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# Effects of Testosterone on Coronary Vasomotor Regulation in Men With Coronary Heart Disease

Carolyn M. Webb, PhD; John G. McNeill, DCRR; Christopher S. Hayward, MB, BS, FRACP; Dominique de Zeigler, MD; Peter Collins, MD, FRCP

**Background**—The increased incidence of coronary artery disease in men compared with premenopausal women suggests a detrimental role of male hormones on the cardiovascular system. However, testosterone has direct relaxing effects on coronary arteries in animals, as shown both in vitro and in vivo. The effect of testosterone on the human coronary circulation remains unknown.

**Methods and Results**—We studied 13 men (aged  $61 \pm 11$  years) with coronary artery disease. They underwent measurement of coronary artery diameter and blood flow after a 3-minute intracoronary infusion of vehicle control (ethanol) followed by 2-minute intracoronary infusions of acetylcholine ( $10^{-7}$  to  $10^{-5}$  mol/L) until peak velocity response. A dose-response curve to 3-minute infusions of testosterone ( $10^{-10}$  to  $10^{-7}$  mol/L) was then determined, and the acetylcholine infusions were repeated. Finally, an intracoronary bolus of isosorbide dinitrate (1000  $\mu$ g) was given. Coronary blood flow was calculated from measurements of blood flow velocity using intracoronary Doppler and coronary artery diameter using quantitative coronary angiography. Testosterone significantly increased coronary artery diameter compared with baseline ( $2.78 \pm 0.74$  mm versus  $2.86 \pm 0.72$  mm [ $P=0.05$ ],  $2.87 \pm 0.71$  mm [ $P=0.038$ ], and  $2.90 \pm 0.75$  mm [ $P=0.005$ ] for baseline versus testosterone  $10^{-9}$  to  $10^{-7}$  mol/L, respectively). A significant increase in coronary blood flow occurred at all concentrations of testosterone compared with baseline (geometric mean [95% CI]: 32 [25, 42] versus 36.3 [27, 48] [ $P=0.006$ ], 35.3 [26, 47] [ $P=0.029$ ], 36.8 [28, 49] [ $P=0.002$ ], and 37 [28, 48] [ $P=0.002$ ] mL/min for baseline versus testosterone  $10^{-10}$  to  $10^{-7}$  mol/L, respectively). No differences existed in coronary diameter or blood flow responses to acetylcholine before versus after testosterone.

**Conclusions**—Short-term intracoronary administration of testosterone, at physiological concentrations, induces coronary artery dilatation and increases coronary blood flow in men with established coronary artery disease. (*Circulation*. 1999;100:1690-1696.)

**Key Words:** coronary arteries ■ testosterone ■ blood flow

In recent years, sex hormones, particularly estrogen, have emerged as important factors for modulating cardiovascular disease.<sup>1,2</sup> Although the incidence of coronary heart disease increases in women after menopause, postmenopausal women have a lower incidence of coronary heart disease and myocardial infarction than men of a similar age. It has been proposed that testosterone may predispose to coronary artery disease and may partially explain the sex difference. However, no direct evidence exists linking testosterone to an increased incidence of coronary heart disease and myocardial infarction.

In men without prior myocardial infarction who are referred for coronary angiography, a significant inverse correlation was found between plasma testosterone levels and extent of coronary artery disease, demonstrating that men with low testosterone levels may be at increased risk for coronary atherosclerosis.<sup>2</sup> Some reports suggest that testos-

terone therapy in men has a beneficial effect on angina pectoris<sup>3,4</sup> and on exercise-induced ST-segment depression in patients with angina pectoris.<sup>5,6</sup> A double-blind study was performed in 50 men who had ST-segment depression after exercise.<sup>6</sup> After 4 to 8 weeks of treatment with testosterone or placebo, a significant decrease in the exercise-induced extent of ST-segment depression occurred with testosterone when compared with placebo. The mechanism(s) by which testosterone decreased postexercise ST-segment depression was not established.

The direct effect of testosterone on coronary circulation in men is unknown. Testosterone induces relaxation in precontracted rabbit coronary arteries and aorta in vitro, with or without endothelium.<sup>7</sup> A high-cholesterol diet and environmental tobacco smoke have detrimental effects on endothelial function in male animals; this effect was exacerbated by testosterone at physiological concentrations.<sup>8</sup> Short-term in-

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From Cardiac Medicine, National Heart and Lung Institute, Imperial College School of Medicine, and Royal Brompton Hospital, London, UK (C.M.W., J.G.M., C.S.H., P.C.), and Columbia Laboratories, Paris, France (D.d.Z.).

