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CLINICAL STUDY

Long-term benefits of testosterone replacement therapy on angina threshold and atheroma in men

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Abstract

Introduction: In short-term studies, testosterone replacement therapy has been shown to protect male subjects from exercise-induced ischaemia and modify cardiovascular risk factors such as insulin resistance, fat mass and lipid profiles.

Methods: This randomised parallel group controlled trial was designed to assess the treatment effect of testosterone therapy (Nebido) compared with placebo in terms of exercise-induced ischaemia, lipid profiles, carotid intima-media thickness (CIMT) and body composition during 12 months treatment in men with low testosterone levels and angina.

Results: A total of 15 men were recruited but 13 ($n=13$) reached adequate duration of follow-up; seven were treated with testosterone and six with placebo. Testosterone increased time to ischaemia (129 ± 48 s versus 12 ± 18, $P=0.02$) and haemoglobin (0.4 ± 0.6 g/dl versus -0.03 ± 0.5, $P=0.04$), and reduced body mass index (-0.3 kg/m^2 versus 1.3 ± 1, $P=0.04$) and triglycerides (-0.36 ± 0.4 mmol/l versus 0.3 ± 1.2, $P=0.05$). The CIMT decreased in the testosterone group more than placebo, but full between group analyses suggested this was only a statistical trend (-0.5 ± 0.1 vs -0.09 ± 0.06, $P=0.16$). There were no significant effects on serum prostate specific antigen, total or high-density lipoprotein cholesterol; or on mood and symptom scores as assessed by Seattle Angina Score and EuroQol.

Conclusion: The protective effect of testosterone on myocardial ischaemia is maintained throughout treatment without decrement. Previously noted potentially beneficial effects of testosterone on body composition were confirmed and there were no adverse effects.

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Introduction

Testosterone is the dominant male sex hormone but in addition it has widespread effects on metabolism and the cardiovascular system. The male-to-female ratio of mortality from vascular disease is ~3:1 throughout the world and this ratio is independent of the background prevalence of vascular disease and of other cardiovascular risk factors (1). Recently, three longitudinal epidemiological studies have found low testosterone levels to be associated with an increased risk of all-cause and cardiovascular mortality (2–4). Similarly, studies of men with prostate cancer treated with anti-androgens have also been found to have increased cardiovascular risk (5). There is a gradual fall in serum testosterone levels after the age of 40 years. Late-onset hypogonadism is defined as an age-related deterioration in androgen production causing low serum testosterone levels combined with symptoms of testosterone deficiency. There is no evidence that elevated or high-normal levels of serum testosterone are associated with the onset of vascular disease (6); indeed, biochemical testosterone deficiency is common in male patients with vascular disease. Testosterone replacement in these subjects can lead potentially to beneficial effects. Physiological testosterone replacement has been reported in a number of trials to improve lipid profiles, increase insulin sensitivity, and reduce visceral fat, clotting and inflammatory profiles (reviewed in (7–9)). Importantly, no adverse effects of testosterone replacement therapy on coagulation have been demonstrated in men with chronic stable angina (10). Furthermore, animal studies have shown that testosterone replacement inhibits the development and progression of atheroma (11). Finally, testosterone has direct effects on vascular tone causing vasodilatation, an increase in coronary blood flow (12, 13) and an increase in the ischaemic threshold in patients with symptoms of stable angina (14, 15). There are, however,

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several questions on the effects of testosterone therapy in men with coronary ischaemia, which remain to be answered.

First, does the effect of testosterone persist in the long-term? Many of the published studies are acute single dose trials and none of the chronic studies have assessed patients formally beyond a few months. Previous clinical studies from our group have been limited to 3 months. There is a concern that in the long term, the vasodilator effects of testosterone replacement on vascular reactivity (16) and the resulting beneficial acute haemodynamic effect would attenuate leading to a drop-off in the clinical effect – as was seen in our 12-month clinical trial of testosterone in heart failure (17). Secondly, does testosterone therapy affect the levels of measurable atheroma? *In vivo* atheroma – measured as carotid intima-media thickness (CIMT) (18) – is a marker of vascular risk and can be used as a surrogate marker of treatment efficacy. Observational studies have consistently shown an inverse relationship between the CIMT and serum testosterone. In follow-up studies, those patients with a low testosterone blood level had bigger changes in CIMT over time, representing increased atheroma progression (19, 20). Prospective animal studies are consistent with this and have shown that physiological androgen replacement inhibits atheroma progression (11, 21–23). There are currently no *in vivo* human data on the effects of androgen therapy on atheroma.

