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The Influence of Orchidectomy on Collagen Glycosylation of Trabecular Bone in Rat[1])

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Summary: This study evaluates the effect of male rat castration on the degree of collagen glycosylation of bone. Twenty 100-day-old male Sprague-Dawley rats were subjected to either orchidectomy (n = 10) or sham operation (n = 10). After surgery animals were divided at random into 2 groups: the first group (5 sham operated and 5 orchidectomized) was sacrificed under anaesthetic at 130 days of age, while the second group (5 sham operated and 5 orchidectomized) was sacrificed at 250 days of age. Femurs and tibiae were separated into cortical and trabecular bone, demineralized, hydrolyzed and analyzed by HPLC for hydroxylysine glycosides and hydroxyproline content. Orchidectomy causes an increased collagen glycosylation only in trabecular bone, as already observed in ovariectomized rats. However, the effect was not seen in the group of 130 day old rats, i. e. 30 days after orchidectomy, but was evident in the 250 day old rats, i. e. at 150 days from castration. These data suggest that collagen glycosylation could also be controlled by testosterone.

Introduction

While there is a great deal of evidence indicating that androgens exert important influences on bone metabolism (1–11), to our knowledge nothing is known of their effect on bone matrix. Estrogens however, have great influences on bone (12–14) and also seem to affect bone collagen glycosylation. As we have shown in a previous work (15), ovariectomy, which in the rat represents mainly an estrogen depletion (16), causes an overglycosylation of collagen of trabecular bone. This finding would appear to be important since changes in matrix composition are certainly relevant to the mechanical properties of bone (17–20). Moreover, this finding may contribute to a better understanding of the pathogenesis of post-menopausal osteoporosis, as it is known that in the first months after ovariectomy there is a marked loss only of the trabecular bone (21).

Following this line of investigation, the present study was undertaken with the aim of establishing whether the effect of orchidectomy on bone collagen glycosylation is similar to that of ovariectomy, and in this paper we report data on the content of hydroxylysine glycosides of collagen of cortical and trabecular bone in orchidectomized rats at 130 and 250 days of age, i. e. at 30 and 150 days from castration.

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Materials and Methods

Animals

All groups of animals were kept in identical conditions and were allowed free access to food and water. The surgical procedures were carried out in accordance with institutionally approved small animal protocols.

Twenty 100-day-old male Sprague-Dawley rats were subjected to either orchidectomy (n = 10) or sham operation (n = 10). Bilateral orchidectomy was carried out under anaesthetic (xylazine hydrochloride, Rompun®, Bayer, 0.15 ml per kg of body weight, plus ketamine hydrochloride, Ketalar®, Parke Davis, 1 ml per kg of body weight). After surgery animals were divided at random into 2 groups: the first group (5 sham operated and 5 orchidectomized) was sacrificed under anaesthetic at 130 days of age (30 days from castration), while the second group (5 sham operated and 5 orchidectomized) was sacrificed at 250 days of age (150 days from surgery).

Tissue preparation

Tibiae and femurs were removed and dissected free of adhering soft tissue. After washing with water, bones were dissected into three anatomically distinct areas: the diaphysis containing only cortical bone and the proximal and distal metaphysis, from which trabecular bone was scraped and pooled. The periosteum was stripped from the diaphysis and cortical bone was fragmented.

Bone treatment prior to analyses

Bones were defatted by sequential treatment in methanol for 16 h followed by chloroform/methanol (2 + 1, by vol.) for 48 h and methanol for a further 16 h and decalcified at 4 °C as previously described (22, 23). Subsequently, bones were extracted with 4 mol/l guanidine hydrochloride to remove soluble matrix macromolecules from the largely insoluble collagenous matrix. Cortical and trabecular bones thus obtained were freeze-dried and then hydrolyzed in 2.5 mol/l NaOH (15 g of bone dry weight per litre) for 22 h and hydroxyproline and hydroxylysine glycosides were measured.

Hydroxylysine glycosides and hydroxyproline measurements

Hydroxylysine glycosides were measured as previously reported (24). Briefly: aliquots of hydrolyzed tissue were derivatized using dansyl chloride following the method of *Gray* (25), and 20 µl were injected into a reversed-phase Ultrasphere-ODS (C_{18} of 5 µm particles) HPLC column of 250 × 4.6 mm i. d. The HPLC Beckman Gold Model was connected with a fluorimeter (Shimadzu RF551), using an excitation wavelength of 366 nm and an emission wavelength of 490 nm. The area of the peaks was calculated by an IBM personal computer. Values of hydroxylysine glycosides were calculated using *L*-lysine as an external standard as previously described (26).

