Effect of testosterone on ex vivo vascular reactivity in man

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ABSTRACT

Testosterone is reported to have an acute vasodilating action in vitro, an effect that may impart a favourable haemodynamic response in patients with chronic heart failure. However, the effect of chronic testosterone exposure on general vascular reactivity is poorly described. In the present study, fresh subcutaneous resistance arteries were obtained from patients with heart failure (n = 10), healthy controls (n = 9) and men with androgen-deficiency (n = 17). All arteries were studied using a wire myograph to examine the effect of cumulative additions of testosterone (1 nmol/l–100 μmol/l) compared with vehicle control following maximal pre-constriction with KCl (1–100 μmol/l). The vascular reactivity of arteries from androgen-deficient patients was examined further by recording tension concentration curves to cumulative additions of noradrenaline (1 nmol/l–100 μmol/l) and U46619 (1–300 nmol/l), followed by relaxation concentration curves to additions of ACh (acetylcholine; 10 nmol/l–30 μmol/l) and SNP (sodium nitroprusside; 10 nmol–30 μmol/l) respectively. In all cases, statistical analysis was performed by ANOVA. Patients with proven androgen-deficiency were treated according to clinical recommendations for a minimum of 3 months and further arteries (n = 19) were taken for experimentation using the same protocol. In all groups, testosterone was confirmed to be an acute concentration-dependent vasodilator at concentrations ≥1 μmol/l (P = 0.0001). The dilating effect of testosterone was augmented in patients with androgen-deficiency prior to treatment, and this effect was abrogated following appropriate testosterone replacement. Testosterone therapy significantly reduced the normal vascular dilating response to ACh and SNP (P < 0.01) and significantly increased the contractile response to noradrenaline (P < 0.01), but not U46619. Testosterone is an acute dose-dependent vasodilator of resistance arteries. Physiological testosterone replacement attenuates general vascular reactivity in androgen-deficient subjects. The numerous perceived benefits of testosterone replacement may be offset by a decline in vascular reactivity and, therefore, further studies and careful monitoring of patients is recommended.

INTRODUCTION

Testosterone is recognized to have important effects on metabolism and vascular behaviour beyond the accepted effects on secondary sexual characteristics. Male sex is a risk factor for vascular disease, but this is not explained by endogenous levels of testosterone or coincident cardiovascular risk factors [1]. High-dose therapy of anabolic androgens is detrimental to the cardiovascular system and causes impaired insulin resistance, dyslipidaemia, reduced vascular reactivity and myocardial hypertrophy [2]; effects that may explain reports of premature myocardial infarction and stroke in subjects using these drugs. However, physiological testosterone therapy

Key words: heart failure, hypogonadism, myography, testosterone, vascular reactivity.
Abbreviations: ACh, acetylcholine; NA, noradrenaline; PSS, physiological saline solution; SNP, sodium nitroprusside.
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is associated with some beneficial effects on the cardiovascular system and has been used with some success to treat the symptoms of patients with stable angina [3] and chronic heart failure [4,5]. In addition, testosterone replacement inhibits atherogenesis in experimental male animals [6], possibly due to effects on atherogenic pro-inflammatory cytokines [7]. The vascular effects of testosterone have been described in part; laboratory investigations on isolated ex vivo vessels have confirmed testosterone to be a vasodilator of pre-constricted arteries in the coronary, pulmonary and mesenteric vascular beds [8–10]. This acute vasodilating effect is endothelium-independent, is not mediated by conversion into 17β-oestradiol by the enzyme aromatase and does not involve the classical genomic androgen receptor [11,12]. In addition, the acute dilating effects of testosterone are not abrogated in the presence of inhibitors of dilator prostanoids, NOS (nitric oxide synthase) or guanylate cyclase, or other endogenous vasodilators [8,13]. Current evidence suggests that testosterone exerts acute vasodilating effects through a transmembrane calcium channel receptor in the smooth muscle cell membrane [14,15]. Although the effects of testosterone in these studies are seen in the supra-physiological (micromolar) range, we have recently confirmed that testosterone has a significant and measurable effect on trans-membrane calcium ion channels in the physiological (nanomolar) range [16] and, indeed, in vivo data exist to support the notion that testosterone within the physiological range is a coronary vasodilator [17]. This vasodilator effect in the coronary, pulmonary and systemic vascular beds may explain some of the benefit derived with testosterone observed in patients with angina [3,18] and heart failure [5]. Heart failure in particular is a syndrome of chronic vasoconstriction and in vivo haemodynamic data show that testosterone increases cardiac output as a consequence of reducing peripheral artery tone [19].