In this study, we aim to address these two issues by examining the clinical effects of testosterone replacement therapy in men with angina over a 12-month-period and assessing the progression of carotid atheroma.

Methods

Subjects

We recruited ambulant males over 20 years of age with stable, chronic angina pectoris for >1 month with proven ST segment depression of >1 mm within 12 min of the Bruce protocol and at least two measurements of early morning serum testosterone <12 nmol/l.

Patients were excluded if they had used androgen therapy or anabolic steroids within 6 months of entry into the study (i.e. screening visit/visit 1) and had any contraindication to treatment with Nebido, prostate specific antigen (PSA) ≥ 4 ng/ml or severe symptomatic benign prostatic hyperplasia. Patients actively or potentially trying to start a family or requiring fertility treatment or with any suspicion of, current, or past history of breast or prostatic carcinoma; myocardial ischaemia (MI), coronary artery bypass surgery or percutaneous coronary intervention (PCI) in the last 3 months or significant hepatic, respiratory, haematological or renal disease were also excluded. Further exclusions included haematocrit $>50\%$ at entry to the study (i.e. screening visit/visit 1), history of drug or alcohol abuse, other trial drugs within 12 weeks, hypercalcaemia, nephrotic-range proteinuria, symptomatic obstructive sleep apnoea syndrome and any electrocardiograph (ECG) abnormalities that preclude ST segment analysis (e.g. left bundle branch block (LBBB), atrial fibrillation (AF)).

Patients were recruited from the ongoing care of cardiologists within the North Trent network. The clinical trial had full Ethics Committee approval and was registered (NCT00131183). Full informed written consent was obtained.

Study design and treatment

This was a double-blind, randomised, parallel group placebo-controlled trial.

Patients were screened and randomised between August 2005 and November 2006. At baseline, subjects completed a medical questionnaire, detailing medical history and concomitant medications. Resting pulse, blood pressure, height and weight were recorded, and body mass index (BMI) was calculated (weight (kg)/height^2 (m^2)). Blood was drawn for serum and haematological assessments. Full blood count, fasting serum glucose and insulin and PSA were performed as standard hospital assays. Total testosterone, SHBG, LH and FSH and high sensitivity CRP (hsCRP) were measured by ELISA (DRG Instruments GmbH, Marburg, Germany) and fasting lipids (Olympic Diagnostics, Hamburg, Germany). Calculated measurements of free and bioavailable testosterone were made using published formulae (24, 25).

Following trial entry, a 2-week single-blind 'run-in' period during which no trial drug therapy was given, was used to allow accurate recording of angina frequency and ensure that baseline exercise parameters were reproducible to within 10% (on the basis of two exercise tests at least 1 week apart). After this, a 12-month treatment period with testosterone or placebo was commenced with assessment of patient response at 3, 6 and 12 months (Table 1). The testosterone/placebo treatment was given in addition to concomitant angina treatment.

Drug treatment for angina was not changed during the study and all exercise tests were performed on anti-anginal therapy. No patient had a coronary intervention during the trial period (surgery or angioplasty).

The primary endpoint was change in time with ST segment depression of >1 mm during Bruce protocol exercise testing. Patients underwent standard treadmill exercise ECG testing by the Bruce protocol. The exercise ECG was analysed by continuous computer monitoring of the ST segments in leads II, V_2 and V_6. The time taken to reach 1 mm or more of horizontal or downsloping ST depression was recorded by a single observer.

Assessments of carotid atheroma were made by a single trained operator (A M) using a dedicated ultrasound laptop with standard software (Sonocalc IMT).

Table 1 Trial programme.

