Journal of Steroid Biochemistry & Molecular Biology 132 (2012) 105–111

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

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

H. Pospíšilová [a], M. Vaňková [a], M. Hill [a], M. Meloun [b], B. Bendlová [a], M. Dušková [a,*], L. Stárka [a]

[a] Institute of Endocrinology, Prague, Czech Republic
[b] Department of Analytical Chemistry, University of Pardubice, Czech Republic

ARTICLE INFO

Article history:
Received 16 November 2011
Received in revised form 21 February 2012
Accepted 26 February 2012

Keywords:
Dihydrotestosterone
Testosterone
Fat deposition
Anthropometric parameters
Insulin resistance

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.

Crown Copyright © 2012 Published by Elsevier Ltd. All rights reserved.

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

* Corresponding author at: Institute of Endocrinology, Národní 8, 11694 Prague, Czech Republic.
E-mail addresses: hpospisilova@endo.cz (H. Pospíšilová), mvankova@endo.cz (M. Vaňková), mhill@endo.cz (M. Hill), milan.meloun@upce.cz (M. Meloun), bbendlova@endo.cz (B. Bendlová), mduskova@endo.cz (M. Dušková), lstarka@endo.cz (L. Stárka).

0960-0760/$ – see front matter. Crown Copyright © 2012 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2012.02.005

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-carboxymethyloxim-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/m²		BMI > 25 kg/m²	
	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.

Acknowledgment

The study was supported by IGA MZ CR grants NS/9831-4 and GAUK 367511.

