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ORIGINAL ARTICLE

Low testosterone levels are associated with coronary artery disease in male patients with angina

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Historically, high androgen levels have been linked with an increased risk for coronary artery disease (CAD). However, more recent data suggest that low androgen levels are associated with adverse cardiovascular risk factors, including an atherogenic lipid profile, obesity and insulin resistance. The aim of the present study was to evaluate the relationship between plasma sex hormone levels and presence and degree of CAD in patients undergoing coronary angiography and in matched controls. We evaluated 129 consecutive male patients (mean age 58 ± 4 years, range 43–72 years) referred for diagnostic coronary angiography because of symptoms suggestive of CAD, but without acute coronary syndromes or prior diagnosis of hypogonadism. Patients were matched with healthy volunteers. Out of 129 patients, 119 had proven CAD; in particular, 32 of them had one, 63 had two and 24 had three vessel disease, respectively. Patients had significantly lower levels of testosterone than controls (9.8 ± 6.5 and 13.5 ± 5.4 nmol/l, $P<0.01$) and higher levels of gonadotrophin (12.0 ± 1.5 vs 6.6 ± 1.9 IU/l and 7.9 ± 2.1 vs 4.4 ± 1.4, $P<0.01$ for follicle-stimulating hormone and luteinizing hormone, respectively). Also, both bioavailable testosterone and plasma oestradiol levels were lower in patients as compared to controls (0.84 ± 0.45 vs 1.19 ± 0.74 nmol/l, $P<0.01$ and 10.7 ± 1.4 vs 13.3 ± 3.5 pg/ml, $P<0.05$). Hormone levels were compared in cases with one, two or three vessel disease showing significant differences associated with increasing severity of coronary disease. An inverse relationship between the degree of CAD and plasma testosterone levels was found ($r=-0.52$, $P<0.01$). In conclusion, patients with CAD have lower testosterone and oestradiol levels than healthy controls. These changes are inversely correlated to the degree of CAD, suggesting that low plasma testosterone may be involved with the increased risk of CAD in men.
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Introduction

Historically, the link between plasma testosterone levels and increased risk of coronary artery disease (CAD) has been attributed, at least in part, to the unfavourable effect of the hormone on lipid metabolism and fibrinolysis.[1–4] The evidence that men have a greater incidence of CAD and myocardial infarction than women of similar age, together with the evidence that android fat distribution is associated with a greater incidence of CAD compared with gynoid distribution, helped to reinforce the belief that high plasma testosterone levels are associated with an increased risk of CAD.[5–9] This belief contrasts with the physiological effects of androgens on the cardiovascular system. In fact, it has been shown that in men testosterone correlates positively with the major stimulator of fibrinolysis, that is, tissue plasminogen activator activity, and inversely with plasminogen activator inhibitor activity and fibrinogen,[3] and that replacement of natural androgens inhibits atheroma formation in castrate male animals.[9] There is also evidence to suggest that low levels of androgens are associated with adverse cardiovascular risk factors, including an atherogenic lipid profile, obesity, insulin resis-

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tance and raised fibrinogen in humans.[10–12] In the past decade, it has been shown that testosterone has vasoactive properties improving both systemic and coronary blood flow.[13,14] Despite these evidences between low levels of androgens, risk factors for coronary heart disease and changes in coronary blood flow, a number of prospective epidemiological studies have failed to show a relationship between baseline testosterone levels and the subsequent development of clinically apparent CAD.[15–18] More recently, English *et al*.[15] have reported that men with proven CAD have lower androgen levels than patients with normal coronary arteriograms. The question rises as to whether patients with different degrees of CAD may have different sex hormone milieu than normal subjects.

The aim of the present study was to evaluate the relationship between plasma sex hormone levels and presence and degree of CAD in patients undergoing coronary angiography and in matched controls.

Methods

Study population
The study population included 138 consecutive male patients referred for diagnostic coronary angiography because of symptoms suggestive of CAD.