Correspondence to Dr Peter Collins, Cardiac Medicine, National Heart and Lung Institute, Imperial College School of Medicine, Dovehouse Street, London SW3 6LY, UK. E-mail peter.collins@ic.ac.uk

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tracoronary infusions of testosterone dilate male and female canine coronary arteries in vivo and increase coronary blood flow, partially by an endothelium-dependent mechanism. ATP-sensitive potassium channels are also involved in the dilator response.

These data suggest a beneficial effect of testosterone on the coronary circulation. We therefore investigated the effects of testosterone on the coronary circulation of men with atherosclerotic coronary artery disease.

## Methods

### Patients

Men aged 35 to 70 years who had angiographically proven coronary artery disease at diagnostic angiography for investigation of stable angina pectoris were enrolled in the study. Patients with primary valvar heart disease, complete heart block, or uncorrected hypokalemia were excluded. All patients gave written informed consent in accordance with the ethical requirements of the Royal Brompton Hospital Ethics Committee.

### Study Design

Cardiac medication was withheld for at least 24 hours before cardiac catheterization, and caffeine-containing beverages were prohibited during this time. After diagnostic coronary angiography and full heparinization, a 0.014-inch Doppler flow wire (Cardiometrics Inc) was positioned in the proximal portion of an unobstructed coronary artery (no lesion >50% occlusive) from which continuous traces of average peak blood flow velocity were recorded. Arterial pressure, heart rate, and ECG were displayed continuously.

### Intracoronary Infusions

A 3-minute intracoronary infusion of vehicle control (ethanol) was given, followed by investigation of endothelium-dependent coronary responses by increasing concentrations of intracoronary acetylcholine (estimated concentrations,  $10^{-7}$  to  $10^{-5}$  mol/L) for 2 minutes each or until peak velocity response. After this, a dose-response curve to testosterone was performed for 3 minutes at each concentration into the right coronary artery with an infusion rate of 1 mL/min (estimated concentration of testosterone: 2.3, 23, 230, and 2300 ng/min) and of 1.5 mL/min into the left coronary artery (estimated concentration of testosterone: 3.45, 34.5, 345, and 3450 ng/min). These doses are approximately equal to the  $10^{-10}$  to  $10^{-7}$  mol/L concentrations of testosterone, respectively, achieved in the coronary blood. Acetylcholine infusions were then repeated. All infusions were given at a rate of 1 mL/min into the ostium of the right coronary artery or 1.5 mL/min into the ostium of the left coronary artery. The study protocol was completed with an intracoronary bolus of 1000  $\mu$ g of isosorbide dinitrate, a non-endothelium-dependent vasodilator. Coronary angiograms were performed at baseline and then immediately after the peak velocity response to each dose of vasoactive substance. A second baseline angiogram (baseline 2) was performed between the first acetylcholine challenge and the commencement of the testosterone infusions. A rest period was observed between each infusion to allow all measured parameters to return to baseline. At baseline 1 and at the end of each testosterone infusion, blood sampling was performed to measure plasma testosterone concentrations (total testosterone) using a standard radioimmunoassay (Abbott IMX System, Abbott Diagnostics Division).

To determine the effect of vehicle control on the acetylcholine response, a 3-minute infusion of vehicle control (0.01 mL of 60% ethanol in 10 mL of blood) was given after the first acetylcholine challenge, as described above, in a similar group of 8 male patients with coronary artery disease. The acetylcholine response was then repeated.

### Testosterone Dilutions

The testosterone dilutions were calculated to give a dose of testosterone in the coronary artery of  $10^{-10}$  to  $10^{-7}$  mol/L (normal range,  $10^{-8}$  to  $5 \times 10^{-8}$  mol/L [3 to 10 ng/mL]) using an assumed coronary blood flow of 80 mL/min in the left coronary arterial tree and 40 mL/min in the right coronary artery. Stock solutions of 2300  $\mu$ g/mL testosterone and vehicle control (ethanol) were provided by Columbia Laboratories (Paris, France). We diluted 0.1 mL of 2300  $\mu$ g/mL testosterone in 10 mL of the patient's blood to give a 23  $\mu$ g/mL ( $10^{-6}$  mol/L) testosterone concentration. This was then diluted further to give testosterone concentrations of  $10^{-7}$  to  $10^{-10}$  mol/L (2.3  $\mu$ g/mL to 2.3 ng/mL) in the coronary blood.

