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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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Resources

AKERSTEDT T, PALMBLAD J, DE LA TORRE B, MARANA R, GILLBERG M: Adrenocortical and gonadal steroids during sleep deprivation. *Sleep* **3(1):** 23-30, 1980.

AMORY JK, ANAWALT BD, MATSUMOTO AM, PAGE ST, BREMNER WJ, WANG C, SWERDLOFF RS, CLARK RV: The effect of 5alpha-reductase inhibition with dutasteride and finasteride on bone mineral density, serum lipoproteins, hemoglobin, prostate specific antigen and sexual function in healthy young men. *J Urol* **179(6):** 2333-2338, 2008.

AUMUELLER G, EICHELER W, RENNEBERG H, ADERMANN K, VILJA P, FORSSMANN WG: Immunocytochemical evidence for differential subcellular localization of 5 alpha-reductase isoenzymes in human tissues. *Acta Anat* **156:** 241–252, 1996.

BÉLANGER C, HOULD FS, LEBEL S, BIRON S, BROCHU G, TCHERNOF A: Omental and subcutaneous adipose tissue steroid levels in obese men. *Steroids* **71(8):** 674-682, 2006.

BLOUIN K, BOIVIN A, TCHERNOF A: Androgens and body fat distribution. *J Steroid Biochem Mol Biol* **108(3-5):** 272-280, 2008.

BLOUIN K, RICHARD C, BROCHU G, HOULD FS, LEBEL S, MARCEAU S, BIRON S, LUU-THE V, TCHERNOF A: Androgen inactivation and steroid-converting enzyme expression in abdominal adipose tissue in men. *J Endocrinol* **191(3):** 637-647, 2006.

BOLDUC C, LAROSE M, YOSHIOKA M, YE P, BELLEAU P, LABRIE C, MORISSETTE J, RAYMOND V, LABRIE F, ST-AMAND J: Effects of dihydrotestosterone on adipose tissue measured by serial analysis of gene expression. *J Mol Endocrinol* **33(2):** 429-444, 2004.

BOLDUC C, YOSHIOKA M, ST-AMAND J: Transcriptoric characterization of long term dihydrotestosterone effects in adipose tissue. *Obesity* **15(5):** 1107-1132, 2007.

BRAMBILLA DJ, MATSUMOTO AM, ARAUJO AB, MCKINLAY JB: The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. *J Clin Endocrinol Metab* **94(3):** 907-913, 2009.

BRUCHOVSKY N, WILSON JD: The conversion of testosterone to 5-alpha-androstan-17-beta-ol-3-one by rat prostate in vivo and in vitro. *J Biol Chem* **243(8):** 2012-2021, 1968.

CHEN W, YANG CC, CHEN GY, WU MC, SHEU HM, TZAI TS: Patients with a large prostate show a higher prevalence of androgenetic alopecia. *Arch Dermatol Res* **296(6):** 245-249, 2004.

DATI E, BARONCELLI GI, MORA S, RUSSO G, BALDINOTTI F, PARRINI D, ERBA P, SIMI P, BERTELLONI S: Body composition and metabolic profile in women with complete androgen insensitivity syndrome. *Sex Dev* **3(4):** 188-193, 2009.

DENTI L, PASOLINI G, CORTELLINI P, SANFELICI L, BENEDETTI R, CECCHETTI A, FERRETTI S, BRUSCHIERI L, ABLONDI F, VALENTI G: Changes in HDL-cholesterol and lipoprotein Lp(a) after 6-month treatment with finasteride in males affected by benign prostatic hyperplasia (BPH). *Atherosclerosis* **152(1):** 159-166, 2000.

DOGRAMACI AC, BALCI DD, BALCI A, KARAZINCIR S, SAVAS N, TOPALOGLU C, YALCIN F: Is androgenetic alopecia a risk for atherosclerosis? *J Eur Acad Dermatol Venereol* **23(6):** 673-677, 2009.

DORFMAN RI, HAMILTON JB: Urinary excretion of androgenic substances after intramuscular and oral administration of testosterone propionate to humans. *J Clin Invest* **18(1):** 67-71, 1939.

DUŠKOVÁ M, ČERMÁKOVÁ I, HILL M, VAŇKOVÁ M, ŠAMALÍKOVÁ P, STÁRKA L: What may be the markers of the male equivalent of polycystic ovary syndrome? *Physiological Research* **53:** 287-294, 2004.