Patients with prior diagnosis of hypogonadism or hypopituitarism or taking drugs that might have affected sex hormone levels as those with high C-reactive protein levels (>5) suggestive of acute infection and/or inflammation were excluded from the study. One hundred and twenty-nine patients (mean age 58 ± 4 years, range 43–72 years), with proven stable CAD and no acute coronary syndromes, were included in the study. Patients were matched with healthy volunteers, recruited from the Hospital medical and non-medical staff, as control group. Patients and controls were excluded if they had major illness within the preceding 3 months or if they had any past history of hypogonadism. Subjects were excluded if they had either a previous cardiovascular event or a diagnosis of coronary or peripheral atherosclerosis and if they had symptoms suggestive of cardiac origin or cardiovascular risk $>20\%$ in 10 years as assessed by the risk charts of the European Society of Cardiology.[19] Patients and controls gave written informed consent to participate in this study that was approved by the local ethics committee.

Evaluations
Baseline data including age, personal and family history of risk factors for CAD were recorded. Blood samples (10 ml of whole blood) were obtained at admission in patients and at recruitment in controls after an overnight fasting at 0800 hours (±60 min), the day before cardiac catheterization in patients and on the day following a non-working day in controls. Whole blood was spun at 3500 r.p.m. for 9 min. The serum obtained was then stored at -80°C for a maximum length of time of 4 weeks. Body mass index (BMI) was calculated by the formula: weight (kg)/height (m).

Laboratory measurement
All samples obtained from the same subject were measured in the same assay. All determinations were performed in duplicate. Serum concentrations of total testosterone were measured by radioimmunoassay (RIA) using a commercial kit (Diagnostic System Laboratories, Webster, TX, USA). The intra- and inter-assay coefficients of the total testosterone assay were 7.5 and 11%, respectively, at the normal adult male range, which in our laboratory was 300–1000 ng/dl. Serum-free testosterone was measured by RIA of the dialysate after an overnight equilibrium dialysis using the same RIA reagents as in the total testosterone assay. The coefficient of variation for free testosterone recovery for increasing doses of total testosterone in the adult male ranged from 10 to 18%. The intra- and inter-assay precisions of free testosterone were 14 and 17%, respectively, at the normal adult male range, which in our laboratory was 200–700 pmol/l. Oestradiol was measured with chemiluminescence (provided by Architect Systems, Abbotts diagnostics, Germany), with a normal adult male range of 0–30 pg/ml; sex hormone-binding globulin levels were measured by immunoradiometric assay (Radim SpA, Pomezia, Italy) with an intra- and inter-assay CV below 6% at the normal adult range, which in our laboratory was 9–55 nmol/l; luteinizing hormone and follicle-stimulating hormone were measured by direct chemiluminescence (ADVIA Centaur, Bayer Co., Germany) with an intra- and inter-assay CV between 6 and 3% at the normal adult range, which in our laboratory was 1.5–9 and 2–11 IU/l, respectively.

Coronary angiography
All patients underwent cardiac catheterization and coronary angiography using the Judkins technique. The presence of CAD was assessed by the investigator performing the diagnostic procedure, whereas the degree of coronary artery stenosis was evaluated using the in-built quantitative coronary angiography software (Philips Integris 5000, Philips Medical Systems, Nederland BV). Significant coronary atherosclerosis was defined by the presence of a stenosis $>50\%$ in the left main or in the principal coronary arteries. Coronary artery score was obtained by multiplying the degree of coronary artery obstruc-

tion by the number of stenosis as reported elsewhere.[19]

Statistical analysis

Data are expressed as mean ± s.d. or percentage where appropriate. Statistical analysis was performed using the GBStat statistical package. The unpaired *t*-test or the χ^2 test was used to test statistical differences in continuous and categorial variables between groups. Comparisons between patients with one-, two- and three-vessel disease were made using one-way analysis of variance. Spearman's correlation test was performed to evaluate statistical correlation between plasma testosterone level and coronary artery score. Statistical significance was accepted when $P \leqslant 0.05$.

