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Research Article Open Access

Obesity, Circulating Androgens and their Precursors

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Abstract

Objective: The association of obesity with a lower circulating testosterone level in men is well documented. However, reports on possible changes in the androgen spectrum in obesity are rare.

Methods: To investigate this phenomenon, serum sex hormone–binding globulin (SHBG), testosterone, dihydrotestosterone, androstenedione, dehydroepiandrosterone and its sulphate, 17α-hydroxypregnenolone, 17α-hydroxyprogesterone and gonadotrophins LH and FSH concentrations were measured in fasting blood samples of 224 men divided into three groups – normal (BMI=18-25, n=109, overweight (BMI 25.10-30, n=78) and obese (BMI=30.1-39, n=37).

Results: A significant decrease in testosterone, dihydrotestosterone, 17α-hydroxypregnenolone, 17α-hydroxyprogesterone and SHBG with increasing body mass index was observed, whereas insignificant changes for dehydroepiandrosterone and its sulphate, androstenedione and gonadotrophins LH and FSH, were found. The ratios of corresponding pairs of steroids were in agreement with the concept that in obesity splitting of the side chain of C_{21}-steroids, and 17β-hydroxysteroid dehydrogenase-reducing activity are decreased. No changes for steroid 5α-reductase or 3β-hydroxysteroid dehydrogenase (HSD3B2) were found.

Conclusion: The findings demonstrate that, in men with increasing body mass index, the formation of C_{19} steroids decreases from their C_{21} precursors and lower 17β-hydroxysteroid dehydrogenase further confines the production of testosterone and dihydrotestosterone.

Keywords: Obesity; Testosterone; Dihydrotestosterone; Androgens; 17α-hydroxyprogesterone; 17α-hydroxypregnenolone

Introduction

Reduced testosterone levels, well into the hypogonadal range, are common in male obesity [1-5]. The mechanism of circulating total testosterone concentration decrease is explained by a high expression of aromatase, the enzyme that converts testosterone to estradiol, in adipose tissue and by the resulting elevated estradiol. Together with the increased leptin and adipokines from fat tissue, this triggers inhibition of the hypothalamic-pituitary-gonadal axis [6, 7]. This results in hypogonadotrophic hypogonadism, which is observed in a large percentage of obese men.

Whereas a handful of publications deal with the relation of testosterone, dihydrotestosterone or estradiol levels to obesity, less attention has been paid to the influence of obesity on androgen metabolism. Some important data were acquired by measuring the intra-adipose metabolism of androgens [8-10]. The activity of enzymes involved in androgen metabolism varies in the different parts of fat tissue and, together with local glucocorticoid activity, constitutes an important factor for fat distribution.

Data concerning circulating androgens and their precursors in obese men, with the exception of that on testosterone or dihydrotestosterone [11], are scarce [12-15]. This short study aims to determine the impact of obesity on the pattern of circulating androgens and to show whether all changes in the concentration of androgens and their precursors 17α-hydroxypregnenolone and 17α-hydroxyprogesterone are proportional to the reduced level of testosterone and if this decline also applies to androgens of mainly adrenal origin.

Materials and Methods

Subjects

A group of 224 healthy (except for their obesity and associated symptoms) men aged 20 to 78 with a broad range of body mass index (BMI) 18 to 39 was enrolled in this study. Anthropometric parameters (i.e. weight, height, BMI) were measured. Blood withdrawal was carried out in fasting subjects in the morning between 7:30 and 8:30 a.m. from the forearm vein. Serum was stored at -80°C until it was processed in the laboratory.

The Ethical Committee approved the study and all patients signed informed consent forms before taking part in the study.

Anthropometric data

Anthropometric data were obtained in a fasting state. Body weight and height were measured in all participants in order to calculate body mass index (BMI). Weight (to the nearest 0.1 kg) and height (to the nearest cm) were measured. Body mass index was calculated as the weight (kg) divided by height squared (m^2).

The group of 224 healthy men was divided into three subgroups according to BMI. The first subgroup consisted of 109 men with BMI between 18 and 25. The second group included 78 men with BMI between 25 and 30. The third subgroup had 37 men with BMI 30 to 39.

Steroid analysis

Laboratory analyses of sex hormone binding globulin (SHBG),

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LH, FSH and steroid hormones: dihydrotestosterone, testosterone, 17α-hydroxyprogesterone, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), 4-androstene-3,17-dione (androstenedione), and 17α-hydroxypregnenolone were carried out.

Serum testosterone was determined by standard radioimmunoassay (RIA) using antiserum anti-testosterone-3-carboxymethyloxim: BSA and testosterone-3-carboxymethyloxim-tyrosylmethyl-ester-[^{125}I] as a tracer. Intra-assay and inter-assay coefficient variants were 7.2% and 10%, respectively, and sensitivity was 0.21 nmol/l. Androstenedione was determined by standard RIA with antiserum anti-androstenedione-6-carboxy-methyloxim: BSA and [^{3}H] androstenedione as tracer. Intra-assay and inter-assay coefficient variants were 8.1% and 10.2% and sensitivity was 0.39 nmol/l. Sexual hormones binding globulin was assayed using an IRMA kit (Orion, Espoo, Finland). Commercial kits (Immunotech, Marseilles, France) were used for the determination of LH, FSH (IRMA kit), 17α-hydroxyprogesterone, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone

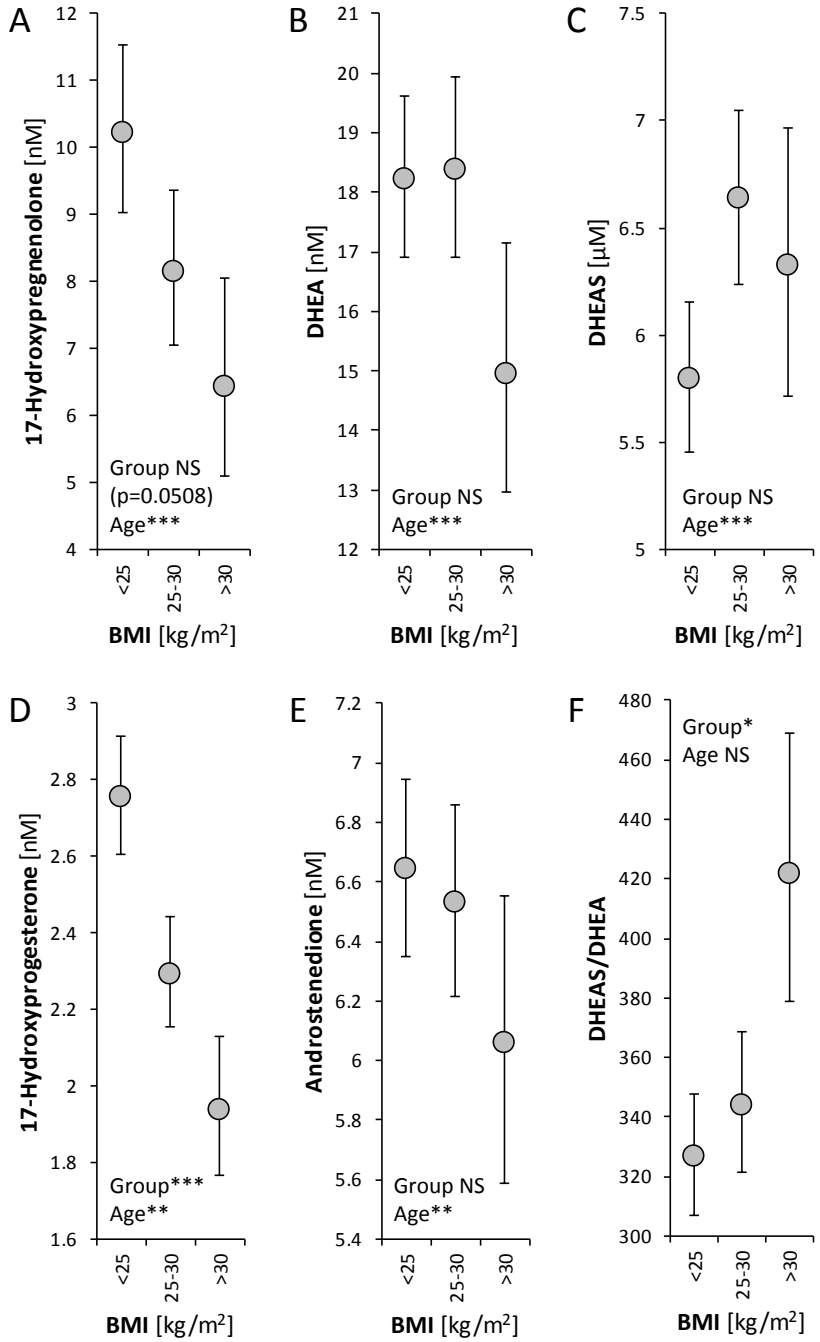

Figure 1: Relationships between steroid levels, DHEAS/DHEA ratio and BMI as evaluated using ANCOVA with BMI group as a main factor and age of the subject as a covariate (for details see statistical data analysis). The circles with error bars represent the group means with their 95% confidence intervals as computed for least significant difference (LSD) multiple comparisons (p=0.05). The confidence values, which do not overlap, represent statistical significant difference between the corresponding groups. Statistical significance: NS=not significant, *p<0.05, **p<0.01, ***p<0.001.

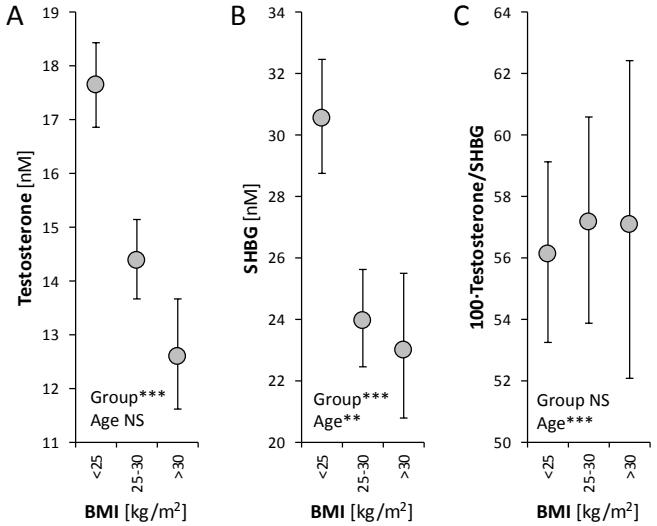

Figure 2: Relationships between levels of testosterone, SHBG, free androgen index (100·testosterone/SHBG), and BMI as evaluated using ANCOVA with BMI group as a main factor and age of the subject as a covariate (for details see statistical data analysis). The circles with error bars represent the group means with their 95% confidence intervals as computed for least significant difference (LSD) multiple comparisons (p=0.05). The confidence values, which do not overlap, represent statistical significant difference between the corresponding groups. Statistical significance: NS=not significant, **p<0.01, ***p<0.001.