Testosterone is lipid-soluble and was prepared in 60% ethanol. Vehicle control was prepared for intracoronary infusion at 1 concentration, which was equivalent to the concentration given at the greatest concentration of testosterone (0.01 mL of 60% ethanol in 10 mL of blood).

### Quantitative Coronary Angiography and Calculation of Flow

Coronary angiograms were acquired and analyzed digitally using a real-time digital image acquisition and analysis system (Digitron III VACI, Siemens AG), as previously described.<sup>9</sup> Measurement of diameter and velocity were made at baseline and at peak velocity change. Diameter was measured  $\approx$ 4 mm distal to the tip of the Doppler wire at the sample volume site by an independent observer. Care was taken to measure diameter at an identical position after each infusion. The wire position did not change in any of the patients studied. A quantitative estimate of coronary blood flow was calculated from Doppler flow velocity and diameter 4 mm distal to the Doppler wire tip using the following equation<sup>10</sup>:

$$Q = \pi(D^2/4)(APV/2)(0.6)$$

where Q is flow (mL/min), D is vessel diameter (mm), and APV is average peak velocity (cm/s).

In addition to measuring local changes in diameter, global diameter changes throughout the entire artery were measured by an independent observer using quantitative coronary angiography. With this analysis, changes in mean coronary diameter were measured, as were responses at the sites of defined focal narrowing and/or dilatation.

### Statistical Analysis

Baseline 1 versus baseline 2 comparison was performed using a paired *t* test or a Wilcoxon matched pairs test when the data were not normally distributed. All other analyses were performed using a 2-way ANOVA with patient and time as factors. In Results, we present comparisons of baseline 1 versus acetylcholine responses before testosterone, baseline 2 versus testosterone, and baseline 2 versus acetylcholine responses after testosterone. We also compared responses to respective doses of acetylcholine before versus after testosterone. The following assumptions were tested: normality of residuals by the Shapiro-Francia *W'* test and equality of variances in the time groups by Bartlett's test. Data are presented as mean  $\pm$  SD or as geometric mean (95% confidence interval [CI]) where data have been log-transformed to normality. *P* < 0.05 was considered significant.

## Results

### Patients

Thirteen men were enrolled in the study; they had a mean age of  $61 \pm 11$  years. Patient characteristics are described in Table 1. Seven patients had 1 significantly diseased coronary artery (stenosis >70%), and 6 patients had 2-vessel disease. The study vessel of all patients was irregular but not significantly obstructed on angiography. The left anterior descending coronary artery was studied in 2 patients, the left circumflex

**TABLE 1. Patient and Control Characteristics**

Characteristic	Patients (n=13)	Controls (n=8)	Normal Range
Age, y	61±11	57±9	
Previous MI, PTCA, or CABG	5	2	
Hypertension	5	2	
Hypercholesterolemia	6	3	
Cigarette smoking, n			
Current	0	1	
Ex	7	2	
Never	6	3	
No. of diseased coronary arteries			
1	7	3	
2	6	5	
Total cholesterol, mmol/L	5±1	5.1±0.9	4–6.5
HDL cholesterol, mmol/L	0.9±0.3	0.8±0.2	1.1–2
Baseline testosterone, nmol/L	11±6	13±10	11–44
Baseline 17β-estradiol, pmol/L	188±77	174±36	<174
Baseline FSH, IU	7±7	6±6	1–12

Values are mean±SD. Normal ranges are from our biochemistry laboratory for men. MI indicates myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass graft surgery; FSH, follicular stimulating hormone; and HDL, high-density lipoprotein.

in 1 patient, and the right coronary artery in 10 patients. Ten patients had focal narrowing in the study vessel between 10% and 40%, with a mean lesion severity of 23±10%.

### Plasma Hormone Concentrations

Plasma testosterone levels in the femoral artery were significantly increased at the greater concentrations of infused testosterone (11±6 versus 14±4, 16±5, 29±16, and 35±15 nmol/L for baseline versus 10<sup>-10</sup> to 10<sup>-7</sup> mol/L testosterone, respectively; *P*=0.40, 0.12, <0.001, and <0.001, respectively). The mean baseline plasma estradiol level was 188±77 pmol/L (range, 112 to 358 pmol/L).

### Coronary Artery Responses to Testosterone

Testosterone (10<sup>-9</sup> to 10<sup>-7</sup> mol/L) significantly increased coronary diameter (Table 2) by 3.1% (*P*=0.05), 3.5% (*P*=0.038), and 4.5% (*P*=0.005), respectively, compared with baseline 2 (Figure). A significant increase occurred in coronary blood flow at all concentrations of testosterone compared with baseline 2 (Table 2). Blood flow was increased by 15.9% (*P*=0.006), 11.9% (*P*=0.029), 16.3% (*P*=0.002), and 17.4% (*P*=0.002) at levels of 10<sup>-10</sup> to 10<sup>-7</sup> mol/L testosterone, respectively (Figure). Velocity was not affected by testosterone at any concentration (Table 2).