The literature is unclear on the effect of chronic testosterone treatment on general vascular reactivity. Vascular reactivity is not easily defined; in health, the vascular system responds quickly to changes in haemodynamic status by an alteration in tone (either by constriction or dilation). An impaired vascular response is a precursor and a marker of atherosclerotic disease. Vascular reactivity can be tested experimentally by stimulating a vessel with known agents and measuring the response from baseline. The effect of testosterone is controversial, as high dose anabolic androgens do consistently appear to worsen vascular reactivity to test agonists in males and females [20,21]. Physiological testosterone therapy also appears to reduce vascular reactivity [22], although when compared with normal controls the effect is actually that of restoring a potentially exaggerated vascular reactivity to normal [23].

The present study has been designed in two parts. The first was to confirm that the reduction of peripheral resistance observed with testosterone therapy in male patients with chronic heart failure in vivo is due to vasodilation in peripheral resistance arteries. The second was to assess whether physiological testosterone therapy given for a minimum of 3 months had any effect on vascular reactivity.

**METHODS**

**Isolation of study vessels**

In all experiments, subcutaneous resistance arteries were taken from study patients for assessment on a wire myograph. All subjects provided written informed consent, and the procedure was approved by the Local Regional Ethics Committee. Resistance vessels were harvested using gluteal skin biopsy. In this procedure, local anaesthetic was injected into the skin and subcutaneous fat on the postero-lateral aspect of the buttock approx. 15 cm below the waist. Using strict aseptic technique, two elliptical incisions were made into the anaesthetized skin, a 2 cm × 1 cm × 1 cm piece of skin and fat was removed and immediately plunged into ice-cold PSS [physiological saline solution; 120 mmol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l CaCl2, 1.17 mmol/l MgSO4, 25 mmol/l NaHCO3, 1.18 KH2PO4, 26.9 μmol/l EDTA and 5.5 mmol/l glucose (pH 7.4)]. The wound was sutured with braided absorbable sutures and sprayed with a liquid plastic wound sealant. Finally, a light dry dressing was applied. Subjects were asked to remain in the Department for 30 min for a period of observation. Fresh tissue was immediately transported to the laboratory and examined under a microscope. Vessels were carefully dissected free and from the surrounding connective tissue and placed in ice-cold PSS, and the vessels were cut into segments < 2.5 mm prior to use.

**Small vessel myography**

Isometric myographic studies were performed using the 610M automated wire myograph (Danish Myo Technology), in which study vessels are held between two jaws, suspended on two wires passed through the lumen of the vessel. The technique of wire myography is commonly utilized in studies of vascular reactivity and has been described in detail previously [9]. In brief, individual resistance vessels were mounted on two 40 μm stainless steel wires in the jaws of the myograph and bathed in 7 ml of PSS. The tension at which the vessel is held has a direct relationship with the response to test agonists. The study vessels were therefore held at a standardized tension of 100 mmHg to mimic mean arterial blood pressure. Length–tension curves were obtained with the myograph software, and normalization of the vessels to physiological tension was performed by distending the vessel in stepwise fashion on the myograph and recording changes in tension readings on the digital
display; the $P_i$ (effective pressure) of the vessel was calculated by the law of Laplace. When a resting tension of 100 mmHg was achieved, the jaws were held in this position for the duration of the experiment, and the vessels were left in PSS to equilibrate for 30 min after the tension was set before experimentation in physiological saline solution. Subsequent responses were recorded as changes in tension transmitted through the myograph pressure transducer.

