

## CLINICAL STUDY

# Testosterone does not adversely affect fibrinogen or tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) levels in 46 men with chronic stable angina

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## Abstract

**Objective:** In women, sex hormones cause increased morbidity and mortality in patients with coronary heart disease (CHD) and adversely affect the coagulation profile. We have studied the effect of physiological testosterone replacement therapy in men on coagulation factor expression, to determine if there is an increased risk of thrombosis.

**Methods:** Double-blind, randomized, placebo-controlled trial of testosterone in 46 men with chronic stable angina. Measurements of free, total and bioavailable testosterone, luteinising hormone (LH) and follicle-stimulating hormone (FSH), estradiol, plasminogen activator inhibitor-1 (PAI-1), fibrinogen, tissue plasminogen activator (tPA) and full blood count were made at 0, 6 and 14 weeks.

**Results:** Bioavailable testosterone levels were:  $2.58 \pm 0.58$  nmol/l at baseline, compared with  $3.35 \pm 0.31$  nmol/l at week 14 ( $P < 0.001$ ) after treatment compared with  $2.6 \pm 0.18$  nmol/l and  $2.44 \pm 0.18$  nmol/l in the placebo group ( $P$  was not significant). There was no change in fibrinogen ( $3.03 \pm 0.18$  g/l at baseline and  $3.02 \pm 0.18$  g/l at week 14,  $P = 0.24$ ), tPA activity ( $26.77 \pm 4.9$  Iu/ml and  $25.67 \pm 4.4$  Iu/ml,  $P = 0.88$ ) or PAI-1 activity ( $0.49 \pm 0.85$  Iu/ml and  $0.36 \pm 0.06$  Iu/ml,  $P = 0.16$ ) with active treatment and no differences between the groups (at week 14,  $P$  value 0.98, 0.59 and 0.8 for fibrinogen, PAI-1 and tPA respectively). Haemoglobin concentration did not change over time, in the testosterone group ( $1.44 \pm 0.02$  g/l and  $1.45 \pm 0.02$  g/l,  $P = 0.22$ ).

**Conclusion:** Physiological testosterone replacement does not adversely affect blood coagulation status.

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## Introduction

Coronary heart disease (CHD) is the commonest cause of death in the UK, with 120 000 people (approximately one in four men and one in six women) dying from CHD in 2001 (1). This gender-specific incidence is consistent between countries and is not due to differences in classical cardiovascular risk factors (2, 3). It has been postulated that the difference observed between the sexes may be due to a cardioprotective effect of endogenous female sex hormones, and that hormone replacement therapy (HRT) might be beneficial in postmenopausal women, thereby reducing the incidence of ischaemic heart disease. This hypothesis would appear to have been supported by observational studies that showed a reduced risk of CHD associated with HRT in women (4, 5). However, three recent large prospective randomized controlled trials of HRT have shown no significant

decrease in the rates of primary or secondary cardiovascular events (6–8). There was however a highly significant increase in venous thromboembolism associated with the use of HRT.

Recently, the role the male sex hormone testosterone plays in CHD has received increasing attention. In men, levels of testosterone fall with increasing age (9, 10) and it is in the elderly population that the highest rates of CHD are found. Low plasma testosterone levels in men are associated with known risk factors for CHD including age, hypertension, obesity, raised fibrinogen, hyperinsulinaemia, diabetes mellitus and adverse lipid profile (11). Males with CHD have lower serum levels of bioavailable testosterone than men of a similar age with normal coronary angiograms (12). In symptomatic patients with angina a number of studies have demonstrated that testosterone treatment prolongs the time to myocardial ischaemia compared

with placebo (13–17). Testosterone treatment reduces diet-induced atherosclerosis in animal studies (18), and human studies have also investigated the relationship between the serum testosterone concentration and atherosclerosis. Two studies have demonstrated an inverse relationship between serum testosterone and the degree of atherosclerosis as determined by carotid artery intima-media thickness on ultrasound (19, 20). Serum testosterone levels were also inversely related in a further study (21), although the degree of atherosclerosis of the abdominal aorta was measured radiographically.