Event	Screening	Baseline	Treatment						End of study
Visit no.	1	2	3	4	5	6	7	8	9
Time point	Week 0	Week 2	Week 8	Week 14	Week 20	Week 28	Week 32	Week 44	After month 12
Patient information/ informed consent	X								
Demographics and medical history	X								
Baseline findings	X	X							
Pulse, blood pressure	X	X		X		X			X
Exercise test	X	X		X		X			X
Fasting blood tests	X			X					X
Serum total testosterone	X	X		X		X			X
Questionnaires		X							X
CIMT assessed		X				X			X
Randomisation		X							
Placebo injection	X								
TU injection/placebo		X	X		X		X	X	
PSA	X			X		X		X	X

Blood tests (high sensitivity CRP, insulin, glucose, lipids). Questionnaires (Seattle Angina Score questionnaire, Beck Depression Inventory, EuroQol). CIMT, carotid intima-media thickness; TU, testosterone undecanoate (Nebido injection); PSA, prostate specific antigen.

Measurements were taken from the left carotid with the patient recumbent. The CIMT was traced in the length of the artery from the common carotid (CCA) into the bulb as far as the internal carotid (ICA). Two automated measurements were automatically generated by the software. The first was the average of mean thickness at each of the three sites (CCA, bulb and ICA) and the second one was the average of the maximum CIMT obtained at each of the three sites (at CCA, bulb and ICA).

Symptoms, quality of life scores and mood were quantified by use of the Seattle Angina Score questionnaire, EuroQol and Beck Depression Inventory, which were completed at trial entry and exit.

Table 2 Baseline data.

	Base group (n=13)	Placebo (n=6)	Testosterone (n=7)	Baseline comparison *P* value
Age (years)	64.8±7.0	67.8±7.9	62.1±5.2	0.17
Total testosterone (nmol/l)	9.9±2.2	10.1±2.8	9.8±1.9	0.8
Sex hormone binding globulin (nmol/l)	26.6±7.4	25±6.1	28±8.3	0.5
Free testosterone (nmol/l)[a]	0.22±0.05	0.23±0.06	0.21±0.04	0.5
Bio-available testosterone[b]	3.3±0.7	3.3±0.8	3.2±0.5	0.7
FSH (nmol/l)	7.0±4.6	10.0±5.3	4.3±1.3	0.05
LH (nmol/l)	4.0±2.6	5.4±1.3	2.7±1.0	0.09
Total cholesterol (mmol/l)	4.1±0.78	4.1±0.9	4.1±0.66	0.9
Prostate specific antigen (μg/l)	1.3±0.94	0.9±0.7	1.6±1.0	0.19
High-sensitivity CRP (μg/ml)	2.8±2.8	3.1±3.9	2.5±1.9	0.7
Total exercise time (s)	368.6±104.1	333.2±143.5	399.1±46.2	0.3
Time to ST segment depression (s)	288.1±92.8	251.2±110.3	319.7±67.5	0.2
Peak METS	7.5±1.6	7.1±2.1	7.9±1.0	0.5
Haemoglobin (g/dl)	14.7±1.1	14.1±1.2	15.2±0.8	0.07
Waist-to-hip ratio	0.97±.06	0.96±0.07	0.98±0.06	0.6
Mass (kg)	86.9±17.0	82.5±18.9	90.6±15.7	0.42
BMI (mass/kg^2)	29.1±5.3	27.6±5.9	30.4±4.7	0.37
CIMT average (mm)	1.02±0.3	1.02±0.29	1.02±0.27	0.9
CIMT max (mm)	1.3±0.35	1.3±0.4	1.3±0.3	0.8
Diabetes	3	2	1	0.6
Myocardial infarction	4	3	1	0.3
β-Blockers	9	4	5	0.9
Nitrates	7	2	5	0.3
Calcium-antagonist	6	2	4	0.6
Other anti-anginal	6	4	2	0.3
CABG/PCI	5/4	4/0	1/4	0.1/0.1

CABG, coronary artery bypass surgery; PCI, percutaneous coronary intervention; CIMT, carotid intima-media thickness.
[a]Free testosterone as calculated by the method of Vermeulen (23).
[b]Bio-available testosterone as calculated by Morris & Malkin (24).

