

Role of Endogenous Androgen Against Insulin Resistance and Atherosclerosis in Men with Type 2 Diabetes

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Abstract: Age-related decline in serum testosterone and dehydroepiandrosterone sulfate concentrations occur in men. Low concentrations of these endogenous androgens have been linked with insulin resistance, which is an important upstream driver for metabolic abnormalities such as hyperglycemia, hypertension, or hyperlipidemia, and increased cardiovascular risk. Moreover, men with diabetes have significantly less circulating androgen than nondiabetic men. Here, we summarize how androgen affects insulin resistance and atherosclerosis in men with type 2 diabetes. Low serum concentrations of endogenous androgens are associated with visceral fat accumulation. Androgen deprivation by castration to treat prostate cancer increases insulin resistance, while testosterone administration in type 2 diabetic men with androgen deficiency improves glucose homeostasis and decreases visceral fat, in addition to alleviating symptoms of androgen deficiency including erectile dysfunction. Androgen correlates inversely with severity of atherosclerosis and has beneficial effects upon vascular reactivity, inflammatory cytokine, adhesion molecules, insulin resistance, serum lipids, and hemostatic factors. Because men with type 2 diabetes have relative hypogonadism, testosterone supplementation could decrease both insulin resistance and atherosclerosis.

Keywords: Testosterone, Dehydroepiandrosterone, Insulin resistance, Atherosclerosis, Type 2 diabetes, Men.

INTRODUCTION

Serum testosterone and dehydroepiandrosterone sulfate (DHEA-S) concentrations decline in older men. Serum androgen concentrations are low during childhood, increasing at puberty until adult concentrations are reached. Beginning at approximately 25 years of age, serum testosterone concentrations then steadily decline by a mean of 1.0% per year. Serum DHEA-S concentrations decline to an even greater extent, by a mean of 2.3% per year [1].

Overt hypogonadism alters body composition and affects mood, cognitive function, sexual function, bone mineral density, and several factors important for cardiovascular disease [2]. Decreased concentrations of testosterone also have been implicated in insulin resistance, which is an important upstream driver for metabolic abnormalities such as hyperglycemia, hypertension, or hyperlipidemia [3,4]. In addition, low concentrations of testosterone are associated with increased risk of cardiovascular disease (CVD) in men [5]. Testosterone replacement in men with symptomatic androgen deficiency is indicated to improve quality of life, maintain lean body mass, preserve bone density, and protect against insulin resistance and atherosclerosis [6,7].

Men with diabetes have significantly lower plasma concentrations of free and total testosterone, as well as DHEA-S, than nondiabetic men [8,9]. CVD is the leading cause of mortality and morbidity in patients with diabetes, who have 2 to 5 times the risk of CVD of the general population, partly because of decreased androgens. Here we summarize how androgen has been found to influence insulin resistance and atherosclerosis in men with type 2 diabetes.

RELATIONSHIPS BETWEEN SERUM TESTOSTERONE CONCENTRATIONS AND INSULIN RESISTANCE

We previously described six patients whose glycemic control was worsened by castration for treatment of prostate cancer, despite increased endogenous insulin secretion [10]. This finding suggested that decreased concentrations of testosterone might contribute importantly to insulin resistance.

Observational Studies

Low testosterone concentrations are expected in men with obesity because of increased conversion of testosterone to estrogen in adipose tissue by the enzyme aromatase, and also because of inhibition of the hypothalamic-pituitary-gonadal axis by leptin [11]. On the other hand, low serum testosterone concentrations exacerbate obesity since sufficient testosterone acts at specific receptors to produce

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lipolytic effects in adipocytes [12]. As suggested by the above observations in therapeutic castration, low concentrations of free and total testosterone in men are associated with hyperinsulinemia [3,4]. Compared to men with normal concentrations of total testosterone, men with low concentrations have significantly higher body mass index (BMI), waist/hip ratio, systolic blood pressure, fasting and postprandial plasma glucose concentrations, and fasting serum insulin and total cholesterol concentrations [3]. Results from the Massachusetts Male Aging Study suggest that low concentrations of testosterone may contribute to development of insulin resistance and subsequent type 2 diabetes [13].

Insulin resistance is central to the metabolic syndrome, which has received increasing attention in the past few years as a concurrence of CVD risk factors including abdominal obesity, impaired glucose tolerance, dyslipidemia, and hypertension [14]. Patients with type 2 diabetes mellitus often show clustering of risk factors, which puts them at particularly high risk for CVD. Laaksonen *et al.* reported that independently of BMI, low serum testosterone concentrations were associated with not only individual components of the metabolic syndrome but also the metabolic syndrome itself [15]. Seidell *et al.* reported that serum free testosterone was associated negatively with visceral fat area [16]. Low serum testosterone concentrations, on the other hand, predict an increase in visceral fat and development of metabolic syndrome in middle-aged men [17,18]. Patients with Klinefelter's syndrome, who have hypergonadotropic hypogonadism, have higher incidence of diabetes mellitus because of insulin resistance [19].

Male patients with absence of estrogen reflecting aromatase deficiency show delayed skeletal maturation, osteopenia, and abnormal glucose and lipid metabolism [20]. Alternatively, estrogen receptor mutations can result in an estrogen resistance syndrome associated with increased fasting glucose concentrations and insulin resistance [21]. Physiologic actions of testosterone in men thus appear to be mediated in part by estrogen receptors following conversion to estrogen by site-specific aromatases in target tissues.

