ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

ORIGINAL ARTICLE

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Keywords:
angina, biomarkers, coronary artery disease, testosterone

Received: 17-Aug-2015
Revised: 17-Feb-2016
Accepted: 22-Feb-2016

doi: 10.1111/andr.12189

Revascularization with percutaneous coronary intervention does not affect androgen status in males with chronic stable angina pectoris

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SUMMARY

There is a clear association between low serum testosterone and coronary artery disease (CAD) in men. Hypotestosteronaemia is associated with accelerated atherosclerosis and a quarter of men with CAD are biochemically hypogonadal. Amongst those with CAD, hypotestosteronaemia is associated with increased mortality. Testosterone vasodilates coronary arteries, and exogenous testosterone reduces ischaemia. Whether hypotestosteronaemia is a cause or a consequence of CAD remains unanswered. The aim of this prospective observational study was to investigate whether coronary revascularization affected androgen status in men with stable angina pectoris. Twenty five men (mean age 62.7, SD 9.18) with angiographically significant CAD and symptomatic angina underwent full coronary revascularization by percutaneous coronary intervention. Androgen status and symptoms of angina, stress, depression and sexual function were assessed before, and at one and 6 months after the coronary revascularization. All patients underwent complete revascularization which was associated with a significant reduction in angina symptoms and ischaemia. No significant difference was seen in total testosterone (11.33 nmol/L baseline; 12.56, 1 month post; 13.04 at 6 months; $p = 0.08$). A significant and sustained rise in sex hormone-binding globulin was seen (33.99 nm/L baseline; 36.11 nm/L 1 month post PCI; 37.94 nm/L at 6 months; $p = 0.03$) Overall, there was no significant alteration in any other marker of androgen status including free testosterone or bioavailable testosterone. There was no change in symptoms of anxiety, depression or sexual function. Coronary revascularization has no sustained effect on androgen status. This supports the hypothesis that hypotestosteronaemia is not a consequence of angina pectoris or myocardial ischaemia.

INTRODUCTION

Irrespective of regional variations in the prevalence of coronary artery disease (CAD), men are more than twice as likely to develop and die from CAD compared with women. Female sex hormones are known to be cardio-protective in women, whereas testosterone is widely regarded as deleterious to the male cardiovascular system. The latter concept is increasingly being challenged. In fact, physiological concentrations of testosterone appear to be cardioprotective in men (Ohlsson *et al.*, 2011). Male CAD is associated with low serum testosterone and one quarter of men presenting with CAD have serum testosterone levels compatible with hypogonadism (Phillips *et al.*, 1994; Akishita *et al.*, 2007; Malkin *et al.*, 2010; Park *et al.*, 2012). Atherosclerosis is accelerated in those with congenital and iatrogenic hypogonadism (The Coronary Drug Research Project, 1973; Bojesen *et al.*, 2006; Tsai *et al.*, 2007), and in those with established CAD, low testosterone is associated with increased mortality and excess adverse cardiovascular events (Wehr *et al.*, 2011; Lerchbaum *et al.*, 2012). Furthermore, testosterone supplementation has been shown to relieve ischaemia using objective functional measures of ischaemia (Rosano *et al.*, 1999; English *et al.*, 2000b), an effect which is greatest in those with the lowest baseline androgen profiles (Malkin *et al.*, 2004; Mathur *et al.*, 2009).

Laboratory studies have demonstrated the vasodilatory effects of testosterone in male animal models (Chou *et al.*, 1996; Yildiz *et al.*, 2005; Seyrek *et al.*, 2007). In the cardiac catheter laboratory, exogenous testosterone administration increases coronary arterial blood flow and cardiac output in areas supplied by unobstructed coronary arteries (Webb *et al.*, 1999; Jones *et al.*, 2002; Thompson *et al.*, 2002). Coronary arterial smooth muscle

contains androgen receptors, activation of which, results in calcium channel antagonism and local nitric oxide release (Jones *et al.*, 2004). However, some remain sceptical regarding testosterone's cardioprotective properties and, to date no studies have prospectively investigated the effects of exogenous testosterone therapy (or replacement in hypogonadal individuals) in men with CAD on survival. Whether hypotestosteronaemia represents a cause or a consequence of CAD remains an important, controversial, and unanswered question. Little has been established regarding causality in this relationship (Morris & Channer, 2012).