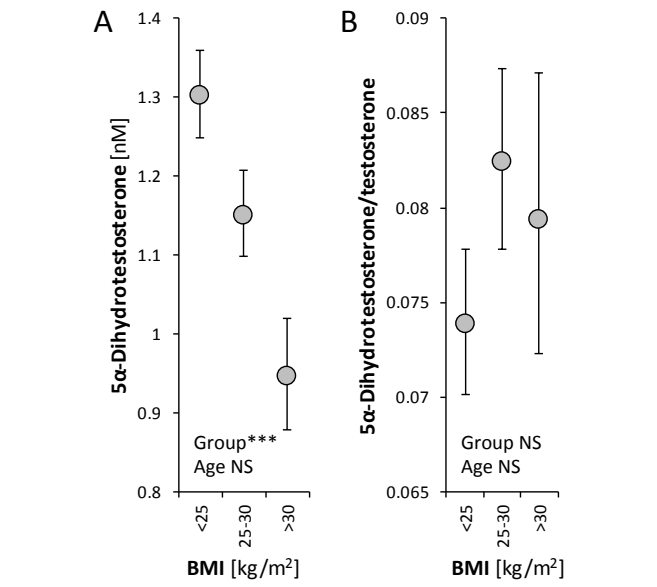

Figure 3: Relationships between levels of 5α-dihydrotestosterone, 5α-dihydrotestosterone/testosterone ratio and BMI as evaluated using ANCOVA with BMI group as a main factor and age of the subject as a covariate (for details see statistical data analysis). The circles with error bars represent the group means with their 95% confidence intervals as computed for least significant difference (LSD) multiple comparisons (p=0.05). The confidence values, which do not overlap, represent statistical significant difference between the corresponding groups. Statistical significance: NS=not significant, ***p<0.001.

sulfate (DHEAS) (RIA kit). Dihydrotestosterone was determined by an original methodology [16,17]. 17α-Hydroxypregnenolone was determined by an in-house RIA method.

Statistical data analysis

To evaluate the relationships between dependent variables, we used the ANCOVA model with BMI group as a main factor and age of the subject as a covariate (age–adjusted ANOVA) followed by least significant difference (LSD) multiple comparisons. The original dependent variables and the covariate were transformed by power transformations to attain a constant variance and symmetric distribution of the data and residuals [18]. Statistical software Statgraphic Centurion version XVI (Herndon, VA, USA) was used for the calculations. The homogeneity of the data and residual were checked as described elsewhere [19].

Results

The comparison of men with normal body mass index, overweight and obese men showed that a significant continuous decrease of parameters for testosterone (Figure 2A), dihydrotestosterone (Figure 3A) and SHBG (Figure 2B) correlates with increasing body mass. Also the circulating both C_{21} androgen precursors, 17α-hydroxyprogesterone and 17α-hydroxypregnenolone, decrease with increasing BMI (Figures 1A and 1D), whereas the changes in androstenedione, DHEA, and DHEAS levels as well as in gonadotrophins do not reach statistical significance (Figures 1B, 1C and 1E). Since the decrease of SHBG (Figure 2B) parallels the decrease of testosterone level (Figure 2A), thus compensating the loss of free testosterone, no change was observed in the free androgen index (Figure 2C).

The dihydrotestosterone : testosterone ratio does not correlate with the degree of obesity (Figure 3B), to the 17α-hydroxyprogesterone : 17α-hydroxpregnenolone ratio or

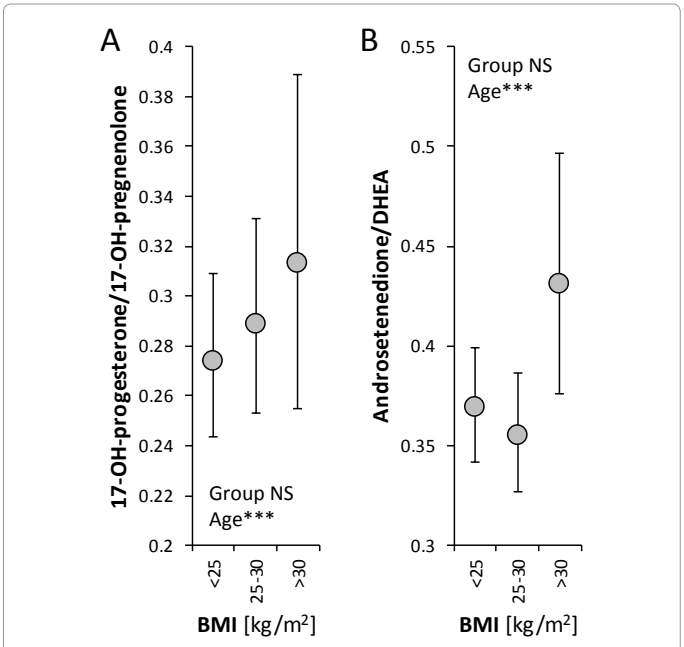

Figure 4: Relationships between steroid ratios reflecting the activity of type 2 3β-hydroxysteroid dehydrogenase (HSD3B2) and BMI as evaluated using ANCOVA with BMI group as a main factor and age of the subject as a covariate (for details see statistical data analysis). The circles with error bars represent the group means with their 95% confidence intervals as computed for least significant difference (LSD) multiple comparisons (p=0.05). The confidence values, which do not overlap, represent statistical significant difference between the corresponding groups. Statistical significance: NS=not significant, ***p<0.001.

androstenedione : dehydroepiandrosterone ratio (Figures 4A and 4B), which demonstrates the undisturbed activity of 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2). On the contrary, it is evident from the DHEA : 17α-hydroxypregnenolone and androstenedione : 17α-hydroxprogesterone ratios (Figure 5A) that the activity of C_{17},C_{20}-lyase (CYP17A1) decreases with the degree of obesity, especially for the Δ^4 pathway (Figure 5B).

The testosterone: androstenedione ratio (Figure 6) decreases significantly, which is in agreement with the decreased activity of 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3).

Multivariate statistical analysis showed that age was a significant factor for the correlation of BMI and the levels of 17α-hydroxypregnenolone, 17α-hydroxyprogesterone, androstenedione, dehydroepiandrosterone and its sulfate and SHBG. The decrease of testosterone and dihydrotestosterone with increasing BMI was independent of age.

Discussion

We can derive from the data that testosterone biosynthesis in overweight and obese men is inhibited already in the step of splitting the side chain of C_{21} steroids and further by the decrease of 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3) reducing activity. The changed ratio of dehydroepiandrosterone sulfate to DHEA, which might be a consequence of decreased sulfatase or increased sulfotransferase or increased secretion of DHEA sulfate from the adrenals, is of interest.

No changes with increasing BMI were observed with regards to steroid 5α-reducatse (SRD5A1) or 3β-hydroxysteroid dehydrogenase (HSD3B2).

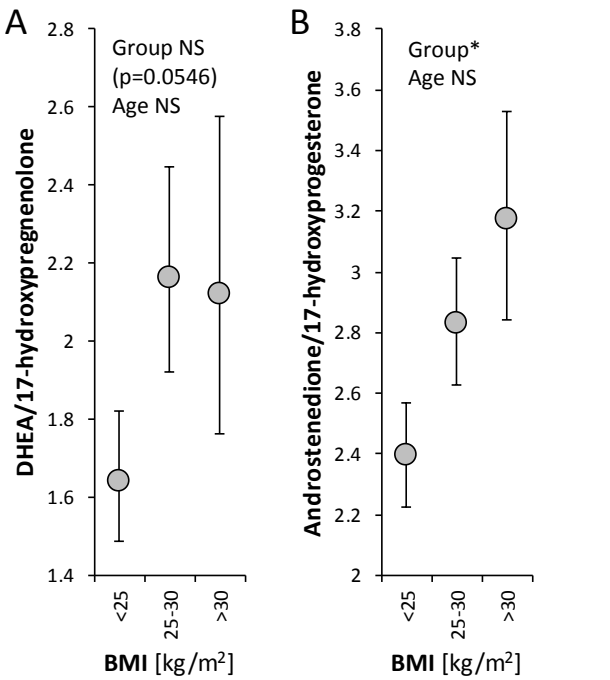

Figure 5: Relationships between steroid ratios reflecting the activity of C17-hydroxylase, C17,20-lyase (CYP17A1) and BMI as evaluated using ANCOVA with BMI group as a main factor and age of the subject as a covariate (for details see statistical data analysis). The circles with error bars represent the group means with their 95% confidence intervals as computed for least significant difference (LSD) multiple comparisons (p=0.05). The confidence, which do not overlap represent statistical significant difference between the corresponding groups. Statistical significance: NS=not significant, *p<0.05.

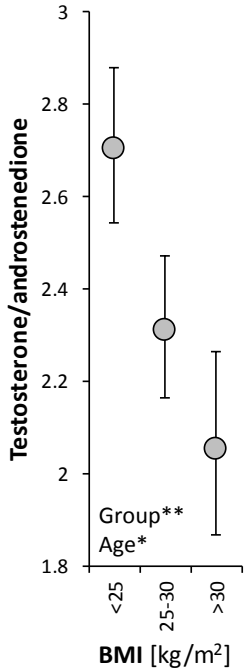

Figure 6: Relationships between steroid ratios reflecting the activity of type 3 17-hydroxysteroid dehydrogenase (HSD17B3) and BMI as evaluated using ANCOVA with BMI group as a main factor and age of the subject as a covariate (for details see statistical data analysis). The circles with error bars represent the group means with their 95% confidence intervals as computed for least significant difference (LSD) multiple comparisons (p=0.05). The confidence, which do not overlap represent statistical significant difference between the corresponding groups. Statistical significance: NS=not significant, *p<0.05, **p<0.01, *p<0.001.

These findings concur with the studies on in vitro metabolism of testosterone in the fat tissue of various localisations. In a study of intra-adipose sex steroid metabolism [9], generalized obesity (BMI) was associated with increased aromatase mRNA and 5α-reductase type 1 levels did not did not predict fat amount or its distribution. This supported the hypothesis that intra-adipose sex steroid metabolism is a determinant of gynoid vs. android patterns of body fat [9].

Modified androgen metabolism pathway influences fat tissue, as androgens modulate adipocyte function and affect the size of adipose tissue compartments in humans. For instance, aldo-keto reductase 1C (AKR1C) enzymes, especially AKR1C2 and AKR1C3, through local synthesis and inactivation of androgens, may be involved in the fine regulation of androgen availability in adipose tissue [10]. Type 3 17β-hydroxysteroid dehydrogenase is co-expressed with aromatase in the abdominal preadipocytes [8].

It could be concluded that in men with increasing body mass index the formation of C_{19} steroids decreases from their C_{21} precursors, and lower 17β-hydroxysteroid dehydrogenase further confines the production of testosterone and dihydrotestosterone.