Results

Out of 129 patients who were eligible for the study and performed coronary angiography, nine patients were found to have completely normal coronary arteries, one patient had a myocardial bridge on the medial left anterior descending artery and 119 had proven CAD. In particular, 32 of them had one, 63 had two and 24 had three vessel disease, respectively. Baseline characteristics of the patients and controls are shown in Table 1. Patients had a greater incidence of coronary risk factors for CAD than controls but similar BMI. Mean differences in hormones levels in patients vs controls are summarized in Table 2. Patients and controls had similar plasma levels of total cholesterol.

Patients had mean lower levels of both testosterone and bioavailable testosterone levels than controls (Table 2). Plasma oestradiol levels were also significantly lower in the cases than in controls (10.7 ± 1.4 and 13.3 ± 3.5 pg/ml; $P<0.05$) (mean ± s.d.) without any difference in the oestradiol:testosterone ratio. Patients had significantly higher plasma gonadotropin levels than control group (Figure 1). Compared with patients with normal coronary arteriograms, patients with coronary atherosclerosis had significantly lower plasma testosterone and bioavailable testosterone levels (9.8 ± 6.5 vs 12.4 + 2.3 nmol/l, $P<0.05$ and 0.84 ± 0.45 vs 1.2 ± 0.52 nmol/l, $P<0.05$, respectively) and a similar oestradiol/testosterone ratio (3.9 ± 0.5 vs 3.8 ± 0.9; P = NS).

In a multivariate analysis, hormone levels were compared in cases with one (no. 32), two (no. 63) or three (no. 24) vessel disease, showing significant differences associated with increasing severity of coronary disease (Figure 2). An inverse relationship

Table 1 Baseline characteristics in patients and controls

	Patients (n = *129*)	*Control group* (n = *110*)
Mean age (years)	58 ± 4	58 + 4 years
Previous MI	56	—
Cholesterol (mg/dl)	247 ± 25	209 + 16
LDL-cholesterol (mg/dl)	156 ± 23	118 + 24
Diabetes	15	—
Cigarette smoking	48	42
BMI (kg/m^2)	26.2 ± 3.1	25.3 ± 5
Family history of CAD	69	37
Arterial hypertension	56	31
CAD	119	—

Abbreviations: BMI, body mass index; CAD, coronary artery disease; LDL, low-density lipoprotein; MI, myocardial infarction.

Table 2 Sex hormones and SHBG in patients and controls

	Patients (n = *129*)	*Control group* (n = *110*)
Testosterone (nmol/l)	9.8 ± 6.5	13.5 ± 5.4*
Oestradiol 17β (pg/ml)	10.7 ± 1.4	13.3 ± 3.5**
Oestradiol/testosterone ($\times 10^{-3}$)	3.9 ± 0.5	3.7 ± 1.4
SHBG (nmol/l)	45 ± 4.1	45 ± 6.6
Bioav T (nmol/l)	0.84 ± 0.45	1.19 ± 0.74*

SHBG, sex hormone binding globulin; T, testosterone.
*$P<0.01$.
**$P<0.05$.

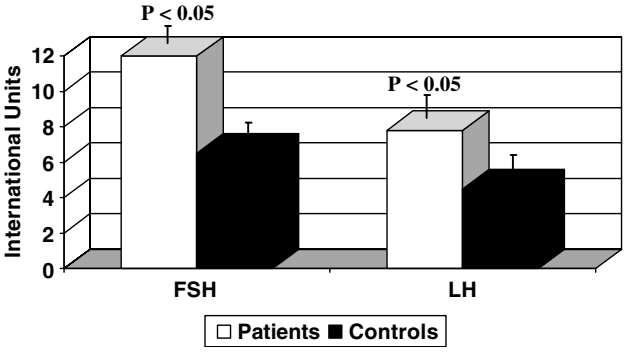

Figure 1 Comparison of gonadotropin (FSH and LH) in patients vs control group.