**Drugs and solutions**

KCl, NA (noradrenaline), ACh (acetylcholine) and SNP (sodium nitroprusside) were dissolved in deionized water to the required concentration. U46619 (a thromboxane A2 analogue) was dissolved in 100 % ethanol. Testosterone was dissolved in 100 % ethanol to the desired concentration. All reagents were purchased from Sigma.

**Myograph protocol**

After 30 min of equilibration, a single dose of 70 $\mu$mol/l KCl was added to test the viability of the vessel (a minimum response of 1 mN was required). The vessels were washed with fresh PSS (7 ml) in the organ bath and left to equilibrate. Further test doses of KCl (70 $\mu$mol/l) were added until similar responses were seen in the vessels. Test additions of NA (70 $\mu$mol/l) and ACh (70 $\mu$mol/l) were made in sequence to ensure the endothelium had not been damaged during vessel dissection and mounting. The increase in tension with NA was recorded in mN, and the relaxation with ACh was recorded as a percentage relaxation from the maximum baseline achieved with NA. The vessels were then washed with further exchanges of PSS in the organ baths.

**Cross-sectional study of healthy men compared with men with chronic heart failure**

A cross-sectional comparison of the *in vitro* heart failure compared with men with chronic heart failure. Cross-sectional study of healthy men after 30 min of equilibration, a single dose of 70 $\mu$mol/l KCl was dissolved in 100 % ethanol. U46619 (a thromboxane A2 analogue) was dissolved in 100 % ethanol. Testosterone was dissolved in 100 % ethanol to the desired concentration. All reagents were purchased from Sigma.

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**Effect of chronic testosterone therapy for 3 months on vascular reactivity**

This was a descriptive study on the effect of testosterone replacement in hypogonadal males. A gluteal skin biopsy was taken on two occasions: prior to clinically indicated androgen replacement and after 3 months of continuous testosterone therapy. Patients were males (> 18 years of age) referred to the Endocrinology Clinic that were found to be androgen-deficient and for whom androgen replacement was deemed clinically indicated by a consultant endocrinologist. Two patients had primary gonadal failure with elevation of gonadotrophins above the normal range. Four patients had hypogonadotropic hypogonadism with low gonadotrophins [one with haemochromatosis and three with no underlying diagnosis and normal pituitary MRI (magnetic resonance imaging)]. The remaining patients ($n = 4$) had a mixed picture of hypogonadism with low serum testosterone and gonadotrophins within the normal range. All patients had total testosterone < 7.5 nmol/l and/or bioavailable testosterone < 2.5 nmol/l or had borderline results but were felt to be clinically hypogonadal because of symptoms.

Patients were excluded if the PSA (prostate specific antigen) was above the age-adjusted normal range or there was a history of malignancy or chronic inflammatory condition.

Androgen replacement was at the discretion of the consultant endocrinologist and was not subject to a trial protocol. Most patients were treated with intra-muscular testosterone esters (Sustanon 100; every 14 days), although two patients were transferred to a transcutaneous soluble testosterone gel preparation (Testagel) shortly before the second biopsy. All medication, including anti-anginal drugs and standard heart failure medication, were
Table 1  Baseline characteristics of the subjects with heart failure and healthy controls

Values are means ± S.E.M. P values were determined using unpaired Student t tests. ACE-I, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subjects with heart failure</th>
<th>Healthy controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Vessels harvested (n)</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.0 ± 5.9</td>
<td>30.5 ± 3.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>14.1 ± 1.4</td>
<td>20.7 ± 1.4</td>
<td>0.005</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/l)</td>
<td>4.5 ± 0.3</td>
<td>7.1 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.7 ± 0.4</td>
<td>4.8 ± 0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>123 ± 5.0</td>
<td>120 ± 4.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>73.3 ± 3.8</td>
<td>81.6 ± 2.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Mean vessel diameter (µm)</td>
<td>399 ± 22.1</td>
<td>451 ± 43.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean vessel load pressure (mmHg)</td>
<td>100.3 ± 1.3</td>
<td>102.8 ± 1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Drugs (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-I/ARB</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blocker/α-blocker</td>
<td>3/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretic</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium antagonist</td>
<td>1</td>
<td></td>
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</tr>
</tbody>
</table>

continued throughout the study and there was no period of pharmacological washout.