Acknowledgement

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The role of non-aromatizable testosterone metabolite in metabolic pathways

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Short title: Dihydrotestosterone and metabolic pathway

Dedication

This paper is dedicated to Professor Luboslav Stárka, MD, DSc

Summary

Dihydrotestosterone (DHT) originates via irreversible reduction of testosterone by catalytic activity of 5α-reductase enzyme and it is demonstratively the most effective androgen. Androgens influence adipose tissue in men either directly by stimulation of the androgen receptor or indirectly, after aromatization, by acting at the estrogen receptor. DHT as a non-aromatizable androgen could be responsible for a male type fat distribution. The theory of non-aromatizable androgens as a potential cause of a male type obesity development has been studied intensively. However, physiological levels of DHT inhibit growth of mature adipocytes. In animal models, substitution of DHT in males after gonadectomy has a positive effect on body composition as a testosterone therapy. Thus, DHT within physiological range positively influences body composition. However, there are pathological conditions with an abundance of DHT, e.g. androgenic alopecia and benign prostatic hyperplasia. These diseases are considered as risk factors for development of metabolic syndrome or atherosclerosis. In obese people, DHT metabolism in adipose tissue is altered. Local abundance of non-aromatizable androgen has a negative effect on adipose tissue and it could be involved in pathogenesis of metabolic and cardiovascular diseases. Increased DHT levels, compared to physiological levels, have negative effect on development of cardiovascular diseases. Difference between the effect of physiological and increased level brings about certain paradox.

Keywords:

non-aromatizable androgen – adipose tissue – metabolic syndrome – atherosclerosis - testosterone

Introduction

DHT was described first in 1930s (Dorfman and Hamilton 1939). For a long time, it was considered an ineffective testosterone metabolite. Its effect was discovered 30 years later (Bruchovsky and Wilson 1968). Nowadays, DHT is known as the most powerful androgen with affinity to the androgen receptor 5× higher than testosterone (T). The androgenic efficacy itself, in comparison to T, is approx. double or triple. Testosterone is sometimes considered to be a DHT effect modulator (attenuation of its effect).

DHT in placental mammals occurs already from 6th week of intrauterine life via irreversible reduction of testosterone by catalytic activity of 5α-reductase enzyme which interferes with metabolism of progesterone, deoxycorticosterone and testosterone, and exists in two isoforms, type I and II. Both isoforms are expressed differently in various tissues and during developmental stages. In humans, type I 5α–reductase is present in sebaceous glands of skin, in liver, muscles and brain; a small amount is also present in the prostate and it may increase in prostate cancer. Type I of 5α–reductase is responsible for approx. ⅓ of circulating DHT. Type II of 5α–reductase is found in prostate, seminal vesicles, epididymis, hair follicles, liver and it is responsible for ⅔ of circulating DHT. Additionally, type III isoform was also found in prostate cancer (Uemura et al. 2003).

DHT plays a role in prenatal differentiation of external genitalia being a regulatory hormone of testicular descent and development of external genitalia, it also affects skin appendages (hair follicle and sebaceous glands), maturation of spermatozoa in epididymis as well as the body composition (Aumueller *et al*. 1996).

It is a known fact that sex steroids influence fat deposition in women and men. Fat distribution is one of the secondary sex characteristics. In men, the fat tends to depose abdominally, they have more visceral fat than premenopausal women. In women, the preferential fat distribution is in gluteofemoral region and body fat portion is overall higher.

Androgens may influence adipose tissue in men either directly by stimulation of androgen receptor or indirectly by influencing estrogen receptor after aromatization. DHT as a non-aromatizable androgen might be responsible for a male type fat distribution.

Influence of DHT physiological level on body composition

Androgens affect body composition in men. During the last 3 decades, several original studies and review articles describing effects of testosterone and its supplementation on body composition were published. Testosterone acts by reducing abdominal fat and by increasing muscle mass.

Supplementation with testosterone, however, brought many controversial outcomes. Blouin *et al.* 2008 in their review article describe as a testosterone physiological window, when its lower as well as higher levels have a negative effect on the body composition and cardiovascular risk. Non-physiological levels are apparently one of the reasons of controversial outcomes.

Much smaller number of studies is dedicated to DHT. In some animal models, effect of DHT mediated only by androgen receptor is used. The quantity of fat, subcutaneously as well as viscerally, correlates with level of both testosterone and DHT (Nielsen *et al.* 2007). DHT as well as testosterone, affect proliferation of fat cells (Singh *et al.* 2003). Their physiological levels therefore have an influence on body composition. It was demonstrated that unlike testosterone, DHT has a positive effect on bone density (Ilangovan *et al.* 2009).

Role of DHT in adipose tissue

Several experimental models are dedicated to effects of DHT in adipose tissue. In cell lines, DHT as well as testosterone affects pluripotent cells by blocking their transformation into adipocyte (Singh *et al.* 2003). In another paper, DHT inhibited the differentiation of

mesenchymal cells and preadipocytes via androgenic receptor but with no influence on their proliferation (Gupta *et al.* 2008). Several animal models were dedicated to effect of DHT on adipose tissue. Two extensive genetic analyses of adipose tissue in gonadectomised male mice after substitution of DHT were carried out. Substitution of DHT improves metabolism of the adipose tissue by numerous mechanisms: stimulation of glycolysis, fatty acids and triacylglyceroles production, lipolysis and cell share reorganization, and cell proliferation and differentiation (Bolduc *et al. 2004,* Bolduc *et al. 2007).* In study Moverare–Skrtic *et al*. only 45ug/day was used for 5 weeks comparing to study Bolduc et al. where 0.1mg /day was administered for 3 weeks. The dosage in study Moverare –Skrtic et al. could have been insufficient for full saturation of DHT in the physiological level which is 0.59 nM (Potter *et al. 2006).*

In visceral fat in obese men, differences in levels and metabolism of DHT were found. Obese men have higher level of DHT in visceral fat than in subcutaneous fat (Bélanger *et al.* 2006). Also, degradation of DHT in omental fat is higher in obese people than in slim people (Blouin *et al.* 2006). A metabolite of DHT, androstane-3 α,17-β-diol-17-glucuronide, in one of the studies, correlated not only with the quantity of fat, but also with central fat distribution, intrahepatic fat, lipid spectrum disorder and insulin resistance (Vandenput *et al.* 2007).

Relation of DHT to cardiovascular diseases risk factors

A number of experimental models deal with influence of DHT on risk factors of cardiovascular diseases. Animal experiments provide evidence of positive effect of DHT levels normalization on cardiovascular risk. In gonadectomised rats, substitution of DHT improves a thrombotic potential of platelets (Li *et al.* 2007); in gonadectomised rabbits, DHT reduces atherosclerosis development through suppression of intimal foam cell formation of

macrophage partly via suppression of lecithin-like oxidized-low-density lipoprotein receptor-1 (Qiu *et al.* 2010).

Studies with cell lines bring findings about the effect of high DHT levels which inhibits growth of smooth muscle cells in cell culture; this inhibition is dose-dependent (Somjen *et al.* 2009). Exogenous administration of DHT in cell culture of human macrophages stimulates expression of proatherogenic genes in male microphages but not in female macrophages (Ng *et al.* 2003). However, DHT dosage used in the study was 10 times higher than DHT physiological level in male plasma which affected the study outcomes.

Yanes et al. monitored influence of DHT on production of aldosterone in the cell line of human adrenocortical cells. Effect of DHT is dose-dependent. Physiological levels of DHT do not alter the secretion of aldosterone. Supraphysiological level of DHT stimulates secretion of aldosterone via its effect on calmodulin/calmodulin-dependent protein kinase and protein kinase C intracellular signalling pathway but independently on classic androgen receptor. According to the authors, supraphysiological levels of androgens may, by means of this mechanism, contribute to development of cardiovascular diseases (Yanes and Romero 2009).

DHT levels and factors influencing DHT levels

Concentration of DHT in male serum is approx. by an order of magnitude lower than concentration of testosterone. Diurnal profile of testosterone is well recognized. The difference between morning and afternoon level of testosterone is up to 25% in young men, decreasing to 10% in older age. Diurnal profile of DHT is similar, however its variations in all age categories are smaller (Brambilla *et al.* 2009). Literary data about DHT/T ratio vary during life. Some studies describe increase of DHT/T during life (Feldman et al. 2002). In other studies, DHT/T remains unchanged (Gray et al. 1991, Maier 2001, Pirke and Doerr 1975). The alteration of the ratio of these two androgens during life, by some authors, is

considered as a cause of the development of benign prostatic hyperplasia and androgenic alopecia (AGA) in the middle age. In our study monitoring serum levels of DHT in 13,152 men during life, we found a constant ratio of a total and free DHT/T since puberty. Before puberty, the dominant androgen is DHT rather than T. These findings indicate that in adulthood, serum levels of DHT in men almost exclusively depend on levels of gonadal testosterone whereas before puberty, may depend on production of androgens in adrenal glands (Stárka *et al.* 2008, Stárka *et al.* 2009).

Considering DHT/T ratio in serum remains constant during life, the role of change of DHT/T in development of AGA and benign prostatic hyperplasia is rather unlikely. The cause is assumed to be in local change of DHT/T in androgen-dependent tissues which however will not be demonstrated in the serum levels of hormones or in the change of tissue sensitivity to the effects of DHT.

Some studies describe geographical and racial differences in DHT levels in various ethnic groups. An extensive American study examined a group of 1899 men aged 30 to 79. The authors did not find a difference between T and SHBG (sex hormone binding globulin); however, after adjustment, they found a higher DHT and lower DHT/T ratio in black people than in white or Hispanic people. This difference could explain racial differences in occurrence of prostatic carcinoma and body composition (Litman *et al.* 2006). In another study, DHT levels in 5,003 men from five continents were described. This study did not prove only racial but also geographical differences in steroid levels which could not be explained by body composition. The geographical differences were expressed more strongly than racial differences. DHT was higher in Japanese people (0.52 ng/ml) and men from Hong Kong (0.45 ng/ml) compared Asian people from the USA (0.34 ng/ml) who had similar levels as white

people (0.36 ng/ml), black people from the USA (0.38 ng/ml) and Swedish people (0.36 ng/ml) (Orwoll et *al.* 2010).

DHT levels may be influenced by some external effects. Sleep deprivation decreases DHT levels but they are corrected after the convalescence. Decrease of androgens is not followed by the decrease of the gonadotropins which remain unchanged (Akerstedt *et al.* 1980, Gonzáles-Santos *et al.* 1989). Combination of physical activity with energetic and sleep deprivation induces decrease of gonadotropins but also decrease of testosterone and DHT (Opstad 1992).