Interventional Studies

Clinical Studies

Treatment with testosterone was shown to increase lean body mass and decrease adipose mass by decreasing proteolysis and protein oxidation while increasing lipolysis and fat oxidation [22]. The role of testosterone in modulating the somatotrophic axis and enhancing growth hormone (GH) secretion is well established [23]. Whether changes in lipolysis and fat oxidation brought about by testosterone therapy are caused by a testosterone effect alone or by a mechanism also involving GH remains unresolved. Testosterone also has been shown to decrease adipose tissue mass by increasing responsiveness to catecholamine-induced lipolysis through up-regulation of β -adrenergic receptor density and postreceptor effects [24].

Young men with high testosterone secretion have low visceral fat, while aging men with low testosterone have abdominal obesity. Testosterone treatment in the latter was followed by a reduction in visceral fat mass according to

computed tomography [25]. Magnetic resonance imaging demonstrated that androgen therapy decreased visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (SAT), and the VAT/SAT ratio [26]. These effects were limited largely to men with low pretreatment testosterone concentrations.

Effects of androgen treatment on insulin sensitivity may be mediated by a change in body composition and by inhibition of lipoprotein lipase activity, resulting in reduced triglyceride uptake and accelerated triglyceride release from abdominal adipose tissue [27]. Reduction in adipose tissue also may decrease circulating free fatty acids, resulting in improvement in insulin sensitivity. Testosterone replacement given to men with symptomatic androgen deficiency resulted in reductions of serum tumor necrosis factor (TNF)- α and interleukin (IL)-1 β compared with placebo, as well as increase in IL-10 [28]. Most studies of effects of androgen replacement on cholesterol profiles show modest reduction of total cholesterol, LDL cholesterol, and HDL cholesterol, according to a recent meta-analysis [29].

Effects of testosterone on insulin sensitivity remain controversial; some studies have suggested that physiologic testosterone replacement improves insulin sensitivity in middle-aged men with low testosterone [6,7]. In contrast, administration of supraphysiologic doses of testosterone to castrated male rats [30] and of anabolic steroid to power lifters [31] has been shown to induce insulin resistance. We believe that both subphysiologic and supraphysiologic testosterone concentrations might adversely affect insulin sensitivity.

Oral testosterone treatment of type 2 diabetic men with androgen deficiency improves glucose homeostasis and body composition (decrease in visceral fat), and improves symptoms of androgen deficiency including erectile dysfunction [32].

Maffei *et al.* reported that in a man with a mutation of the aromatase gene, estrogen treatment decreased extent of acanthosis nigricans, insulin resistance, and steatohepatitis, coupled with better glycemic control and disappearance of two carotid plaques [33].

Deprivation

We have previously described six patients in whom glycemic control was worsened, in spite of increased endogenous insulin secretion, by castration for treatment of prostate cancer [10]. This finding suggested that decreased concentrations of testosterone might play an important role in insulin resistance. Inaba *et al.* similarly reported two patients who showed marked hyperglycemia after androgen-deprivation therapy for prostate cancer [34], but showed better glycemic control when given an insulin sensitizer, thiazolidinedione.

Smith *et al.* reported that gonadotropin releasing hormone (GnRH) agonists increased weight and percentage fat body mass while decreasing percentage lean body mass and muscle size in men with prostate cancer [35]. The increased adiposity resulted primarily from accumulation of subcutaneous rather than intraabdominal adipose tissue. In a few human studies, elderly men with hypogonadism induced

with GnRH agonists developed significant increases in serum TNF- α and IL-6 [36].

Animal Studies

Castrated male rats have decreased insulin sensitivity that is improved by low-dose testosterone administration [30]. Both excessive and deficient testosterone appear to result in insulin resistance in the male rat. Castration of male mice was reported to increase the endogenous TNF- α production elicited by administration of bacterial endotoxin; this response was suppressed by subsequent testosterone replacement [37].

Male aromatase-knockout mice appear to develop glucose intolerance and insulin resistance. Treatment of such mice with estrogen improved glucose metabolism [38]. Alternatively, male estrogen receptor-knockout mice result in marked increase in white adipose tissue, insulin resistance, impaired glucose tolerance, and altered energy metabolism [39].

RELATIONSHIPS BETWEEN SERUM DEHYDRO-EPIANDROSTERONE SULFATE CONCENTRATION AND INSULIN RESISTANCE

DHEA and its sulfate ester DHEA-S together represent the most abundant adrenally produced steroid, a weak androgen contributing to androgenicity mainly after peripheral conversion to more potent androgens, testosterone and dihydrotestosterone. DHEA-S, which is converted to the active form DHEA in a linear manner, represents the circulating hormonal pool of DHEA and is a good marker for DHEA availability.

Increasing evidence suggests that DHEA may have a number of clinical uses. After passage of the US Dietary Supplement Health and Education Act of 1994, DHEA became widely available, finding a growing popular market as a “fountain of youth” or “super hormone” expected to prevent cancer, heart disease, and type 2 diabetes; to delay aging; to prevent or slow progression of Alzheimer’s disease; to boost libido; and to alleviate autoimmune disease [40].

Men with diabetes have significantly lower plasma concentrations of DHEA than nondiabetic men [9,41]. Decreased DHEA and DHEA-S concentrations in patients with type 2 diabetes are associated with low activity of 17, 20-lyase, an adrenal steroidogenic enzyme [41]. Increased risk for CVD in diabetic men could be mediated in part by low concentrations of DHEA.