The majority of cases of myocardial infarction (MI) are caused by thrombotic coronary occlusion. The thrombotic process is complicated with various pro- and anti-thrombotic mediators determining the coagulation status. The major pro-thrombotic factors are plasminogen activator inhibitor-1 (PAI-1), fibrinogen, alpha-2-antiplasmin and factor VIIc, whilst tissue plasminogen activator (tPA), protein C and anti-thrombin III are important anti-thrombotic agents. It has been shown that PAI-1 is of particular importance in CHD, with elevated levels predicting MI and progression of atherosclerosis in stable CHD patients (22, 23). Cross-sectional studies have reported a positive association between testosterone levels and tPA and a negative association with PAI-1, VIIc and fibrinogen (14, 15, 24). Replacement of testosterone in hypogonadal men and treatment of normal men with dehydroepiandrosterone (DHEA) reduces PAI-1 serum levels, whilst testosterone administration in healthy men also reduces plasma levels of the acute phase protein fibrinogen (23), which is an independent risk factor for CAD (25).

In this study we have evaluated the role of 3 months of physiological testosterone therapy upon fibrinogen, PAI-1 and tPA activity levels in men with chronic stable angina.

## Subjects and methods

### Methods

This was a double-blind, randomized, placebo-controlled, add-on trial. Subjects entered an initial 2-week, single-blind, placebo run-in period, followed by double-blind randomization to active or placebo treatment for 12 weeks. Subjects applied two 2.5 mg self-adhesive active testosterone or placebo patches each night before retiring. The main results have previously been reported (26). Measurements of free, total and bioavailable testosterone and PAI-1 activity, fibrinogen and tPA activity were undertaken at week 0, 6 and 14.

### Subjects

Sixty one men with CHD (>70% stenosis of a major coronary artery at coronary angiography, previous proven

MI, or typical symptoms of angina pectoris and a 'double positive' (>1 mm downsloping ST-segment (the segment between the S and the T wave on the ECG) depression associated with chest pain) treadmill exercise test) were recruited. All patients gave written informed consent, and the study was approved by the local ethics committee. No changes were made to anti-anginal medication for 4 weeks before or during the trial. Patients were excluded if they had a prostate specific antigen (PSA) level above the normal range or any other contraindication to testosterone therapy. Subjects with severe hypertension (blood pressure >180/114 mmHg), significant arrhythmias or who had left main stem (or equivalent) stenosis, a coronary or cerebrovascular event, or had taken other trial drugs in the last 3 months were also excluded.

Of the 61 patients screened, 53 entered the single-blind placebo run-in phase. Of these 53, 50 patients completed the single-blind run-in phase. Twenty-five patients were randomized to each group. Three patients were withdrawn from the active treatment arm: one suffered a MI, one had severe skin irritation, and one had an elective coronary angioplasty performed earlier than expected. One patient withdrew from the placebo arm complaining of depression. All early withdrawals after the placebo run-in occurred before the first assessment of double-blind treatment; therefore, these subjects were excluded from the final analysis. Twenty-two patients completed active treatment and 24 completed placebo treatment; all included in the final analysis (Table 1).

### Trial drug

Testosterone was given via a transdermal patch delivery system (Andropatch, GlaxoSmithKline). Identical placebo patches were manufactured by Thera-Tech Inc. (Bloomington, IL, USA) Subjects applied two 2.5 mg patches at night, a dose that has previously been shown to raise levels of testosterone to within the normal range in 93% of hypogonadal men and to mimic the normal diurnal variation in hormone levels seen *in vivo* (27).

### Patient assessment

Patients were assessed at week 0, 6 and 14 and measurements were made of free, total and bioavailable testosterone, sex hormone binding globulin (SHBG), estradiol, luteinising hormone (LH), follicle-stimulating hormone (FSH), full blood count and the fibrinolytic markers, fibrinogen, tPA activity and PAI-1 activity. PSA was measured at the beginning and end of the trial. A medical history was taken, documenting risk factors for CAD and drug history. Baseline recordings were also taken of body mass index, waist to hip ratio, systolic BP, diastolic BP, cardiac output and pulse.