Aerobic exercise for 1 year time period increases levels of DHT and SHBG but does change levels of T, estradiol and 3α-androstanediol glucuronide (Hawkins *et al.* 2008). One of the studies monitored effect of a 3-week diet enhanced with creatine in rugby players versus placebo. Creatine increased DHT levels but T level remained unchanged (van der Merwe *et al.* 2009). Above mentioned studies show that some food stimuli, stress or physical activity may change androgen levels and ratios which explain geographical differences among the androgens. These changes should also be considered in interpreting of the studies outcomes comparing influence of individual factors on disease development.

Androgenic alopecia (AGA) as a condition with DHT abundance

AGA is the most common form of hair loss in men. Occurrence of the first AGA symptoms is in 20 % of 20 year-old men rising by 10 % with every decade. As a premature alopecia is denoted a fully apparent baldness before 35th year of age. Androgens control hair growth all over the body; their effect varies in different parts of the body: occipital scalp, eyebrows and eyelashes are insensitive to androgens. In other parts, androgen effect on the hair growth is opposite; on the chin, chest, axilla, pubic area and extremities, the hair follicles are stimulated by a higher level of androgens to be transformed into terminal follicles. In men with a

hereditary predisposition to baldness, follicles are inhibited on the frontal and parietal scalp. Why hair responds differently to androgens in various parts of the body has been a subject of various hypotheses, but no convincing reason is known yet. The cause is seen in different density of receptors for androgens, increased production of DHT, reduced metabolic degradation of androgens and also other factors (Kaufman 2002).

The essential role of DHT for hair growth and AGA development is confirmed by Imperato-McGinley syndrome caused by mutation of gene for type II of 5α–reductase, which prevents expression of this enzyme and sufficient production of DHT. Men with this syndrome do not suffer from enlarged prostate and do not become bald (Imperato-McGinley et *al.* 1974). Another evidence is that follicles or skin samples taken from bald spots in AGA have a higher content of DHT than in men without bald. There are not many findings about the role of I type 5α–reductase for hair growth, however its level in sebaceous glands is high, especially in acne prone areas. Clinical evidence of role of DHT was shown also by studies focused on the use of 5α–reductase inhibitors in treatment of AGA either localized on vertex (Finasteride Male Pattern Hair Loss Study Group 2002) or manifested by frontal hair line retreat.

AGA as a symptom of increased androgen activity has been intensively studied to be a possible risk factor of some diseases. In the literature, there has been described a higher risk of both benign hyperplasia (Oh *et al.* 1998, Chen *et al.* 2004) and prostate carcinoma (Hawk *et al.* 2000, Gilles *et al.* 2002), i.e. a prostate disease; prostate, similarly to hair follicles, is more influenced by DHT than T. In some studies, the relation of AGA and prostate carcinoma was not confirmed (Hsieh *et al.* 1999).

Premature AGA is also associated with higher occurrence of obesity (Hirsso *et al.* 2007). Several studies bring evidence on AGA as an independent risk factor of cardiovascular and metabolic diseases Trevisan *et al.* 1993, Ford *et al.* 1996, Herrera *et al.* 1995, Lesko *et al.* 1993, Sasmaz *et al.* 1999, Lotufo *et al.* 2000, Matilainen *et al.* 2000, Dušková *et al.* 2004,

González-González *et al.* 2008, Dogramaci *et al.* 2009). Some studies, however, face methodical problems, e.g. small number of probands which raises doubts about these results. In an extensive epidemiological study, including 5,056 men from 45 to 64 years of age, no relation between AGA and myocardial infarction or between AGA and intimomedial thickness as a marker of symptomatic atherosclerosis was proved (Shahar *et al.* 2008). The key problem of this study is that it did not monitor the start of hair loss, for it is apparently only premature AGA that is related to the mentioned diseases. It should be also mentioned that changes in prostate as well as changes in metabolic parameters and cardiovascular risk factors, in men with a premature AGA, are expected to change significantly in older age. Local abundance of DHT could play a role in development of both premature AGA and male type obesity.

Conditions with DHT deficiency

Naturally, there are several situations with reduced effect of androgens, the first one is a complete androgen insensitivity syndrome. Girls with this syndrome have genotype 46XY. One of the studies dealt with metabolic parameters and body composition in women with this syndrome. Higher prevalence of obesity, dyslipidemia and insulin resistance were found (Dati *et al.* 2009).

The problem of this study is a small number of probands due to rarity of this syndrome. There is an animal model for this syndrome, the knock-out mice for AR receptor are obese at unchanged food habits but their lipid spectrum remains unchanged (Sato *et al.* 2003).

Another natural model related directly to DHT is the above mentioned Imperato-McGinley syndrome. Affected individuals produce testosterone in normal or even in slightly elevated quantity but do not convert it to DHT sufficiently. Homozygous patients with a male karyotype are born with a phenotype as a specific type of hermafroditism and look rather like

girls until adolescence. During puberty, due to influence of increasing levels of testosterone, the virilisation starts: normal libido, stabilization of male phenotype, sparse beard and a scanty body hair; in older age, they are not affected by prostate growth or baldness (Imperato-McGinley et *al.* 1974). In the literature, there are no references to their body composition or cardiovascular risks.

Polymorphism of the gene for 5 α-reductase was studied in relation to peripheral arterial disease. Significant relation between the polymorphism of the gene for 5 α-reductase of type I associated with lower activity of this enzyme and peripheral arterial disease was found. Lower DHT level could therefore predispose to peripheral arterial disease (Signorelli *et al.* 2008). A question remains how this polymorphism is manifested when 5 α-reductase of type I is responsible for only ⅓ of DHT.

Finasteride treatment is an artificially created model of lower DHT levels. The key problem of finasteride treatment as a model of lower DHT levels effects is that medication is prescribed in patients with DHT abundance. Finasteride as a 5 α-reductase blocker is used in treatment of benign prostatic hyperplasia and its indication has recently been extended to the treatment of AGA in a lower total daily dose.

Therefore this model accumulates a double effect: a long term exposition to higher DHT level and also reducing their levels with finasteride which is relatively short. Two studies dealt with administering the treatment to healthy individuals without differentiation whether the probands had DHT abundance or not. Gormley *et al.* 1990 did not observe changes in lipid profile after a short term use of finasteride in higher and low dosage. Amory *et al.* 2008 during administering of finasteride or dutasteride, did not find significant influence on lipid metabolism in healthy men with a long term use. Another two studies monitored effect of finasteride in patients with DHT abundance. Denti *et al.* 2000 observed increase of levels of HDL-cholesterol and lipoproteins after a 6-months treatment in patients with benign prostatic

hyperplasia. In our study, we have found elevation of cholesterol, HDL, LDL after 3, 6, 8 months with normalization of all parameters after 1-year treatment. In patients with AGA, decrease of insulin resistance in the insulin tolerance test after 1- year finasteride treatment, was observed (Dušková *et al.* 2009).

Although this model does not seem appropriate for studying effects of lower DHT levels, it could be suitable for a long term monitoring of possible changes in metabolic parameters by decrease in DHT levels in patients exposed to higher DHT levels. The studies published so far are short term and involved a small number of probands.

Conclusion

DHT as well as testosterone has its physiological range when it reduces the content of body fat. Decreased or increased DHT levels are detrimental to adipose tissue. This physiological range of DHT could form the window of physiological function. DHT as the most powerful androgen, influencing only the androgen receptor, could be responsible for male type of fat deposition. The actual fat distribution type is not a risk factor for obesity development and it has a neutral relation to cardiovascular diseases; however, the situation is different in case of fat abundance, where the localization does play a role. This finding is supported by studies on a positive effect of DHT substitution on body composition in experiments with gonadectomised animals. Therefore it is necessary to distinguish the effect of DHT in physiological window which is positive on body composition, and on the cardiovascular risk, from effects of higher DHT levels which can affect obesity development. Different effects of individual DHT levels create a paradox which is left out in some studies. Like the male type of fat deposition or physiological DHT level are not risk factors for cardiovascular diseases, a shift from the physiological window is negative and may contribute to their development.

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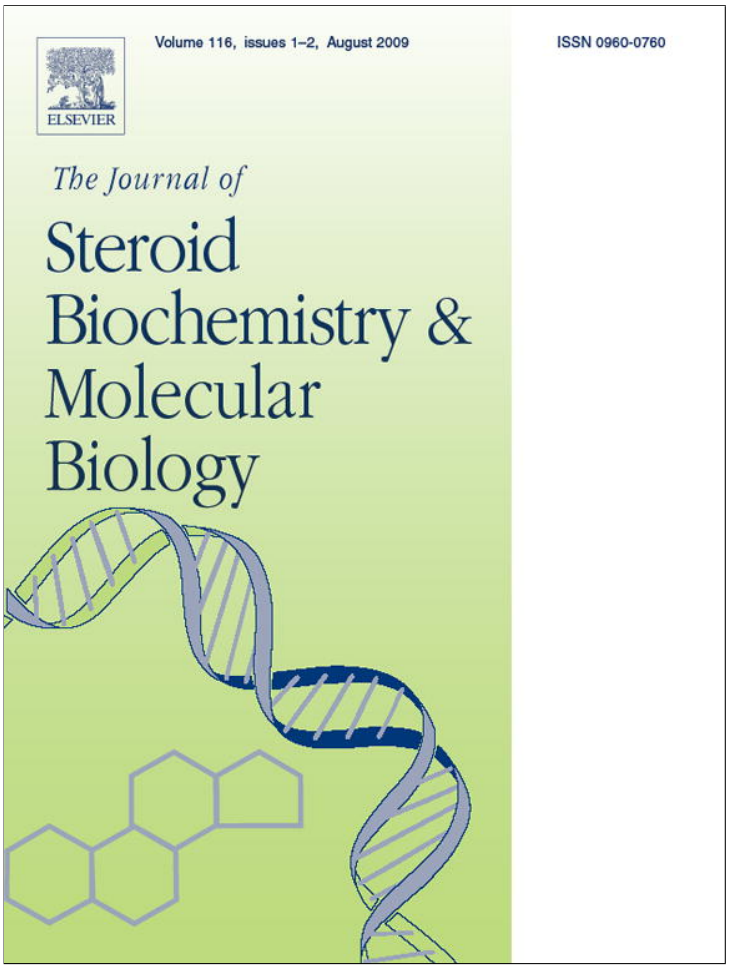

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Free testosterone and free dihydrotestosterone throughout the life span of men

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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.