Observational Studies

Low DHEA concentrations are associated with development of central obesity [42], while decreased serum concentrations of DHEA may contribute to insulin resistance [43]. Epidemiologic studies linking low serum DHEA concentrations with conditions characterized by insulin resistance and hyperinsulinemia offer additional supportive evidence [44].

Conversely, insulin may reduce serum concentrations of DHEA and DHEA-S by both inhibiting their production and promoting their clearance [45]. Experimentally induced hyperinsulinemia lowers serum adrenal androgens [46].

Administration of metformin is reported to increase serum DHEA-S secondarily to alleviation of hyperinsulinemia [47]. One factor responsible for the epidemiologically demonstrated association between hyperinsulinemia and atherosclerosis may be insulin-mediated suppression of serum DHEA and DHEA-S concentrations.

Interventional Studies

Clinical Studies

Villareal and Holloszy reported a significant increase in an insulin sensitivity index in response to DHEA in the elderly [48]. DHEA treatment can reduce body weight and serum TNF- α [49], and also may increase insulin sensitivity [43] and slow progression of type 2 diabetes [43], although some studies have reported conflicting findings concerning effects of DHEA supplementation on insulin sensitivity [50,51].

Serum estradiol concentrations and free testosterone concentrations increase after DHEA administration, although not significantly [52]. Whether DHEA exerts its effects mainly by direct action or by peripheral bioconversion to androgens and estrogens remains an unsettled matter.

Animal Studies

DHEA, a precursor of both estradiol and testosterone, has been shown to have an antiobesity effect in several species. Administration of DHEA has been reported to reduce obesity [53], hyperlipidemia, severity of diabetes [54], and atherosclerosis [55] in obese rodents. Genetically diabetic (db/db) mice develop obesity and glucose intolerance associated with insulin resistance, and subsequently exhibit β cell necrosis and islet atrophy. Supplementing their diet with DHEA prevented these pathologic changes and effected rapid remission of hyperglycemia, β cell dysfunction, and insulin resistance [56]. Adiponectin gene expression in adipose tissue and serum adiponectin levels were elevated in DHEA-treated rats by activation of peroxisome proliferators-activated receptor γ (PPAR γ) [57].

RELATIONSHIPS BETWEEN SERUM TESTOSTERONE CONCENTRATION AND ATHEROSCLEROSIS

We recently demonstrated a negative association between serum testosterone concentration and carotid atherosclerosis, determined by ultrasonographically evaluated intima-media thickness (IMT) and plaque score, in men with type 2 diabetes [58]. In addition, IMT and plaque score were significantly greater in patients with lower concentrations of free testosterone (<10 pg/ml) than in patients with higher concentrations of free testosterone.

Observational Studies

Low testosterone concentrations in men have been found to be associated with increased risk of CVD [59]. Significant negative correlations have been detected between serum testosterone concentration and measures of both extent and severity of coronary artery disease in angiographic studies [60]. A recent study from Denmark determined a relationship between low concentrations of testosterone and acute ischemic stroke, with total testosterone concentration

showing inverse association with stroke severity, infarct size, and mortality [61]. However, other studies examining the association between low concentrations of testosterone and CVD mortality have been inconclusive. In disagreement with several reports suggesting that low concentrations of testosterone are associated with increased risk of CVD in men, some investigators have found no significant association between total testosterone concentration and prevalence of CVD [62]. Our own study found that serum testosterone concentrations did not differ significantly between patients with and without clinical CVD; however, serum free testosterone concentrations correlated negatively with ultrasonographically evaluated mean IMT and plaque score, which are early markers of atherosclerosis even prior to clinical manifestations. In the same study, the association of low free testosterone concentration with severity of carotid atherosclerosis was stronger than that observed for total testosterone concentration.

Testosterone exhibits a favorable effect upon vascular reactivity, inflammatory cytokine production, adhesion molecule expression, insulin resistance, serum lipid concentrations, and hemostatic factors [37], although data concerning the relationship between androgens and lipids are to some extent contradictory. Cross-sectional studies have demonstrated a positive correlation between HDL cholesterol and testosterone, but inconsistent relationships between testosterone and total cholesterol, LDL cholesterol, and triglycerides [63].

Men with diabetes have significantly lower plasma concentrations of free and total testosterone than nondiabetic men. Increased risk for CVD in diabetic men could be mediated partially through low concentrations of testosterone.

Interventional Studies

Clinical Studies

Short-term intracoronary testosterone administration at physiologic concentrations was found to induce coronary artery dilatation and increase coronary blood flow in men with established coronary artery disease [64]. In addition, short-term administration of intravenous testosterone improves exercise tolerance and reduces the angina threshold in men with coronary artery disease [65,66]. Testosterone treatment enhances endothelium-dependent as well as endothelium-independent vasodilatation [67]. In addition, testosterone attenuates expression of vascular cell adhesion molecule (VCAM)-1 in human umbilical vein endothelial cells (HUVEC), thus inhibiting adhesion of monocytes to endothelial cells [68]. The last of the above effects is mediated by endothelial cell aromatase, which converts testosterone to estradiol. Estrogen may aid in maintenance of vascular function in men. A direct effect of estrogen on vascular cells has been demonstrated [69]. A male patient with estrogen deficiency showed marked impairment of endothelium-dependent vasodilatation of the brachial artery [70], suggesting that low-dose estrogen might offer cardioprotection. Indeed, a low-dose estrogen regimen given to Finnish men with prostate cancer resulted in significant lowering of cardiovascular mortality [71]. In men, estrogens also may offer a degree of protection against CVD by

influencing the lipid profile [72]. On the other hand, induced hypogonadism to treat prostate cancer increases augmentation of central arterial pressure, suggesting large-artery stiffening [73].

