (Fig. 6).

Discussion

Multiple factors are known to determine the bone mass. Genetic influences, nutritional factors such as protein and calcium intake, physical activity and endocrine status have been shown to play key roles in humans[21]. Changes in body composition (lean and fat mass) and BMC have been repeatedly reported being associated with a decrease in the estrogen level in women[2,22]. In men, a decreased androgen level is associated with decreased lean body mass and an increase in fat mass is associated with aging[12]. Acquired hypogonadism has also been found to induce similar modifications[13]. The beneficial effects of androgen replacement therapy on bone and body composition have been studied by Katznelson et al. in hypogonadic men whose age ranged from 22 to 69 years old[14]. Testosterone was found to reduce bone remodeling and fat mass and conversely to increase muscle mass in hypogonadic patients[23].

The ovariectomized rat model (OVX) has been favored for post-menopausal osteoporosis research by the Food and Drug Administration and the World Health Organization[24-27]. The validation of animal models has led to a renewed interest in non-invasive measures of bone mass and body composition in small animals using DXA[28]. DXA was found to be a sensitive and reproducible technique to measure bone loss in small animals although the relationships between BMC and ash weight are somewhat influenced by the animal or bone weight[29,30]. The aged male rat appears a useful model for studying the age-related changes in body composition and bone or muscular mass[31,32].

Our results in the mature rat (>19 weeks) are in agreement with those of Vanderschueren[32]. There is a significant reduction in the lean mass and BMC while fat mass remained unchanged. On the other hand, ORX rats had a reduction in body weight after 16 weeks. Noticeable differences exist in the literature when considering the age, the weight and the species of animals at ORX.

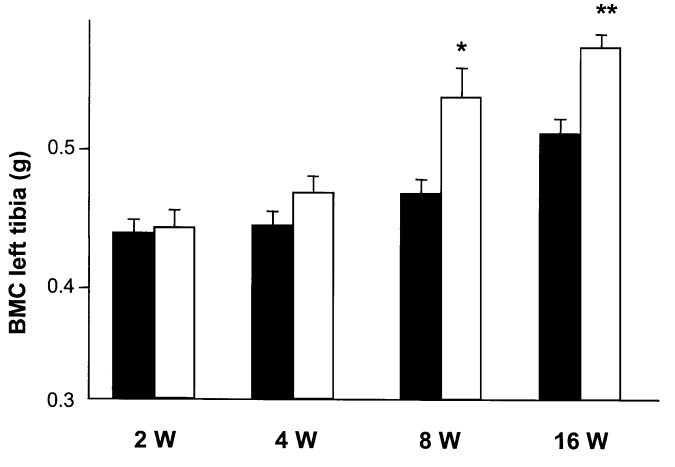

Figure 4. Time evolution of the BMC of the left tibia measured by DXA in SHAM rats: hollow bar and ORX rats: solid bar. * significant difference: $p<0.01$, ** significant difference: $p<0.006$.

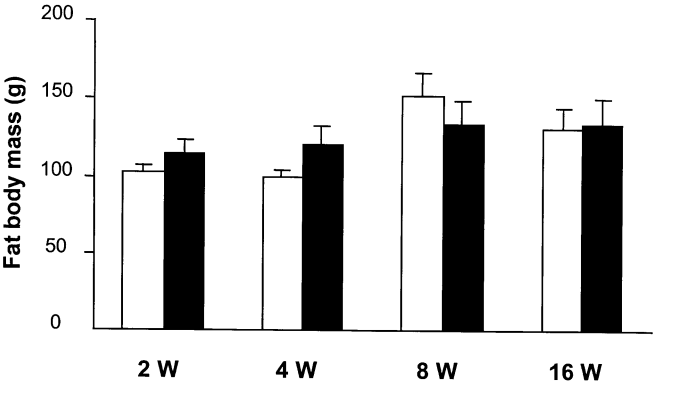

Figure 6. Time evolution of fat mass measured by DXA in SHAM rats: hollow bar and ORX rats: solid bar.

Makan et al reported that lean and fat mass measured by DXA were more accurate and reproducible in animals weighing between 150 to 600 grams[28]. Aged animals should be preferred because there is no longer interference between the androgen deficiency and bone growth. In the present series, we have found no modification of the fat mass after ORX. This discrepancy with human studies performed in aged men[10-12,14] could be explained by a study of too short duration to induce significant changes in the rat.

The effects of ORX on bone were assessed by DXA and histomorphometry in 12 week old rats by Rosen et al,[33]. They found both methods valuable to detect the reduction of bone mass, but the histomorphometric changes were much more marked (10 times). In the present study, a reduction in bone mass was evidenced on the whole skeleton and on the isolated tibia only at 8 weeks. Our findings are in accordance with those reported by others in the ORX rat model[31,34,35]. In the present study, loss of lean mass could be detected as early as 2 weeks post-ORX, while whole body BMC was reduced from 8 weeks; this seems to indicate that loss of lean mass precedes that of bone. Similar results were reported in the rat by the group of Jee & Yao et al[36,37], who found that making ORX rats rise to erect bipedal stance increases muscle mass and partially prevents cancellous bone mass in the tibia metaphysis and cortical tibial shaft[36,37]. Our results fit in well with the Utah paradigm's thesis that "muscle strength strongly influences and may even dominate control of post-natal changes in the strength of load-bearing bones"[38,39]. The most important and significant modifications after ORX seem to be the reduction in the lean mass. This result agrees with human studies from Smerdely et al. which concluded that measurements of weight and lean mass are the main predictors of bone mass in healthy, community-dwelling older men[40].

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