

## CLINICAL STUDY

# Effect of testosterone replacement therapy on arterial stiffness in older hypogonadal men

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## Abstract

**Objective:** To assess arterial stiffness in a cohort of hypogonadal males and to investigate the effect of testosterone replacement therapy on arterial properties in this specific group.

**Design:** Eighteen male patients with untreated acquired hypogonadism due to either adult-onset idiopathic hypogonadotropic hypogonadism ( $n=9$ ) or pituitary tumor ( $n=9$ ) and 12 age-, sex- and weight-matched eugonadal healthy controls were recruited for the study. Arterial properties, plasma glucose, lipid profile, total, and bioavailable testosterone (BT) levels were measured in fasting state. In the hypogonadal subjects, the effect of transdermal testosterone replacement therapy on arterial properties was studied by repeat noninvasive measurements at baseline, as well as 48 h and 90 days following the initiation of treatment.

**Methods:** Arterial stiffness was evaluated using applanation tonometry and pulse wave analysis by three different standard devices that assess various measures of arterial stiffness: pulse wave velocity (PWV), augmentation index (AIx), and large/small artery compliance (C1 and C2).

**Results:** Age- and blood pressure-adjusted PWV was significantly higher in hypogonadal men ( $8.90 \pm 2.29$  vs  $6.78 \pm 1.16$  m/s in the control group;  $P=0.025$ ). Testosterone therapy increased BT level from  $2.01 \pm 1.04$  to  $4.68 \pm 2.43$  and  $7.83 \pm 6.2$  nmol/l after 48 h and 3 months respectively ( $P=0.001$ ). PWV decreased from  $8.9 \pm 2.29$  to  $8.24 \pm 1.39$  and  $8.25 \pm 1.82$  m/s after 48 h and 3 months of treatment respectively ( $P=0.03$ ).

**Conclusions:** Male hypogonadism is associated with increased PWV, which is rapidly but incompletely ameliorated by normalization of circulating testosterone levels.

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## Introduction

Androgens appear to exert numerous effects on cardiovascular health, potentially operating through metabolic, hormonal, functional, and structural modulation of the arterial system, apparently eliciting both beneficial and deleterious responses. Low testosterone level is associated with insulin resistance and abdominal obesity (1–3). On the other hand, pharmacological suppression of testosterone levels by GnRH analogues reportedly increases HDL cholesterol (HDL-C) levels, an effect that is abolished by administration of testosterone (4, 5). Castration apparently attenuates and testosterone repletion increases mRNA expression of angiotensinogen and renin in kidneys of spontaneously hypertensive rats (6). Supraphysiologic doses of testosterone used in the treatment of female-to-male transsexuals increased plasma endothelin level compared with untreated females (7).

Diverse functional properties have been ascribed to the action of androgens in the vasculature. For example, patients with low testosterone level reportedly exhibit improved vascular endothelial function as measured by endothelium-dependent dilatation (8). Malkin and colleagues recently showed that while testosterone functions as an acute vasodilator in human s.c. arteries *ex vivo*, testosterone replacement in men with androgen deficiency impairs the vasodilator effects to acetylcholine (9). Unrelated to the latter observation, androgens apparently possess direct vasodilator properties in a variety of arterial beds, including the coronary and pulmonary circulation (10). These effects may be independent of a functional androgen receptor and gene transcription, as testosterone appears to induce vasodilation presumably by blocking a membrane-associated calcium channel in vascular smooth muscle cells (11) and/or opening of a potassium channel (12). Testosterone confers symptomatic benefits in patients with

coronary heart disease and heart failure (13, 14) apparently through its action as a vasodilator. Testosterone can also increase nitric oxide availability and improve flow-mediated dilation in hypogonadal men (15).

That androgen deficiency may adversely impact vascular structure in human subjects can be indirectly inferred from observations linking male hypogonadism to increased risk of cardiovascular mortality (16). In the experimental setting, testosterone may retard atherogenesis through both aromatization to estradiol and activation of nonclassical androgen receptors (17). Just as estradiol, testosterone was shown to exert anti-atherogenic effects in the cholesterol-fed rabbit (18). Finally, pharmacological suppression of circulating testosterone in men with prostate cancer can result in increased arterial stiffness (19, 20), thus indirectly suggesting the induction of structural changes.

Because low androgen levels in older men are now recognized as a fairly common finding, we set out to assess the possibility that androgen replacement therapy favorably affects arterial function in this setting. Among the various noninvasively determined parameters of arterial function, pulse wave velocity (PWV) appears not only to detect the presence of vascular disease, but also to predict future cardiovascular events, independent of established cardiovascular risk factors, as has been recently shown in hypertensive patients (21), type 2 diabetic patients (22), elderly subjects (23), and apparently healthy individuals (24).

**Methods**

**Subjects and study design**

Eighteen males with untreated acquired hypogonadism aged  $62.5 \pm 8.33$  years (means  $\pm$  s.d.) and 12 age-, sex-, and weight-matched controls ( $59.8 \pm 8.67$  years;  $P = NS$ ) participated in this study. All hypogonadal patients suffered from hypogonadotropic hypogonadism, which was either secondary to pituitary tumor ( $n=9$ ) or reflected adult-onset acquired idiopathic hypogonadotropic hypogonadism. The latter condition was defined by the combination of adult life appearance of clinical symptom/s associated with hypogonadism, the presence of low serum bioavailable testosterone (BT) unaccompanied by increased circulating LH, and the absence of abnormal finding on pituitary imaging (magnetic resonance imaging or CT scan). The specific details of these subjects are provided in Table 1.