DUŠKOVÁ M, HILL M, STÁRKA L: Changes of metabolic profile in men treated for androgenetic alopecia with 1 mg finasteride. *Endocr Regul* **44(1):** 3-8, 2010.

FELDMAN HA, LONGCOPE C, DERBY CA, JOHANNES CB, ARAUJO AB, COVIELLO AD, BREMNER WJ, MCKINLAY JB: 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):** 589-598, 2002.

FINASTERIDE MALE PATTERN HAIR LOSS STUDY GROUP: Long-term (5-year) multinational experience with finasteride 1 mg in the treatment of men with androgenetic alopecia. *Eur J Dermatol* **12:** 38-49, 2002.

FORD ES, FREEDMAN DS, BYERS T: Baldness and ischemic heart disease in a national sample of men. *Amer J Epidemiol* **143:** 651-657, 1996.

GILLES GG, SEVERI G, SINCLAIR R , ENGLISH DR, MCCREDIE MR, JOHNSON W, BOYLE P, HOPPER JL: Androgenetic alopecia and prostate cancer: findings from an Australian case-control study. *Cancer Epidemiol Biomarkers Prev* **11(6):** 549, 2002.

GONZALEZ-GONZALEZ JG, MANCILLAS-ADAME LG, FERNANDEZ-REYES M, GOMEZ-FLORES M, LAVALLE-GONZALEZ FJ, OCAMPO-CANDIANI J, VILLARREAL-PEREZ JZ: Androgenetic alopecia and insulin resistance in young men. *Clin Endocrinol* **71(4):** 494-499, 2009.

GONZÁLEZ-SANTOS MR, GAJÁ-RODRÍGUEZ OV, ALONSO-URIARTE R, SOJO-ARANDA I, CORTÉS-GALLEGOS V: Sleep deprivation and adaptive hormonal responses of healthy men. *Arch Androl* **22(3):** 203-207, 1989.

GORMLEY GJ, STONER E, RITTMASTER RS, GREGG H, THOMPSON DL, LASSETER KC, VLASSES PH, STEIN EA: Effects of finasteride (MK-906), a 5 alpha-reductase inhibitor, on circulating androgens in male volunteers. *J Clin Endocrinol Metab* **70(4):** 136-141, 1990.

GRAY A, FELDMAN HA, MCKINLAY JB, LONGCOPE C: Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. *J Clin Endocrinol Metab* **73(5):** 1016-1025, 1991.

GUPTA V, BHASIN S, GUO W, SINGH R, MIKI R, CHAUHAN P, CHOONG K, TCHKONIA T, LEBRASSEUR NK, FLANAGAN JN, HAMILTON JA, VIERECK JC, NARULA NS, KIRKLAND JL, JASUJA R: Effects of dihydrotestosterone on differentiation and proliferation of human mesenchymal stem cells and preadipocytes. *Mol Cell Endocrinol* **296(1-2):** 32-40, 2008.

HAWK W, BRESLOW RA, GRAUBARD BI: Male pattern baldness and clinical prostate cancer in the epidemiologic follow-up of the first National Health and Nutrition Examination survey. *Cancer Epidemiol Biomerkers Prev* **9:** 523-527, 2000.

HAWKINS VN, FOSTER-SCHUBERT K, CHUBAK J, SORENSEN B, ULRICH CM, STANCYZK FZ, PLYMATE S, STANFORD J, WHITE E, POTTER JD, MCTIERNAN A: Effect of exercise on serum sex hormones in men: a 12-month randomized clinical trial. *Med Sci Sports Exerc* **40(2):** 223-233, 2008.

HERRERA CR, D´AGOSTINO RB, GERTSMAN BB, BOSCOO LA, BELANGER AJ: Baldness nad coronary heart disease rates in men from the Farmingham Study. *Am. J Epidemiol* **142:** 828-833, 1995.

HIRSSO P, RAJALA U, HILTUNEN L, JOKELAINEN J, KEINÄNEN-KIUKAANNIEMI S, NÄYHÄ S: Obesity and low-grade inflammation among young Finnish men with early-onset alopecia. *Dermatology* **214(2):** 125-129, 2007.