### Coronary Artery Responses to Acetylcholine Before and After Testosterone

Coronary artery diameter at the Doppler wire tip did not change after acetylcholine infusion compared with baseline, and no difference existed in this diameter response to acetylcholine before and after testosterone (Table 2). Coronary velocity and flow were significantly increased by 10<sup>-6</sup> and 10<sup>-5</sup> mol/L acetylcholine compared with baseline 1 (all

*P*<0.001; Table 2), but no difference existed in the velocity or flow responses before and after testosterone.

### Mean Coronary Artery Diameter Changes

The mean diameter of the entire study vessel was measured in response to the maximum dose of acetylcholine infused in 11 patients. Acetylcholine (10<sup>-5</sup> mol/L) induced a significant increase in mean diameter compared with baseline (3.01±0.68 versus 3.14±0.63 mm for baseline 1 versus acetylcholine 10<sup>-5</sup> mol/L; *P*<0.05); however, no difference existed in this response before and after testosterone infusion (3.14±0.63 versus 3.07±0.64 mm). In 8 areas of focal narrowing (mean severity, 23±10%), the diameter response to acetylcholine (10<sup>-5</sup> mol/L) was unchanged before and after testosterone (2.46±0.7 mm versus 2.46±0.86 mm). In 11 areas of dilatation to acetylcholine (10<sup>-5</sup> mol/L), the dilator response was the same before and after infusions of testosterone (4.01±0.82 mm versus 3.85±0.79 mm).

### Coronary Artery Responses to Vehicle Control and Isosorbide Dinitrate

Vehicle control did not change coronary artery diameter or velocity or blood flow compared with baseline 1 or baseline 2 (Table 2). Isosorbide dinitrate significantly increased coronary velocity, diameter and flow compared with baseline 2 (*P*<0.001, *P*=0.005, and *P*<0.001, respectively; Table 2).

### Systemic Hemodynamics

Table 2 shows that mean arterial pressure and heart rate did not change significantly throughout the study.

### Controls

#### Characteristics

Eight controls were enrolled (mean age, 57±9 years), and all had coronary atherosclerosis (Table 1). Three controls had 1-vessel disease, and 5 had 2-vessel disease. The left circumflex coronary artery was studied in 1 control patient, and in 7 controls, the right coronary artery was studied. No differences existed between the patients and controls with respect to age, baseline plasma testosterone concentration, or factors that might affect endothelial function, such as lipid profile, blood pressure, heart rate, or coronary atherosclerosis (Table 1). Seven controls had focal narrowing in the study vessel of between 10% and 40%, with a mean lesion severity of 21±9%.

#### Coronary Artery Vasoreactivity

Vehicle control did not affect coronary velocity, diameter, or blood flow compared with baseline (Table 3). No differences existed in the velocity, diameter, or blood flow responses to acetylcholine before and after vehicle control (Table 3). Mean diameter was measured in response to the maximum dose of acetylcholine infused in 8 patients. Acetylcholine (10<sup>-5</sup> mol/L) did not significantly change mean diameter compared with baseline 1 (3.22±0.56 mm versus 3.31±0.6 mm), and no differences existed in this response before and after infusions of vehicle control (3.31±0.6 mm versus 3.27±0.58 mm). In 7 areas of focal narrowing (mean severity, 21±9%), the diameter response to acetylcholine (10<sup>-5</sup> mol/L) was unchanged before and after vehicle control

TABLE 2. Effects of Testosterone on Coronary Vasoreactivity and Systemic Hemodynamics