**Table 1** Baseline characteristics of 46 men with CHD before testosterone (active) or placebo treatment. Values are means  $\pm$  S.E.M. unless otherwise stated.

Baseline characteristics	Active treatment (n = 22)	Placebo treatment (n = 24)
Age, years	62 $\pm$ 2	62 $\pm$ 2
Diagnosis of CAD, n (%)		
Angiogram	14 (64)	18 (75)
Previous MI	3 (14)	2 (8)
Angina + ETT	5 (22)	4 (17)
Risk Factors, n (%)		
Hypertension	4 (18)	14 (58)*
Diabetes mellitus	2 (9)	5 (21)
Family history	8 (36)	13 (54)
Current smoker	1 (4)	4 (16)
Hypercholesterolaemia at presentation	15 (68)	18 (75)
Concurrent medication, n (%)		
NSAID (Aspirin)	22 (100)	24 (100)
B-blocker	18 (81)	20 (83)
Long-acting nitrate	11 (50)	11 (45)
Calcium channel blocker	11 (50)	12 (50)
Potassium channel opener	1 (4)	3 (12)
Statin	13 (59)	17 (70)
ACE-I	3 (13)	5 (20)
Diuretics	2 (9)	9 (37)**
Baseline recordings		
Body mass index, kg/m <sup>2</sup>	27.7 $\pm$ 0.7	28.4 $\pm$ 0.8
Waist-to-hip ratio	0.96 $\pm$ 0.1	0.98 $\pm$ 0.01
Systolic BP, mmHg	131 $\pm$ 4	142 $\pm$ 4 ***
Diastolic BP, mmHg	78 $\pm$ 2	80 $\pm$ 2.3
Cardiac output, l/min	3.8 $\pm$ 0.2	3.7 $\pm$ 0.3
Pulse, bpm	61 $\pm$ 3	63 $\pm$ 2

CAD, coronary heart disease; MI, myocardial infarction; ETT, exercise treadmill testing; ACE-I, angiotensin converting enzyme-inhibitor; BP, blood pressure.

\* $\chi^2 = 7.8$ ,  $P < 0.01$ ; \*\* $\chi^2 = 5.1$ ,  $P < 0.05$ ; \*\*\* $P < 0.05$ .

### Laboratory measurements

Serum-total and -free testosterone were measured using radio-immuno-assay (Coat-A-Count, Diagnostic Products Corporation, Tyne and Wear, UK), with inter-assay coefficient of variation 5.5% at 16.3 pmol/l, 3.4% at 147 pmol/l, 11.2% at 3.6 nmol/l and 6.1% at 13.1 nmol/l. Percentage bioavailable testosterone was assayed using an adaptation of the methods described by Tremblay & Dube (24), where 3H-labelled testosterone radioactive tracer was measured in the supernatant fraction following ammonium sulphate precipitation of sex hormone binding globulin. The concentration of bioavailable testosterone was then calculated from the percentage of the total steroid. Inter-assay variation was less than 8% throughout the range of the assay. Estradiol was measured by an automated competitive enzyme-immuno-assay (Immulite, Diagnostic Products Corporation, Los Angeles, USA), with inter-assay coefficient of variation 16.7% at 242 pmol/l, 9.1% at 2420 pmol/l and 10% at 5381 pmol/l. LH and FSH

were measured using an automated micro-particle-immuno-assay (Abbott AxSYM). Inter-assay coefficient of variation was 8.0% at 4.5 Iu/l, 6.3% at 17.1 Iu/l, 12.0% at 41.7 Iu/l and 6.3% at 8.9 Iu/l, 7.7% at 19.4 Iu/l and 6.8% at 41.7 Iu/l. Fibrinogen levels were analyzed using a method modified from the Von Clauss assay. The fibrinogen concentration affects the time taken for fibrin formation after dade thrombin is added to dilute plasma. The time taken for clot formation to occur was measured on the Clauss CA6000 machine, this was then converted to a fibrinogen concentration using a calibration curve. Inter-assay coefficient of variation was < 9%. Measurement of the activity levels of tPA and PAI-1 involved taking blood samples into Stabilyte tubes that were placed immediately on ice to reduce the blood pH to 5.9. This inhibited PAI-1/tPA complex formation, thereby preserving blood levels. Levels were using the Biopool Chromolize bio-functional immunosorbent assay (Biopool International, Venova, USA/Umea, Sweden). Inter-assay coefficients of variation for tPA were 5.2% at mid range and 5.3% at the lower range of the assay and for PAI-1 they were 16.9% at 2 Iu/ml, 4.6% at 22 Iu/ml and 3.6 at 36 Iu/ml. Haemoglobin concentration was measured using the fully automated Coulter STKS system.