This study addresses this question by investigating whether the treatment of coronary stenosis; improving myocardial ischaemia; and alleviation of the clinical symptom of angina, leads to an increase in circulating serum testosterone levels. A positive effect supports the hypothesis that hypotestosteronaemia is a consequence of CAD as opposed to an aetiological factor. It is also possible that low testosterone levels are because of inflammation from atherosclerosis and/or visceral fat in these patients and therefore unaffected by revascularization. Low testosterone levels are associated with a more favourable cytokine profile, which is associated with atherogenesis (Pugh *et al.*, 2003). Correcting angina may increase physical and sexual activity and lead to weight loss which may all increase testosterone levels.

Research question: Does alleviation of myocardial ischaemia and symptomatic angina pectoris increase testosterone levels in men with angina pectoris?

METHODS

Study design and ethics

This was a single centre, prospective, observational study carried out at the South Yorkshire Cardiothoracic Centre, Sheffield, UK. Favourable opinion was granted by the National Health Service National Research Ethics Service (Ref 06/Q2308/138).

Patient population

Male patients referred for elective coronary PCI were recruited. The sample size was chosen to have 80% power at the two sided 5% level to detect a difference of 0.5 nmol L^{-1} in bioavailable testosterone with SD of 1.0 nmol L^{-1}. Inclusion criteria were as follows: symptomatic angina of greater than 3 months' duration, a positive functional study for myocardial ischaemia and angiographic evidence of coronary artery stenosis ≥70% who were suitable for full revascularization by PCI. Exclusion criteria were: previous myocardial infarction, previous PCI or CABG, ventricular dysfunction, left main coronary artery stenosis, chronic total occlusion of any vessel, treatment within 2 years with glucocorticoid therapy, spironolactone, digoxin, androgen or oestrogen therapy, renal insufficiency, and a CRP level of greater than 10 mg/L. Informed consent was gained from each participant prior to entry to the study.

Clinical protocol

Participants underwent an initial assessment 1 month prior to the date of the PCI (time point 1, TP1). All patients had venous blood samples drawn between 08.00 and 10.00 for measurement of total serum testosterone (TT) and serum sex hormone-binding globulin (SHBG). These measurements were repeated on the morning of the PCI, again between 08.00 and 10.00 prior to arrival at the catheter laboratory (time point 2, TP2). Participants were advised to eat a light breakfast on the morning of any study contact prior to venepuncture.

PCI was carried out at the discretion of the operator according to usual clinical practice. Any patient who at the time of the procedure was deemed to be unsuitable for full percutaneous revascularization, or where the final result was sub-optimal was withdrawn from the study. Post-operative care, medical therapy and discharge arrangements proceeded in accordance with standard practice. All patients received dual antiplatelet therapy (aspirin 75 mg daily, plus either clopidogrel 75 mg daily or prasugrel 10 mg daily) for a period of 12 months post PCI, as well as other medications, including lipid lowering therapy. No changes to lipid lowering therapy were made during the course of the study period.

Subjects were recalled at 1 month (time point 3, TP3) and 6 months (time point 4, TP4) after PCI for repeat measurements of the blood indices. Blood samples were also drawn at the same time of day and sent for immediate analysis.

A sub-set (n = 15) of the subjects were invited to complete three symptom questionnaires at the index visit (TP1) and again at the final study visit (TP4). These standard, validated questionnaires were designed to establish whether there was any subjective change in angina Seattle Angina Questionnaire (SAQ) (Spertus *et al.*, 1995), anxiety and depression Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983) and sexual function Brief Sexual Function Inventory (BSFI) (O'Leary *et al.*, 1995). A separate cohort also underwent body fat analysis at TP1, using the Bodystat 1500 bioelectrical impedance body composition analyser single frequency (Bodystat Ltd, Douglas, Isle of Man), and repeated exercise tolerance tests (ETT) at TP3 and TP4.