The experiment protocol was similar to that described above. Test doses of KCl (70 mmol/l), NA (70 mmol/l) and ACh (70 mmol/l) were used to confirm viability and presence of endothelium. The effect of testosterone on maximally pre-constricted arteries was compared with the ethanol vehicle control using the methodology as above. In addition to these studies, concentration–tension curves were recorded for cumulative additions of NA (1 nmol–100 µmol/l), followed by a concentration–relaxation curve to cumulative addition of ACh (10 nmol–30 µmol/l). After rinsing with PSS, a further concentration–tension curve was constructed to U46619 (1–300 nmol/l), followed by a final concentration–relaxation curve to SNP (10 nmol–30 µmol/l).

Data and statistical analysis

Data are means ± S.E.M., unless otherwise stated. Data were tested against a normal distribution with Kolmogorov–Smirnov tests; two and three group comparisons of myography data were performed utilizing a univariate ANOVA as part of the SPSS version 11.5 package. Post-hoc analyses were performed with Mann–Whitney U tests, where appropriate. Comparisons of the baseline data were performed using unpaired Student’s t tests.

RESULTS

Cross-sectional study of men with heart failure compared with healthy controls

The baseline data for the heart failure and control groups are shown in Table 1. The heart failure group were significantly older and their serum androgen levels were lower than those of the healthy controls.

There was no significant difference in the vessel responses to test additions of 70 µmol/l NA and 70 µmol/l ACh between the groups. The response of maximally pre-constricted vessels to testosterone or ethanol vehicle control is shown in Figure 1. Testosterone caused a dose-dependent acute vasodilation of the resistance arteries, although the effect was only seen in the micromolar (supra-physiological) range. There was no difference in the response to testosterone of vessels from patients with heart failure and healthy controls.

![Figure 1](image_url)  Dose–response curves for testosterone compared with vehicle control in vessels obtained from subjects with confirmed heart failure and healthy controls

Values are means ± S.E.M. CHF, vessels from subjects with confirmed heart failure (n = 10); healthy, vessels taken from healthy volunteers (n = 9); (T+), testosterone concentration–response curve; (C), ethanol control response curve; % relaxation, relaxation from maximal pre-constricted baseline.
Table 2  Patient characteristics before and after 3 months of testosterone administration in eight androgen-deficient men
Values are means ± S.E.M. P values were determined using unpaired Student t tests. ACE-I, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.5 ± 3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.9 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>146 ± 2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>79.3 ± 3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>5.6 ± 0.4</td>
<td>28 ± 3.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/l)</td>
<td>2.6 ± 0.4</td>
<td>7.3 ± 0.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sex-hormone-binding hormone (nmol/l)</td>
<td>33.7 ± 7.5</td>
<td>24 ± 3.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Vessels harvested (n)</td>
<td>17</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Vessel diameter (µm)</td>
<td>440 ± 17.1</td>
<td>334 ± 14.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Vessel tension (mmHg)</td>
<td>102 ± 0.7</td>
<td>103 ± 0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Co-morbid disease (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drugs (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-I/ARB</td>
<td>3</td>
<td></td>
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</tr>
<tr>
<td>β-Blocker</td>
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<tr>
<td>Calcium antagonist</td>
<td>4</td>
<td></td>
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</tbody>
</table>

Effect of chronic testosterone therapy for 3 months on vascular reactivity
Ten patients were recruited to the study and agreed to a baseline gluteal biopsy prior to treatment with testosterone therapy and a further biopsy after a minimum of 3 months of treatment. We failed to harvest any vessels from two of these patients and therefore these men where excluded from the analysis. Baseline and hormone data on the eight remaining men from whom the vessels were taken are shown in Table 2. Total testosterone and bioavailable testosterone increased appropriately with testosterone therapy, and the vessels taken in the follow-up biopsies had a statistically smaller diameter than the vessels taken from the initial biopsy.