Table 3 Treatment effect.

Parameter	**Treatment effect visit 4** (week 14)			**Treatment effect visit 6** (week 28)			**Treatment effect visit 8** (week 52)			
	Placebo	Testosterone	*P*	Placebo	Testosterone	*P*	Placebo	Testosterone	*P*	**ANOVA treatment effect**
Exercise time (s)	−27.8±56.2	54.3±41.1	0.01	13.5±32.0	55.2±49.6	0.1	30.1±46.0	64.5±13.3	0.1	0.07
Time ST depression (s)	0.33±24.3	75.4±36.5	0.001	0.5±17.7	109.9±62.6	0.002	11.5±17.6	129.3±48.0	0.0001	0.02
Peak METS	−0.57±0.86	1.0±0.72	0.004	0.18±0.66	1.2±1.0	0.06	0.32±0.64	1.4±0.53	0.007	0.02
Prostate specific antigen (μg/l)	−0.017±0.41	−0.01±0.48	0.98	0.00001±0.2	0.33±0.6	0.2	0.1±0.15	0.2±0.61	0.6	0.26
C-reactive protein (μg/ml)	0.2±2.8	0.6±2.1	0.76	−0.46±1.97	0.06±0.9	0.54	1.1±2.2	0.17±0.86	0.38	0.9
Haemoglobin (g/dl)	−0.8±0.6	0.8±0.7	0.05	−0.1±0.7	0.8±0.48	0.02	−0.03±0.5	0.38±0.62	0.2	0.04
Haematocrit (%)	−0.003±0.018	0.03±0.03	0.034	−0.003±0.2	0.03±0.03	0.05	−0.003±0.02	0.01±0.03	0.23	0.03
Mass (kg)	2.75±1.25	1.0±0.64	0.008	3.2±1.8	0.29±1.5	0.009	3.5±2.3	−0.93±3.6	0.02	0.06
Body mass index (mass/kg^2)	1.0±0.69	0.34±0.3	0.04	1.3±0.75	0.23±0.46	0.009	1.3±1.0	−0.29±1.3	0.03	0.04
Waist-to-hip ratio	0.01±0.05	−0.01±0.035	0.3	0.02±0.05	−0.01±0.03	0.15	−0.005±0.04	−0.01±0.04	0.7	0.09
Total testosterone (nmol/l)	−1.0±1.04	6.2±6.7	0.02	−0.03±4.3	9.2±8.5	0.35	−2.0±1.5	6.2±8.3	0.04	0.01
FSH (nmol/l)	0.27±1.6	−3.2±2.2	0.008	0.6±2.0	−2.9±2.2	0.01	0.3±2.3	−1.4±2.8	0.24	0.03
LH (nmol/l	1.3±2.1	−2.1±1.5	0.005	0.13±1.3	−1.96±1.68	0.03	−0.07±1.7	−1.3±1.2	0.25	0.06
Total cholesterol (mmol/l)	−0.25±0.54	−0.16±0.43	0.74	0.07±1.1	−0.17±0.48	0.6	0.18±1.1	−0.29±0.58	0.37	0.3
HDL (mmol/±l)	−0.82±0.17	0.22±0.53	0.2	0.02±0.24	−0.11±0.15	0.23	0.08±0.22	−0.06±0.28	0.34	0.85
Triglycerides (mmol/l)	0.21±0.59	−0.06±0.35	0.32	0.2±1.2	−0.1±0.57	0.56	0.27±1.2	−0.36±0.4	0.27	0.05
CIMT average				−0.068±0.05	−0.32±0.13	0.12	−0.043±0.034	−0.48±0.1	0.002	0.16
CIMT max				−0.11±0.65	−0.37±0.16	0.18	−0.09±0.06	−0.58±0.11	0.004	0.18
Glucose (mmol/l)	0.07±3	0.9±1.7	0.5	0.65±1.9	0.6±1.2	0.9	0.8±2.8	−0.2±2.8	0.5	0.9
Insulin (mIU/l)	51.9±67.5	224±379	0.3	106±132	114±133	0.9	366±563	108±132	0.3	0.5

HDL, high-density lipoprotein; CIMT, carotid intima-media thickness.

