Dihydrotestosterone and testosterone throughout the life span of Czech 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 (T) in aging men is well known, controversial data can be found in literature concerning the age dependence of dihydrotestosterone (DHT) levels. Data from the database of the Institute of Endocrinology served as the basis for the definition of the life span curve for the ratio dihydrotestosterone : testosterone.

METHODS*:* The results of testosterone and dihydrotestosterone obtained immunoassays from 10 251 male patients were used in the construction of the curve.

RESULTS*:* The data show that after a peak of DHT:T in infancy and a subsequent decrease in puberty, the ratio of both androgens remains practically without change from approx. 20 years of age till old age.

CONCLUSION*:* High DHT:T ratio in infancy decreases at puberty and throughout the entire reproductive period of life this ration remains practically constant.

INTRODUCTION

For some androgen-dependent functions, testosterone is a pro-hormone, peripherally converted to 5α-dihydrotestosterone (DHT) by the action of 5α-reductases type 1 and type 2. Dihydrotestosterone, the most physiologically potent endogenous androgen, cannot be aromatized into estrogen and in males plays a key role in many physiological and pathological events. 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. The affected 46XY individuals have elevated plasma testosterone levels, decreased levels of DHT and elevated testosterone/DHT ratios. They have ambiguous external genitalia at birth so that they are believed to be girls and are often raised as such. Virilization occurs at puberty along with the increase of testosterone production and frequently with a gender role change. The prostate in adulthood is 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 micropenis, which can in some cases be corrected by dihydrotestosterone treatment. In normal individuals, higher local concentrations of dihydrotestosterone play a key role in the development of benign prostate hyperplasia and probably also of prostate carcinoma [8,11,15,18,29,] and also in androgenic alopecia [2], hirsutism and acne. Until now, the physiological effects of DHT on rat bone growth zone chondrocytes, spermatogenesis, especially on maturation of spermatozoa in epididymis, on sexual brain differentiation and its action as neuroactive

ORIGINAL ARTICLE

steroid or association of its higher levels with homosexuality is not well understood in detail and deserves further study [28]. Recent preclinical data indicate that the subsequent 3α-reduction of DHT produces steroid metabolites with rapid non-genomic effects on brain function and behavior, primarily via an enhancement of γ-aminobutyric acid (GABA)-ergic inhibitory neurotransmission. Consistent with their ability to enhance the action of GABA at GABA(A) receptors, these neuroactive steroid derivatives possess anticonvulsant, antidepressant and anxiolytic effects in addition to altering aspects of sexual- and alcohol-related behaviors [7]. In the brain of laboratory animals, DHT played a central role in this respect [30].

It is widely believed that benign prostate hyperplasia (BPH) is associated with aging. In addition, the incidence androgenic alopecia increases with aging. 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 [3]. 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 [12, 17, 24], others report a decrease [4,9,10] or even an increase [6] 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 question therefore arises whether or not a correlation exists between dihydrotestosterone to testosterone ratio and aging.

AIM OF THE STUDY

The goal of our study was to determine the serum levels of testosterone and dihydrotestosterone as a function of age in a population of Czech men consisting of outpatients of the Institute of Endocrinology and to find whether changes in the ratio of DHT to testosterone are connected to the periods in which clinical symptoms of relative DHT excess appear, such as balding or prostate disorders.

SUBJECTS

We examined the relevant data from 1994–2007 from the database of the Institute of Endocrinology which included 10251 men, treated as out-patients. From the cohort, data was recorded for 3076 men on the serum concentration of both DHT and testosterone. Men treated with testosterone or 5α-reductase inhibitors, which evidently influence the ratio of the investigated steroids, were excluded. 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 8–10 A.M. and the serum samples were then stored at –20°C until analyzed in the laboratory.