### Statistical analysis

We confirmed the data were parametric using Kolmogorov-Smirnov test. In subsequent analysis, unpaired, paired Student's *t*-test or ANCOVA was used as appropriate. For analysis of differences over time, time point and group were entered as fixed factors and baseline values as a covariate to adjust for slight differences in baseline values. Unless otherwise stated data are expressed as means  $\pm$  S.E.M. Statistical significance was accepted at  $P < 0.05$ .

## Results

### Safety data

The transdermal testosterone delivery system was well tolerated, with the main side effect being skin irritation. Eleven patients reported this on active treatment and six on placebo. One patient, who was awaiting coronary revascularization, suffered a myocardial infarction whilst on active treatment. There was no significant change in PSA in either group.

### Patient demographics

Patients in the active and placebo-treated groups were well matched, with similar ages and proportions in the diagnosis of CAD. However the placebo group had a higher incidence of risk factors for CAD, higher systolic blood pressure and higher diuretic use (Table 1).

### Hormone levels

The hormone levels at baseline and weeks 6 and 14 are shown in Table 2. At baseline, the mean testosterone levels were at the lower limit of normal in both groups (bioavailable testosterone =  $2.58 \pm 0.28$  nmol/l in the active group compared with  $2.60 \pm 0.18$  nmol/l in the placebo group, normal range  $> 2.5$  nmol/l). Active treatment led to a significant increase in the level of testosterone, which peaked at week 6 and waned slightly by week 14 (Table 2). These changes were reflected in changes in the levels of gonadotrophins (Table 2). There were no significant changes in any serum measurements in the placebo group (Table 2).

### Fibrinogen, tPA and PAI-1 levels

The coagulation marker levels are shown at baseline and week 6 and 14 in Table 3. There was no change in the levels of fibrinogen, tPA activity or PAI-1 activity over time, with no differences between the testosterone and placebo-treated groups (Table 3).

### Discussion

In this study we have shown that administration of physiological doses of supplemental testosterone to men with chronic stable angina does not adversely

affect the coagulation system, indeed no significant changes were found in the levels of tPA and PAI-1 activities or fibrinogen level. This is the first study to assess the influence of physiological doses of testosterone upon haemostatic factors in men with CHD.

Previous studies have suggested that testosterone may be associated with a beneficial effect upon coagulation. Philips *et al.* (28) studied the association between testosterone levels and fibrinolytic factors in men undergoing angiography for chest pain or an abnormal exercise treadmill test, but excluding patients with previous MI. An estimate of the degree of CAD was taken from the angiogram and this was compared with hormone and fibrinolytic marker levels. Results showed that the testosterone level was correlated negatively with CAD and PAI-1 (28). A subgroup analysis showed a negative association with fibrinogen when patients with hypertension, diabetes or major medical disorders were excluded (28). Another study investigated the relationship between testosterone levels, fibrinolytic activity and lipid levels, in hyperlipidaemic men (29). It was reported that testosterone was positively associated with tPA activity and inversely associated with PAI-1 activity and fibrinogen levels (29). Similarly a potentially beneficial effect of testosterone upon coagulation was reported in men in the period after an acute MI (30). Both total and bioavailable testosterone fell transiently in the first 24 h post-infarction. In contrast, following thrombolysis there was a rise in

**Table 2** Levels of total, free and total testosterone, LH, FSH and Estradiol in male CHD patients, at different time points, during testosterone (active) or placebo treatment. Values are means  $\pm$  S.E.M. unless otherwise stated.