	Diameter, mm	Velocity, cm/s	Flow, mL/min	Mean Arterial Pressure, mm Hg	Heart Rate, beats/min
Baseline 1	2.93 (0.72)	16 (10)	40 (13)	79 (9)	62 (9)
Vehicle control	2.89 (0.74)	20 (16, 25)	38 (30)	81 (9)	63 (8)
ACh 10 <sup>-7</sup> mol/L	3.04 (0.77)	23 (5)	52* (22)	79 (9)	61 (8)
ACh 10 <sup>-6</sup> mol/L	3.01 (0.78)	34‡ (8)	75.9‡ (51)	80 (10)	63 (15)
ACh 10 <sup>-5</sup> mol/L	2.92 (0.85)	49‡ (13)	89‡ (39)	80 (10)	60 (9)
Baseline 2	2.78 (0.74)	20 (7)	35 (15)	79 (11)	61 (9)
Testosterone 10 <sup>-10</sup> mol/L	2.82 (0.7)	22 (10)	40† (20)	79 (8)	61 (9)
Testosterone 10 <sup>-9</sup> mol/L	2.86* (0.72)	21 (9)	39* (21)	79 (10)	59 (9)
Testosterone 10 <sup>-8</sup> mol/L	2.87* (0.71)	22 (9)	41† (20)	80 (9)	63 (12)
Testosterone 10 <sup>-7</sup> mol/L	2.9† (0.75)	22 (10)	41† (20)	81 (9)	59 (11)
ACh 10 <sup>-7</sup> mol/L	2.85 (0.72)	22 (6)	48* (22)	81 (9)	60 (10)
ACh 10 <sup>-6</sup> mol/L	2.88 (0.67)	28‡ (8)	68‡ (32)	81 (9)	60 (11)
ACh 10 <sup>-5</sup> mol/L	2.92 (0.72)	49‡ (8)	103‡ (41)	82 (8)	60 (9)
Isosorbide dinitrate	3.1† (0.71)	43‡ (18)	98‡ (60)	82 (8)	65 (17)

Values are mean (SD). ACh indicates acetylcholine.

\* $P < 0.05$ , † $P < 0.01$ , and ‡ $P < 0.001$  compared with most recent baseline.

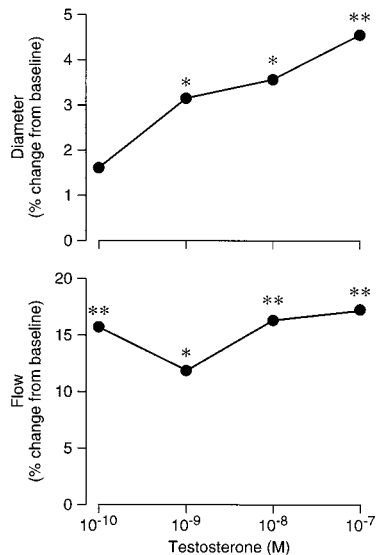
( $2.65 \pm 0.46$  mm versus  $2.58 \pm 0.48$  mm). In 8 areas of dilatation to acetylcholine ( $10^{-5}$  mol/L), the dilator response was the same before and after infusions of vehicle control ( $4.6 \pm 1.2$  mm versus  $4.5 \pm 1.23$  mm). Systemic blood pressure and heart rate did not change after infusion of vehicle control compared with baseline (Table 3).

### Discussion

We demonstrated that testosterone, administered acutely at physiological (and greater) concentrations, induces coronary artery dilatation (up to 4.5%) and increases coronary blood flow (up to 17.4%) in men with coronary atherosclerosis. Testosterone did not alter acetylcholine-induced increases in coronary blood flow or areas of constriction or dilatation induced by acetylcholine, which suggests that it had no measurable effect on stimulated endothelial nitric oxide. These findings are similar to those found in vitro in which testosterone-induced coronary relaxation was shown to be independent of the endothelium,<sup>7</sup> with the possible involvement of potassium channel modulation. The effect of testosterone on coronary artery diameter and blood flow are

approximately half that demonstrated after intracoronary diltiazem administration, where diameter was increased by 10% and flow by 30%.<sup>11</sup> Nicorandil, a potassium channel activator, exerts its vasodilator actions through a mechanism similar to that of testosterone, via activation of ATP-sensitive potassium channels. In a study in patients with normal coronary arteries and coronary artery disease, this agent increased proximal coronary artery diameter by 13%. The dose of nicorandil infused into these coronary arteries was 0.5 mg, a pharmacological dose.<sup>12</sup>

In an animal model, short-term intracoronary infusions of testosterone ( $10^{-7}$  and  $10^{-6}$  mol/L) increased coronary blood flow, similar to the findings of the present study.<sup>13</sup> Inhibition of nitric oxide synthase by L-NAME significantly attenuated testosterone-induced increases in blood flow ( $P = 0.04$ ), indicating an endothelium-dependent effect of testosterone on this calculated parameter. However, a high concentration of testosterone was used to achieve this effect ( $10^{-6}$  mol/L), and the attenuation of cross-sectional area and velocity were not statistically significant at the 5% level (both  $P = 0.06$ ). These authors<sup>13</sup> could not distinguish between a direct effect of