LABORATORY METHODS

Testosterone: Testosterone levels were determined as described elsewhere [13]. Radioimmunoassay was carried out after diethyl-ether extraction using rabbit polyclonal antiserum against testosterone-3-CMO:BSA and radioiodine labeled testosterone-tyrosin methylester as a tracer [14]. 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) [13]. Intra-assay and inter-assay coefficients of variation for the method were 8.7% and 12.1%, respectively.

STATISTICAL ANALYSIS

A polynomial regression was used in the assessment of age dependence of the steroids and DHT to testosterone ratio. The optimum degree of polynomial was determined using a mean square error of prediction and a correlation coefficient of polynomial regression adjusted to degrees of freedom. The quality of the polynomial fit was checked using a lack of fit test. In addition, age dependence was evaluated using one-way ANOVA followed by least significant difference 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 regression and ANOVA [19]. In both methods, the non-homogeneities were detected using an approach as described elsewhere [20,21] and the computations were performed from the data without non-homogeneities never representing more than 5% of the data.

RESULTS

The course of testosterone and dihydrotestosterone concentrations with age is shown in the Fig. 1 and 2 and the course of the ratio of DHT to testosterone in Fig 3. The changes in testosterone in regards to age are generally in agreement with other data on testosterone decline with age [5,16]. A slight but significant increase was obvious in the age groups 45–50 and 50–55 years, i.e. at the age of evolving andropause. Other authors also have described a similar increase of testosterone serum levels in analogical age group [1]. DHT showed a similar

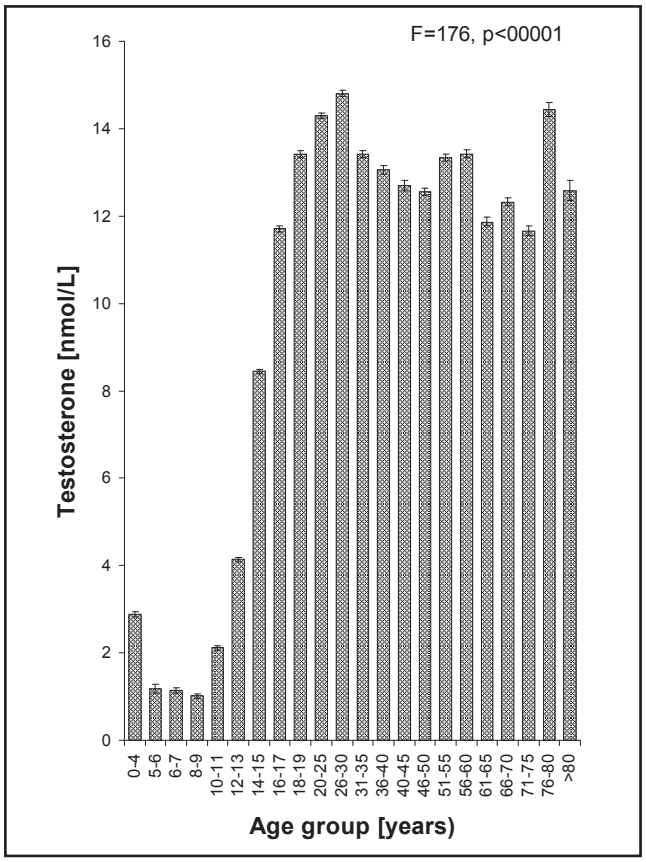

Fig. 1. The course of testosterone serum concentrations over the life span of Czech men. F = F-ratio of variability = factor or interaction to the unexplained variability.

