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Free testosterone and free dihydrotestosterone throughout the life span of men

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ABSTRACT

Objective: The dihydrotestosterone/testosterone ratio seems to be an important factor in the expression of androgenic activity, especially in the prostate and pilosebaceous unit. Whereas the decline of testosterone in aging men is well known, controversial data can be found concerning the age dependence of dihydrotestosterone levels. Hormonal values from our database served for the construction of the life span curve of free dihydrotestosterone/free testosterone ratio.
Methods: The results of testosterone, dihydrotestosterone and SHBG determination obtained by immunoassays from 13,152 male patients were used for the calculation of free steroid content and the construction of the age dependence curves.
Results: After initial high free dihydrotestosterone: free testosterone ratio in infancy it decreases at the start of puberty and remains practically without change from approx. 20 years of age till senescence.
Conclusion: The course of free dihydrotestosterone/free testosterone ratio demonstrates the role of dihydrotestosterone for androgen functions especially in prepubertal age.

1. Introduction

5α-Dihydrotestosterone (DHT) is the most powerful naturally occurring androgen with three to six times higher biopotency than of testosterone. For some androgen-dependent functions, testosterone is a pro-hormone, peripherally converted to 5α-dihydrotestosterone by the action of 5α-reductases type 1 and type 2. A natural model of DHT deprivation is the Imperato-McGinley syndrome, in which mutations in type 2 isoenzyme of steroid 5α-reductase cause male pseudohermaphroditism. In normal men dihydrotestosterone plays a key role in the prostate growth and also in hormonal regulation of the pilosebaceous unit. DHT and its metabolites are strongly associated with several metabolic risk factors in men.

There is general consensus that aging is also associated with a decrease in the concentration of circulating testosterone in the prevailing part of male population. On the other hand, some limited and confusing data concerning age dependence of DHT concentrations can be found in literature. While some authors report no change [1–3], others report a decrease [4–6] or even an increase [7] in the concentrations of circulating DHT. As some authors suggest, testosterone could even exert protective effects to the action of dihydrotestosterone, especially in the prostate. The course of total DHT:total T ratio throughout the life span in men showed in our previous study [8] no decline or increase in higher age groups. Recently, Mazer underlines the importance of measuring free DHT in various conditions [9]. Now we use our database for the comparison of free DHT and free testosterone and its age dependence.

2. Subjects, materials and methods

2.1. Subjects

We examined the relevant data from the database of the Institute of Endocrinology obtained in the period 1994–2007, which included 13,152 men, treated as outpatients. From the cohort, data on the serum concentration of DHT were recorded for 6643 men, of testosterone for 6886 men and of SHBG for 4175 men. In 2665 patients, all three parameters were available after exclusion of men treated with testosterone or 5α-reductase inhibitors. We did not define any other exclusion criteria, as our aim was to include a cohort of patients as similar as possible to the spectrum of the clients of our Institute, which cares mainly for patients with thyroid diseases, diabetes and other metabolic disorders and obesity. As concerns the ethnic origin of the men, all of them were Caucasian (white). Blood samples were obtained from the cubital vein, between the hours of 8 and 10 A.M. and the serum samples were then stored at −20 °C until analyzed in the laboratory.

2.2. Laboratory methods

Testosterone. Testosterone levels were determined as described elsewhere [10]. Radioimmunoassay was carried out after diethyl-

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ether extraction using rabbit polyclonal antiserum against testosterone-3-CMO:BSA and radioiodine labeled testosterone-tyrosin methylester as a tracer. Intra-assay and inter-assay coefficients of variation for the method were 8.2% and 10.7%, respectively.

Dihydrotestosterone (17β-hydroxy-5α-androstan-3-one). Radioimmunoassay of dihydrotestosterone after diethyl-ether extraction after $KMnO_4$-oxidation of cross-reacting 4-en-3-oxosteroids was carried out using rabbit antiserum against dihydrotestosterone-3-CMO:BSA and [^{3}H]dihydrotestosterone as a tracer (Amersham, UK) [11]. Intra-assay and inter-assay coefficients of variation for the method were 8.7% and 12.1%, respectively.

SHBG was determined by IRMA immunoassay I using commercial kit Orion, Finland. Intra-assay CV = 6.1%, inter-assay CV = 7.9%

All analyses were carried out on analyzer Stratec (France).