HSIEH CC, THANOS A, MITROPOULOS D, DELIVELIOTIS C, MANTZOROS CS, TRICHOPOULOS D: Risk factors for prostate cancer: a case-control study in Greece. *Int J Cancer* **80(5):** 699-703, 1999.

ILANGOVAN R, SITTADJODY S, BALAGANESH M, SIVAKUMAR R, RAVI SANKAR B, BALASUBRAMANIAN K, SRINIVASAN S, SUBRAMANIAN C, THOMPSON DM, QUEIMADO L, SRINIVASAN N: Dihydrotestosterone is a determinant of calcaneal bone mineral density in men. *J Steroid Biochem Mol Biol* **117(4-5):** 132-138, 2009.

IMPERATO-MCGINLEY J, GUERRERO L, GAUTIER T, PETERSON RE: Steroid 5α-reductase deficiency in man: an inherited form of male pseudohermaphroditism. *Science* **186:** 1213-1215, 1974.

KAUFMAN, KD: Androgens and alopecia. *Molecular and Cellular Endocrinology* **198(1):** 89-95, 2002.

LESKO SM, ROSENBERG L, SHAPIRO S: A case-control study of baldness in relation to myocardial infarction in men. *J Amer Med Assoc* **269:** 998-1003, 1993.

LI S, LI X, LI J, DENG X, LI Y, CONG Y: Experimental arterial thrombosis regulated by androgen and its receptor via modulation of platelet activation. *Thromb Res* **121(1):** 127-134, 2007.

LITMAN HJ, BHASIN S, LINK CL, ARAUJO AB, MCKINLAY JB: Serum androgen levels in black, Hispanic, and white men. *J Clin Endocrinol Metab* **91(11):** 4326-4334, 2006.

LOTUFO PA, CHAE CU, AJANI UA , HENNEKENS CH, MANSON JE: Male pattern baldness and coronary heart disease. The Physicians´Health Study. *Arch Intern Med* **160:** 165-171, 2000.

MAIER U: Hormone profile in the aging male. *Sien Med Wochenschr* **151 (18-20):** 422-425, 2001.

MATILAINEN V, KOSKELA P, KEINANEN-KIUKAANNIEMI S: Early anderogenetic alopecia as a marker of insulin resistance. *Lancet* **356:** 1165- 1166, 2000.

MOVERARE-SKRTIC S, VENKEN K, ANDERSSON N, LINDBERG MK, SVENSSON J, SWANSON C, VANDERSCHUEREN D, OSCARSSON J, GUSTAFSSON JA, OHLSSON

C: Dihydrotestosterone treatment results in obesity and altered lipid metabolism in orchidectomized mice. *Obesity* **16:** 662-672, 2006.

NG MK, QUINN CM, MCCROHON JA, NAKHLA S, JESSUP W, HANDELSMAN DJ, CELERMAJER DS, DEATH AK: 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):** 1306-1313, 2003.

NIELSEN TL, HAGEN C, WRAAE K, BRIXEN K, PETERSEN PH, HAUG E, LARSEN R, ANDERSEN M: Visceral and 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):** 2696-2705, 2007.

OH BR, KIM SJ, MOON JD, KIM HN, KWON YH, RYU SB, PARK YI: Association of benign prostatic hyperplasia with male pattern baldness. *Urology* **51:** 744-748, 1998.

OPSTAD PK: The hypothalamo-pituitary regulation of androgen secretion in young men after prolonged physical stress combined with energy and sleep deprivation. *Acta Endocrinol (Copenh)* **127(3):** 231-236, 1992.

ORWOLL ES, NIELSON CM, LABRIE F, BARRETT-CONNOR E, CAULEY JA, CUMMINGS SR, ENSRUD K, KARLSSON M, LAU E, LEUNG PC, LUNGGREN O, MELLSTRÖM D, PATRICK AL, STEFANICK ML, NAKAMURA K, YOSHIMURA N, ZMUDA J, VANDENPUT L, OHLSSON C: for the Osteoporotic Fractures in Men (MrOS) Research Group: Evidence for Geographical and Racial Variation in Serum Sex Steroid Levels in Older Men. *J Clin Endocrinol Metab* **28:** 2010 .

PIRKE KM, DOERR P : Age related changes in free plasma testosterone, dihydrotestosterone and oestradiol. *Acta Endocrinol (Copenh)* **80(1):** 171-178, 1975.

