

LOW LEVELS OF TESTOSTERONE INDUCE THE GENERATION OF FREE RADICALS AS WELL AS APOPTOSIS IN THE ANAL SPHINCTER, WHICH CAN BE AVOIDED THROUGH ALPHA TOCOPHEROL SUPPLEMENTATION

Hypothesis / aims of study

Fecal incontinence is one of the major public health concern in societies undergoing an aging process. One of its causes is apoptosis of the muscle cells of the anal sphincter. Because apoptosis is age-dependent, it is believed that aging is one of the main causes of sphincter deficiency. Results from a study on fragments of human anorectal tissue have shown intense expression of androgen and estrogen receptors in the tissues of the anal sphincter complex, thus indicating that this is a target organ for sex steroid hormones¹

The physiopathology of incontinence is often described in terms of the damage that occurs at tissue or cellular level. However, little is known about the physiopathological basis for sphincter dysfunction at molecular level. Most hypotheses point towards aging as the oxidative stress mechanism. Antioxidant agents are capable of limiting or preventing the harmful effects of free radicals. However, as the aging process develops, these mechanisms diminish and oxidative damage becomes prevalent².

In the light of the above points, the present study had the aims of contributing towards understanding the manifestations of the anal sphincter in the presence of low levels of sex hormones and evaluating the beneficial effect of alpha-tocopherol for avoiding oxidative stress induced by orchiectomy.

Study design, materials and methods

The project was approved by the University Ethical Committee.

Forty male rats of wistar breed weighing 250-300g were used, divided into four groups: **group I** – subjected to a *sham* procedure; **group II** – subjected to bilateral orchiectomy and sacrificed eight weeks after the procedure; **group III** – subjected to bilateral orchiectomy with alpha-tocopherol supplementation for four weeks preceding the procedure; and **group IV** – subjected to bilateral orchiectomy with alpha-tocopherol supplementation for four weeks preceding the procedure and for eight weeks afterwards. At the end of the experiment, the sphincter complex was dissected and analyzed stereologically, with evaluation of the volumetric density of the collagen as well as muscle fibers. The presence of oxidative stress was determined by means of assaying the tissue level of 8-isprostane and occurrences of apoptosis were evaluated by means of identifying active caspase-3 through immunohistochemical analysis. Variance analysis with Dunnett's post-test was used for the statistical analysis.

Results

The volumetric concentrations of collagen were 21,59% ($\pm 1,53$), 49,27% ($\pm 4,6$), 38,6% ($\pm 4,28$) and 16,93% ($\pm 1,5$), in groups I, II, III and IV, respectively. Regarding the volumetric density of muscle fibers the concentration was 75,56% ($\pm 3,27$), 52,43% ($\pm 6,28$), 62,00% ($\pm 4,88$) and 83,07% ($\pm 1,50$), respectively. The statistical analysis demonstrated that the collagen fibers concentration was statistically greater in group I and IV compared to groups II and III ($p = 0.0012$) (figure I). On the other hand the muscle fibers concentration was higher in group I and IV compared to groups II and III ($p = 0.003$). Vitamin supplementation for twelve weeks presented the highest protection against the damage caused by castration. Analysis of 8-isoprostane levels showed high concentrations of oxygen-reactive species in group II, in the other groups ($p < 0,004$). Groups III and IV presented active caspase-3 levels that were lower than in group II ($p < 0,021$) (figure II_{a,b}), thus corroborating the hypothesis that alpha-tocopherol has a protective effect in relation to apoptosis induced by oxidative stress.

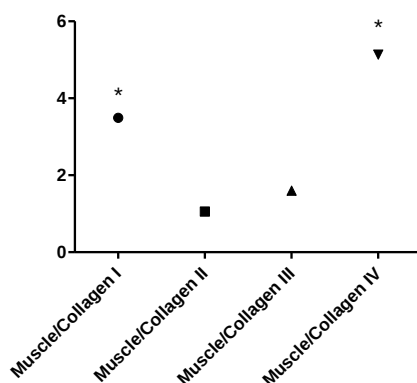


Figure I: The ratio smooth muscle – collagen fibers in the anal sphincter in different groups. (* $p < 0.05$ groups I and IV compared to groups II and III).

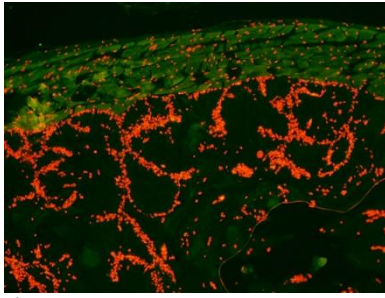


Figure II_a

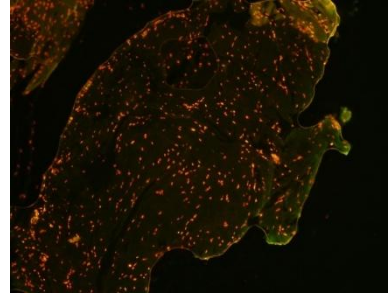


Figure II_b

Figure II. Microscopic fluorescence immunohistochemistry for the detection of active caspase-3. The positive reaction is visualized in green. Nuclei are observed in red. Increase 10x, orchiectomized group (a) and orchiectomized with alpha-tocopherol supplementation for four weeks preceding and for eight weeks after the procedure (b).

Interpretation of results

Castration of male rats caused oxidative stress in the anal sphincter complex, thus demonstrating that low testosterone levels lead to the development of oxidative stress and induction of apoptosis in the musculature, with consequently increased collagen deposition. Alpha-tocopherol supplementation avoided the generation of free radicals and the fibrosis that they cause in muscle tissue, and also presented a protective effect with regard to apoptosis induced by oxidative stress in castrated rats.

Concluding message

From a broader perspective, it is suggested that low testosterone levels are associated with the induction of free radical formation. It is further suggested that the use of antioxidants has a protective role in situations of hypogonadism, thereby avoiding or minimizing the effects of anal sphincter dysfunctions coming from muscle damage.

References

¹ Eur J Obstet Gynecol Reprod Biol (1998) 77(2);211-6

² J Gerontol A Biol Sci Med Sci 2006;61(5):427-34

<i>Specify source of funding or grant</i>	NON
<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	ANIMAL
<i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i>	Yes
<i>Name of ethics committee</i>	COMMITTEE OF ETHICAL IN RESEARCH OF FEDERAL UNIVERSITY OF SÃO PAULO/EPM