	Baseline	Week 6	Week 14	P value
Total testosterone (RR 7.5–37.0), nmol/l				
Active	13.55 $\pm$ 0.78	22.34 $\pm$ 1.19	18.57 $\pm$ 1.6	< 0.0001
Placebo	12.38 $\pm$ 0.72	11.35 $\pm$ 0.76	12.23 $\pm$ 0.89	
P	0.3	< 0.001*	< 0.001*	
Free testosterone (RR 37.4–138.7), pmol/l				
Active	45.68 $\pm$ 2.42	84.70 $\pm$ 4.89	72.56 $\pm$ 5.8	< 0.0001
Placebo	46.36 $\pm$ 2.54	44.86 $\pm$ 2.76	48.69 $\pm$ 3.29	
P	0.9	< 0.0001*	< 0.001*	
Bioavailable testosterone (RR > 2.5), nmol/l				
Active	2.58 $\pm$ 0.28	4.34 $\pm$ 0.26	3.35 $\pm$ 0.31	< 0.001
Placebo	2.6 $\pm$ 0.18	2.42 $\pm$ 0.22	2.44 $\pm$ 0.18	
P	0.5	< 0.0001*	0.01*	
LH (RR 1.3–9.1), IU/l				
Active	4.49 $\pm$ 0.61	1.95 $\pm$ 0.35	2.72 $\pm$ 0.67	< 0.0001
Placebo	5.28 $\pm$ 0.58	5.46 $\pm$ 0.61	5.15 $\pm$ 0.55	
P	0.358	< 0.0001*	< 0.005*	
FSH (RR 1.7–12.6), IU/l				
Active	6.43 $\pm$ 0.91	3.22 $\pm$ 0.59	3.29 $\pm$ 0.74	< 0.0001
Placebo	6.88 $\pm$ 0.91	6.98 $\pm$ 0.91	7.0 $\pm$ 0.88	
P	0.7	0.001*	< 0.005*	
Estradiol (RR < 150), pmol/l				
Active	70.27 $\pm$ 6.05	80.50 $\pm$ 6.6	77.68 $\pm$ 4.8	0.301
Placebo	67.75 $\pm$ 4.0	72.13 $\pm$ 4.2	76.46 $\pm$ 3.8	
P	0.7	0.3	0.8	

RR; reference range; LH, luteinising hormone; FSH, follicle-stimulating hormone. Probability values across the groups calculated with ANCOVA; between the 2 groups, with Student's *t*-test for independent variables. \*Statistically significant; *P* < 0.05.

**Table 3** Levels of fibrinogen, PAI-1, tPA and haemoglobin in male CHD patients, at different time points, during testosterone (active) or placebo treatment. Values are means  $\pm$  S.E.M. unless otherwise stated.

	Baseline	Week 6	Week 14	P
Fibrinogen, g/l				
Active	3.03 $\pm$ 0.18	3.0 $\pm$ 0.19	3.02 $\pm$ 0.18	0.24
Placebo	3.37 $\pm$ 0.17	3.36 $\pm$ 0.21	3.02 $\pm$ 0.19	
P	0.19	0.22	0.98	
PAI-1, lu/ml				
Active	26.77 $\pm$ 4.9	28.80 $\pm$ 4.84	25.67 $\pm$ 4.38	0.16
Placebo	29.13 $\pm$ 3.57	35.27 $\pm$ 5.20	28.99 $\pm$ 4.25	
P	0.7	0.37	0.59	
tPA, lu/ml				
Active	0.49 $\pm$ 0.85	0.39 $\pm$ 0.11	0.36 $\pm$ 0.06	0.88
Placebo	0.42 $\pm$ 0.86	0.32 $\pm$ 0.68	0.33 $\pm$ 0.07	
P	0.6	0.59	0.8	
Haemoglobin, g/l				
Active	1.44 $\pm$ 0.02	1.43 $\pm$ 0.02	1.45 $\pm$ 0.02	0.22
Placebo	1.47 $\pm$ 0.02	1.44 $\pm$ 0.02	1.45 $\pm$ 0.02	
P	0.82	0.89	0.83	

PAI-1, Plasminogen activator inhibitor-1; tPA, tissue plasminogen activator. Probability values across the groups calculated with ANCOVA; between the 2 groups, with Student's *t*-test for independent variables.