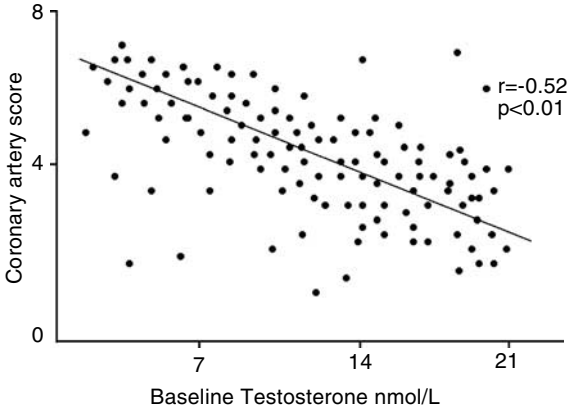

Figure 2 Relationship between testosterone (nmol/l) and coronary artery score in patients affected by CAD.

was found between plasma testosterone levels and coronary artery score ($r = -0.52$, $P < 0.01$). Following correction for age and BMI, levels of total, bioavailable and free testosterone, and free androgen index remained significantly lower in patients than in controls (data not shown).

Discussion

Our study shows that men with proven CAD have significantly lower serum levels of androgens compared to normal controls, and that these lower androgen levels are likely to be related to testicular dysregulation. Our findings are confirmatory in that the beneficial effects of testosterone on myocardial ischaemia have been already shown.[20–23] We have also found that among patients with CAD, those with lower androgen levels have a greater amount of coronary atherosclerosis, thereby suggesting a possible pathogenetic role of low testosterone levels in the development of CAD. Whether this effect is related to a direct effect of androgens on the progression of atherosclerosis or whether it is dependent on the effect of androgens on surrogate markers of atherosclerosis is still a matter of speculation. Previous data from observational studies lack to show a positive relationship between testosterone and CAD to demonstrate that high levels of this androgen may be a risk factor, but seem to suggest that patients with CAD may have lower testosterone levels.[10] On the other hand, clinical studies investigating the effect of testosterone administration exclude a possible causal relationship between testosterone exposure and development of CAD, and suggest a potential beneficial effects on myocardial ischemia.[10] Our group has shown that acute intravenous administration of testosterone, in patients with proven CAD, improves exercise-induced myocardial ischaemia.[4] In that study, although the effect seemed to be more evident in those patients with lower plasma level of the hormone, testosterone had an anti-ischaemic effect also in patients with normal plasma testosterone levels. Pugh *et al*.[24] have evaluated the effect of acute testosterone administration and have found that testosterone increases cardiac output acutely, apparently via reduction of left ventricular afterload.

The concept that chronic testosterone supplementation adversely affects the plasma lipoprotein profile and increase the risk of atherosclerotic heart disease is not supported by data gathered in patients in whom a physiological supplementation of testosterone was obtained.[25,26] Studies using supraphysiological doses of testosterone and non-aromatizable androgens in athletes or in female to male transexuals showed a decrease in plasma high-density lipoprotein (HDL)-c levels,[27–31] and physiological testosterone replacement in older men has been associated with no decrease in plasma HDL-c.[32–35] On the other hand, a recent study on transsexual, healthy, non-obese, young subjects does not show unequivocally that high-dose testosterone administration increases cardiovascular risk, as testosterone had a neutral effects on blood pressure and insulin sensitivity.[36] Furthermore, cross-sectional studies of middle-aged men have found a direct relationship between serum testosterone levels and plasma HDL-c concentrations and an inverse correlation between serum testosterone levels and visceral fat volume.[37] These data suggest that testosterone levels in the range that is mid-normal is related to an optimal cardiovascular risk profile at any age, and that testosterone concentration either above or below the physiological normal range may increase the risk of atherosclerotic heart disease.[37]