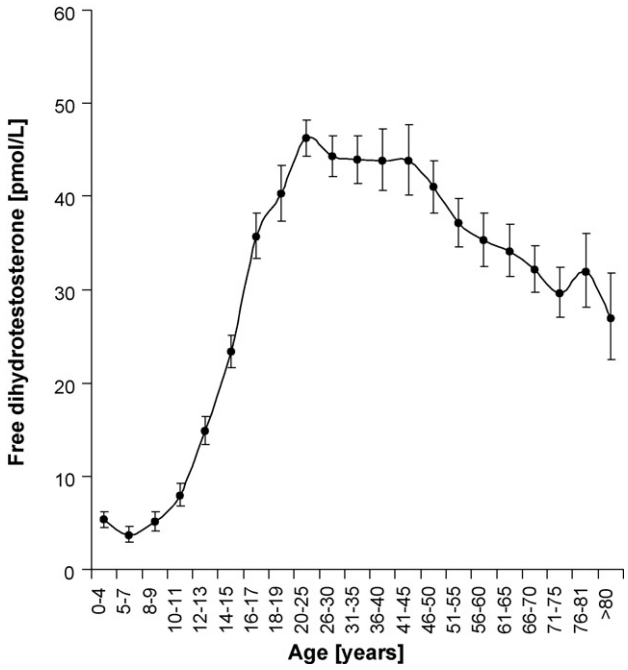

Fig. 1. The course of free testosterone serum concentrations over the life span of Czech men as evaluated using ANOVA followed by Bonferroni multiple comparisons (vs. control, $p < 0.05$). The empty circles with error bars represent re-transformed mean values with their 95% confidence intervals. The 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.

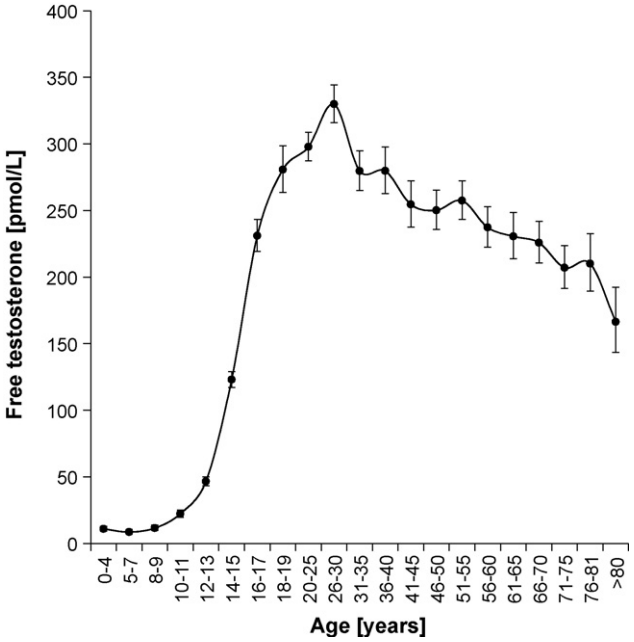

Fig. 2. The course of free dihydrotestosterone serum concentrations over the life span of Czech men as evaluated using ANOVA followed by Bonferroni multiple comparisons (vs. control, $p < 0.05$). The drawings and symbols are the same as for Fig. 1. Bonferroni multiple comparisons showed homogeneity within 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-

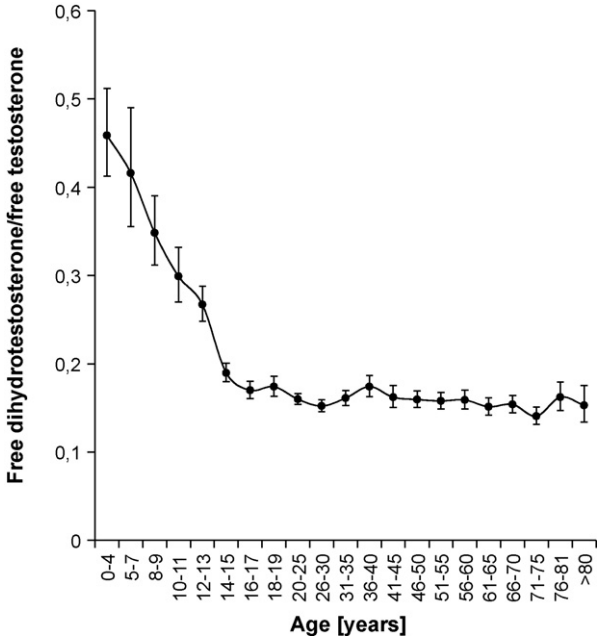

Fig. 3. The course of the ratio of serum concentrations of free dihydrotestosterone to free testosterone over the life span of Czech men as evaluated using ANOVA followed by Bonferroni multiple comparisons (vs. control, $p < 0.05$). The drawings and symbols are the same as for Fig. 1. Bonferroni multiple 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.

Acknowledgement

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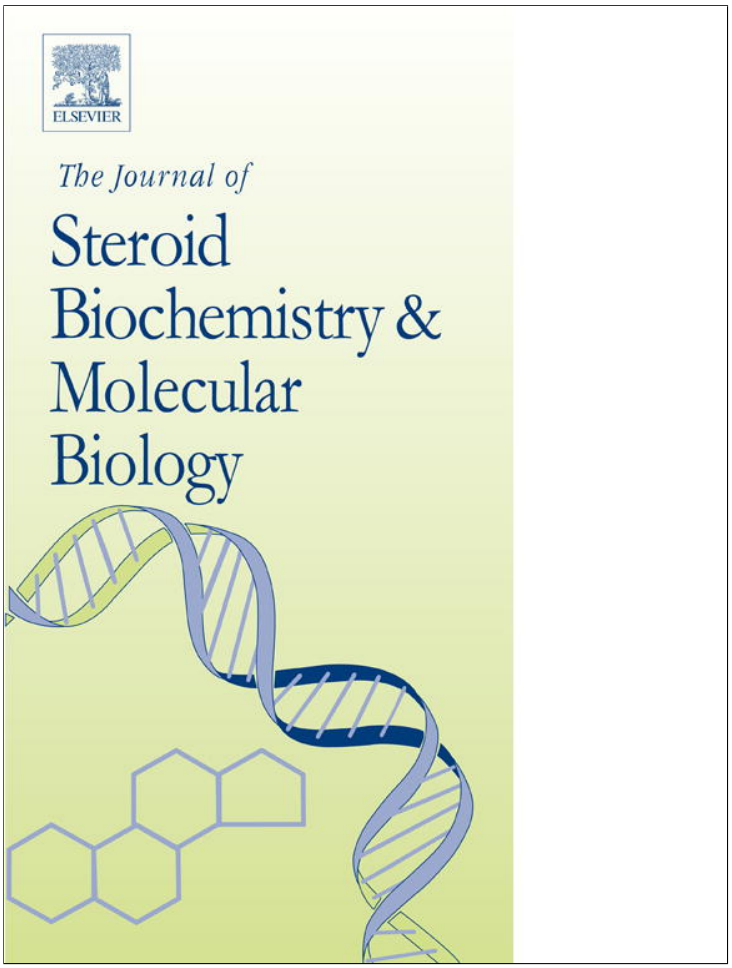

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The differences between aromatizable and non-aromatizable androgens in relation to body composition and metabolic syndrome risk factors in men

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ABSTRACT

The relationships between the parameters of metabolic syndrome and non-aromatizable metabolites of testosterone have been discussed in literature. Some papers describe these metabolites as one of the possible causes of male-type obesity. On the contrary, other studies show a protective influence of dihydrotestosterone on visceral obesity.

The aim of this study to analyse the relationship between anthropometric parameters, lipid spectrum, glycemia and the level of endogenous testosterone and dihydrotestosterone, and to compare the effects of these androgens. Our population-based study involved 232 healthy men ranging from 20 to 78 years with BMI 18 to 39 kg/m^2. Serum testosterone, dihydrotestosterone and sex hormone binding globulin SHBG levels, lipid spectrum, glucose metabolism parameters were measured and the oral glucose tolerance test was carried out in all subjects. Their anthropometric parameters (weight, height, waist, hips, waist-to-hip ratio, 14 skin folds) and body composition parameters were determined and calculated by the Antropo program. Multiple regression analysis showed a correlation between hormonal levels, esp. of testosterone and dihydrotestosterone, and the anthropometric data, lipid spectrum and parameters of glucose regulation. Low testosterone and/or dihydrotestosterone was correlated to a higher body-mass index, fat content, waist diameter, total-, HDL-, LDL-cholesterol and triglycerides, fasting glucose, insulin resistance and lower muscle and bone mass. In addition, statistical analysis using multivariate regression with reduction in dimensionality did not discover any striking difference between aromatizable and non-aromatizable androgens in their association to lipid and glucose metabolism parameters in healthy, normosthenic men. In conclusion, the association of endogenous testosterone and dihydrotestosterone to anthropometric data, lipid spectrum and insulin sensitivity are of the same quality; however, the effect of the circulating levels of dihydrotestosterone is quantitatively smaller.

1. Introduction

Fat distribution is one of the secondary sexual characteristics. Men have a tendency to deposit fat abdominally and have a greater amount of visceral fat than premenopausal women. This type of fat deposit is associated to a higher risk of diabetes mellitus and cardiovascular diseases. In women the preferential fat distribution is gluteofemoral and women have a greater percentage of body fat in total. Androgens can affect fat tissue formation and localization in men through the androgenic receptor or indirectly after aromatization by stimulation of the estrogenic receptor. Dihydrotestosterone (DHT) is an androgen with the greatest effect; its affinity to the androgen receptor (AR) is about five times higher compared to testosterone (T). The DHT–AR complex has a longer half-life and a higher DNA binding affinity than the T–AR complex. Therefore, the effective dose of DHT, required to activate an androgen responsive marker gene by 50%, is about 10-fold lower than that required to achieve the same level of induction with T [1]. The actual androgenic efficiency within the target tissues is about two or three times higher [2].

The concentration of DHT in men's serum is one order of magnitude lower than the concentration of T. In the literature the data on DHT-to-T ratio differ [3,4]. In our previous study on DHT levels over a lifetime we found a constant ratio of both total and free DHT/T over a lifetime starting with puberty [5,6].

DHT plays a key role in prenatal differentiation of external genitalia. It is a control hormone for the descent of the testes and differentiation and development of external genitalia and prostate development and growth. DHT effects are important for spermatozoid maturation in epididymis [7]. DHT also influences the

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skin adnexa (hair follicle and sebaceous glands) and plays a role in the development of androgenic alopecia. Androgenic alopecia, as a symptom of dihydrotestosterone abundance, is related to a higher occurrence of prostate hyperplasia and prostate carcinoma [8–10] and it is also considered a risk factor for cardiovascular and metabolic diseases [11–13].

The syndrome of Imperato-McGinley can serve as a natural model of DHT-insufficiency [14].

DHT is irreplaceable by T in the effects on external genitalia development, prostate development and on skin adnexa. In other roles both hormones are similar. DHT, contrary to testosterone, is a non-aromatizable androgen and so its effects cannot be explained by its transformation to estrogens. Several papers have discussed the effect of dihydrotestosterone on some anthropometric indicators and metabolic parameters and especially on male fat deposition [15–19].

In our study we tried to answer the question of whether endogenous DHT has the same or a different effect on body composition, glucose tolerance and lipid spectrum than testosterone, and whether both hormones are identical in this respect.