Response to NA
Resistance vessels taken after testosterone replacement appeared to be more sensitive to NA (Figure 2). The P value derived from an ANOVA indicated significance throughout the dose–response curve, although individual post-hoc analysis of the response at the range of concentrations found no significant difference at each point. Therefore the apparent increased sensitivity to NA is present throughout the range of responses tested.

Response to ACh
Resistance vessels taken from men after androgen replacement appeared to be less sensitive to the dilating property of ACh (Figure 3). Again, individual post-hoc tests at single concentrations were not significantly different, although the P value derived from an ANOVA reflected an effect throughout the dose–response curve.

Response to U46619
There was no significant difference in the contractile response of the vessels in the study groups to U46619 (Figure 4).

Response to SNP
As with the response to ACh, vessels taken after testosterone treatment were less sensitive to the dilating effect of SNP (Figure 5). Post-hoc tests were only significant at the lower concentrations (30–100 nmol/l; Figure 5).

Response to testosterone
Consistent with previous results in this and other studies [8–16], testosterone was found to be an acute dose-dependent dilator of pre-constricted resistance vessels.

Figure 2  NA dose–response curves in vessels obtained before and after 3 months of testosterone therapy
Values are means ± S.E.M. P < 0.009 between the curves, as determined by ANOVA.
Figure 3  ACh dose–response curves in vessels obtained before and after 3 months of testosterone therapy
Values are means ± S.E.M. $P < 0.0002$ between the curves, as determined by ANOVA. % relaxation, relaxation from maximal pre-constricted baseline.

Figure 4  U46619 dose–response curves in vessels obtained before and after 3 months of testosterone therapy
Values are means ± S.E.M. $P = 0.6$ between the curves, as determined by ANOVA.

Figure 5  SNP dose–response curves in vessels obtained before and after 3 months of testosterone therapy
Values are means ± S.E.M. $P < 0.01$ between the curves, as determined by ANOVA. % relaxation, relaxation from maximal pre-constricted baseline.

However, the effect in the vessels from androgen-deficient men taken before testosterone replacement was augmented compared with that of healthy controls and patients with heart failure described in the cross-sectional element of the present study. Figure 6 shows the pooled data for the testosterone dose–response curves for the three groups (healthy controls, heart failure patients and androgen-deficient patients prior to treatment). There was a clear and statistically significant difference between the curve for the androgen-deficient men and the other
The results described in the present study are novel as this is the first time that small vessel myography has been used to test the effect of androgens in human subcutaneous resistance vessels. The ‘before and after’ treatment study on vascular reactivity has never been performed using the myograph technique, although several studies have used non-invasive assessment with ultrasound Doppler [22,23].

**Response to testosterone**

The present results have confirmed an acute dilating property of testosterone in three different populations of men, including a heart failure group. Although the concentration of testosterone required to induce this effect was supra-physiological, this phenomenon is common to myograph experiments. To develop a stable baseline, the vessels are intensely pre-constricted with very high levels of constrictor agonists (in the present study, KCl was used) and, as a consequence, very high levels of testosterone are required to have an effect. It is well recognized that discrepancies exist between the concentrations of agents required to produce effects in vitro and in vivo. For example, chromatokalin (a potassium-channel opener) induces a reduction in peripheral vascular reactivity in a concentration that is non-physiological, but this effect occurs at high concentrations that make the myograph technique impractical.

**DISCUSSION**

The results described in the present study are novel as this is the first time that small vessel myography has been used to test the effect of androgens in human subcutaneous resistance vessels. The ‘before and after’ treatment study on vascular reactivity has never been performed using the myograph technique, although several studies have used non-invasive assessment with ultrasound Doppler [22,23].

Two groups. Testosterone began to exert an effect at the same concentration (1 µmol/l), but the dilatory response was greater at each subsequent concentration.