Testosterone-induced increases in coronary artery diameter (top) and blood flow (bottom). \* $P < 0.05$  and \*\* $P < 0.01$  compared with baseline 2.

testosterone on nitric oxide synthase or a flow-mediated effect of testosterone on the endothelium. Testosterone-induced changes in coronary velocity and flow were significantly attenuated in resistance vessels by glibenclamide ( $P = 0.03$  and  $0.02$ , respectively), indicating inhibition of ATP-sensitive potassium channels by testosterone. Although acetylcholine had no effect on epicardial coronary artery diameter in the present study, it did increase blood flow velocity and blood flow volume in a dose-dependent manner, suggesting that functional endothelium exists in resistance

vessels. The fact that this response was not different after administration of testosterone does not preclude involvement of the endothelium in testosterone-induced increases in blood flow. In vitro<sup>7</sup> and in vivo<sup>13</sup> animal data would suggest that testosterone stimulates epicardial coronary dilatation, independent of the endothelium, possibly by effects on ion channels on the vascular smooth muscle plasma membrane, such as ATP-sensitive potassium channels. However, it is impossible to rule out an indirect flow-mediated endothelium-dependent effect of testosterone on blood flow response without the use of an inhibitor of nitric oxide.

We assessed the mean coronary artery diameter response to acetylcholine throughout the length of the study arteries and found no significant difference in mean diameter response to acetylcholine before versus after the testosterone infusions. Areas of focal narrowing and areas that dilated to acetylcholine before exposure to testosterone did not react differently before versus after exposure to testosterone. This reinforces our suggestion that testosterone does not enhance endothelial function in sites of constriction or dilatation to acetylcholine.

Testosterone is converted to  $17\beta$ -estradiol by the enzyme aromatase. It is possible that estradiol may account for the vascular effects of testosterone; however, the evidence to date does not support this potential mechanism. Inhibition of both the testosterone and estrogen receptors does not affect testosterone-induced coronary relaxation in vitro<sup>7</sup> or coronary dilatation and increases in blood flow in vivo.<sup>13</sup> Androgen receptors have been identified in ventricular and atrial myocytes, endothelial cells, and vascular smooth muscle cells of some mammalian species<sup>14-16</sup>; however, at present, no information exists regarding the presence of androgen receptors in the coronary arteries of humans. Also, it has been shown in a

**TABLE 3. Effects of Vehicle Control on Coronary Vasoreactivity and Systemic Hemodynamics**

	Diameter, mm	Velocity, cm/s	Flow, mL/min	Mean Arterial Pressure, mm Hg	Heart Rate, beats/min
Baseline	2.91 (0.47)	16.1 (5.8)	36 (13)	77 (14)	68 (7)
ACh 10 <sup>-7</sup> mol/L	2.97 (0.38)	23 (12)	52 (33)	78 (13)	66 (8)
ACh 10 <sup>-6</sup> mol/L	2.9 (0.41)	34 (21)	72† (52)	74 (14)	64 (9)
ACh 10 <sup>-5</sup> mol/L	2.82 (0.43)	49 (26)	99† (69)	80 (13)	64 (6)
Vehicle control	2.89 (0.5)	17 (5.6)	35 (19)	75 (12)	65 (5)
ACh 10 <sup>-7</sup> mol/L	2.86 (0.42)	22 (10)	44 (27)	79 (18)	65 (7)
ACh 10 <sup>-6</sup> mol/L	2.77 (0.51)	28 (10)	55 (31)	76 (10)	64 (9)
ACh 10 <sup>-5</sup> mol/L	2.85 (0.48)	49 (23)	104 (79)	75 (13)	63 (12)
Isosorbide dinitrate	3.12 (0.57)	37* (14)	94* (55)	77 (14)	68 (9)

Values are mean (SD). ACh indicates acetylcholine.  
\* $P < 0.01$ , † $P < 0.001$  compared with baseline.

number of studies that estrogen does not have a direct relaxing effect on human coronary arteries in vivo.<sup>9,17</sup>