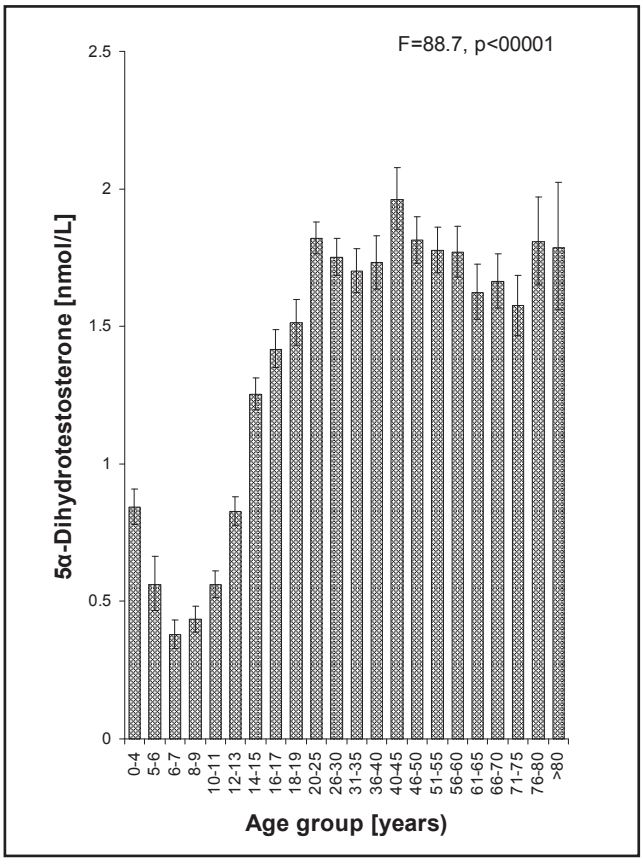

Fig. 2. The course of dihydrotestosterone serum concentrations over the life span of Czech men. F = F-ratio of variability = factor or interaction to the unexplained variability.

course, with a less pronounced increase before andropause. The curve of DHT to testosterone ratio showed higher values, i.e. prevailing DHT over testosterone in childhood but practically constant course from adulthood to senescence. However in the oldest subjects it indicated a decrease, which was observed in a selected population with signs of longevity.

DISCUSSION

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 [4,9,10] but others observed no significant change [12,17,24] in aging men. Longitudinal results from the Massachusetts male aging study reported increasing DHT concentration in aged men [6]. This phenomenon could be linked to substantial change of DHT:testosterone ratio in old age. Our results, obtained from a representative group of the Middle-European population, show a DHT to testosterone ratio, which is almost constant.

In mammalian species the course of DHT to testosterone ratio is far from uniform. In fallow deer [26] during the annual cycle individual T and DHT profiles showed opposite relationships, in crab-eating macaque

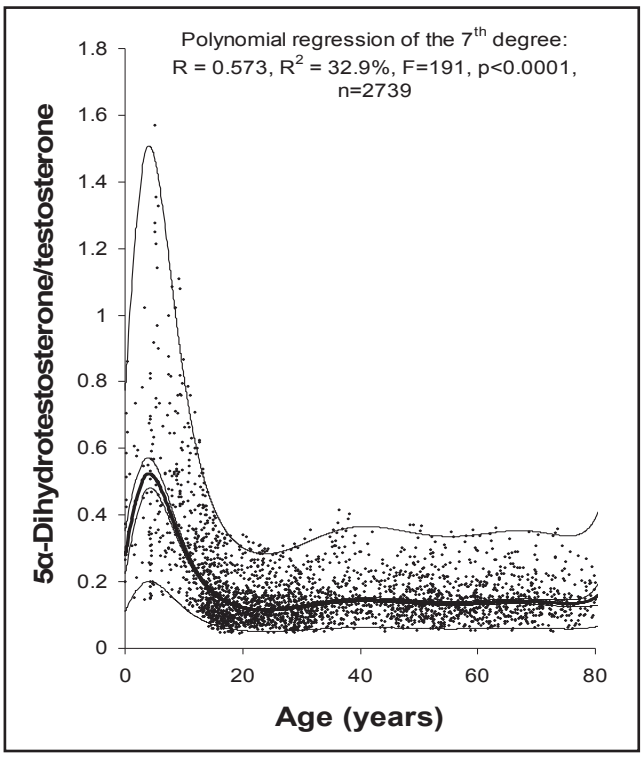

Fig.3. Age-dependent course of DHT:testosterone ratio in 3076 men. F = F-ratio of variability = factor or interaction to the unexplained variability.