The effect of chronic testosterone replacement on testosterone vascular response is shown in Figure 7. This curve demonstrates the difference between testosterone and the control vehicle (ethanol). Following androgen replacement, the response to testosterone is attenuated and is restored to the response observed in vessels from eugonadal healthy males and men with stable heart failure (approx. 100% relaxation at 100 µmol/l testosterone).
response due to peripheral dilation at concentrations 2–3 × 10⁻⁸ mol/l in vivo, whereas in vitro dilatory effects are only seen at concentrations at 1 × 10⁻⁶ mol/l [12].

Testosterone has been shown to increase cardiac output and reduce peripheral vascular resistance in men with stable heart failure [19] and, in this in vivo study, a noticeable effect was seen at high physiological concentrations of testosterone (40–60 nmol/l). There is now good evidence that testosterone inhibits voltage-gated calcium channels at physiological concentrations (to a low nanomolar range) using a patch-clamping technique [16]. Although the concentration of testosterone in the present myograph study is an order of magnitude greater than those in the in vivo study, it is still likely that the prime reason for the reduction of peripheral artery tone in vivo was due to testosterone-induced vasodilation.

The augmented response of vessels to testosterone from androgen-deficient men has never been reported previously in a myograph-based study. Few studies have reported the effect of gonadal status on the acute vascular response to testosterone. Jones et al. [12] found that the dose–response curve to testosterone was similar in the testicular-feminized mouse compared with littermate controls. The animals in this study [12] had significantly lower testosterone levels and lacked a functioning androgen receptor. Clinical studies in hypogonadal patients (exclusively using ultrasound analysis) have not investigated the acute vascular effect of testosterone. Extensive evidence has shown that the acute diluting effect of testosterone is due to an interaction with a vascular smooth muscle non-genomic trans-membrane-bound calcium channel [8,15,16]. From the results of the present study, it can be postulated that this channel is up-regulated or more sensitive in a state of androgen deficiency and that this effect is reversed with testosterone replacement. As testicular-feminized mice (with low androgen levels) have a similar dose–response curve to testosterone as littermate controls with normal androgen levels, this suggests that the classical androgen receptor is not involved in this augmented response. We did not assess the effect of aminoglutethamide (an aromatase inhibitor that inhibits the conversion of testosterone into 17β-oestradiol) and, as such, we cannot comment on whether this may have affected the response. However, in previous ex vivo studies, the acute vascular actions of testosterone have been found to be unaffected by aminoglutethamide [8,10] and it is unlikely that aromatase conversion into 17β-oestradiol is relevant to the vascular responses seen in our present data.

Response to experimental vasoactive compounds

The results of the present study suggest that high-dose (but physiological) androgen replacement increases the vasoconstrictor response to NA and reduces the dilating response to ACh and SNP. One factor in the present study that may be relevant is that the mean vessel diameter after treatment was significantly lower than before (334 ± 14.9 µm compared with 440 ± 17.1 µm respectively; P < 0.0001). The absolute difference in size is relatively small, and vessels < 600 µmol/l are anatomically recognized as functional resistance vessels with broadly consistent responses. It is recognized that vessels of different size may have variable sensitivity to vasoactive agonists (although not to testosterone). A previous study [24] suggested that the dilating action of ACh is less in large resistance vessels (> 500 µmol/l) than in smaller resistance vessels (< 200 µmol/l) taken from the same sample, due to a relative lack of muscarinic receptors in larger vessels. In fact, in the present study, the dilating action of ACh was greater in the larger vessel group (mean, 440 ± 17.1 µm), suggesting that these vessels expressed adequate muscarinic receptors to permit fair comparison.