The inverse relationship between testosterone levels and coronary atherosclerosis found in our study suggests a possible protective role of the hormone on the progression of atherosclerosis. The data described here do not explain any testicular dysregulation but just an association with reduced testosterone levels if compared with the control group. Previous studies have investigated the effect of androgens on the development and progression of experimentally induced atherosclerosis in different animal models with diet- or injury-induced atherosclerosis.[38–47] Larsen *et al*.[44] investigated the effect of intramuscular testosterone enhantate in castrated male rabbits and found no difference in the cholesterol content of abdominal aorta lesion after 17 weeks. Alexandersen *et al*.[40] found in the same animal model that androgen replacement reduces total cholesterol and prevents the development of atherosclerosis, suggesting that testosterone might be atheroprotective, acting independently on both plasma lipid profile and cell proliferation. Adams *et al*.,[45] who evaluated the effect of testosterone administration in female ovariectomized monkeys, found that the experimentally induced male plasma androgen pattern results in an exacerbation of diet-induced progression of atherosclerosis.[45] These conflicting results from animal studies reflect the existence of many different mechanisms in the pathophysiology of atherosclerosis that could be influenced by androgens, the sexually dimorphic response to atherogenic triggers, as well as the gender-specific response to sex steroid.[10] Although it is difficult to translate from animals studies the potential effect of sex hormones in humans, the animal data suggest that low testosterone levels may favour the development of coronary atherosclerosis in men, whereas in women this latter is consistent with a state of relative hyperandrogenism.[47] On the other hand, it can be postulated that endothelial dysfunction of testicular vessels can be another, maybe early, manifestation of atherosclerosis. In such case, this ubiquitous manifestation of CAD may be responsible for testicular dysfunction and

androgen deficiency rather than being the result of them.

Levels of testosterone decline with age in healthy subjects, but data in coronary patients are limited. It is known that late-onset hypogonadism (LOH)[48] is associated with endothelial and erectile dysfunction (ED) and with several cardiovascular risk factors, including dyslipidaemia, adverse clotting profiles, obesity and insulin resistance, probably because hypogonadal men have a higher visceral fat mass than age-matched eugonadal control, all of them are often reversed by testosterone administration.[49–51] The occurrence of hypogonadism in our patients is consistent with the presence of LOH, associated with higher gonadotrophin levels. We could not search for a direct relationship between the use of lipid-lowering drugs, that is, statins, and the observed reduction of sex hormone levels, but it has been recently reported in a meta-analysis that testosterone replacement therapy may delay time to ischaemia and is associated with potentially beneficial reductions of total cholesterol, fat mass and fat-free mass.[52] It is therefore plausible to evaluate the potential benefits of androgen replacement therapy also in patients with CAD and lower testosterone levels.

A possible limitation of our study may be related to the selection of the control group. Subjects selected as controls in our study have several differences in baseline risk factors compared to patients with CAD. However, these are the differences that do exist between patients with CAD and the general population. Furthermore, our control group did not undergo cardiac catheterization for the exclusion of possible CAD, causing a possible grey zone of potential unknown CAD. The reason why we choose to use consecutive subjects without clinical evidence of cardiovascular disease instead of patients with normal coronary arteriograms as a control group is related to the fact that patients with normal coronary arteriograms undergoing coronary angiography often have a higher degree of cardiovascular risk than the general population, whereas the aim of our study was to address whether sex hormones are different in normal subjects and in patients with coronary atherosclerosis. Previous studies have shown, however, that patients with proven CAD have lower plasma testosterone levels compared to patients with symptoms suggestive of a possible cardiac origin but with normal coronary arteriograms.[15] Although limited in the sample size, similar findings were observed in our patients with normal coronary arteriograms that showed significantly lower plasma testosterone levels than patients with proven coronary atherosclerosis. Another possible limitation of the study is represented by the lack of data on the prevalence of ED in our cardiac patients. However, it is known that risk factors for ED (hypertension, diabetes, smoking and lipid abnormality) are also risk factors for CAD. Recent studies reported that ED is extremely common in men with chronic CAD (affecting approximately 75%), yet most cardiologists do not ask about it.[53] Based on how common ED is in patients with chronic stable CAD, we could speculate that most of patients included in our study might be considered as potentially affected by some degree of endothelial dysfunction of cavernous arteries hence affecting their overall sexual capacity.