We demonstrated direct coronary effects of testosterone at physiological concentrations (adult male normal range is  $\approx 10^{-9}$  to  $10^{-8}$  mol/L)<sup>18</sup> in humans. Previous studies (described above) showed significant effects of testosterone at pharmacological concentrations in vitro ( $10^{-6}$  and  $10^{-5}$  mol/L)<sup>7</sup> and near-physiological concentrations in vivo ( $10^{-7}$  mol/L).<sup>13</sup> Interestingly, the mean baseline testosterone level of the men included in the present study was at the lower end of the normal range (mean,  $11 \pm 6$  nmol/L; range, 1 to 26 nmol/L), which reinforces the observation of Phillips et al<sup>2</sup> that low plasma testosterone may be a risk factor for coronary heart disease. Indeed, 7 of the 13 men had plasma testosterone levels  $< 11$  nmol/L. Serum testosterone decreases with age,<sup>19</sup> and bioavailable testosterone is decreased in older men.<sup>20</sup> Concurrently, an apparent stimulation of gonadotrophin release occurs, with increased levels of follicular-stimulating hormone and luteinizing hormone in elderly men.<sup>21</sup> The patients in the current study demonstrated relatively low testosterone levels for men of a younger age; therefore, it would be plausible that, in our study group, coronary artery disease was not simply a function of age but may also be related to serum testosterone levels. Separate analyses of flow responses in our subjects with normal and low baseline testosterone levels showed no significant correlation between baseline plasma testosterone levels and flow response to infused testosterone (data not shown). This may be due to small numbers ( $n=7$  and  $n=6$ , respectively) or to the fact that the normal group had testosterone levels at the lower end of the normal range.

Dose is an important consideration regarding long-term testosterone administration. High concentrations of testosterone have detrimental effects on atheroma progression,<sup>22</sup> plasma lipids,<sup>23</sup> and hemostatic factors,<sup>2,24,25</sup> and they increase the risk of myocardial infarction and stroke.<sup>26</sup> However, recently published data show a beneficial effect of testosterone on atheroma development in rabbits.<sup>27</sup> The natural androgens testosterone and dehydroepiandrosterone, at physiological concentrations, produced this effect, which was only partially mediated through a beneficial effect on lipid profile. The results of the present study are important because they show the effects of low-dose, physiological levels of testosterone on coronary vasomotion in humans. The results are also important because testosterone is present in both men and women and, therefore, the results may be pertinent to both sexes. Further studies will be needed to investigate the effects of long-term, low-dose testosterone administration on coronary reactivity and risk factors for coronary artery disease in men and the role of testosterone in coronary physiology and pathophysiology in women.

### Limitations

We found no effect of testosterone on acetylcholine-induced increases in blood flow, indicating a lack of effect of testosterone on endothelium-dependent responses. To prove a direct effect of testosterone on nitric oxide synthase, however, experiments inhibiting endothelium-derived nitric oxide synthesis would need to be performed. These further exper-

iments would still not exclude the possibility that testosterone may affect the endothelium indirectly via a shear stress-mediated effect.

Diameter responses to testosterone were compared with baseline 2, which revealed a testosterone-induced dilatation. Although the diameter did not return to the original baseline value, baseline 1 and baseline 2 were not significantly different for any measured variable. A carry-over effect of the acetylcholine may account for the decreased coronary diameter at baseline 2; however, this effect has not been reported in previous, similar studies using estrogen,<sup>9,17</sup> and it did not affect the blood flow measurement at baseline 2 in the present study.

Due to the complexity of the study protocol, a number of statistical comparisons were performed. This could call into question statistical significance set at the 5% level. However, with particular regard to the effect of testosterone on coronary blood flow for 3 of the 4 data points, the significance levels were at the 1% level. As multiple comparisons were not performed, we did not need Bonferroni correction of data. The methodology used in the present study, including the statistical analysis, is very similar to that used in previous intracoronary studies of estrogen.<sup>9,17</sup>

### Conclusions

We demonstrated, for the first time, a direct effect of physiological doses of testosterone in human coronary circulation, which resulted in coronary artery dilatation and increases in coronary blood flow. This observation may have important clinical implications if testosterone can be shown to improve myocardial ischemia and long-term outcomes in men with established coronary heart disease.

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### References

- Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. *Prev Med.* 1991;20:47–63.
- Phillips GB, Pinkernell BH, Jing TY. The association of hypotestosteronemia with coronary artery disease in men. *Arterioscler Thromb.* 1994;14:701–706.
- Levine SA, Likoff WB. The therapeutic value of testosterone propionate in angina pectoris. *N Engl J Med.* 1943;229:770–772.
- Lesser MA. Testosterone propionate therapy in one hundred cases of angina pectoris. *J Clin Endocrinol.* 1946;6:549–557.
- Webb CM, Adamson DL, de Ziegler D, Collins P. Effect of acute testosterone on myocardial ischemia in men with coronary artery disease. *Am J Cardiol.* 1999;83:437–439.
- Jaffe MD. Effect of testosterone cypionate on postexercise ST segment depression. *Br Heart J.* 1977;39:1217–1222.
- Yue P, Chatterjee K, Beale C, Poole-Wilson PA, Collins P. Testosterone relaxes rabbit coronary arteries and aorta. *Circulation.* 1995;91:1154–1160.
- Hutchison SJ, Sudhir K, Chou TM, Sievers RE, Zhu BQ, Sun YP, Deedwania PC, Glantz SA, Parmley WW, Chatterjee K. Testosterone worsens endothelial dysfunction associated with hypercholesterolemia