testosterone values continued to increase, reaching adult values at about 5–6 years of age, whereas DHT levels tended to stabilize from 4–5 years [22]. In rats testosterone was low (less than 1 ng/ml) until the 42nd day of life; adult levels (3–4 ng/ml) were attained at the 62nd day and declined gradually with advanced age. 5α-Dihydrotestosterone (DHT) did not change markedly (90–160 pg/ml) from prepubertal to advanced age [27]. Serum DHT of elephants reflected testosterone levels, except that the striking elevation of testosterone in Asian elephant bulls during musth was not paralleled by equal increases in DHT levels [25].

The DHT to testosterone ratio might be of importance in local functions, for which testosterone is supposed as weaker androgen protective to DHT action, as in the case of prostate proliferation or of the pilosebaceous gland. Recently, statistical analysis of the results of Vandepute et al. [31] indicated that DHT, but not testosterone, was independently negatively associated with different measures of fat mass and insulin resistance ($P<0.001$) in humans. Conversely, in castrated mice DHT treatment resulted in obesity, associated with reduced energy expenditure and fat oxidation [23]. However, DHT did not affect food consumption or locomotor activity. Nevertheless, it should be emphasized hat the circulating levels of both androgens need not necessarily express their local proportions in tissues or at the active sites of hormone action.