Hydroxyproline measurements were performed by HPLC following the method of *Teerlink* et al. (27). The amount of hydroxylysine glycosides is expressed as a molar ratio with hydroxyproline. Hydroxyproline content is expressed as mmol of hydroxyproline per g of bone dry weight.

Testosterone measurements

Serum testosterone was measured by radioimmunoassay (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA).

Statistics

Statistical differences between groups were evaluated by unpaired t-test.

Results

Body weight

Body weight of the two groups of animals was similar at the beginning of the experiment. As shown in figure 1, at 130 days of age, body weight increased by 115 ± 11 g for sham operated and by 85 ± 14 g for orchidectomized animals (p = 0.005 vs castrated). At 250 days of age, sham animals had gained 206 ± 58 g and orchidectomized 138 ± 30 g (p = 0.005 vs castrated).

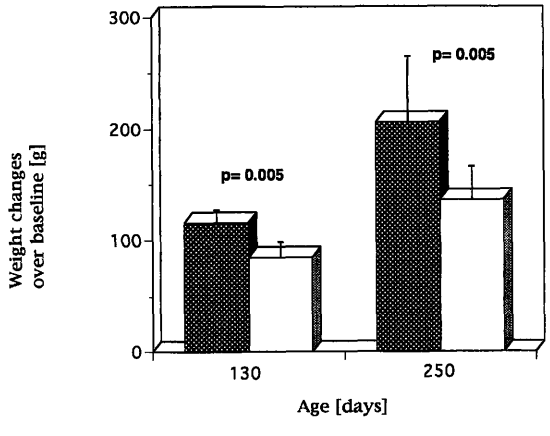

Fig. 1 Rat's body weight. Increment from baseline values in body weight at 130 and 250 days of age of sham operated (■) and orchidectomized (□) rats. Values are expressed as mean ± SD.

Serum testosterone concentration

Testosterone was measured only in rats of 250 days of age. The average value of testosterone in sham animals was 0.49 ± 0.22 µg/l (range 0.22−0.75), while values were not detectable in orchidectomized rats.

Hydroxyproline content

The hydroxyproline content of cortical and trabecular bone collagen at the two time points of the experiment, both in sham operated and in orchidectomized rats, was not significantly different, as shown in table 1.

Tab. 1 Hydroxyproline content of cortical and trabecular bone collagen in sham operated and orchidectomized rats at 130 and 250 days of age.

	Hydroxyproline (mol/kg bone dry weight)[a]	
	Cortical	Trabecular
Age 130 days		
Sham operated	0.50 ± 0.05	0.50 ± 0.07
Orchidectomized	0.52 ± 0.08	0.51 ± 0.04
Age 250 days		
Sham operated	0.48 ± 0.05	0.53 ± 0.04
Orchidectomized	0.49 ± 0.04	0.51 ± 0.01

[a] $\bar{x}$ ± SD

Glycosylation

As shown in figure 2, glycosylation in cortical and trabecular bone, both in sham and in orchidectomized animals, significantly increases with age.

Cortical bone does not show any significant statistical difference in the content of glycosides of hydroxylysine

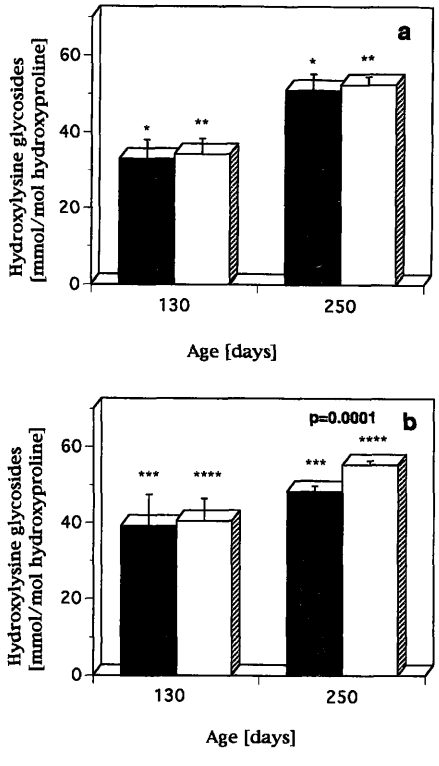

Fig. 2 Bone collagen glycosylation. Hydroxylysine glycosides content (mmol/mol hydroxyproline) at 130 and 250 days of age of sham operated (■) and orchidectomized (□) rats. (**a:** cortical bone collagen; **b:** trabecular bone collagen). Values are expressed as mean ± SD.
* p = 0.0003; ** p = 0.0001; *** p = 0.043; **** p = 0.0005.

between sham operated and orchidectomized animals, at either experimental timepoint (fig. 2a). On the contrary, as shown in figure 2b, at 250 days of age trabecular bone collagen of orchidectomized animals has a higher glycosylation compared to collagen of sham operated animals (orchidectomized vs. sham operated $p = 0.0001$).