In conclusion, our data confirm that a significant relationship exists between testosterone plasma level and the severity of coronary atherosclerosis, suggesting that low testosterone levels may be one of the causes rather than the consequence of cardiovascular disease in men. These data complement those reported in larger studies[54] and suggest the importance to evaluate the androgen status in patients with CAD and the eventual need of androgen replacement therapy.

References

1 Sorva R, Taskinen MR, Perheentupa J, Nikkila EA. Testosterone substitution increases the activity of lipoprotein lipase and hepatic lipase in hypogonadal males. *Atherosclerosis* 1988; **69**: 191–197.
2 Jones DB, Higgins B, Billet JS, Price WH, Edwards CR, Beastall GH *et al.* The effect of testosterone replacement on plasma lipids and apolipoproteins. *Eur J Clin Invest* 1989; **19**: 438–441.
3 Glueck CJ, Glueck HI, Stroop D, Speirs J, Hamer T, Tracy T. Endogenous testosterone, fibrinolysis, and coronary heart disease risk in hyperlipidemic men. *J Lab Clin Med* 1993; **122**: 412–420.
4 Rosano GMC, Leonardo F, Pagnotta P, Pelliccia F, Panina G, Cerquetani E *et al.* Acute anti-ischemic effect of testosterone in men with coronary artery disease. *Circulation* 1999; **99**: 1666–1670.
5 Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow up of the Framingham population. *Am J Cardiol* 1986; **111**: 383–390.
6 Freedman DS, Jacobsen SJ, Barboriak JJ, Sobocinski KA, Anderson AJ, Kissebah AH *et al.* Body fat distribution and male/female differences in lipids and lipoproteins. *Circulation* 1990; **81**: 1498–1506.
7 Seidell JC, Cigolini M, Charzewska J, Ellsinger BM, Bjorntorp P, Hautvast JG *et al.* fat distribution and gender differences in serum lipids in men and women from four European communities. *Atherosclerosis* 1991; **87**: 203–210.
8 Larsson B, Bengtsson C, Bjorntorp P, Lapidus L, Sjostrom L, Svardsudd K *et al.* Is abdominal body fat distribution a major explanation for the sex differences in the incidence of myocardial infarction? The study of men born in 1913 and the study in women, Goteborg, Sweden. *Am J Epidemiol* 1992; **135**: 266–273.
9 Alexandersen P, Haarbo J, Byrjalsen I, Lawaetz H, Christiansen C. Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits. *Circ Res* 1999; **84**(7): 813–819.
10 Wu FCW, von Eckardstein A. Androgens and coronary artery disease. *Endocr Rev* 2003; **24**: 183–217.
11 Malkin CJ, Pugh PJ, Jones RD, Jones TH, Channer KS. Testosterone as a protective factor against atherosclerosis – immunomodulation and influence upon plaque development and stability. *J Endocrinol* 2003; **178**: 373–380.