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REFERENCES

1 Andersson A-M, Jensen T, Juul A, et al. Secular decline in male testosterone sex hormone binding globulin serum levels in Danish population surveys. J Clin Endocrinol Metab 2007; **92**(12): 4696–4705.
2 Bayne EK, Flanagan J, Einstein M, et al.. Immunohistochemical localization of types 1 and 2 5alpha-reductase in human scalp. Br J Dermatol. 1999; **141**(3): 481–91.
3 Bhasin S, Cunningham GR, Hayes FJ, et al. Testosterone therapy in adult men with androgen deficiency syndromes: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2006, **91**(6), p.1995–2010.
4 Drafta D, Schindler AE, Stroe E, Neacşu E. Age-related changes of plasma steroids in normal adult males. J Steroid Biochem. 1982; **17**(6): 683–7.
5 Elmlinger MW, Dengler T, Weinstock C, Kuehnel W. Endocrine alterations in the aging male. Clin Chem Lab Med. 2003; **41**(7): 934–41.
6 Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, et al. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. J Clin Endocrinol Metab. 2002; **87**(2): 589–98
7 Finn DA, Beadles-Bohling AS, Beckley EH, Ford MM, Gililland KR, Gorin-Meyer RE, et al. A new look at the 5alpha-reductase inhibitor finasteride. CNS Drug Rev. 2006; **12**(1): 53–76.
8 Friedman AE. The estradiol-dihydrotestosterone model of prostate cancer. Theor Biol Med Model. 2005; **2**(1): 10.
9 Ghanadian R, Puah CM. Age-related changes of serum 5alpha-androstane-3alpha, 17beta-diol in normal men. Gerontology. 1981; **27**(5): 281–5.
10 Giusti G, Gonnelli P, Borrelli D, Fiorelli G, Forti G, Pazzagli M, et al. Age-related secretion of androstenedione, testosterone and dihydrotestosterone by the human testis. Exp Gerontol. 1975; **10**(5): 241–5
11 Giwercman YL, Abrahamsson PA, Giwercman A, et al. The 5alpha-reductase type II A49T and V89L high-activity allelic variants are more common in men with prostate cancer compared with the general population. Eur Urol. 2005; **48**(4): 679–85.
12 Gray A, Feldman HA, McKinlay JB, Longcope C. Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. J Clin Endocrinol Metab. 1991; **73**(5): 1016–25.
13 Hampl R, Dvořák P, Lukešová Š, Kozák I, Chrpová M, Stárka L. The use of iodinated steroids as radioligand for testosterone radioimmunoassay. J. Steroid Biochem. 1978; **9**: 771–773.
14 Hampl R, Putz Z, Stárka L. Radioimunological determination of dihydrotestosterone and its importance for laboratory diagnostic. (In Czech). Biochem. Clin. Bohemoslov. 1990; **19**, 157–163.
15 Heráček J, Urban M, Sachová J, et al. The endocrine profiles in men with localised and locally advanced prostate cancer treated with radical prostatectomy. Neuro Endocrinol Lett 2007; **28** (1): 101–107.
16 Li JY, Li XY, Li M, Zhang GK, Ma FL, Liu ZM, et al.. Decline of serum levels of free testosterone in aging healthy Chinese men. Aging Male. 2005; **8**(3–4): 203–6
17 Maier U. Hormone profile in the aging man. Wien Med Wochenschr. 2001; **151**(18–20): 422–5.
18 Marks LS, Hess DL, Dorey FJ, Macairan ML. Prostatic tissue testosterone and dihydrotestosterone in African-American and white men. Urology. 2006; **68**(2): 337–41.
19 Meloun M, Hill M, Militky J, Kupka K. Transformation in the PC-aided biochemical data analysis. Clin Chem Lab Med. 2000; **38:** 553–9.
20 Meloun M, Militky J, Hill M, Brereton RG. Crucial problems in regression modelling and their solutions. Analyst . 2002; **127:** 433–50.
21 Meloun M, Hill M, Militky J, Vrbikova J, Stanicka S, Skrha J. New methodology of influential point detection in regression model building for the prediction of metabolic clearance rate of glucose. Clin Chem Lab Med. 2004; .**42:** 311–22,
22 Meusy-Dessolle N, Dang DC. Plasma concentrations of testosterone, dihydrotestosterone, delta 4-androstenedione, dehydroepiandrosterone and oestradiol-17 beta in the crab-eating monkey (Macaca fascicularis) from birth to adulthood. J Reprod Fertil. 1985; **74**(2): 347–59.
23 Movérare-Skrtic S, Venken K, Andersson N, Lindberg MK, Svensson J, Swanson C, et al. Dihydrotestosterone Treatment Results in Obesity and Altered Lipid Metabolism in Orchidectomized Mice Obesity 2006; **14**: 662–672 .
24 Pirke KM, Doerr P. Age related changes in free plasma testosterone, dihydrotestosterone and oestradiol. Acta Endocrinol (Copenh). 1975; **80**(1): 171–8.
25 Rasmussen LE, Buss IO, Hess DL, Schmidt MJ.Testosterone and dihydrotestosterone concentrations in elephant serum and temporal gland secretions. Biol Reprod. 1984; **30**(2): 352–62.
26 Rolf HJ, Fischer K. Serum testosterone (T) and 5-alpha-dihydrotestosterone (DHT) in male fallow deer (Dama dama L.): seasonality and age dependence. Comp Biochem Physiol A. 1990; **95**(3): 445–52.
27 Saksena SK, Lau IF. Variations in serum androgens, estrogens, progestins, gonadotropins and prolactin levels in male rats from prepubertal to advanced age. Exp Aging Res. 1979; **5**(3): 179–94.
28 Stárka L.: Dihydrotestosterone and inhibitors of steroid 5alfa-reductase. (in Czech) Urolog listy 2007; **5**(3): 11–16.
29 Titus MA, Schell MJ, Lih FB,et al. Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. Clin Cancer Res. 2005; **11**(13): 4653–7.
30 Torres JM, Ortega E. Steroid 5alpha-reductase isozymes in the adult female rat brain: central role of dihydrotestosterone. Mol Endocrinol. 2006; **36**(2): 239–45.
31 Vandenput L, Mellström D, Lorentzon M, Swanson C, Karlsson MK, Brandberg J, et al.. Androgens and glucuronidated androgen metabolites are associated with metabolic risk factors in men. J Clin Endocrinol Metab. 2007; **92**(11): 4130–7.