Animal Studies

In an animal model, castration increased aortic atheroma formation while testosterone replacement ameliorated this effect [74]. Physiologic testosterone supplementation also has been shown to reduce atherosclerosis in orchidectomized LDL-receptor knockout mice fed a cholesterol-enriched diet [75]. Increased aortic lesion formation was reported in orchidectomized male mice, while a reduction in plaque development was demonstrated in orchidectomized mice receiving physiologic testosterone supplementation. This effect was not observed in animals treated simultaneously with testosterone and aromatase inhibitor, which suggests that conversion to estrogen is responsible for testosterone-mediated attenuation of atherosclerosis. Aortic nitric oxide release may be impaired in male estrogen-receptor knockout mouse [76]. In isolated coronary, femoral, and pulmonary arteries, testosterone showed a dose-dependent vasodilatory effect, caused by direct actions on vascular smooth muscle, *via* either potassium [77] or calcium channels [78].

RELATIONSHIPS BETWEEN SERUM DEHYDRO-EPIANDROSTERONE SULFATE CONCENTRATION AND ATHEROSCLEROSIS

Decreased serum DHEA concentrations may be associated with increased risk of CVD in men. We evaluated relationships between serum DHEA-S concentration and carotid atherosclerosis in men with type 2 diabetes. Serum DHEA-S concentrations were measured in 206 consecutive men with type 2 diabetes. Serum DHEA-S concentration was associated negatively with carotid atherosclerosis determined by ultrasonographically evaluated IMT and plaque score in men with type 2 diabetes [79].

Observational Studies

A few prospective clinical trials [80], and some cross-sectional studies [81] suggest that DHEA has a beneficial effect against development of atherosclerosis and/or its clinical manifestations in men. While DHEA is considered to have a protective effect against coronary artery disease and was found to inhibit atherosclerosis and plaque progression in an experimental model [82,83], some studies failed to find an association between decreased DHEA concentration and atherosclerosis [84,85].

Our study in men with type 2 diabetes demonstrated that serum DHEA-S concentrations were significantly lower in patients with than without CVD, in addition to finding a negative correlation of serum DHEA-S concentrations with ultrasonographically evaluated mean IMT and plaque score, which are early preclinical markers of atherosclerosis. Advanced age is one of the strongest predictors of coronary artery disease; a decline in DHEA concentration with age may partly explain age-related increases in risk of CVD.

Both light-to-moderate alcohol consumption and higher serum concentrations of DHEA are associated with reduced CVD mortality, raising the possibility of DHEA as a causal

intermediate in the relationship between alcohol consumption and CVD. We therefore investigated relationships between alcohol consumption, serum DHEA-S concentration, and carotid atherosclerosis as evaluated by carotid ultrasonography in 404 consecutive men with type 2 diabetes [86]. Serum DHEA-S concentrations also were higher in light-to-moderate drinkers than in nondrinkers. Carotid IMT and plaque score were lower in light-to-moderate drinkers than in nondrinkers. We concluded that higher serum DHEA-S concentrations in light-to-moderate drinkers may represent part of the link between light-to-moderate alcohol consumption and lower CVD mortality.

As, both elevated urinary albumin excretion and low serum concentrations of DHEA are associated with increased CVD mortality [87], DHEA might be a causal intermediate linking urinary albumin excretion to CVD. Accordingly, we investigated relationships of urinary albumin excretion to serum DHEA-S concentration in 357 consecutive men with type 2 diabetes [88]. Serum DHEA-S concentration correlated inversely with log (urinary albumin excretion), which suggests that serum DHEA-S concentration may represent part of the link between elevated urinary albumin excretion and higher CVD mortality in male patients with type 2 diabetes.

Interventional Studies

Clinical Studies

DHEA may exert its antiatherogenic action by several mechanisms: amelioration of insulin resistance, inhibition of differentiation and proliferation of smooth muscle cells and fibroblasts [89]; a hypolipidemic effect [90]; decreases in platelet aggregation [91] and in plasminogen activator inhibitor [43]; and enhanced endothelial function [43] and vascular contractility [92]. DHEA also reduces atherogenic cytokines such as IL-6 [93] and TNF- α [49].

Low-dose DHEA supplementation (25 mg/day) was shown to improve vascular endothelial function. Flow-mediated dilatation of brachial artery after transient occlusion, expressed as a percent change from the baseline arterial diameter, increased with DHEA supplementation in men with hypercholesterolemia [43].

Because DHEA is metabolized enzymatically to both androgens and estrogens, whether DHEA exerts effects directly or only after conversion to androgens or to estrogens is not clear. However, DHEA has been shown to inhibit human vascular smooth muscle cell proliferation by mechanisms independent of either androgen or estrogen receptors [94].

Animal Studies

Experimental studies [82,83] suggest that DHEA could have a beneficial effect against development of atherosclerosis. Severe atherosclerosis was found in rabbits after aortic endothelial injury induced with a balloon catheter plus feeding a 2% cholesterol diet. However, rabbits additionally receiving DHEA showed almost a 50% reduction in plaque [83]. These beneficial actions were not attributable to differences in body weight gain, food intake, total plasma cholesterol, or distribution of cholesterol among VLDL, LDL, or HDL fractions. DHEA administration in a hyper-

cholesterolemic rabbit model of heterotopic cardiac transplantation significantly retarded progression of accelerated atherosclerosis in both the transplanted heart and the native heart [82].

