

Testicular and blood steroid levels in aged men

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SUMMARY

In this study, we have evaluated the hypophyso-gonadal axis in three groups of men aged 60-69, 70-79 and 80-91 years by measuring the intratesticular concentrations of several steroids (pregnenolone, progesterone, DHEA, DHEA-S, testosterone, estradiol) and serum levels of FSH, LH, testosterone, estradiol and sex hormone binding globulin (SHBG). The histological examination of testes revealed normal spermatogenesis in all examined samples. No significant changes in serum hormone and SHBG concentrations as well as in testicular steroid contents among the three groups of patients were found. However, the mean serum SHBG level was three times higher in the oldest men than in other groups and a positive correlation between patient's age and serum SHBG was observed. Therefore, the bioavailability of estradiol in the oldest men was likely diminished. Consequently, the hormonal status in aged men is rather unchanged but great variations observed between patients imply special cautious when the SHBG and estradiol levels are concerned. *Reproductive Biology*, 2004 4(3): 299-304.

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INTRODUCTION

The mammalian testis is a complex organ synthesizing steroid hormones and producing spermatozoa. Normal testicular development and maintenance of spermatogenesis are controlled by gonadotropins and testosterone, effects of which are modulated by locally-produced factors including estrogens [for review see: 2, 4]. It is well known that in mammals, testicular aromatase is mainly localized in Leydig cells. However, there is now evidence that germ cells represent half of the testicular source of estrogens [3].

The aromatase, responsible for irreversible transformation of androgens into estrogens, plays a role in development, sexual differentiation, reproduction and behaviour. The enzyme is also involved in the regulation of bone and lipid metabolisms, brain functions and cancer development. It is difficult to find a tissue devoid of aromatase gene expression [14] and estrogen receptors [13]. Therefore, estrogens, in addition to affect gonadal functions, exert important effects on a broad array of tissues including bones, blood vessels and central nervous system either in post-menopausal women or aged men. In order to bring insight concerning the role that these female hormones may play in male physiological functions, the purpose of the present study was to determine the concentrations of steroids and gonadotropins in testicular tissues and blood serum in aged patients.

MATERIALS AND METHODS

The study was performed in agreement with the local ethical committee and the international ethical rules. The testicular tissue was collected from patients assigned to one of three groups of men (group I: 60-69 years-old, n=11; group II: 70-79 years-old, n=15; group III: 80-89 years-old, n=11) undergoing orchidectomy for prostatic cancer therapy. None of the patients had received any hormonal treatment prior surgery. Since neither atrophy nor abnormal development was observed in patients' gonads, the testes were assumed to be "normal" or "control".

Pieces of testicular tissues were fixed in a Bouin's Hollande solution for histological studies [11]. Concentrations of serum gonadotropins (LH and FSH), sex steroids (testosterone, estradiol) and sex hormone binding globulin (SHBG) were measured [8] using kits from bioMerieux (France). Testicular testosterone, estradiol, pregnenolone, progesterone, dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) were measured after extraction with diethylether/choloroform using highly specific antibodies [7-8]. All data were expressed as means \pm SEM. Statistical comparisons of data have been done using two-way ANOVA and Tukey test. Pearson's correlation analysis was performed by Sigmastat 3.0 software.

RESULTS AND DISCUSSION

The histological examination of testes revealed normal spermatogenesis in all examined samples except a progressive fibrosis which was more visible along the advanced age in the interstitial tissue (data not shown). No significant changes in serum hormone and SHBG concentrations among the three groups of patients were found. Nevertheless, the mean serum SHBG level was 3-times higher in group III compared to the two other groups of men (tab. 1). The concentrations of testicular steroids are depicted in Table 2. The mean levels of the main steroids produced by human testis (pregnenolone, DHEA, DHEA-S and testosterone) as well as those of estradiol were within normal range. No differences among groups were found.