The trial drug used in this study is a novel long-acting depot preparation of testosterone requiring only four injections per year with an additional loading dose after the first 6 weeks, or matching placebo. The i.m. application of the trial drug, Nebido, which contains 1000 mg testosterone undecanoate in 4 ml castor oil, has been shown to maintain the testosterone level within the physiological range for about 12 weeks while largely avoiding non-physiological troughs and peaks (26).

Statistical analysis and power calculation

The study was powered on the basis of the primary endpoint. We envisaged that most of the patients recruited for this trial would have moderate-to-severe angina. From previous studies in a similar patient group (14, 15), we anticipated a treatment effect of around 75 ± 50 s improvement in time to 1 mm ST segment depression. Therefore, assuming a treatment effect delta of 75 ± 50 s, for a parallel placebo controlled trial with 5% significance and 80% power, seven patients would be required in each arm. Randomisation was determined by computer-generated random numbers. All data were tested against a normal distribution with Kolmogorov–Smirnov tests. Data are presented as mean $\pm$ S.D. with analysis based on intention to treat with data carried over from previous visits in the case of patient withdrawal. The treatment effect was defined as the difference between the active treatment delta from baseline and placebo treatment delta from baseline. Data sampled at baseline, 3, 6 and 12 months were analysed by univariate analysis of the variance using a general linear model (SPSS version 14). The dependent variable tested was the change from baseline (e.g. time to ST segment depression); the fixed variables were treatment group and time of sampling. The model incorporated an adjustment for subject effect since the baseline covariate was confounded with the subject variable. The main outcome was the effect of testosterone treatment on time to ischaemic threshold and *post-hoc* *t*-tests were calculated for the primary outcome. Parameters sampled twice (questionnaire data) were analysed with the Mann–Whitney *U*-test. Correlations were tested with Pearson's correlation coefficient for parametric data. Baseline data were compared with unpaired *t*-tests for parametric data and Fisher's exact test for 2×2 categorical data. In all analyses significance was sought at the 5% level.

Results

A total of 15 patients were randomised following the run-in period, seven to placebo and eight to testosterone. Two patients withdrew early in the trial before any outcome assessment could be made, therefore complete data was available on 13 patients. Nebido injections were well tolerated and no patient suffered any major adverse event or unplanned hospital admission. Baseline data are shown in Table 2. The two groups were well matched. The gonadotrophin levels were higher in the placebo group because two patients were found to have manifest hypergonadotrophic hypogonadism with elevated FSH and LH levels. All other patients had a mixed picture with low total testosterone and gonadotrophins in the normal range. Results are presented in Table 3; the treatment effect given as change from the baseline of each time point and analysis of the variance for the full-time period are presented separately. Total testosterone increased and gonadotrophins decreased appropriately with testosterone therapy. Testosterone levels in the treatment group were elevated but maintained within the physiological normal range. The mean trough levels of total testosterone achieved in the treatment arm were $+16.1 \pm 6.7$ nmol/l at week 14, $+19.0 \pm 7.8$ nmol/l at week 26 and $+16.0 \pm 7.3$ nmol/l at week 52. The highest single level of total testosterone was 32 nmol/l measured in one patient at week 14. Maximal exercise time, time to 1 mm ST depression and peak metabolic equivalents (METS) achieved all increased testosterone treatment compared with placebo, although a significant effect was only maintained for the whole year in time to ST depression and peak METS. At 12 months, the effective increase in time to ST depression was 117.8 ± 21 s giving 95% confidence limits of (72–164 s; Fig. 1). The increase in time to ST segment depression correlated positively with change in total testosterone at all points of assessment ($rP < 0.8$, $P < 0.01$ in each case). Testosterone treatment was associated with small but significant increases in haemoglobin and haematocrit and small decreases in BMI and serum triglycerides; the decreases in total mass and waist-to-hip ratio approached but did not meet acceptable levels of significance. There were no differences in the effect of testosterone treatment on hsCRP, total cholesterol, high-density lipoprotein cholesterol, fasting glucose and insulin or