CLINICAL IMPLICATIONS AND CONCLUSIONS

Because men with type 2 diabetes have relative hypogonadism [8,9], supplementation with androgen might reduce both insulin resistance and atherosclerosis. Evaluation of elderly men with physiologic testosterone deficiency are less clear-cut, and an appropriate biochemical cut-off below which replacement should be offered to such patients has not been clearly defined. Testosterone undecanoate and testosterone buciclate, which currently are undergoing evaluation for use in clinical practice, appear promising as longer-acting preparations providing a less variable testosterone concentration [63]. No current evidence suggests that androgen replacement in hypogonadal men causes an increase in prostate cancer, although studies addressing this issue have been small and retrospective. We suspect that both sub-physiologic and supraphysiologic testosterone concentrations are likely to adversely affect insulin sensitivity. Weight loss in obese men has been shown to increase serum testosterone [95]. Life-style changes could be a logical first-line treatment in many ventrally obese men with andropause. Weight loss is important in prevention of progressive metabolic decompensation and CVD associated with obesity and the metabolic syndrome. While a number of preparations are available for testosterone replacement, little is known about suitability or efficacy of individual preparations for protection from insulin resistance or atherosclerosis. Long-term trials of testosterone replacement therapy also are needed to assess both efficacy and safety.

REFERENCES

- [1] Blouin K, Despres J-P, Couillard C, *et al.* Contribution of age and declining androgen levels to features of the metabolic syndrome in men. *Metabolism* 2005; 54: 1034-1040.
- [2] Howell S, Shalet S. Testosterone deficiency and replacement. *Horm Res* 2001; 56: 86-92.
- [3] Simon D, Charles MA, Nahoul K, *et al.* Association between plasma total testosterone and cardiovascular risk factors in healthy adult men: The Telecom Study. *J Clin Endocrinol Metab* 1997; 82: 682-685.
- [4] Haffner SM, Karhapaa P, Mykkanen L, Laakso M. Insulin resistance, body fat distribution and sex hormones in men. *Diabetes* 1994; 43: 12-19.
- [5] English KM, Steeds R, Jones TH, Channer KS. Testosterone and coronary heart disease: is there a link? *Q J Med* 1997; 90: 787-791.
- [6] Bhasin S. Effects of testosterone administration on fat distribution, insulin sensitivity, and atherosclerosis progression. *Clin Infect Dis* 2003; 37: S142-S149.
- [7] Marin P, Holmang S, Jonsson L *et al.* The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. *Int J Obes* 1992; 16: 991-997.
- [8] Andersson B, Marin P, Lissner L, Vermeulen A, Bjorntorp P. Testosterone concentrations in women and men with NIDDM. *Diabetes Care* 1994; 17: 405-411.
- [9] Barrett-Connor E. Lower endogenous androgen levels and dyslipidaemia in men with NIDDM. *Ann Intern Med* 1992; 117: 807-811.
- [10] Fukui M, Koyama M, Nakagawa Y, Itoh Y, Nakamura N, Kondo M. Castration and diabetes. *Diabetes Care* 2000; 23: 1032-1033.
- [11] Jockenhovel F, Blum WF, Vogel E, *et al.* Testosterone substitution normalizes elevated serum leptin levels in hypogonadal men. *J Clin Endocrinol Metab* 1997; 82: 2510-2513.
- [12] Xu X, De Pergola G, Eriksson PS, *et al.* Postreceptor events involved in the up-regulation of beta-adrenergic receptor mediated