2. Materials and methods

A group of 232 healthy men (except of obesity and associated symptoms) at the age of 20–78 with a broad range of body mass index (BMI) 18–39 was enrolled in this study. Anthropometric parameters (*i.e.* weight, height, waist, hips, waist-to-hip ratio, 14 skin folds, BMI, percentage representation of muscle and fat tissue) were measured. Laboratory analyses of metabolic parameters (lipid spectrum – triglycerides, total cholesterol, HDL, LDL, glucose metabolism parameters – glycemia, immunoreactive insulin – IRI, C-peptide, oral glucose tolerance test (oGTT)) and steroid hormones dihydrotestosterone (DHT), testosterone (T), 17alpha-hydroxy-progesterone (17-OH), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), 4-androstene-3,17-dione (A2), LH, FSH, 17alpha-hydroxy-pregnenolone (Preg17) and sex hormone binding globulin (SHBG) were also carried out. The overall characteristics of the male volunteers in subgroups of lean and obese participants are listed in Table 1.

The Ethical Committee approved the study and all patients signed informed consent form before taking part in the study.

2.1. Anthropometric data

Anthropometric data were obtained in a fasting state. Body weight, height, waist and hip circumferences were measured in all participants in order to calculate body mass index (BMI) and to evaluate visceral fat accumulation by means of waist circumference, waist-to-hip ratio (WHR). Furthermore, 14 skin folds (c1–c14) were measured. Body composition (% of subcutaneous fat mass, % of muscle mass, and % of bone mass from the total body weight) was then calculated using the ANTROPO program [20]. Weight (to the nearest 0.1 kg) and height (to the nearest cm) were measured. Circumferences were measured in a standing position, waist in halfway between the lower ribs and the crest of the pelvis and hip circumference at the level of the greater trochanters. Body mass index (BMI) was calculated as the weight (kg) divided by height squared (m^2) and waist-to-hip ratio (WHR) as waist divided by hip circumference.

2.2. Biochemical analysis

After an overnight fast, venous blood samples were obtained in order to determine biochemical parameters. The blood glucose level was measured by the glucose oxidase method (Beckman Glucose Analyzer 2). Glycosylated proteins (Glykop) (spectrophotometric redox reaction using nitro blue tetrazolium as a sensitive redox indicator for the specific quantification of fructosamine in alkaline solution) were determined. Immunoreactive insulin (IRI) was assayed using an immunoradiometric assay and serum levels of C-peptide were evaluated by the immunoradiometric assay (Immunotech IRMA, Marseilles, France). Total cholesterol (Merckotest, CHOD-PAP-Method), high-density lipoprotein cholesterol (HDL, Merck System Cholesterol, CHOD-PAP-Method), and triglyceride concentrations (Merck System, GPO-PAP-Method) were measured in serum using the analyzer Merck (Vitalab Eclipse). Low-density lipoprotein cholesterol (LDL) levels were calculated as: LDL = total cholesterol-(TG/2.2)-HDL. The 3-h oral glucose tolerance test (oGTT) with 75 g of glucose load was performed in all subjects.

2.3. Steroid analysis

Serum testosterone was determined by standard radioimmunoassay (RIA) using antiserum anti-testosterone-3-carboxymethyloxim: BSA and testosterone-3-carboxymethyl-oxim-tyrosylmethyl-ester-[^{125}I] as a tracer. Intra-assay and inter-assay coefficients variants were 7.2% and 10%, respectively, and sensitivity was 0.21 nmol/l. Androstenedione was determined by standard RIA with antiserum anti-androstenedione-6-carboxymethyloxim: BSA and [^{3}H] androstenedione as tracer. Intra-assay and inter-assay coefficients variants were 8.1% and 10.2% and sensitivity was 0.39 nmol/l. Sexual hormones binding globulin was assayed by IRMA kit (Orion, Espoo, Finland). Commercial kits (Immunotech, Marseilles, France) were used for the determination of LH, FSH (IRMA kit), 17-hydroxyprogesterone (Prog17), DHEA and DHEAS (RIA kit). DHT was determined by original methodology [21]. 17-Hydroxy-5-pregnenolone (Preg17) was determined by an in house RIA method.

2.4. Statistical data analysis

To eliminate skewed data distribution and heteroscedasticity, the original data was transformed to a Gaussian distribution by a Box-Cox transformation before further processing using the statistical software Statgraphics Centurion, version XVI from Statpoint Inc. (Herndon, VA, USA). The differences between the groups with successful and unsuccessful treatment were evaluated by age-adjusted ANCOVA.

To simultaneously evaluate the relationships between anthropometric indices and markers of insulin resistance on the one hand (matrix **X**), and steroids and related substances on the other hand (matrix **Y**), to compare the predictive value of individual variables and to explain the structure in the data, we applied multivariate regression with reduction of dimensionality, known as bidirectional orthogonal projections, to latent structures (O2PLS). The O2PLS method is bidirectional and enables the prediction of variables constituting the matrix **Y** from variables constituting the matrix **X** and *vice versa*. The predictivity of individual variables for the model may be simply expressed as a correlation of the variable with a common predictive component. The predictive component extracts variability from the **X** and **Y**, which is shared between **X** and **Y** from variability within the matrixes **X** and **Y**, which is separated into the orthogonal components.

The transformed data underwent processing by the O2PLS method, which is effective in coping with the problem of severe multicollinearity within the matrixes of both dependent and independent variables. The O2PLS enabled us to find the variables with high predictive value for the description of the relationships

Table 1
Characterization of the male volunteers. Summary statistics of anthropometric characteristics and laboratory indices of the lean and obese subgroup.

Variable	BMI ≤ 25 kg/m^2		BMI > 25 kg/m^2	
	Mean (SD)	Median (quartiles)	Mean (SD)	Median (quartiles)
Age [years]	30.5 (11)	26.5 (23.7, 34.2)	46.6 (17.3)	45.7 (32.1, 60.3)
BMI [kg/m2]	22.5 (2.79)	22.8 (21.4, 24)	29.6 (4.82)	28.2 (26.7, 31.4)
TV [cm]	181 (18.2)	181 (177, 185)	179 (17.8)	180 (173, 183)
Abdomen [cm]	82.7 (9.78)	82.2 (78.3, 86.6)	103 (15)	101 (94.4, 108)
Hip [cm]	95.9 (9.88)	96 (93.1, 98.8)	107 (12.3)	105 (101, 110)
Waist [cm]	79.4 (9.28)	78.8 (74.9, 83.4)	100 (15.1)	97.8 (91.1, 106)
Bone [kg]	12.8 (1.9)	12.8 (11.8, 13.6)	13.5 (2.08)	13.5 (12.2, 14.6)
Bone [%]	17.4 (2.41)	17.3 (16.2, 18.5)	14.5 (2.09)	14.5 (13.5, 15.5)
Muscle [kg]	34.2 (4.79)	33.9 (31.5, 36.5)	39 (6.31)	38.8 (35.4, 42.4)
Muscle [%]	46.5 (5.75)	46.6 (44.1, 48.8)	41.7 (5.84)	41.6 (39.5, 43.6)
Fat [kg]	10.2 (4.11)	9.32 (6.91, 12.9)	20.2 (7.91)	19 (14.8, 24.8)
Fat [%]	13.6 (4.78)	12.7 (9.87, 16.8)	21.3 (6.7)	20.7 (16.4, 25.6)
Weight [kg]	73.9 (10.2)	73.8 (69.1, 79)	94.6 (15.7)	92.6 (84.3, 100)
c1 [cm] (cheek skin fold)	6.85 (3.26)	6 (4.5, 9)	16 (5.73)	15.8 (12, 20)
c2 [cm] (chin skin fold)	9.6 (4.7)	8.45 (5.63, 13)	18.2 (6.45)	18.3 (13.5, 21.9)
c3 [cm] (chest skin fold 1)	14.6 (6.36)	14 (9, 19)	27.8 (10.3)	27 (23, 33.5)
c4 [cm] (chest skin fold 2)	7.05 (3.01)	7 (5, 8)	11.5 (5.5)	10 (8, 13)
c5 [cm] (hip skin fold)	3.22 (1.67)	3 (2, 4)	7.43 (4.25)	6.5 (4, 10)
c6 [cm] (abdomen skin fold)	3.18 (1.77)	2.5 (2, 3.5)	6.44 (3.5)	6 (4, 8.38)
c7 [cm] (patellar skin fold)	9.2 (4.82)	8.5 (5.63, 12)	14.5 (7.09)	12.8 (9.5, 18)
c8 [cm] (biceps skin fold)	10.3 (4.15)	9.5 (7, 12.4)	20.1 (6.76)	20 (16.5, 22.9)
c9 [cm] (forearm skin fold)	7.16 (3.34)	6.5 (5, 9)	11.3 (5.26)	10 (8, 13.9)
c10 [cm] (triceps skin fold)	13.3 (5.21)	13 (10, 16)	19.2 (8.45)	18.5 (12, 23.9)
c11 [cm] (back skin fold)	7.06 (3.46)	6 (4.5, 9.88)	9.72 (4.6)	9 (6.5, 12)
c12 [cm] (calf skin fold 1)	5.61 (1.56)	5.5 (4.63, 6.5)	8.05 (2.53)	8 (6.5, 9)
c13 [cm] (thigh skin fold)	4.19 (2.27)	3.75 (2.5, 5)	8.71 (3.91)	8 (6, 11)
c14 [cm] (calf skin fold 2)	4.98 (3.06)	4 (3, 6)	8.49 (5.24)	7 (4.5, 11)
BPS [mm Hg]	121 (18.4)	120 (111, 129)	134 (23.4)	130 (120, 145)
BPD [mm Hg]	72.9 (11.3)	72 (66.5, 78.5)	79.9 (14.3)	80 (71, 89)
glOGTT0 [mM]	4.86 (0.855)	4.7 (4.5, 5.1)	6.37 (2.77)	5.3 (4.7, 7)
cpep0 [nM]	0.526 (0.189)	0.485 (0.4, 0.63)	0.892 (0.373)	0.805 (0.61, 1.11)
IRI0 [mIU/L]	5.46 (3.06)	4.72 (3.3, 6.85)	11.4 (9.48)	8.9 (6.3, 12.1)
IRI180 [mIU/L]	4.33 (4.32)	3.3 (2.38, 4.7)	7.86 (7.03)	5.6 (3.7, 9.5)
TG [mM]	0.934 (0.412)	0.78 (0.63, 1.18)	1.89 (1.58)	1.62 (1.14, 2.22)
CH [mM]	4.25 (0.907)	4.15 (3.65, 4.73)	4.94 (1.05)	4.96 (4.36, 5.51)
HDL [mM]	1.39 (0.335)	1.35 (1.18, 1.58)	1.12 (0.305)	1.08 (0.92, 1.32)
LDL [mM]	2.43 (0.812)	2.33 (1.85, 2.93)	2.98 (0.859)	2.9 (2.52, 3.51)
Glykop [%]	1.1 (0.166)	1.07 (1, 1.14)	1.21 (0.251)	1.13 (1.05, 1.31)
Dihydrotestosterone (DHT) [nM]	2.17 (8.58)	1.32 (1.12, 1.58)	1.93 (8.8)	1.07 (0.863, 1.3)
Testosterone (T) [nM]	18.5 (6.05)	17.4 (13.8, 22.4)	14.4 (5.03)	13.8 (11.1, 17.2)
17-OH-progesterone [nM]	3.37 (3)	2.89 (2.32, 3.75)	2.48 (2.08)	2.09 (1.52, 2.84)
DHEAS [nM]	7.28 (3.65)	6.64 (5.49, 9.17)	5.99 (3.36)	6.04 (3.55, 7.81)
DHEA [nM]	23.9 (12.7)	21.5 (14.9, 31)	16.6 (10.8)	14.9 (8.29, 21.5)
Androstenedione (A2) [nM]	7.81 (9.42)	7.08 (5.42, 8.38)	6.33 (1.95)	6.02 (5.1, 7.53)
LH [IU/L]	5.01 (3.53)	4.35 (3.3, 5.52)	5.13 (3.46)	4.2 (3.3, 5.98)
FSH [IU/L]	4.77 (6.27)	3.85 (2.77, 4.83)	6.67 (7.03)	4.65 (3.2, 7.38)
SHBG [nM]	31.3 (14.3)	29.5 (21.6, 37.2)	30.2 (24.6)	25 (18.9, 33.8)
17-OH-pregnenolone [nM]	15.9 (12.7)	13 (7.05, 21.9)	9.22 (9.12)	6 (2.73, 11.8)