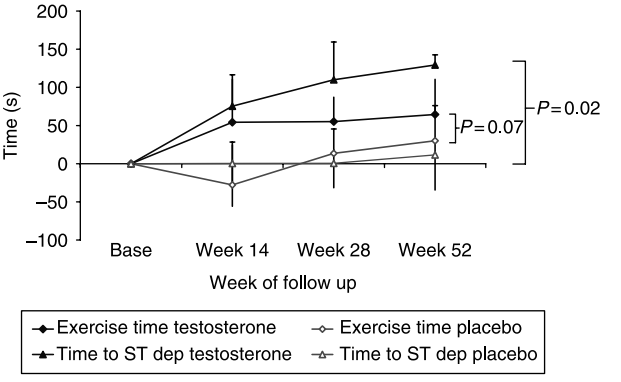

Figure 1 Impact on exercise time and time to 1 mm ST segment depression (analysis by ANOVA).

serum PSA but serum triglycerides in the treatment group were noted to decrease.

There was no difference between the testosterone treatment group and placebo on quality of life measures. Treatment effect as measured by EuroQol (-0.9 ± 1.9, $P=0.3$), Beck Depression Inventory (0.8 ± 4.8, $P=0.3$) and Seattle Angina Score (2.1 ± 7.6, $P=0.1$) appeared unchanged.

In both measures of carotid atheroma (CIMT), the testosterone group appeared to have greater reductions of CIMT than the placebo treated group, however in the full ANOVA analysis the effect of testosterone treatment on CIMT only approached required levels of significance ($P=0.16$ and $P=0.18$).

Discussion

In this study, we have demonstrated that testosterone therapy significantly delays time- to exercise-induced MI. This is consistent with the findings in our previous studies; however, in the present study the clinical effect was maintained and sustained for a longer period of 12 months. This is an important point since previous studies of the effect of testosterone on MI have been of short duration – in some cases comprising only a single i.v. dose of testosterone (27). No trial has followed up patients beyond 3 months (15). There did not appear to be a decrement in the treatment effect of testosterone on ischaemia, a concern that was raised in our previous trials of testosterone in heart failure (17) and vascular reactivity (16). Therefore, we conclude that the anti-ischaemic effect of testosterone is consistent and maintained without tachyphylaxis for as long as the treatment is continued. Recent *in vitro* studies have demonstrated that testosterone inhibits L-type calcium channels (28), which is the same site of action as the dihydropyridine calcium antagonist nifedipine, and we postulate that this may be the mechanism of its anti-ischaemic action (29, 30). Its inhibitory effect appears to be of the same order of magnitude as nifedipine.

Our data on the effect of testosterone therapy on carotid atheroma are of significant potential interest. Numerous animal models have demonstrated that physiological testosterone replacement therapy prevents progression of established atheroma and development of nascent atheroma (11, 21). This outcome has never been reported in prospective randomised clinical trials of testosterone in humans. Our data show that CIMT measures during replacement therapy with testosterone were reduced compared with placebo, although the effect did not meet accepted standards of clinical significance. It is likely that our data are considerably underpowered for such an outcome since the changes in CIMT in absolute terms were small and the technique is subject to considerable inter- and intra-observer variability. Although in this study our reductions in CIMT are not statistically significant, they support the need for a prospective evaluation of testosterone therapy on the effect of atheroma in humans.

In conclusion, the anti-ischaemic effect of testosterone in men is consistent (in numerous studies) and durable. The next question to answer is whether the testosterone also reduces vascular events and atheroma progression.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. The authors designed, conducted and analysed the results and wrote the paper. The financial sponsors had no editorial input over this manuscript.

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Author contribution statement

Atish Mathur screened and recruited the study patients with the help of Basil Saeed and Rangasamy Muthusamy.

Chris Malkin collected and analysed the trial data and wrote the first draft of the paper.

T Hugh Jones helped design trial protocol, obtained funding and edited the final manuscript.

Kevin Channer designed protocol, obtained ethics, regulatory approval and funding, edited the final manuscript and is the guarantor and corresponding author of the paper.

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