Discussion

In a previous work (24) we were able to demonstrate that the extent of collagen glycosylation is different between the sexes, i. e. greater in females than in males. Such differences have been interpreted as indicating that sex hormones may influence collagen glycosylation to a greater or lesser degree.

In another work (15) we have seen that ovariectomy increases collagen glycosylation of trabecular bone and the results of the present investigation show that orchidectomy has the same effect. However, in addition to similarities, some differences between sexes, both in the extent and time of collagen over-glycosylation, can be observed. In fact, castration leads to a greater extent of collagen over-glycosylation in the female than in the male rat in a shorter time, suggesting an inhibitory capacity of both sex hormones on collagen glycosylation, greater for estrogens than for testosterone. However, since testosterone is aromatized into estradiol (28) and osteoblasts express receptors for both androgens and estrogens (1–3, 29), it is not possible to exclude that in the male rat collagen glycosylation could be also controlled by estrogens. To this regard, in a recent work, *Vanderschueren* and co-workers have shown that a selective blockade of aromatase activity has effects similar to castration on the skeleton of male rats (30). Whether collagen over-glycosylation is mediated by direct inhibition of glycosyl-transferases or by the promotion of the folding of α chains into triple helix, thus decreasing the time for glycosyl-transferases to act (31), is not yet possible to establish.

In the male rat, over-glycosylation cannot be observed at 30 days from orchidectomy, but it can be detected at 150 days from surgery. This second time interval in our experiment was chosen since a report (32) showed that in old male rats which had virtually stopped growing, androgen deficiency leads to a condition similar to that observed in menopause, and at 250 days of age the growth rate of the animals used in this experiment tends to be null (33). Although we lack other experimental times between these and, therefore, we cannot establish the exact time when over-glycosylation occurs, we may conclude that in the male rat the effect of castration is delayed in comparison to the female rat.

The reason why only osteoblasts from trabecular bone are susceptible to the action of orchidectomy is not yet clear. It might be thought that collagen over-glycosylation is a result of increased bone turnover following castration. However, this possibility has been ruled out since no effect can be seen at 130 days (after 30 days of surgery), but it is evident at 250 days of age. Bone turnover increases immediately after orchidectomy (34–36) and therefore if over-glycosylation were the consequence of orchidectomy, we would expect to see it already in rats at 130 days of age.

Interestingly, a significant increase in glycosylation in both cortical and trabecular bone collagen in sham operated and orchidectomized animals occurs with age as shown in figure 2. This is also present in female rats at 100 and 300 days old (author's data not published) and although no explanations about the reasons and the mechanism at the moment are available, this observation is in agreement with the acknowledged consideration that increased protein glycosylation is one of the features of aging (37–41).

From our studies, it appears that the degree of collagen post-translational modification differs between bone compartments in accordance with their function and that they are susceptible to the action of systemic factors in different ways (24). As already reported (17, 36, 42), the site at which the trabecular bone is sampled can show significant differences. Therefore, it cannot be excluded that glycosylation might be different in different parts of the skeleton, according to the mechanical or the metabolic function of the specific site. Thus, for example, the proximal and distal metaphysis of femur, which have a mechanical and a metabolic function, respectively (43–45), might be differently glycosylated in accordance with our observation that bone which exerts a mechanical function is less glycosylated than bone with a mainly metabolic function, e. g. cortical and trabecular bone, respectively (24). However, in the rat we are unable to study separately trabecular bone from proximal and distal metaphysis, since the amount of material which can be collected from each site is not sufficient to carry out the measurements.

In conclusion, in this work we have shown that orchidectomy affects collagen glycosylation of trabecular bone suggesting that it could also be controlled by testosterone, thus both sex hormones would seem to influence this post-translational modification. Whether testosterone acts directly or after its aromatization into estradiol remains to be established.

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