Blood samples were analysed using the Elecsys electrochemiluminescence Testosterone II assay (Roche Diagnostics GMBH, Basel, Switzerland), with an expected normal range of 8.64–29.0 nmol/L for testosterone and 19.3–76.4 nmol/L for SHBG in males over 50 years old. Free testosterone (FT) was calculated by the Vermeulen equation (Vermeulen *et al.*, 1999). Bioavailable testosterone (BioT) was calculated using the formula described by Morris *et al.* (2004).

Statistical analysis

Statistical analysis was performed using IBM SPSS for Windows version 21.0 (IBM Corporation, Armonk, NY, USA). A Shapiro–Wilks test and visual inspection of Q–Q plots were performed to confirm that the data were normally distributed. Repeated measures ANOVA using the general linear model, using a Greenhouse-Geisser correction was used to establish whether there was a significant change in TT, FT and BioT during the course of the study. Wilcoxon signed rank analysis was performed on the questionnaire responses to establish any significant change in responses from baseline to post-PCI.

RESULTS

Subject demographics

A total of 30 male patients between the age of 18 and 75 years were recruited. Twenty-five patients completed the study. Five

patients did not attend follow-up appointments. Baseline characteristics are shown in Table 1.

Full revascularization was satisfactorily achieved in all patients. All stents used with the exception of one were drug eluting. No patients required repeat revascularization, and there were no deaths during the study period. Five subjects performed poorly during ETTs as a result of musculoskeletal pain, dyspnoea and poor fitness and hence were not asked to complete a second study. One declined a second ETT. Three post-PCI exercise tests demonstrated evidence of ischaemic ECG changes, these patients all underwent early repeat angiography demonstrating no evidence of flow-limiting stenoses in major epicardial arteries.

Androgen results

At baseline, 12 subjects (46%) demonstrated a measured serum total testosterone of lower than 11 nmol/L. Although there was a trend of a reduction in androgenization from baseline to the date of PCI, only SHBG showed a significant and sustained rise over the study period (Table 2, Figs 1–4). There were no other significant changes in between individual time points. For those with a low testosterone estimation at baseline ($n = 12$), no significant change in any androgen marker was seen ($p = 0.618$ for TT, $p = 0.858$ for SHBG).

Questionnaire data (TP1 and TP4)

Valid paired responses were collected for 15 participants (Table 3). On the HADS scale, no significant difference was seen in overall scores ($p = 0.74$), demonstrating no significant change in indicators of anxiety or depression from the time of recruitment to 6 months post PCI.

A significant improvement was seen in the Seattle Angina Questionnaire, both in the overall questionnaire ($p < 0.001$) and in the activity domains ($p = 0.001$).

Fourteen respondents answered the BSFI questionnaire (one declined), and no significant difference was observed between time points ($p = 0.052$) for indicators of sexual function.

Table 1 Subject demographics

	Mean (SD)
Age (years)	62.7 (9.2)
Mean ETT time at TP3 ($n = 15$) (min)	8.3 (2.6)
Mean ETT time at TP4 ($n = 9$) (min)	9.6 (1.4)
Height at TP1 ($n = 21$) (cm)	174 (5.1)
Weight at TP1 ($n = 21$) (kg)	83 (11.7)
BMI at TP1 ($n = 21$) (kg/m^2)	27.5 (3.2)
Body fat at TP1 ($n = 23$)	21.2% (11.4%)
Number of stents deployed	1.9 (1.4)

BMI, body mass index; ETT, exercise tolerance test.

DISCUSSION

The results of this observational study demonstrate a significant and sustained increase in SHBG levels from PCI up to 6 months post procedure, but not in the other androgen levels measured. Questionnaire respondents reported an improvement in angina symptoms after revascularization but there was no change in the anxiety and depression scale scores, or improvement in sexual function.

Consistent with other studies in this population, mean testosterone and SHBG levels were at the lower end of those observed in age-matched populations, although not at a level at which testosterone replacement is recommended (Dai *et al.*, 1981; Ross *et al.*, 1986; English *et al.*, 2000a; Bhasin *et al.*, 2006; Rosano *et al.*, 2007; Spitzer *et al.*, 2013). 46% of subjects demonstrated a TT compatible with a diagnosis of hypogonadism at baseline, however, it must be noted that this study was not powered to perform subgroup analysis. The mean patient age for this study was 62.7 years, and it is well recognized that serum testosterone levels decline with advancing age (Kaufman & Vermeulen, 1997).