- lipolysis by testosterone in rat white adipocytes. *Endocrinology* 1993; 132: 1651-1657.
- [13] Stellato RK, Feldman HA, Hamdy O, Horton ES, McKinlay JB. Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men. *Diabetes Care* 2000; 23: 490-494.
- [14] Alberti KG, Zimmet P, Shaw J, for the IDF Epidemiology Task Force Consensus Group. The metabolic syndrome – a new worldwide definition. *Lancet* 2005; 366: 1059-1062.
- [15] Laaksonen DE, Niskanen L, Punnonen K, *et al.* Sex hormone, inflammation and the metabolic syndrome: a population-based study. *Eur J Endocrinol* 2003; 149: 601-608.
- [16] Seidell JC, Bjorntorp P, Sjostrom L, Kvist H, Sannerstedt R. Visceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels. *Metabolism* 1990; 39: 897-901.
- [17] Laaksonen DE, Niskanen L, Punnonen K, *et al.* Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. *Diabetes Care* 2004; 27: 1036-1041.
- [18] Laaksonen DE, Niskanen L, Punnonen K, *et al.* The metabolic syndrome and smoking in relation to hypogonadism in middle-aged men: a prospective cohort study. *J Clin Endocrinol Metab* 2005; 90: 712-719.
- [19] Pei D, Sheu WHH, Jeng CY, Liao WK, Fuh MMT. Insulin resistance in patients with Klinefelter's syndrome and idiopathic gonadotropin deficiency. *J Formos Med Assoc* 1998; 97: 534-540.
- [20] Faustini-Fustini M, Rochira V, Carani C. Oestrogen deficiency in men: where are we today? *Eur J Endocrinol* 1999; 140: 111-129.
- [21] Smith EP, Boyd J, Frank GR, *et al.* Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med* 1994; 331: 1056-1061.
- [22] Arslanian S, Suprasongsin C. Testosterone treatment in adolescents with delayed puberty: changes in body composition, protein, fat, and glucose metabolism. *J Clin Endocrinol Metab* 1997; 82: 3213-3220.
- [23] Eakman GD, Dallas JS, Ponder SW, Keenan BS. The effects of testosterone and dihydrotestosterone on hypothalamic regulation of growth hormone secretion. *J Clin Endocrinol Metab* 1996; 81: 1217-1223.
- [24] Xu X, Depergola G, Eriksson PS, *et al.* Post-receptor events involved in the up-regulation of receptor mediated lipolysis by testosterone in rat white adipocytes. *Endocrinology* 1993; 132: 1651-1657.
- [25] Marin P, Holmang S, Jonsson L, *et al.* The effects of testosterone treatment on body composition and metabolism in middle aged obese men. *Int J Obes* 1992; 16: 991-997.
- [26] Schroeder ET, Zheng L, Ong MD, *et al.* Effects of androgen therapy on adipose tissue and metabolism in older men. *J Clin Endocrinol Metab* 2004; 89: 4863-4872.
- [27] Betancourt-Albrecht M, Cunningham GR. Hypogonadism and diabetes. *Int J Impot Res* 2003; 15: S14-S20.
- [28] Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH. The effect of testosterone on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *J Clin Endocrinol Metab* 2004; 89: 3313-3318.
- [29] Whitsel EA, Boyko EJ, Matsumoto AM, Anawalt BD, Siscovick DS. Intramuscular testosterone esters and plasma lipids in hypogonadal men: a meta-analysis. *Am J Med* 2001; 111: 261-269.
- [30] Holmang A, Bjorntorp P. The effects of testosterone on insulin sensitivity in male rats. *Acta Physiol Scand* 1992; 146: 505-510.
- [31] Cohen JC, Hickman R. Insulin resistance and diminished glucose tolerance in powerlifters ingesting anabolic steroids. *J Clin Endocrinol Metab* 1987; 64: 960-963.
- [32] Boyanov MA, Boneva Z, Christov VG. Testosterone supplementation in men with type 2 diabetes, visceral obesity and partial androgen deficiency. *Aging Male* 2003; 6: 1-7.
- [33] Maffei L, Murata Y, Rochira V, *et al.* Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment. *J Clin Endocrinol Metab* 2004; 89: 61-70.
- [34] Inaba M, Otani Y, Nishimura K, *et al.* Marked hyperglycemia after androgen-deprivation therapy for prostate cancer and usefulness of pioglitazone for its treatment. *Metabolism* 2005; 54: 55-59.
- [35] Smith MR, Finkelstein JS, McGovern FJ, *et al.* Changes in body composition during androgen deprivation therapy for prostate cancer. *J Clin Endocrinol Metab* 2002; 87: 599-603.