Abbreviations: DHT, dihydrotestosterone; T, testosterone; Prog, progesterone; A2, androstenedione; DHEA, dehydroepiandrosterone; Prog17, 17-hydroxyprogesterone; Preg17, 17-hydroxypregnenolone; c1–c14, skin folds; BPS, blood pressure systolic; BPD, blood pressure diastolic; glORTT0, fasting glucose; cpep0, fasting C-peptide; IRI0, fasting imunoreactive insulin; TG, triglycerides; Chol, total cholesterol; Glykop, glycated proteins.

between **X** and **Y** and to find the structure of these relationships. The O2PLS model may be expressed as follows:

$$\mathbf{X} = \mathbf{T}_p + \mathbf{T}_0\mathbf{P}_0 + \mathbf{E}$$

$$\mathbf{Y} = \mathbf{U}_p\mathbf{Q}_p + \mathbf{U}_0\mathbf{Q}_0 + \mathbf{F}$$

where **X** is the matrix with *l* independent variables and *i* subjects, **Y** is the matrix of *m* dependent variables and *i* subjects. $\mathbf{T}_p$ and $\mathbf{T}_0$ represent the matrixes of component scores from the predictive and orthogonal components, respectively, extracted from **X**. $\mathbf{P}_p$, and $\mathbf{P}_0$ represent the matrixes of component loadings from the predictive and orthogonal component, respectively extracted from **X**. Similarly, $\mathbf{U}_p$ and $\mathbf{U}_0$ represent the matrixes of component scores from the predictive and orthogonal component, respectively, extracted from **Y**.

$\mathbf{Q}_p$ and $\mathbf{Q}_0$ represent the matrixes of component loadings from the predictive and orthogonal component extracted from **Y**. **E** and **F** are error terms.

We have tested the relevance of individual variables for the model using a criterion Variable Importance (VIP). Only the variables that showed significant relevance for the first and/or the second predictive component were included in the model. Similarly, the relevant number of predictive components was tested using a criterion Prediction Error Sum of Squares (PRESS).

The statistical software SIMCA-P+ Version 12.0.0.0 from Umetrics (Umeå, Sweden) was used for data analysis. The software enabled us to find the number of the relevant components utilizing the prediction error sum of squares and also allowed the detection of multivariate non-homogeneities and testing of multivariate normal distribution and homoscedasticity [22,23].

3. Results

In our study we have proved the close relationship between dihydrotestosterone and testosterone regarding the effect on body composition and main metabolic parameters. Comparing the hormone levels and anthropometric parameters, we found a negative correlation of both androgens between the age, weight, skin folds, waist, hips, waist-to-hip ratio.

Multiple regression analysis shows the correlation of steroids to single variable (Tables 2–4). The relation of steroids to body mass composition, BMI, fat mass, bone mass and muscle mass is shown in Table 2, the relation to glucose metabolism parameters fasting glucose, fasting C-peptide, fasting insulin in Table 3 and to lipid composition as total cholesterol, LDL cholesterol, HDL cholesterol and, triglycerides in Table 4. A positive correlation of bone mass and muscle mass on one side and the T, DHT and SHBG levels and negative correlation of androgen status with BMI and fat mass was demonstrated as expected. Insulin and C-peptide levels were negatively associated with both testosterone and dihydrotestosterone, but glucose concentration had only a weak negative correlation to dihydrotestosterone. A negative correlation of total cholesterol, HDL, LDL cholesterol and triglycerides and both androgens was found.

The regression coefficients of the relation of testosterone and dihydrotestosterone to the other variables are very similar except for the coefficients of T and DHT to fat mass (Table 2) and HDL-and LDL-cholesterol (Table 4). Testosterone seems to have a more effective influence on these parameters than dihydrotestosterone.

In conclusion, the effects of testosterone and dihydrotestosterone on anthropometric data, glucose control and lipid spectrum are the same in quality; however, the effect of the circulating levels of dihydrotestosterone is quantitatively smaller.

We also monitored DHT/T ratio with lean and obese men. This ratio was constant and did not change with body mass index (not shown here).

Multivariate regression analysis discovers the mutual relations of the components. The 1st principal component (Table 5) shows that androgens and their precursors are negatively correlated with parameters of metabolic syndrome. In accordance with this finding, the FSH and parameters of metabolic syndrome are correlated positively. Furthermore, there is a positive correlation between SHBG and parameters of metabolic syndrome. Surprisingly, 17-hydroxyprogesterone (Prog17) shows both the highest component loading for the 1st predictive component as well as the ratio of the component to its 95% confidence interval. While the first column in Table 5 (component loadings for the predictive components expressed as regression coefficients) represents the influence of the variable, the parameter ratio of the regression coefficient to its 95% confidence interval (in the next column 2 in Table 5) demonstrate the statistical significance of the component loading for the variable. The most influential parameter from matrix X is the waist; however, the most significant one is skin fold c1 (cheek fold), probably due to greater inter-individual variability in the waist. In general, the active androgens (T, DHT) show lower importance compared to steroids primarily of adrenal origin (Prog17, Preg17 and DHEA).

Under normal physiological conditions with unmanipulated levels of androgens we have found a negative correlation between weight, skin folds, waist, hips, waist-to-hips ratio, BMI, total cholesterol, HDL-, LDL-cholesterol and insulin resistance on one side and testosterone (T) and dihydrotestosterone (DHT) levels and SHBG on the other side. Alternatively, we have found and muscle mass on one side and DHT and T levels and SHBG on the other side.

Table 2
Relationship between hormones and body composition as evaluated by multiple regression derived from the O2PLS model.

Variable	Explained variable: BMI Explained var. = 27.2% (25.7%)		Explained variable: abdomen Explained var. = 28.3% (26.9%)		Explained variable: hip Explained var. = 20.3% (19.2%)		Explained variable: waist Explained var. = 31.4% (29.9%)		Explained variable: bone [%] Explained var. = 22.9% (21.8%)		Explained variable: muscle [%] Explained var. = 20.5% (19.2%)		Explained variable: fat [%] Explained var. = 27.2% (25.3%)	
	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI
DHT	−0.124**	−2.19	−0.127**	−2.23	−0.115**	−2.17	−0.126**	−2.17	0.113**	2.13	0.125**	2.64	−0.139**	−2.89
T	−0.140**	−3.38	−0.141**	−3.79	−0.133**	−5.75	−0.136**	−3.52	0.137**	4.12	0.155**	4.22	−0.189**	−3.79
Prog17	−0.155**	−5.29	−0.162**	−5.05	−0.140**	−5.49	−0.164**	−4.52	0.132**	6.61	0.142**	5.73	−0.139**	−2.78
DHEA	−0.106**	−3.84	−0.121**	−4.25	−0.086**	−3.46	−0.131**	−4.21	0.062**	2.64	0.055*	1.40	0.005	0.09
A2	−0.053*	−1.40	−0.062**	−1.66	−0.042*	−1.08	−0.068**	−2.00	0.027	0.76	0.022	0.51	0.015	0.27
FSH	0.043*	1.07	0.054*	1.48	0.031	0.73	0.060**	1.67	−0.015	−0.45	−0.007	−0.23	−0.038	−0.93
SHBG	−0.123**	−2.41	−0.114**	−2.55	−0.127**	−3.19	−0.102**	−2.19	0.147**	2.57	0.177**	3.45	−0.261**	−6.60
Preg17	−0.099**	−3.02	−0.111**	−3.01	−0.083**	−3.85	−0.118**	−3.14	0.064**	1.88	0.061**	1.61	−0.018	−0.26

Abbreviations: as in Table 1.
* $p < 0.05$.
** $p < 0.01$.

Table 3
Relationship between hormones and glucose metabolism as evaluated by multiple regression derived from the O2PLS model.

Variable	Explained variable: glOGTT0 Explained var. = 16.3% (14.8%)		Explained variable: cpep0 Explained var. = 16.8% (15%)		Explained variable: IRI0 Explained var. = 13.2% (12.2%)	
	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI
DHT	−0.046*	−1.20	−0.108**	−2.95	−0.078**	−2.66
T	−0.019	−0.42	−0.126**	−3.56	−0.105**	−2.20
Prog 17	−0.091*	−1.57	−0.129**	−4.44	−0.080**	−2.20
DHEA	−0.163**	−4.85	−0.074*	−1.22	−0.004	−0.07
A2	−0.095**	−2.86	−0.035*	−1.54	0.004	0.14
FSH	0.103**	3.73	0.025	0.61	−0.016	−0.39
SHBG	0.078*	1.28	−0.125**	−2.32	−0.140**	−2.55
Preg17	−0.131**	−2.47	−0.072**	−1.77	−0.015	−0.27

Abbreviation: as in Table 1
* $p < 0.05$.
** $p < 0.01$.