Clinical records, SAQ results and exercise tolerance testing data show that PCI was successful in restoring blood flow and improving or alleviating the symptoms of angina. This is consistent with current evidence in the management of stable symptomatic CAD (Strauss *et al.*, 1995; Boden *et al.*, 2007). There was no evidence that the rates of reported anxiety and depression symptoms were reduced following PCI. Sexual function was similarly unaltered.

The pattern of change in serum TT, FT and SHBG levels was interesting. The magnitude of change seen between time points 1 & 2 was larger than expected given the controlled sample timing, and there was a wide range of individual values demonstrated at all points. It is possible that the dip in TT and FT levels seen at TP2 may represent an anticipatory stress response prior to the PCI procedure (Smith *et al.*, 2005, 2006). Endogenous testosterone is largely (50–70%) transported tightly bound to SHBG. The purpose of SHBG is thought to be to maintain the hormone in a biologically inactive state until presentation at the target cell. A further 20–30% circulates bound loosely to albumin and only 1–3% of the hormone is free in the serum. The physiologically active hormone (bioavailable testosterone) consists of the albumin-bound and free portions of the hormone. Testosterone has a circadian and circannual variation with peak levels in the morning and in the spring-summer (Dai *et al.*, 1981; Clair *et al.*, 1985; Dabbs, 1990; Brambilla *et al.*, 2009). Sampling in this study was performed throughout the year, and not confined to a single season. The performance data from the assay report an intermediate precision coefficient of variance of no more than 8.4%, and hence the observed variations are not attributed to analytical inaccuracy.

Table 2 Androgen status [mean (SD)]

	TP1 ($n = 26$)	TP2 ($n = 30$)	TP3 ($n = 27$)	TP4 ($n = 25$)	p Value
Total testosterone (nmol/L)	11.8 (4.2)	11.33 (4.66)	12.56 (5.63)	13.04 (4.92)	0.08
SHBG (nmol/L)	33.99 (10.70)	33.85 (10.74)	36.11 (11.65)	37.94 (13.80)	0.03
Free testosterone (Vermeulen) (nmol/L)	0.240 (0.09)	0.223 (0.08)	0.251 (0.09)	0.243 (0.08)	0.186
BioT	3.72 (1.18)	3.42 (1.30)	4.01 (1.27)	3.88 (1.29)	0.107

Total testosterone, SHBG, free testosterone, LNBioT.

Figure 1 Total testosterone. Small, non-significant rise in total testosterone across the study period.

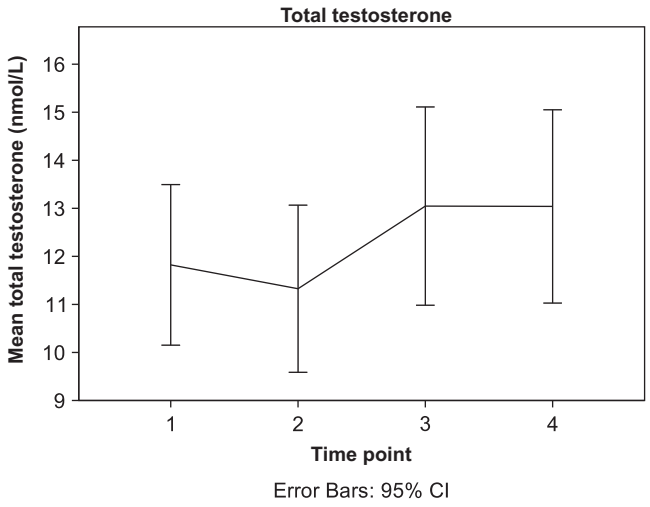


Figure 2 Sex hormone-binding globulin (SHBN). Sustained rise in SHBG across the study period.