12 Wynne FL, Khalil RA. Testosterone and coronary vascular tone: implications in coronary artery disease. *J Endocrinol Invest* 2003; **26**: 181–186.
13 Deenadayalu VP, White RE, Stallone JN, Gao X, Garcia AJ. Testosterone relaxes coronary arteries by opening the large-conductance, calcium-activated potassium channel. *Am J Physiol Heart Circ Physiol* 2001; **281**: H1720–H1727.
14 Aversa A, Fierro V, Malizia F, Greco EA, Canale D, Macchia E *et al.* A double-blind, randomized, placebo-controlled cross-over study on the effects of testosterone (Testogel) plus sildenafil on cavernous arteries and psychological correlates in men with mild hypogonadism and erectile dysfunction. *J Sex Med* 2006; **3**(s3): 182.
15 English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS. Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms. *Eur Heart J* 2000; **21**: 890–894.
16 Cauley JA, Gutai JP, Kuller LH, Dai WS. Usefulness of sex steroid hormone levels in predicting coronary artery disease in men. *Am J Cardiol* 1987; **60**: 771–777.
17 Barrett-Connor E, Khaw KT. Endogenous sex hormones and cardiovascular disease in men. A prospective population-based study. *Circulation* 1988; **78**: 539–545.
18 Yarnell JW, Beswick AD, Sweetnam PM, Riad-Fahmy D. Endogenous sex hormones and ischemic heart disease in men. The Caerphilly Prospective Study. *Arterioscler Thromb* 1993; **13**: 517–520.
19 De Backer C, Ambrosioni E, Borch-Johnsen K, Brotous C, Cifkova R, Dallougeville J *et al.* European guidelines on cardiovascular disease prevention in clinical practice. *Eur J Cardiovasc Prevent Rehabil* 2003; **10**(Suppl 1): S1–S78.
20 Webb CM, Adamson DL, de Zeigler D, Collins P. Effect of acute testosterone on myocardial ischemia in men with coronary artery disease. *Am J Cardiol* 1999; **83**: 437–439, , A9..
21 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.
22 English KM, Steeds RP, Jones TH, Diver MJ, Channer KS. Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: a randomized, double-blind, placebo-controlled study. *Circulation* 2000; **102**: 1906–1911.
23 Malkin CJ, Pugh PJ, Morris PD, Kerry KE, Jones RD, Jones TH Channer KS. Testosterone replacement in hypogonadal men with angina improves ischaemic threshold and quality of life. *Heart* 2004; **90**: 871–876.
24 Pugh PJ, Jones H, Channer KS. Acute haemodynamic effects of testosterone in men with chronic heart failure. *Eur Heart J* 2003; **24**: 909–915.
25 Rolf C, Nieschlag E. Potential adverse effects of long-term testosterone therapy. *Baillieres Clin Endocrinol Metab* 1998; **12**: 521–534.
26 Alexandersen P, Haarbo J, Christiansen C. The relationship of natural androgens to coronary heart disease in males: a review. *Atherosclerosis* 1996; **125**: 1–13.
27 Hurley BF, Seals DR, Hagberg JM, Goldberg AC, Ostrove SM, Holloszy JO *et al.* High-density-lipoprotein cholesterol in bodybuilders vs powerlifters. Negative effects of androgen use. *JAMA* 1984; **252**: 507–513.
28 Thompson PD, Cullinane EM, Sady SP, Chenevert C, Saritelli AL, Sady MA *et al.* Contrasting effects of testosterone and stanozolol on serum lipoprotein levels. *JAMA* 1989; **261**: 1165–1168.
29 Crist DM, Peake GT, Stackpole PJ. Lipemic and lipoproteinemic effects of natural and synthetic androgens in humans. *Clin Exp Pharmacol Physiol* 1986; **13**: 513–518.
30 Taggart HM, Applebaum-Bowden D, Haffner S, Warnick GR, Cheung MC, Albers JJ *et al.* Reduction in high density lipoproteins by anabolic steroid (stanozolol) therapy for postmenopausal osteoporosis. *Metabolism* 1982; **31**: 1147–1152.
31 Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J *et al.* The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *N Engl J Med* 1996; **335**: 1–7.
32 Sih R, Morley JE, Kaiser FE, Perry III HM, Patrick P, Ross C. Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. *J Clin Endocrinol Metab* 1997; **82**: 1661–1667.
33 Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Lenrow DA *et al.* Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. *J Clin Endocrinol Metab* 1999; **84**: 2647–2653.