Free testosterone was calculated from the equation $fT = \left(\sqrt{A(A/4)+(T/23.3)10^{-18}}-(A/2)\right)10^{12}$ for free testosterone and $fDHT = \left(\sqrt{B(B/4)+(1/1.304)\times(DHT/23.3)10^{-18}}-(B/2)\right)10^{12}$ for free dihydrotestosterone according to Vermeulen et al. [12] with the use of association constants for DHT as found by Sodergard et al. [13], where $A = ((\mathrm{SHBG}-\mathrm{T}+23.3)/23.3)10^{-9}$ and $B = A/1.346$.

2.3. Statistical analysis

The age dependence was evaluated using one-way ANOVA followed by Bonferroni multiple comparisons. Respecting the skewed data distribution in all dependent variables, the data were transformed by a power transformation to obtain symmetry in the distribution of studentized residuals in ANOVA [14]. The non-homogeneities were detected using an approach as described elsewhere [15] and the computations were performed from the data without non-homogeneities never representing more than 5% of the data.

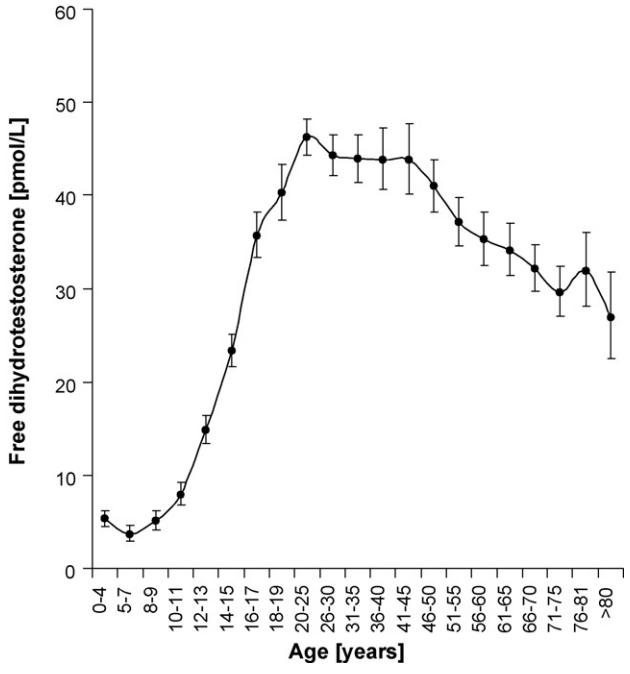

Fig. 1. The course of free testosterone serum concentrations over the life span of Czech men as evaluated using ANOVA followed by Bonferroni multiple comparisons (vs. control, $p < 0.05$). The empty circles with error bars represent re-transformed mean values with their 95% confidence intervals. The full, dashed and dotted lines represent group medians, quartiles and 10/90th percentiles, respectively. Bonferroni multiple comparisons showed homogeneity within 0 and 9 years of age followed by significantly increasing s-shaped trend within 10th and 30th years of age and then by a significant but slow decline from the 30th years of age to senescence.

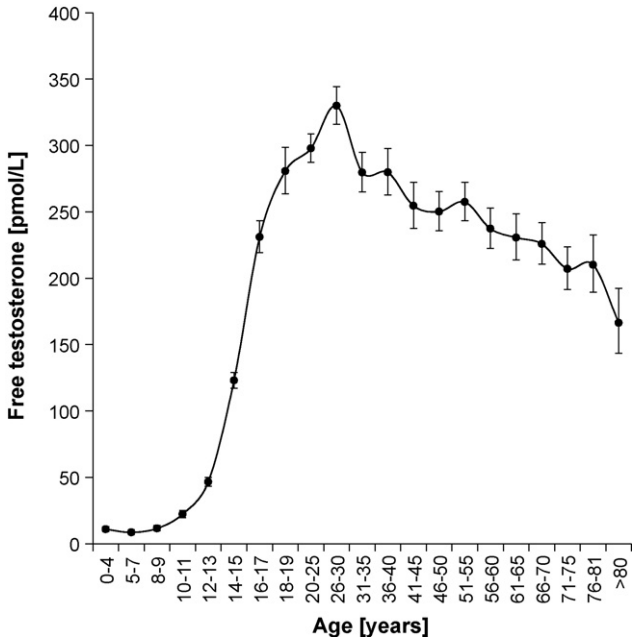

Fig. 2. The course of free dihydrotestosterone serum concentrations over the life span of Czech men as evaluated using ANOVA followed by Bonferroni multiple comparisons (vs. control, $p < 0.05$). The drawings and symbols are the same as for Fig. 1. Bonferroni multiple comparisons showed homogeneity within 0 and 9 years of age followed by significantly increasing s-shaped trend within 10th and 25th years of age and then by a significant but slow decline from the 25th years of age to senescence.