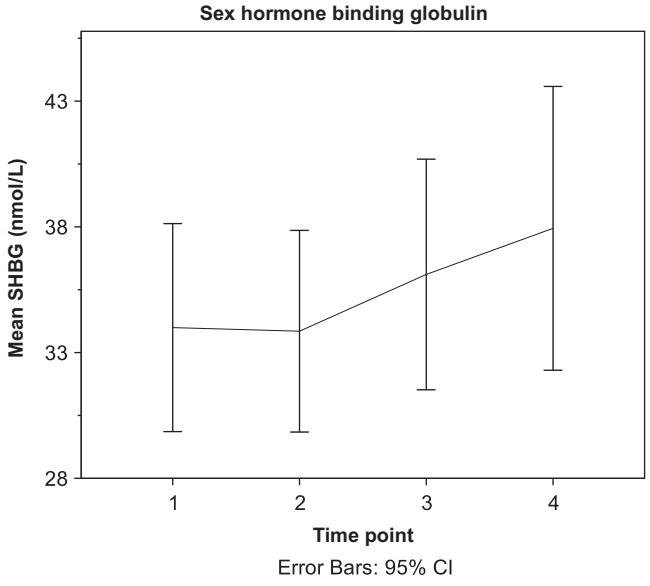


Figure 3 Free testosterone (Vermeulen). No significant change in Free T across the study period.

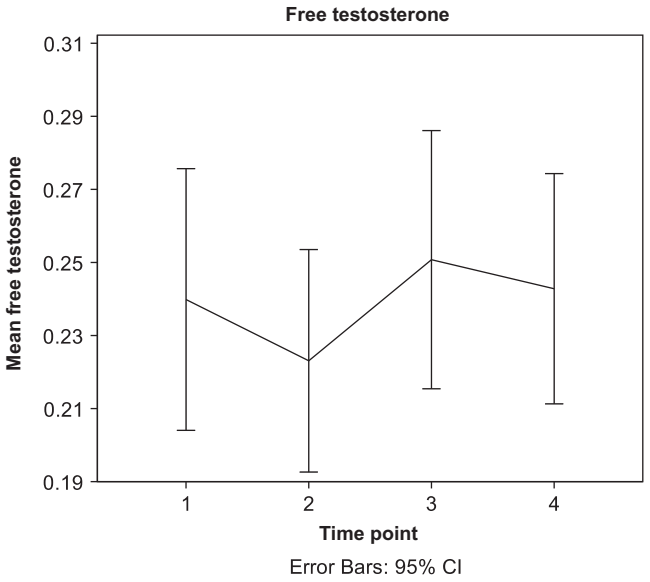
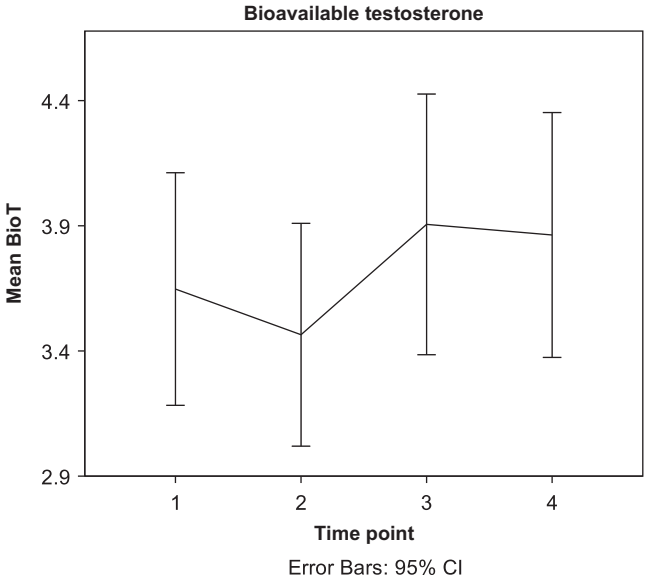


Figure 4 Bioavailable testosterone. No significant change in bioavailable testosterone across the study period.



Testosterone measurements were all taken within a 2-h period during the day (between 08.00 and 10.00) because the diurnal variation in testosterone levels is well recognized, with mid to late afternoon levels 30–35% lower than levels at 08.00 in young men, and at least 10% in older men (Clair *et al.*, 1985; Diver *et al.*, 2003; Brambilla *et al.*, 2009). SHBG levels may show a similar but smaller pattern of diurnal variation (Plymate *et al.*, 1989; Diver *et al.*, 2003).