4. Discussion

The higher incidence of cardiovascular diseases in men than in women of reproductive age initially led to the assumption that testosterone is a risk factor regarding cardiovascular diseases. Yet this has not been proven. On the contrary, low (or in some cases high) testosterone levels are connected with visceral obesity, metabolic syndrome, diabetes mellitus and cardiovascular diseases. Testosterone supplementation did not bring uniform results [24]. The so-called physiological window of testosterone has been described where both lower and higher T levels have a negative impact on body composition and cardiovascular risk. However, it is generally accepted that low serum testosterone is associated with increased adiposity, an adverse metabolic risk profile, atherosclerosis and cardiovascular risk [25–27], which only partially can be corrected by the administration of exogenous testosterone to hypotestosteronemic men. This has been confirmed also by the present study, which ascertained positive correlation of both androgens, T and DHT, with bone mass, muscle, HDL-cholesterol and negative correlation with anthropometric parameters of obesity, LDL-cholesterol, total cholesterol, triglycerides, fasting glycemia, insulin and C-peptide. This is valid under normal physiological condition without any intervention in the natural levels of testosterone and dihydrotestosterone.

Several experimental models focus on DHT influence on cardiovascular diseases risk factors. Experiments on animals point to the effect of the DHT level on reduction of cardiovascular risk [28,29]. Experiments with cell lines provide proof of the effect of the high DHT level inhibiting the growth of vessel smooth muscular cells in cell culture; this inhibition is dose dependant [30]. Exogenous DHT delivery to human macrophage cell culture is proatherogenic [31]. However, the dose of DHT used in the experiments was ten times higher than a physiologic level in plasma with men, which confirms the negative effect of high DHT levels that is dose dependant.

Yanes et al. [32] monitored DHT effect on aldosterone production in cell culture and proved that supraphysiologic androgen levels can, according to the authors, contribute to the development of cardiovascular diseases.

The DHT effect on adipose tissue was examined by several animal models. Two large genetic adipose tissue analyses of gonadectomized male mice after DHT substitution proved that several genes for glycolysis and lipogenesis are regulated by DHT [17,33]. The results of Bolduc et al. [17] suggest that chronic androgen treatment may help to improve metabolic profile by regulating various critical pathways involved in adipose tissue physiology. In addition, several genes associated with a healthier metabolic profile, such as adiponectin and CD36 antigen, were up-regulated by 21 days of DHT treatment. The experiments on mice of Movérare-Skrtic et al. [19] showed that DHT treatment resulted in obesity, associated with reduced energy expenditure and fat oxidation. In contrast, DHT did not affect food consumption or locomotor activity. Furthermore, DHT treatment resulted in increased high-density lipoprotein-cholesterol and triglyceride levels associated with markedly decreased 7alpha-hydroxylase gene expression, indicating decreased bile acid production.

Both testosterone and DHT block the transformation of pluripotent cell/into adipose cell [16].

Some studies have proved a different DHT metabolism in adipose tissue in obese and lean patients. Differences in DHT levels and metabolism in visceral fat of obese men have been found. DHT levels were higher in visceral fat than in subcutaneous fat of obese men [34]. In comparison with lean men in obese men a greater DHT

Table 4
Relationship between hormones and lipid markers as evaluated by multiple regression derived from the O2PLS model.

Variable	Explained variable: TG Explained var. = 12.8% (10.9%)		Explained variable: Chol Explained var. = 9.1% (7.6%)		Explained variable: HDL Explained var. = 10.4% (8.2%)		Explained variable: LDL Explained var. = 8.6% (7.6%)	
	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI	Regression coefficient	Regression coefficient/95% CI
DHT	−0.077**	−2.52	−0.084**	−2.46	−0.087**	−2.18	−0.088**	−1.91
T	−0.112**	−2.10	−0.108**	−2.36	−0.098**	−4.24	−0.097**	−5.67
Prog17	−0.071**	−1.61	−0.091**	−2.94	−0.108**	−4.05	−0.112**	−3.87
DHEA	0.027	0.39	−0.021	−0.33	−0.073*	−1.52	−0.083**	−1.84
A2	0.024	0.58	−0.005	−0.15	−0.036*	−1.34	−0.043*	−1.53
FSH	−0.039	−0.79	−0.006	−0.13	0.030	0.80	0.037	0.98
SHBG	−0.170**	−2.73	−0.133**	−1.99	−0.087**	−1.83	−0.079*	−1.40
Preg17	0.009	0.14	−0.029	−0.51	−0.069**	−1.79	−0.076**	−2.26

Abbreviation: as in Table 1
* $p < 0.05$.
** $p < 0.01$.

Table 5
The relationships between steroids, related substances (matrix Y) and anthropometric and metabolic parameters (matrix Y) as evaluated by multivariant regression analysis.

	Variable	Predictive component 1 Explained variability = 14.5% (13.5%)				Variable	Predictive component 1 Explained variability = 14.5% (13.5%)		
		Parameter[a]	Parameter/95% CI[b]	R^c			Parameter[a]	Parameter/95% CI[b]	R^c
X	DHT	0.367	3.70	0.602**	**Y**	c10	−0.124	−0.84	−0.283
	T	0.391	3.83	0.641**		c11	−0.115	−0.62	−0.195
	Prog17	0.450	7.05	0.737**		c12	−0.135	−1.01	−0.371*
	DHEA	0.395	4.47	0.643**		c13	−0.172	−1.32	−0.504*
	A2	0.255	4.39	0.419**		c14	−0.144	−0.79	−0.379
	FSH	−0.218	−2.13	−0.356**		BPS	−0.086	−0.50	−0.254
	SHBG	0.294	1.66	0.481**		BPD	−0.101	−0.55	−0.326
	Preg17	0.410	3.75	0.672**		glOGTT0	−0.100	−0.50	−0.327
Y	Age	−0.128	−0.71	−0.475		glOGTT60	−0.095	−0.29	−0.196
	BMI [kg/m^2]	−0.185	−1.54	−0.523*		glOGTT90	−0.134	−0.46	−0.322
	Abdomen	−0.194	−1.35	−0.533*		glOGTT120	−0.125	−0.45	−0.275
	Hip	−0.169	−1.02	−0.452*		glOGTT150	−0.109	−0.41	−0.267
	Waist	−0.194	−1.29	−0.559*		cpep0	−0.157	−0.99	−0.394
	Bone [kg]	−0.058	−0.33	−0.150		cpep60	−0.101	−0.52	−0.220
	Bone [%]	0.160	2.03	0.458**		cpep90	−0.117	−0.50	−0.268
	Muscle [kg]	−0.077	−0.38	−0.234		cpep120	−0.135	−0.59	−0.286
	Muscle [%]	0.174	1.14	0.449*		cpep150	−0.145	−0.61	−0.354
	Fat [kg]	−0.191	−1.75	−0.483**		cpep180	−0.155	−0.65	−0.344
	Fat [%]	−0.177	−1.22	−0.433*		IRI0	−0.135	−0.80	−0.362
	Weight	−0.175	−1.16	−0.486*		IRI60	−0.092	−0.46	−0.199
	c1	−0.190	−2.22	−0.547**		IRI90	−0.113	−0.56	−0.234
	c2	−0.179	−2.15	−0.437**		IRI120	−0.130	−0.59	−0.238
	c3	−0.180	−2.02	−0.456**		IRI150	−0.133	−0.73	−0.301
	c4	−0.151	−0.98	−0.434		IRI180	−0.127	−0.90	−0.306
	c5	−0.183	−1.33	−0.476*		TG	−0.132	−0.91	−0.349
	c6	−0.167	−1.12	−0.448*		Chol	−0.097	−0.57	−0.286
	c7	−0.141	−0.99	−0.313		HDL	0.110	0.72	0.311
	c8	−0.191	−1.58	−0.533*		LDL	−0.091	−0.66	−0.268
	c9	−0.144	−0.65	−0.363		Glykop	−0.058	−0.61	−0.204

[a] Component loadings for the predictive components expressed as regression coefficients.
[b] Confidence interval.
[c] Component loadings for the predictive components expressed as correlation coefficients of individual variables with the predictive components.
* $p < 0.05$.
** $p < 0.01$.
Values in parentheses represent explained variability after cross-validation procedure
Statistical evaluation shows that most of the variability shared between Y and X are explained by the 1st predictive component (14.5% of the total variability). The second component explains only 1.5% of the total variability and can be hardly interpreted. The 1st principal component shows that androgens and their precursors are negatively correlated with parameters of metabolic syndrome.
Abbreviations: DHT, dihydrotestosterone; T, testosterone; Prog, progesterone; A2, androstenedione; DHEA, dehydroepiandrosterone; Prog17, 17-hydroxyprogesterone; Preg17, 17-hydroxypregnenolone; c1–c14, skin folds; BPS, blood pressure systolic; BPD, blood pressure diastolic; glORTT0–180, glucose at oral tolerance test (ORTT) at 0–180 min; cpep0–cpep180, C-peptide at ORTT at 0–180 min; IRI0–IRI180, imunoreactive insulin; TG, triglycerides; Chol, total cholesterol; Glykop, glycated proteins.

degradation in omental fat has been observed [35]. It is the DHT metabolite androstan-3α,17β-diol-17-glucoronide that correlated positively not only with the amount of fat, but also with the central fat distribution, intrahepatic fat, risk type of lipid spectrum and insulin resistance [18].

Some hypotheses presume that the change of androgen ratio in favor of DHT can occur, along with the effect on obesity development. In our study we monitored DHT/T ratio with slender and obese men. This ratio stayed constant. Both androgens have the same affect, both with slender and obese men. No ratio change has been detected.

In the present study no essential differences between the association of testosterone and dihydrotestosterone in respect to body composition and anthropometric data, lipid spectrum and glucose regulation parameters could be discovered. Notwithstanding, it is possible that under the manipulated condition with either blocked DHT formation or DHT or testosterone administration a specific effect on fat formation or localization of the deposition could be detected.

5. Conclusion

Comparing hormone levels with anthropometric data during our study, we did not prove any differences in the effects of aromatizable and non-aromatizable steroids. Both steroids correlate so closely with each other with regards to anthropometric characteristics that we can entertain the possibility of a substitution of one for another concerning the effect on body composition. That means that the physiologic DHT levels are equivalent to testosterone in their effect on body composition and that both steroids can be substituted. However, this does not apply to other effects of these two steroids, such as their role in intrauterine evolution or their influence on skin adnexa, or to the situation when the levels of testosterone or dihydrotestosterone are manipulated by administration of the hormones or their modulators.

Testosterone has beneficial effects on body composition and glycaemic control in hypogonadal man. There is consistent evidence from randomized trials that testosterone therapy alters body composition in a metabolic favourable manner, but changes are modest and have not consistently translated in insulin resistance and improvements in glucose metabolism [24,36,37]. There were attempts to induce in practice substitution of hypogonadism by transdermal dihydrotestosterone treatment [38–40], which found application also in misuse in anabolic doping [41]. However, in light of present results it seems that dihydrotestosterone brings no advantage in comparison with testosterone as far to the beneficial effects on metabolic parameters and body composition concerns when the physiological levels of the androgens are maintained.

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