The results of the present study are compatible with the literature. The cross-sectional study of Herman et al. [25] found that androgen-deprived prostate cancer patients (either via orchidectomy or gonadotrophin-releasing hormone antagonists) had increased flow (predominantly ACh)-mediated dilation of the brachial artery. Zitzman et al. [23] found an exaggerated flow-mediated response in 36 hypogonadal men compared with controls; this response was normalized by androgen-replacement therapy and the flow-mediated response after androgen replacement was reduced to the level observed in the control group. A similar response was reported by Sader et al. [22] (although with no control group) and by Ebenbichler et al. [20], although the latter was in male athletes using high-dose anabolic steroids. Two studies in men with vascular disease are at variance with the studies described above. Kang et al. [26] reported that 12 weeks of oral testosterone therapy increased flow- and nitrate-mediated brachial artery dilation in 18 men with coronary disease compared with placebo; however, the post-treatment testosterone levels did not increase to a significant level in the treatment group. Ong et al. [27] reported large increases in flow-mediated dilation following acute testosterone exposure, but only at very high doses well beyond the physiological range. In summary, there are therefore four clinical studies in which a higher androgen status is associated with a reduced flow-mediated response. These studies are consistent with the reduced dilatory effect of ACh following androgen replacement shown in the present study. It is important to note that, although the response may be reduced following androgen replacement, this may not be abnormal, as the study by Zitzman et al. [23] elegantly demonstrated that the response was reduced only to the level of healthy controls. There are no control data in any of the other in vivo studies. Unfortunately, of the clinical studies cited above, only three [20,22,26] tested
the effect of testosterone therapy on nitrate-mediated dilation. Two found no significant difference, although the nitrate response in the study by Ebenblicher et al. [20] was diminished overall, and the unusual results from the study by Kang et al. [26] have been discussed and make this study difficult to judge. The effect of androgen therapy on nitrate vascular response is inconsistently reported, with two studies reporting no effect [20,22] and two a reduction [21,28]. There are at least three studies on male to female trans-sexuals in which a greater nitrate-mediated response is reported [29–31]; however, these effects may be due to vascular effects of oestrogen.

The results of the present study also found an augmented response of vessels to the contractile agonist NA, but not to the synthetic prostaglandin analogue U46619. With the present results, we cannot account for this difference, as these two contractile agents have different mechanisms of action. NA acts on both the α₁-adrenoceptor with G-protein-coupled activation of intracellular signalling mechanisms and also by receptor-operated calcium channels. U46619 is a thromboxane A₂ agonist and exerts its action via prostanoid receptors gated to receptor-operated calcium channels. It could be postulated that chronic testosterone therapy has effects on the NA contractile pathway, but not the U46619 pathway; however, the evidence based on the effect of testosterone therapy on the contractile response to test agonists is limited and highly conflicting, and there are no human data. Schror et al. [32] reported that testosterone treatment induced a significantly greater coronary artery constriction to U46619 in a perfused guinea pig heart model, whereas Farhat et al. [33] treated male and female pigs with subcutaneous testosterone for 2 weeks and found that there was an elevated response to prostaglandin F₂α (an analogue of U46619) and KCl in female pigs, but only to KCl in male pigs. This study is difficult to assess, because the testosterone levels in female animals increased, whereas the testosterone levels in male animals were reduced by approx. 90%. The only study describing the effects of testosterone on vascular response to NA used isolated rat thoracic aortae and found that testosterone incubation attenuated the contraction induced by NA and KCl [34]; however, this study reported the effect of incubation and not chronic exposure.

Conclusions
The cross-sectional data confirm for the first time that testosterone is a vasodilator of pre-constricted subcutaneous resistance arteries. The dilating effect was acute and dose-dependent at concentrations in the micromolar range. The response of vessels taken from men with heart failure and healthy controls was similar. In vessels taken from men with androgen deficiency, testosterone induced vasodilation at a similar range of concentrations to the effect seen in healthy controls and heart failure patients, but the response to testosterone was augmented. Furthermore, this effect was abolished by physiological androgen replacement. Androgen-replacement therapy also induced changes in the vessel response to other vasoactive agonists, specifically an increased response to the contractile properties of NA and a reduced response to the dilating properties of SNP and ACh. It appears that androgen status seems to affect the response to testosterone and that testosterone therapy reduces the response to certain vasoactive compounds. At present, we cannot provide evidence for the mechanism of this response, but these exploratory data confirm that even physiological testosterone therapy has effects on vascular reactivity.

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