- [36] Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *J Clin Endocrinol Metab* 2004; 89: 3313-3318.
- [37] Jones RD, Nettleship JE, Kapoor D, Jones HT, Channer KS. Testosterone and atherosclerosis in aging men: purported association and clinical implications. *Am J Cardiovasc Drugs* 2005; 5: 141-154.
- [38] Takeda K, Toda K, Saibara T, *et al.* Progressive development of insulin resistance phenotype in male mice with complete aromatase (CYP19) deficiency. *J Endocrinol* 2003; 176: 237-246.
- [39] Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-knockout mice. *Proc Natl Acad Sci USA* 2000; 97: 12729-12734.
- [40] Nawata H, Yanase T, Goto K, Okabe T, Ashida K. Mechanism of action of anti-aging DHEA-S and the replacement of DHEA-S. *Mech Ageing Dev* 2002; 123: 1101-1106.
- [41] Ueshiba H, Shimizu Y, Hiroi N, *et al.* Decreased steroidogenic enzyme 17, 20-lyase and increased 17-hydroxylase activities in type 2 diabetes mellitus. *Eur J Endocrinol* 2002; 146: 375-380.
- [42] Haffner SM, Valdez RA, Stern MP, Katz MS. Obesity, body fat distribution and sex hormones in men. *Int J Obes Relat Metab Disord* 1993; 17: 643-649.
- [43] Kawano H, Yasue H, Kitagawa A, *et al.* Dehydroepiandrosterone supplementation improves endothelial function and insulin sensitivity in men. *J Clin Endocrinol Metab* 2003; 88: 3190-3195.
- [44] Haffner SM, Valdez RA, Mykkanen L, Stern MP, Katz MS. Decreased testosterone and dehydroepiandrosterone sulfate concentrations are associated with increased insulin and glucose concentrations in nondiabetic men. *Metabolism* 1994; 43: 599-603.
- [45] Nestler JE, Clore JN, Blackard WG. Dehydroepiandrosterone: the "missing link" between hyperinsulinemia and atherosclerosis? *FASEB J* 1992; 6: 3073-3075.
- [46] Nestler JE, McClanahan MA, Clore JN, Blackard WG. Insulin inhibits adrenal 17, 20-lyase activity in man. *J Clin Endocrinol Metab* 1992; 74: 362-367.
- [47] Nestler JE, Beer NA, Jakubowicz DJ, Beer RM. Effects of a reduction in circulating insulin by metformin on serum dehydroepiandrosterone sulfate in nondiabetic men. *J Clin Endocrinol Metab* 1994; 78: 549-554.
- [48] Villareal DT, Holloszy JO. Effect of DHEA on abdominal fat and insulin action in elderly women and men. *JAMA* 2004; 292: 2243-2248.
- [49] Kimura M, Tanaka S, Yamada Y, Kiuchi Y, Yamakawa T, Sekihara H. Dehydroepiandrosterone decreases serum tumor necrosis factor- α and restores insulin sensitivity: independent effect from secondary weight reduction in genetically obese Zucker fatty rats. *Endocrinology* 1998; 139: 3249-3253.
- [50] Usiskin KS, Butterworth S, Clore JN, *et al.* Lack of effect of dehydroepiandrosterone in obese men. *Int J Obes* 1990; 14: 457-463.
- [51] Nestler JE, Barlascini CO, Clore JN, Blackard WG. Dehydroepiandrosterone reduces serum low density lipoprotein levels and body fat but does not alter insulin sensitivity in normal men. *J Clin Endocrinol Metab* 1988; 66: 57-61.
- [52] Arlt W, Haas J, Callies F, *et al.* Biotransformation of oral dehydroepiandrosterone in elderly men: significant increase in circulating estrogens. *J Clin Endocrinol Metab* 1999; 84: 2170-2176.
- [53] Yen TT, Allan JA, Pearson DV, Acton JM, Greenberg MM. Prevention of obesity in Avy/a mice by dehydroepiandrosterone. *Lipids* 1977; 12: 409-413.
- [54] Coleman DL, Schwizer RW, Leithr EH. Effect of genetic background on the therapeutic effects of dehydroepiandrosterone (DHEA) in diabetes-obesity mutants and aged normal mice. *Diabetes* 1984; 33: 26-32.
- [55] Gordon GB, Bush DE, Weisman HF. Reduction of atherosclerosis by administration of dehydroepiandrosterone. *J Clin Invest* 1988; 82: 712-720.
- [56] Coleman DL, Leiter EH, Schwizer RW. Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. *Diabetes* 1982; 31: 830-833.
- [57] Karbowska J, Kochan Z. Effect of DHEA on endocrine functions of adipose tissue, the involvement of PPAR. *Biochem Pharmacol* 2005; 70: 249-257.