No correlation was found between age of patients and serum level of estradiol ($r = - 0.155$, $p = 0.193$) or testosterone ($r = - 0.165$, $p = 0.259$). Similarly, serum SHBG concentration was not correlated with estradiol ($r = - 0.093$, $p = 0.622$) or testosterone ($r = - 0.187$, $p = 0.324$). A positive correlation was found between patient's age and serum level of SHBG ($r = 0.371$, $p < 0.05$).

There were no significant changes between the three groups of examined men regardless of the parameter measured and statistical test used. Nevertheless, the SHBG levels were increased in the oldest men which is in agreement with our correlated data and previous results [9, 16]. Our results demonstrated that the testicular source of androgens is not significantly de-

Table 1. Serum gonadotropin, SHBG and steroid levels in aged men

Parameters	Gr. I (60-69 years) n=11	Gr. II (70-79 years) n=15	Gr. III (80-91 years) n=11
FSH (mIU/ml)	8.71 ± 2.10	8.27 ± 0.99	10.27 ± 1.31
LH (mIU/ml)	10.77 ± 1.21	8.66 ± 1.21	8.86 ± 1.30
Testosterone (ng/ml)	4.24 ± 0.45	4.09 ± 0.52	3.22 ± 0.48
Estradiol (pg/ml)	24.46 ± 2.32	20.83 ± 2.51	18.60 ± 1.99
SHBG (ng/ml)	2.76 ± 0.16	2.61 ± 0.25	7.66 ± 1.78

Data are expressed as means±SEM

Table 2. Intratesticular concentrations of steroids (ng/g tissue) in the three groups of aged men

Parameters	Gr. I (60-69 years) n=11	Gr. II (70-79 years) n=15	Gr. III (80-91 years) n=11
Pregnenolone	165.54 ± 30.85	147.58 ± 18.09	147.79 ± 12.69
Progesterone	90.70 ± 12.93	93.38 ± 9.99	85.82 ± 6.55
DHEA	235.38 ± 47.04	193.50 ± 37.79	285.17 ± 67.22
DHEA-S	254.90 ± 37.44	247.09 ± 27.49	239.89 ± 38.83
Testosterone	408.50 ± 84.27	562.34 ± 72.30	461.77 ± 73.55
Estradiol	20.25 ± 8.04	4.59 ± 0.47	16.21 ± 5.17

Data are expressed as means±SEM

creased in aged men. Therefore, these male hormones are available for the aromatase activity in both testicular [2-3] and extragonadal tissues including adipose tissue which is more developed in aged men [14, 16]. However, since SHBG levels increase according to age [6, 9, 16], the bioavailability of serum testosterone diminishes.

The role of estrogens in male physiology is still a matter of debates, even though specific estrogen receptors (ER α and ER β) are distributed in many organs [13]. Severe bone problems are observed both in men with aromatase

deficiency (for review see: [1]) and aged men [16]. Aromatase is present in bones [14] and estrogens play a positive role in regulating osteoporosis. There is evidence showing a clear association between estradiol levels and vertebrates fractures [5, 15]. In addition to their effects on the skeleton, estrogens in males appear to be important for controlling the vascular functions and atherosclerosis [12]. Moreover, the non-genomic action of estrogens should also be considered since membrane-initiated signaling was found to affect almost all cellular processes *via* kinase pathways, protooncogenes and even nuclear responses [10].

There is now a general agreement that bioavailable estrogens are significant not only in the development and maintenance of male reproductive organs but also in various physiological processes in males, involving liver, bone, lipid metabolism, adipose tissue, vascular compartment and central nervous system. A lot of studies are focused on the role of estrogens in pathological processes such as osteoporosis, atherosclerosis and Alzheimer's disease in aged humans. Even despite the lack of significant changes in estradiol testicular and plasma levels, the bioavailability of estradiol may be decreased, taking into account the tendency in the increase of serum SHBG of elderly men. This possibility should be considered before any hormonal supplementation.

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