3. Results

The courses of free dihydrotestosterone and free testosterone concentrations with age are shown in Figs. 1 and 2 and the course of the ratio of DHT to testosterone in Fig. 3. Free DHT showed a sim-

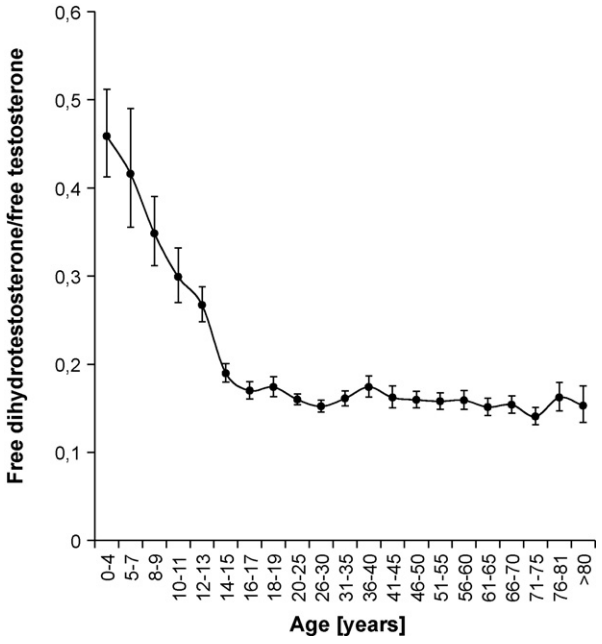

Fig. 3. The course of the ratio of serum concentrations of free dihydrotestosterone to free testosterone over the life span of Czech men as evaluated using ANOVA followed by Bonferroni multiple comparisons (vs. control, $p < 0.05$). The drawings and symbols are the same as for Fig. 1. Bonferroni multiple comparisons showed homogeneity within 0 and 9 years of age followed by significantly decreasing s-shaped trend within 10th and 30th years of age and then by a plateau from the 30th years of age to senescence.

ilar course as free testosterone. The values of DHT to testosterone ratio were higher till the start of puberty, i.e. prevailing activity of DHT over testosterone in childhood but practically a constant course from adulthood to senescence was confirmed.

4. Discussion

The changes in testosterone in regards to age are generally in agreement with other data on total testosterone [16–24] and especially on free testosterone [20,22] decline with increasing age. Free DHT until now has not been included in the list of analyts required by clinical endocrinologist, however, its importance is underlined by Mazer [9] who recently reported an alternative calculation of its values from values of total DHT and SHBG.

In contrast to the well-known decline of testosterone concentrations over the life span of men, there are confusing data about the age dependence of dihydrotestosterone levels. Some authors report a decline in DHT levels [4–6] but others observed no significant change [1,3,19] in aging men. Longitudinal results from the Massachusetts male aging study reported increasing DHT concentration in aged men [7]. Until now, no values of free DHT were reported in comparison with free testosterone in the course of the life span in men. Our results, obtained from a representative group of the Middle-European population, show the ratio of free DHT to free testosterone, which demonstrates a dominant activity of DHT over T in childhood till puberty. In the course of puberty it changes in favour to free testosterone and than remains almost constant in adult men till senescence.

This is in full agreement with the clinical features of the Imperato-MacGinnley syndrome. The affected 46XY individuals have elevated plasma testosterone levels, decreased levels of DHT and elevated testosterone/DHT ratios. Due to the insufficient DHT levels during fetal development they have ambiguous external genitalia at birth so that they are believed to be girls and are often raised as such. A dramatic change in virilization occurs as late as in puberty along with the increase of testosterone production and frequently with a gender role change and full masculinisation. However, the prostate in adulthood remains small and rudimentary, and facial and body hair is absent or decreased and balding is also absent. Partial deficiency of 5α-reductase is related to the development of some forms of micropenis, which can be, in some cases, corrected by dihydrotestosterone treatment.

The DHT to testosterone ratio might be of importance in local functions, for which testosterone is supposed as weaker protective androgen to DHT action, as in the case of prostate proliferation or of the pilosebaceous gland. Recently, statistical analysis of the results of Vandenput et al. [25] indicated that DHT, but not testosterone, was independently negatively associated with different measures of fat mass and insulin resistance in humans. Conversely, in castrated mice DHT treatment resulted in obesity, associated with reduced energy expenditure and fat oxidation [26]. However, DHT did not affect food consumption or locomotor activity. Nevertheless, it should be emphasized that the circulating levels of both androgens need not necessarily express their local proportions in tissues or at the active sites of hormone action.

Conflict of interest

The authors declare no conflict of interest.

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