34 Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Holmes JH *et al.* Effect of testosterone treatment on bone mineral density in men over 65 years of age. *J Clin Endocrinol Metab* 1999; **84**: 1966–1972.
35 Tenover JS. Effects of testosterone supplementation in the aging male. *J Clin Endocrinol Metab* 1992; **75**: 1092–1098.
36 Elbers JM, Giltay EJ, Teerlink T, Scheffer PG, Asscheman H, Seidell JC *et al.* Effects of sex steroids on components of the insulin resistance syndrome in transsexual subjects. *Clin Endocrinol (Oxford)* 2003; **58**: 562–571.
37 Bhasin S. Effects of testosterone administration on fat distribution, insulin sensitivity, and atherosclerosis progression. *Clin Infect Dis* 2003; **37**(Suppl 2): S142–S149.
38 Toda T, Toda Y, Cho BH, Kummerow FA. Ultrastructure of newly formed vessels in thrombi. Endothelium formation in human umbilical artery. *Acta Pathol Jpn* 1984; **34**: 529–536.
39 Bruck B, Brehme U, Gugel N, Hanke S, Finking G, Lutz C *et al.* Gender-specific differences in the effects of testosterone and estrogen on the development of atherosclerosis in rabbits. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2192–2199.
40 Alexandersen P, Haarbo J, Byrialsen I, Lawaetz H, Christiansen C. Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits. *Circ Res* 1999; **84**: 813–819.
41 Obasanjo IO, Clarkson TB, Weaver DS. Effects of the anabolic steroid nandrolone decanoate on plasma lipids and coronary arteries of female cynomolgus macaques. *Metabolism* 1996; **45**: 463–468.
42 Elhage R, Arnal JF, Pieraggi MT, Duverger N, Fievet C, Faye JC *et al.* 17 Beta-estradiol prevents fatty streak formation in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2679–2684.
43 Von Dehn G, von Dehn O, Volker W, Langer C, Weinbauer GF, Behre HM *et al.* Atherosclerosis in apolipoprotein E-deficient mice is decreased by the suppression of endogenous sex hormones. *Horm Metab Res* 2001; **33**: 110–114.
44 Larsen BA, Nordestgaard BG, Stender S, Kjeldsen K. Effect of testosterone on atherogenesis in cholesterol-fed rabbits with similar plasma cholesterol levels. *Atherosclerosis* 1993; **99**: 79–86.
45 Adams MR, Williams JK, Kaplan JR. Effects of androgens on coronary artery atherosclerosis and atherosclerosis-related impairment of vascular responsiveness. *Arterioscler Thromb Vasc Biol* 1995; **15**: 562–570.
46 Fogelberg M, Bjiorkhem I, Diczfalusy U, Henriksson P. Stanozolol and experimental atherosclerosis: atherosclerotic development and blood lipids during anabolic steroid therapy of New Zealand white rabbits. *Scand J Clin Lab Invest* 1990; **50**: 693–696.
47 Chen SJ, Li HB, Durand Joparil S, Chen YF. Estrogen reduces myointimal proliferation after balloon injury of rat carotid artery. *Circulation* 1996; **93**: 577–584.
48 Nieschlag E, Swerdloff R, Behre HM, Gooren LJ, Kaufman JN, Legros JJ *et al.* Investigation, treatment and monitoring of late-onset hypogonadism in males: ISA, ISSAM, and EAU recommendations. *Int J Androl* 2005; **28**: 125–127.
49 Aversa A, Isidori AM, Greco EA, Giannetta E, Gianfrilli D, Spera E *et al.* Hormonal supplementation and erectile dysfunction. *Eur Urol* 2004; **45**: 535–538.
50 Rosano GC. Androgens and coronary artery disease. A sex specific effect of sex hormones? *Eur Heart J* 2000; **21**: 868–871.

51 Negri M, Sheiban I, Arigliano PL, Tonni S, Montresor G, Carlini S *et al.* Interrelation between angiographic severity of coronary artery disease and plasma levels of insulin, C-peptide and plasminogen activator inhibitor-1. *Am J Cardiol* 1993; **72**: 397–401.
52 Isidori AM, Giannetta E, Greco EA, Gianfrilli D, Bonifacio V, Isidori A *et al.* Effects of testosterone on body composition, serum lipid profile in middle-aged men: a meta-analysis. *Clin Endocrinol* 2005; **63**: 280–293.
53 Kloner RA, Mullin SH, Shook T, Matthews R, Mayeda G, Burstein S *et al.* Erectile dysfunction in the cardiac patient: how common and should we treat? *J Urol* 2003; **170**(Part 2): S46–S50.
54 Hak AE, Witteman JCM, de Jong FH, Geerlings MI, Hofman A, Pols HAP. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: The Rotterdam Study. *J Clin Endocrinol Metab* 2002; **87**: 3632–3639.