- [58] Fukui M, Kitagawa Y, Nakamura N, *et al.* Association between serum testosterone concentration and carotid atherosclerosis in men with type 2 diabetes. *Diabetes Care* 2003; 26: 1869-1873.
- [59] English KM, Steeds R, Jones TH, Channer KS. Testosterone and coronary heart disease: is there a link? *Q J Med* 1997; 90: 787-791.
- [60] Phillips GB, Pinkernell BH, Jing TY. The association of hypotestosteronaemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 1994; 14: 701-706.
- [61] Jeppeson LL, Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS, Winther K. Decreased testosterone in men with acute ischaemic stroke. *Arterioscler Thromb Vasc Biol* 1996; 16: 749-754.
- [62] Barrett-Connor E, Khaw KT. Endogenous sex hormones and cardiovascular disease in men. A prospective population based study. *Circulation* 1988; 78: 539-545.
- [63] Howell S, Shalet S. Testosterone deficiency and replacement. *Horm Res* 2001; 56: S86-S92.
- [64] Webb CM, McNeill JG, Hayward CS, de Zeigler D, Collins P. Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. *Circulation* 1999; 100: 1690-1696.
- [65] Webb CM, Adamson DL, de Zeigler D, Collins P. Effects of testosterone on myocardial ischemia in men with coronary artery disease. *Am J Cardiol* 1999; 83: 437-439.
- [66] Rosano GMC, Leonardo F, Pagnotta P, *et al.* Acute anti-ischaemic effect of testosterone in men with coronary artery disease. *Circulation* 1999; 99: 1666-1670.
- [67] Kang SM, Jang Y, Kim JY, *et al.* Effect of oral administration of testosterone on brachial arterial vasoreactivity in men with coronary artery disease. *Am J Cardiol* 2002; 89: 862-864.
- [68] Mukherjee TK, Dinh H, Chaudhuri G, Nathan L. Testosterone attenuates expression of vascular cell adhesion molecule-1 by conversion to estradiol by aromatase in endothelial cells: implications in atherosclerosis. *Proc Natl Acad Sci USA* 2002; 99: 4055-4060.
- [69] Sudhir K, Komesaroff P. Cardiovascular actions of estrogens in men. *J Clin Endocrinol Metab* 1999; 84: 3411-3415.
- [70] Sudhir K, Chou TM, Messina LM, *et al.* Endothelial dysfunction in a man with disruptive mutation in oestrogen-receptor gene. *Lancet* 1997; 349: 1146-1147.
- [71] Aro J. Cardiovascular and all-cause mortality in prostatic cancer patients treated with estrogens or orchiectomy as compared to the standard population. *Prostate* 1991; 18: 131-137.
- [72] Blum A, Cannon III RO. Effects of oestrogens and selective oestrogen receptor modulators on serum lipoproteins and vascular function. *Curr Opin Lipidol* 1998; 9: 575-586.
- [73] Smith JC, Bennett S, Evans LM, *et al.* The effects of induced hypogonadism on arterial stiffness, body composition, and metabolic parameters in males with prostate cancer. *J Clin Endocrinol Metab* 2001; 86: 4261-4267.
- [74] Alexandersen P, Haarbo J, Byrjalsen I, Lawaetz H, Christiansen C. Natural androgens inhibit male atherosclerosis. A study of castrated, cholesterol fed rabbits. *Circ Res* 1999; 84: 813-819.
- [75] Nathan L, Shi WB, Dinh H, *et al.* Testosterone inhibits early atherogenesis by conversion to estradiol: critical role of aromatase. *Proc Natl Acad Sci USA* 2001; 98: 3589-3593.
- [76] Rubanyi GM, Freay AD, Kauser K, *et al.* Vascular estrogen receptors and endothelium-derived nitric oxide production in the mouse aorta. Gender difference and effect of estrogen receptor gene disruption. *J Clin Invest* 1997; 99: 2429-2437.
- [77] Deenadayalu VP, White RE, Stallone JN, *et al.* Testosterone relaxes coronary arteries by opening the large-conductance, calcium-activated potassium channel. *Am J Physiol* 2001; 281: H1720-H1727.
- [78] English KM, Jones RD, Jones TH, *et al.* Testosterone acts as a coronary vasodilator by a calcium channel antagonist action. *J Endocrinol Invest* 2002; 25: 455-458.
- [79] Fukui M, Kitagawa Y, Nakamura N, *et al.* Serum dehydroepiandrosterone sulfate concentration and carotid atherosclerosis in men with type 2 diabetes. *Atherosclerosis* 2005; 181: 339-344.
- [80] Hak AE, Witteman JC, deJong FH, Geerlings MI, Hofman A, Pols HA. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *J Clin Endocrinol Metab* 2002; 87: 3632-3639.
- [81] Herrington DM, Gordon GB, Achuff SC, *et al.* Plasma dehydroepiandrosterone and dehydroepiandrosterone sulfate in patients undergoing diagnostic coronary angiography. *J Am Coll Cardiol* 1990; 16: 862-870.
- [82] Eich DM, Nestler JE, Johnson DE, *et al.* Inhibition of accelerated coronary atherosclerosis with dehydroepiandrosterone in the heterotopic rabbit model of cardiac transplantation. *Circulation* 1993; 87: 261-269.
- [83] Gordon GB, Bush DE, Weisman HF. Reduction of atherosclerosis by administration of dehydroepiandrosterone. A study in the hypercholesterolemic New Zealand white rabbit with aortic intimal injury. *J Clin Invest* 1988; 82: 712-720.
- [84] Haffner SM, Moss SE, Klein BE, Klein R. Sex hormones and DHEA-SO₄ in relation to ischemic heart disease mortality in diabetic subjects. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Diabetes Care* 1996; 19: 1045-1050.
- [85] Kiechl S, Willeit J, Bonora E, Schwarz S, Xu Q. No association between dehydroepiandrosterone sulfate and development of atherosclerosis in a prospective population study (Bruneck Study). *Arterioscler Thromb Vasc Biol* 2000; 20: 1094-1100.
- [86] Fukui M, Kitagawa Y, Nakamura N, Kadono M, Hasegawa G, Yoshikawa T. Association between alcohol consumption and serum dehydroepiandrosterone sulfate concentration in men with type 2 diabetes: a link to decreased cardiovascular risk. *Diabetic Med* 2005; 22: 1446-1450.
- [87] Dinneen SF, Gerstein HC. The association of microalbuminuria and mortality in non-insulin-dependent diabetes mellitus: a systematic overview of the literature. *Arch Intern Med* 1997; 157: 1413-1418.
- [88] Fukui M, Kitagawa Y, Nakamura N, Kadono M, Hasegawa G, Yoshikawa T. Association between urinary albumin excretion and serum dehydroepiandrosterone sulfate concentration in male patients with type 2 diabetes. *Diabetes Care* 2004; 27: 2893-2897.
- [89] Furutama D, Fukui R, Amakawa M, Ohsawa N. Inhibition of migration and proliferation of vascular smooth muscle cells by dehydroepiandrosterone sulfate. *Biochim Biophys Acta* 1998; 1406: 107-114.
- [90] Okamoto K. Relationship between dehydroepiandrosterone sulfate and serum lipid levels in Japanese men. *J Epidemiol* 1996; 6: 63-67.
- [91] Jesse RL, Loesser K, Eich DM, Qian YZ, Hess ML, Nestler JE. Dehydroepiandrosterone inhibits human platelet aggregation *in vitro* and *in vivo*. *Ann N Y Acad Sci* 1995; 774: 281-290.
- [92] Barbagallo M, Shan J, Pang PK, Resnick LM. Effects of dehydroepiandrosterone sulfate on cellular calcium responsiveness and vascular contractility. *Hypertension* 1995; 26: 1065-1069.
- [93] Straub RH, Konecna L, Hrach S, *et al.* Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man *in vitro*: possible link between endocrinosenescence and immunosenescence. *J Clin Endocrinol Metab* 1988; 83: 2012-2017.
- [94] Williams MR, Ling S, Dawood T, *et al.* Dehydroepiandrosterone inhibits human vascular smooth muscle cell proliferation independent of ARs and ERs. *J Clin Endocrinol Metab* 2002; 87: 176-181.
- [95] Niskanen L, Laaksonen DE, Punnonen K, Mustajoki P, Kaukua J, Rissanen A. Changes in sex hormone-binding globulin and testosterone during weight loss and weight maintenance in abdominally obese men with the metabolic syndrome. *Diabetes Obes Metab* 2004; 6: 208-215.