References

[1] M. Kaufman, L. Pinsky, M. Trifo, R. Lumberoso, N. Sabaghian, et al., Kinetic evidence for a unique testosterone-receptor complex in 5alpha-reductase deficiency of its formation, J. Steroid Biochem. Mol. Biol. 45 (1993) 467–476.
[2] M. Dušková, H. Pospíšilová, The role of non-aromatizable testosterone metabolite in metabolic pathways, Physiol. Res. 60 (2011) 253–261.
[3] H.A. Feldman, C. Longcope, C.A. Derby, C.B. Johannes, A.B. Araujo, A.D. Coviello, W.J. Bremner, J.B. McKinlay, 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. 87 (2) (2002) 589–598.
[4] A. Gray, H.A. Feldman, J.B. McKinlay, C. Longcope, Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study, J. Clin. Endocrinol. Metab. 73 (5) (1991) 1016–1025.
[5] L. Stárka, M. Dusková, M. Hill, Dihydrotestosterone and testosterone throughout the life span of Czech men, Neuro. Endocrinol. Lett. 29 (2) (2008) 201–204.
[6] L. Stárka, H. Pospísilová, M. Hill, Free testosterone and free dihydrotestosterone throughout the life span of men, J. Steroid Biochem. Mol. Biol. 116 (1–2) (2009) 118–120.
[7] G. Aumueller, W. Eicheler, H. Renneberg, K. Adermann, P. Vilja, W.G. Forssmann, Immunocytochemical evidence for differential subcellular localization of 5 alpha-reductase isoenzymes in human tissues, Acta Anat. 156 (1996) 241–252.
[8] B.R. Oh, S.J. Kim, J.D. Moon, H.N. Kim, Y.H. Kwon, S.B. Ryu, Y.I. Park, Association of benign prostatic hyperplasia with male pattern baldness, Urology 51 (1998) 744–748.
[9] W. Chen, C.C. Yang, G.Y. Chen, M.C. Wu, H.M. Sheu, T.S. Tzai, Patients with a large prostate show a higher prevalence of androgenetic alopecia, Arch. Dermatol. Res. 296 (6) (2004) 245–249.
[10] W. Hawk, R.A. Breslow, B.I. Graubard, Male pattern baldness and clinical prostate cancer in the epidemiologic follow-up of the first National Health and Nutrition Examination survey, Cancer Epidemiol. Biomarkers Prev. 9 (2000) 523–527.
[11] P.A. Lotufo, C.U. Chae, U.A. Ajani, C.H. Hennekens, J.E. Manson, Male pattern baldness and coronary heart disease. The Physicians' Health Study, Arch. Int. Med. 160 (2000) 165–171.
[12] V. Matilainen, P. Koskela, S. Keinanen-Kiukaanniemi, Early anderogenetic alopecia as a marker of insulin resistance, Lancet 356 (2000) 1165–1166.
[13] L. Stárka, M. Dušková, I. Čermáková, J. Vrbíková, M. Hill, Premature androgenic alopecia and insulin resistance. Male equivalent of polycystic ovary syndrome? Endocr. Regul. 39 (2005) 127–131.
[14] J. Imperato-McGinley, L. Guerrero, T. Gautier, R.E. Peterson, Steroid 5α-reductase deficiency in man: an inherited form of male pseudohermaphroditism, Science 186 (1974) 1213–1215.
[15] T.L. Nielsen, C. Hagen, K. Wraae, K. Brixen, P.H. Petersen, E. Haug, R. Larsen, M. Andersen, Visceral, subcutaneous adipose tissue assessed by magnetic resonance imaging in relation to circulating androgens, sex hormone-binding globulin, and luteinizing hormone in young men, J. Clin. Endocrinol. Metab. 92 (7) (2007) 2696–2705.
[16] R. Singh, J.N. Artaza, W.E. Taylor, N.F. Gonzalez-Cadavid, S. Bhasin, Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway, Endocrinology 144 (11) (2003) 5081–5088.
[17] C. Bolduc, M. Yoshioka, J. St-Amand, Transcriptoric characterization of long term dihydrotestosterone effects in adipose tissue, Obesity 15 (5) (2007) 1107–1132.
[18] L. Vandenput, D. Mellstrom, M. Lorentzon, C. Swanson, M.K. Karlsson, J. Brandberg, L. Lonn, E. Orwoll, U. Smith, F. Labrie, O. Ljunggren, A. Tivesten, C. Ohlsson, Androgens and glucuronidated androgen metabolites are associated with metabolic risk factors in men, J. Clin. Endocrinol. Metab. 92 (11) (2007) 4130–4137.
[19] S. Movérare-Skrtic, K. Venken, N. Andersson, M.K. Lindberg, J. Svensson, C. Swanson, D. Vanderschueren, J. Oscarsson, J.A. Gustafsson, C. Ohlsson, Dihydrotestosterone treatment results in obesity and altered lipid metabolism in orchidectomized mice, Obesity 14 (2006) 662–672.
[20] P. Bláha, ANTROPO-ein Programm für automatische Bearbeitung anthropologischer Daten, Wiss. Zeitsch. Humboldt-Univ. Berlin 5 (1991) 153–156.
[21] R. Hampl, Z. Putz, L. Stárka, Radioimunological determination of dihydrotestosterone and its importance for laboratory diagnostic, Biochem. Clin. Bohemoslov. 19 (1990) 157–163.