- and environmental tobacco smoke exposure in male rabbit aorta. *J Am Coll Cardiol*. 1997;29:800–807.
9. Collins P, Rosano GMC, Sarrel PM, Ulrich L, Adamopoulos S, Beale CM, McNeill J, Poole-Wilson PA. Estradiol-17 $\beta$  attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. *Circulation*. 1995;92:24–30.
  10. Doucette JW, Corl PD, Payne HM, Flynn AE, Goto M, Nassi M, Segal J. Validation of a Doppler guide wire for intravascular measurement of coronary artery flow velocity. *Circulation*. 1992;85:1899–1911.
  11. Vrolix MC, Sionis D, Piessens J, Van Lierde J, Willems JL, De Geest H. Changes in human coronary flow reserve after administration of intracoronary diltiazem. *J Cardiovasc Pharmacol*. 1991;18(suppl 9):S64–S67.
  12. Haager PK, Klues HG, Schmidt M, vom Dahl J, Hanrath P. Effects of nitroglycerin and nicorandil on regional poststenotic quantitative coronary blood flow in coronary artery disease: a combined digital quantitative angiographic and intracoronary Doppler study. *J Cardiovasc Pharmacol*. 1999;33:126–134.
  13. Chou TM, Sudhir K, Hutchison SJ, Ko E, Amidon TM, Collins P, Chatterjee K. Testosterone induces dilation of canine coronary conductance and resistance arteries in vivo. *Circulation*. 1996;94:2614–2619.
  14. McGill HC Jr, Sheridan PJ. Nuclear uptake of sex steroid hormones in the cardiovascular system of the baboon. *Circ Res*. 1981;48:238–244.
  15. Lin AL, Gonzalez R Jr, Shain SA. Androgen directs apparent cytoplasmic and nuclear distribution of rat cardiovascular androgen receptors. *Arteriosclerosis*. 1985;5:659–667.
  16. Higashiura K, Mathur RS, Halushka PV. Gender-related differences in androgen regulation of thromboxane A2 receptors in rat aortic smooth-muscle cells. *J Cardiovasc Pharmacol*. 1997;29:311–315.
  17. Gilligan DM, Quyyumi AA, Cannon RO III. Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. *Circulation*. 1994;89:2545–2551.
  18. Hassan T. *A Guide to Medical Endocrinology*. London: MacMillan; 1985.
  19. Gray A, Feldman HA, McKinlay JB, Longcope C. Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. *J Clin Endocrinol Metab*. 1991;73:1016–1025.
  20. Nankin HR, Calkins JH. Decreased bioavailable testosterone in aging normal and impotent men. *J Clin Endocrinol Metab*. 1986;63:1418–1420.
  21. Harman SM, Tsitouras PD, Costa PT. Reproductive hormones in aging men, II: basal pituitary gonadotropins and gonadotropin responses to luteinizing hormone-releasing hormone. *J Clin Endocrinol Metab*. 1982;54:547–551.
  22. Adams MR, Williams JK, Kaplan JR. Effects of androgens on coronary artery atherosclerosis and atherosclerosis-related impairment of vascular responsiveness. *Arterioscler Thromb Vasc Biol*. 1995;15:562–570.
  23. Thompson PD, Cullinane EM, Sady SP, Chenevert C, Saritelli AL, Sady MA, Herbert PN. Contrasting effects of testosterone and stanozolol on serum lipoprotein levels. *JAMA*. 1989;261:1165–1168.
  24. Ajayi AA, Mathur R, Halushka PV. Testosterone increases human platelet thromboxane A2 receptor density and aggregation responses. *Circulation*. 1995;91:2742–2747.
  25. Heller RF, Meade TW, Haines AP, Stirling Y, Miller NE, Lewis B. Inter-relationships between factor VII, serum testosterone and plasma lipoproteins. *Thromb Res*. 1982;28:423–425.
  26. Bagatell CJ, Bremner WJ. Androgens in men: uses and abuses. *N Engl J Med*. 1996;334:707–714.
  27. Alexandersen P, Haarbo J, Byrjalsen I, Lawaetz H, Christiansen C. Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits. *Circ Res*. 1999;84:813–819.