POTTER LK, ZAGER MG, BARTON HA: Mathematical model for the androgenic regulation of the prostate in intact and castrated adult male rats. *Am J Physiol Endocrinol Metab* **291(5):** 952-964. 2006.

QIU Y, YANASE T, HU H, TANAKA T, NISHI Y, LIU M, SUEISHI K, SAWAMURA T, NAWATA H: Dihydrotestosterone suppresses foam cell formation and attenuates atherosclerosis development. *Endocrinology* **151(7):** 3307-3316, 2010.

SASMAZ S, SENOL M, OZCAN A, DOGAN G, TUNCER C, AKYOL O, SENER S: The risk of coronary heart disease in men with androgenetic alopecia. *J Eur Acad Dermatol Venereol* **12(2):** 123-125, 1999.

SATO T, MATSUMOTO T, YAMADA T, WATANABE T, KAWANO H, KATO S: Late onset of obesity in male androgen receptor-deficient (AR KO) mice. *Biochem Biophys Res Commun* **300(1):** 167-171, 2003.

SCATÀ MC, NAPOLITANO F, CASU S, CARTA A, DE MATTEIS G, SIGNORELLI F, ANNICCHIARICO G, CATILLO G, MOIOLI B: Ovine acyl CoA:diacylglycerol acyltransferase 1- molecular characterization, polymorphisms and association with milk traits. *Anim Genet* **40(5):** 737-742, 2009.

SHAHAR E, HEISS G, ROSAMOND WD, SZKLO M: Baldness and myocardial infarction in men: the atherosclerosis risk in communities study. *Am J Epidemiol* **167(6):** 676-683, 2008.

SIGNORELLI SS, BARRESI V, MUSSO N, ANZALDI M, CROCE E, FIORE V, CONDORELLI DF: Polymorphisms of steroid 5-alpha-reductase type I (SRD5A1) gene are associated to peripheral arterial disease. *J Endocrinol Invest* **31(12):** 1092-1097, 2008.

SINGH R, ARTAZA JN, TAYLOR WE, GONZALEZ-CADAVID NF, BHASIN S: Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. *Endokrinology* **144(11):** 5081-5088, 2003.

SOMJEN D, KOHEN F, GAYER B, KNOLL E, MANY A, STERN N: 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):** 171-176, 2009.

STÁRKA L, POSPÍSILOVÁ H, HILL M: Free testosterone and free dihydrotestosterone throughout the life span of men. J Steroid Biochem Mol Biol 116(1-2): 118-120, 2009.

STÁRKA L, DUSKOVÁ M, HILL M: Dihydrotestosterone and testosterone throughout the life span of Czech men. *Neuro Endocrinol Lett* **29(2):** 201-204, 2008.

TREVISAN M, FARINARO E, KROGH V, JOSSA F, GIUMETTI D, FUSCO G, PANICO S, MELLONE C, FRASCATORE S, SCOTTONI A, ET AL. Baldness and coronary heart disease risk factors. *J Clin Epidemiol* **46(10):** 1213-1218, 1993.

UEMURA M, TAMURA K, CHUNG S, HONMA S, OKUYAMA A, NAKAMURA Y, NAKAGAWA H: Novel 5 alpha-steroid reductase (SRD5A3, type-3) is overexpressed in hormone-refractory prostate cancer. *Cancer Sci* **99(1):** 81-86, 2008.

VANDENPUT L, MELLSTROM D, LORENTZON M, SWANSON C, KARLSSON MK, BRANDBERG J, LONN L, ORWOLL E, SMITH U, LABRIE F, LJUNGGREN O, TIVESTEN A, OHLSSON C: Androgens and glucoronidated androgen metabolites are associated with metabolic risk factors in men. *J Clin Endocrinol Metab* **92(11):** 4130-4137, 2007.

VAN DER MERWE J, BROOKS NE, MYBURGH KH: Three weeks of creatine monohydrate supplementation affects dihydrotestosterone to testosterone ratio in college-aged rugby players. *Clin J Sport Med* **19(5):** 399-404, 2009.

YANES LL, ROMERO DG: Dihydrotestosterone stimulates aldosterone secretion by H295R human adrenocortical cells. *Mol Cell Endocrinol* **303(1-2):** 50-56, 2009.