[22] J. Trygg, S. Wold, Orthogonal projections to latent structure, J. Chemometrics 16 (2002) 119–128.
[23] J. Trygg, E. Holme, T. Lundstedt, Chemometrics in metabonomics, J. Proteome Res. 6 (2) (2007) 469–479.
[24] K. Blouin, A. Boivin, A. Tchernof, Androgens and body fat distribution, J. Steroid. Biochem. Mol. Biol. 108 (3–5) (2008) 272–280.
[25] S.T. Page, J.K. Amory, F. DuBois Bowman, D. Bradley, D. Anawalt, M. Matsumoto, W.J. Brenner, J.L. Tenover, Exogenous testosterone (T) alone or with finasteride increases physical performance, grip strength, and lean body mass in older men with low serum T, J. Clin. Endocrinol. Metab. 90 (2005) 1502–1510.
[26] S. Basaria, A.D. Cobiello, T.G. Travison, T.W. Storer, W.E. Farwell, A.M. Jette, et al., Adverse effects associated with testosterone administration, N. Engl. J. Med. 363 (2) (2010) 109–122.
[27] C. Ohlsson, E. Barret-Connor, S. Bhasin, E. Orwoll, F. Labrie, M.K. Larlsson, O. Ljunggren, L. Vandenput, D. Mellstrom, A. Tiveste, High serum testosterone is associated with reduced risk of cardiovascular events in elderly men. The MrOS (Osteoporotic fractures in men) study in Sweden, J. Am. Coll. Cardiol. 58 (16) (2011) 1674–1681.
[28] S. Li, X. Li, J. Li, X. Deng, Y. Li, Y. Cong, Experimental arterial thrombosis regulated by androgen and its receptor via modulation of platelet activation, Thromb. Res. 121 (1) (2007) 127–134.
[29] Y. Qiu, T. Yanase, H. Hu, T. Tanaka, Y. Nishi, M. Liu, K. Sueishi, T. Sawamura, H. Nawata, Dihydrotestosterone suppresses foam cell formation and attenuates atherosclerosis development, Endocrinology 151 (7) (2010) 3307–3316.
[30] D. Somjen, F. Kohen, B. Gayer, E. Knoll, A. Many, N. Stern, Dihydrotestosterone and estradiol-17beta mutually neutralize their inhibitory effects on human vascular smooth muscle cell growth in vitro, J. Steroid. Biochem. Mol. Biol. 113 (3–5) (2009) 171–176.
[31] M.K. Ng, C.M. Quinn, J.A. McCrohon, S. Nakhla, W. Jessup, D.J. Handelsman, D.S. Celermajer, A.K. Death, Androgens up-regulate atherosclerosis-related genes in macrophages from males but not females: molecular insights into gender differences in atherosclerosis, J. Am. Coll. Cardiol. 42 (7) (2003) 1306–1313.
[32] L.L. Yanes, D.G. Romero, Dihydrotestosterone stimulates aldosterone secretion by H295R human adrenocortical cells, Mol. Cell. Endocrinol. 303 (1–2) (2009) 50–56.
[33] C. Bolduc, M. Larose, M. Yoshioka, P. Ye, P. Belleau, C. Labrie, J. Morissette, V. Raymond, F. Labrie, J. St-Amand, Effects of dihydrotestosterone on adipose tissue measured by serial analysis of gene expression, J. Mol. Endocrinol. 33 (2) (2004) 429–444.
[34] C. Bélanger, F.S. Hould, S. Lebel, S. Biron, G. Brochu, A. Tchernof, Omental and subcutaneous adipose tissue steroid levels in obese men, Steroids 71 (8) (2006) 674–682.
[35] K. Blouin, C. Richard, G. Brochu, F.S. Hould, S. Lebel, S. Marceau, S. Biron, V. Luu, A. Tchernof, Androgen inactivation and steroid-converting enzyme expression in abdominal adipose tissue in men, J. Endocrinol. 191 (3) (2006) 637–647.
[36] M. Grossmann, Low testosterone in men with type 2 diabetes: significance and treatment, J. Clin. Endocrinol. Metab. 98 (6) (2011) 2341–2353.
[37] P. Dandona, S. Dhindsa, Update: hypogonadotropic hypogonadism in type 2 diabetes and obesity, J. Clin. Endocrinol. Metab. 96 (9) (2011) 4078–4088.
[38] L.P. Ly, M. Jimenez, T.N. Zhuang, D.S. Celermajer, A.J. Conway, et al., A double-blind, placebo-controlled, randomized trial of transdermal dihydrotestosterone gel on muscular strength, mobility, and quality of life in older men with partial androgen deficiency, J. Clin. Endocrinol. Metab. 86 (2001) 4078–4088.
[39] P. Kunelius, O. Lukkarinen, M.L. Hannuksela, O. Itkonen, J.S. Tapanainen, The effects of transdermal dihydrotestosterone in the aging male: a prospective. randomized, double-blind study, J. Clin. Endocrinol. Metab. 87 (2002) 1467–1472.
[40] M.Y. Roth, S.T. Page, A role for dihydrotestosterone treatment in older men? Asian J. Androl. 13 (2011) 199–200.
[41] A. Fabregat, O.J. Pozo, P. Van Renterghem, P. Van Eenoo, J. Marcos, J. Segura, R. Ventura, Detection of dihydrotestosterone gel, oral dehydroepiandrosterone, and testosterone gel misuse through the quantification of testosterone metabolites released after alkaline treatment, Drug Test Anal. 3 (11–12) (2011) 828–883.