The rise in SHBG levels during the study, which was significant and sustained may be explained by the alleviation in angina resulting in increased physical activity, weight loss and increased skeletal muscle activity. Performance in the exercise tests performed post PCI was good for those subjects that completed them when compared with an asymptomatic population (Leon *et al.*, 1981). All subjects had a positive exercise test for ischaemia prior to entry into the study, whereas only three who completed the subsequent assessments demonstrated ischaemic ECG changes. The subjects were overweight at baseline (mean BMI 27.5), and although this was not measured subsequently, any reduction in this may have contributed to the increase in SHBG. SHBG has been associated with increasing age and intake of dietary fibre (Longcope *et al.*, 2000). It also shows a negative correlation with abdominal obesity (Gapstur *et al.*, 2002). Subjects were enrolled in the study for a total of 7 months, which is unlikely to be contributory, however, changes in diet because of increased awareness of cardiovascular health cannot be excluded, as these were not controlled for in this observational study.

Limitations of this study include the relatively modest sample size and failure to record details of usual diet (although a

Table 3 Questionnaire responses [mean (SD)]

	TP1	TP4	*p*
Hospital Anxiety and Depression Score	2.8 (0.7)	2.9 (0.5)	0.739
Seattle Angina Questionnaire (overall)	3.7 (1.6)	4.5 (1.1)	0.001
Seattle Angina Questionnaire (activity)	3.8 (1.6)	4.4 (1.2)	<0.001
Brief Sexual Function Inventory	2.4 (1.0)	2.9 (1.0)	0.052

light breakfast on the morning of sampling was advised), and the presence of diabetes mellitus or smoking, both of which can impact upon testosterone levels (Svartberg & Jorde, 2007; Ukkola *et al.*, 2013). The interaction between diet and testosterone levels is poorly understood. Studies have reported higher testosterone concentrations on a high carbohydrate diet compared with a high protein diet after 10 days. Postprandial testosterone concentrations have also been shown to be significantly decreased after a fat-rich meal (Volek *et al.*, 2001). As described above, a diet high in fibre is associated with increased SHBG levels.

The lack of sustained increase in total testosterone levels does not conclusively demonstrate that low serum testosterone is not a direct consequence of coronary artery atherosclerosis. PCI is a mechanical macrovascular treatment which does not in itself reverse or remove the underlying atherosclerotic process. All the patients in the study cohort were on optimal medical therapy prior to referral for PCI and initial measurement. Another possible factor is that in males with established, angiographically significant coronary disease may have a degree of microvascular disease, or myocardial ischaemic conditioning, which could influence testosterone levels. It may still be the case that the presence of CAD has a causative role in hypotestosteronaemia, but that macrovascular revascularization cannot reverse this. Given the other experimental evidence available however, it would seem more likely that low testosterone is a cause of CAD rather than the reverse. This work is hypothesis generating but further work is required before the long-standing *cause or consequence* question is conclusively answered.

CONCLUSIONS

This observational study did not demonstrate any sustained change in androgen status in a group of males with CAD undergoing revascularization by PCI. PCI was successful in fully restoring epicardial coronary flow and alleviating the clinical symptoms of angina pectoris in all cases. In failing to demonstrate an effect on androgenization, this supports the hypothesis that low serum testosterone may be a causative or at least contributory factor in the development of CAD rather than a consequence, although this cannot be inferred conclusively from these data.

STATEMENT OF FUNDING

Biochemical assays for this study were funded by the Royal Hallamshire Hospital Cardiology Research Fund. No other sources of funding were used.

CONFLICTS OF INTEREST

The authors report no financial relationships or conflicts of interest regarding the content herein.

STATEMENT OF CONTRIBUTION

JNG, TN, PDM, KSC and EDG were involved in the setup and implementation of this study, including protocol design and ethical application preparation. JNG, PC, YP were involved in data collection. JNG, YP, PDM and EDG were involved in the statistical analysis of data.

All authors have been involved in the preparation of this manuscript and have approved the final version.

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