PAI-1 activity, which could lead to a relatively pro-thrombotic state (30). The greater the fall in testosterone following MI, the higher PAI-1 activity and lower tPA activity (30), suggesting a similar interaction between testosterone, PAI-1 and tPA. In addition, the anti-thrombotic influence of testosterone maybe potentially lost following an MI (30).

Supraphysiological testosterone therapy is also reported to modulate coagulation factor expression. In a study of 32 healthy men who received 200 mg testosterone oethanthate by weekly intramuscular injection for 52 weeks in a trial of hormonal male contraception (31), testosterone therapy resulted in a reduction in fibrinogen and PAI-1 activity, together with decreases in serum concentrations of the pro-thrombotic factors protein C and protein S (31). There was no change in the level of tPA, although an increase in antithrombin III activity was observed (31). Apart from fibrinogen, the observed alterations in haemostatic factors returned to pretreatment levels during continued treatment. An increase in both haemoglobin and haematocrit was noticed which was sustained throughout the study period (31). It was concluded that supraphysiological doses of testosterone do not result in a pro-thrombotic state, which is supported by our current study. The lack of effect upon fibrinogen and PAI-1 activity in the current study is likely to be a consequence of the smaller, physiological doses of testosterone that were used, but these more accurately reflect serum levels generated by testosterone replacement.

These studies have investigated the potential anti-thrombotic role of testosterone and the relationship with CHD. HRT has previously been hypothesized to

reduce the incidence of CHD in postmenopausal women. However the Heart and Estrogen/Progestin Replacement study (HERS) (6) reported an increase in vascular events and death in the first year of treatment, which has been postulated to be due to the increased risk of arterial thrombosis leading to MI or stroke. One theory used to explain the unexpected increased vascular risk was that the HRT preparation used in these trials combined estrogens and progestones, with the latter confounding any potential benefit of the former. However, this was later disproved in the ESPRIT trial (32), in which women who had had their first MI where randomized to either estradiol or placebo, no beneficial effect in the risk of secondary cardiovascular events was found. The risk of venous thromboembolism is also increased with female HRT. The Women's Health Initiative (WHI) trial (7) reported a two-fold increase in vascular events and death, with marked similarity to the results of HERS/HERS II (6, 8). The risk was highest in the period immediately after commencing HRT and this is thought to be due to an enhancement of a pre-existing pro-thrombotic abnormality in some women. For example, one of the commonest pro-thrombotic disorders is factor V Leiden deficiency, occurring in 2–6% of the general population. This was associated with a 15 fold increased risk of venous thrombosis in a study carried out in Oxford (33) and similar figures were found in the HERS study (6).

Arterial and venous thromboembolism is also increased with the oral contraceptive pill (OCP). An increased risk of ischaemic stroke and MI was first reported soon after the introduction of the OCP in 1962 (34) and 1963 (35). More recent studies have confirmed this increased relative risk with the World Health Organization collaborative study (36) finding a five-fold increase in the risk of MI and a three-fold increased risk of ischaemic stroke in OCP users. The RATIO study (37) found a two-fold increase in the risk of MI. However, the risk was substantially increased by the presence of additional risk factors (six-fold for hypertensive OCP users, 13.6-fold for smoking OCP users and 17.4-fold for diabetic OCP users). Venous thromboembolism has also been shown to be increased with the OCP, between two- and six-fold (38) and as with HRT, this risk is greatly increased in patients with familial thrombophilia, with Factor V Leiden deficiency increasing the risk 20- to 30-fold (39).

The effect of male and female sex hormones in cardiovascular disease would appear to be different. The increased risk of arterial and venous thromboembolism associated with HRT and the OCP, may not occur with testosterone replacement therapy in men, since physiological doses of testosterone do not adversely affect blood coagulation factor expression. This negative finding is important, since recent research has shown that testosterone is beneficial in

patients with angina (13–17) and chronic heart failure (40, 41). The findings of this study suggest that male hormone replacement therapy may provide cardiovascular benefit without adversely affecting blood coagulation.

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