Individuals with coexisting hypopituitarism, secondary to the presence of pituitary adenoma (three with hypoadrenalism and four with hypothyroidism) were receiving adequate replacement therapy for associated hormonal deficiencies and were considered hormonally stable and well controlled for at least 6 months prior to the initiation of this study. In this respect, none of the

**Table 1** Clinical and biochemical features of the patients with acquired idiopathic hypogonadotropic hypogonadism.

| Patient number | Presenting symptoms                        | Age (years) | Serum LH (mIU/ml) | Serum FSH (mIU/ml) | Serum PRL (mIU/l) | Serum BT (nmol/l) | Coexisting disorders                       | Medications  |
|----------------|--|-------------|-------------------|--------------------|-------------------|-------------------|--|--|
| 1              | Erectile dysfunction                       | 54          | 3.5               | 3.1                | 131               | 2.32              | Hypertension                               | Enalapril, hydrochlorothiazide   |
| 2              | Weakness; depression; erectile dysfunction | 77          | 1.05              | 2.12               | 267               | 1.35              |  |  |
| 3              | Sexual dysfunction                         | 61          | 4.1               | 6.9                | 85                | 2.6               | Depression gastroesophageal reflux disease | Paroxetine, lansoprazole   |
| 4              | Loss of libido                             | 69          | 4.1               | 6.5                | 124               | 3.16              | Primary hypothyroidism                     | Levothyroxine  |
| 5              | Gynecomastia; erectile dysfunction         | 52          | 2.5               | 9.0                | 191               | 2.74              | Primary hypothyroidism; allergic rhinitis  | Levothyroxine, loratadine  |
| 6              | Osteoporosis                               | 70          | 1.36              | 6.74               | 199               | 1.59              | Diabetes mellitus; hypertension            | Aspirin, metformin, amlodipine, enalapril, bisoprolol                                      |
| 7              | Loss of libido                             | 64          | 3.07              | 7.32               | 149               | 2.15              | Hyperlipidemia                             | Atorvastatin   |
| 8              | Osteoporosis                               | 64          | 2.8               | 7.7                | 372               | 1.9               | Hypertension                               | Cilazapril, hydrochlorothiazide, amlodipine, calcium and vitamin D                         |
| 9              | Loss of libido; osteoporosis               | 61          | 3.1               | 8.8                | 98                | 2.04              | Hyperlipidemia; hypertension               | Alendronate, calcium and vitamin D, cilazapril, hydrochlorothiazide, ciprofibrate, aspirin |

patients was either tested or treated for GH deficiency. Exclusion criteria included severely uncontrolled diabetes mellitus (HgbA1C above 10%), severe liver and kidney disease, hemoglobin > 16 g/dl; hematocrit > 50%, and history of hormone-dependent cancer.

Five patients with hypogonadism and two controls also suffered from diabetes mellitus or prediabetes, as defined by fasting plasma glucose (FPG) 100–125 mg/dl or 2-h glucose of 140 mg/dl but < 200 mg/dl upon challenge with standard (75 g) oral glucose tolerance test. None of the participating subjects, however, had HgbA1C > 6.9%. The control and hypogonadal groups were also not different in terms of fasting glucose or HgbA1C levels ( $115 \pm 1.4$  vs  $112 \pm 6.3$  mg/dl and  $6.45 \pm 0.49$  vs  $6.28 \pm 0.4\%$  for the control and hypogonadal groups respectively;  $P = \text{NS}$  for both comparisons). Additionally, ten patients with hypogonadism and eight controls had been on antihypertensive medication. For both conditions, medications had been stable for at least 3 months prior to and throughout the duration of the study. Tobacco smokers were not included in this study.

Most hypogonadal men had received no previous androgen treatment ( $n = 11$ ), and those who had previously been treated ( $n = 7$ ) had discontinued testosterone ester injections for at least 6 weeks prior to their enrolment in the study. Subjects were studied as outpatients, and were free of any acute illness during the study period. All hypogonadal patients had a normal rectal examination and prostate-specific antigen (PSA) level of less than  $3.5 \mu\text{g/l}$ .

Measurement of vascular function and metabolic parameters (lipid profile, glucose, total testosterone, and BT) was performed in all subjects at baseline in the fasting state. Immediately after obtaining baseline laboratory and arterial function studies, hypogonadal patients started individualized replacement therapy with testosterone (AndroGel T gel 1%, Laboratories Besins International, Montrouge, France, 25–50 mg daily as a starting dose). Dosage adjustments were made at 6-week intervals according to BT level. The gel was applied in the morning after a shower, and subjects were instructed to avoid showering until 6 h after the application. Patients applied T gel at a different site each day (right and left upper arms/shoulder or right and left abdomen). Measurements of vascular function were repeated after 48 h of treatment to assess the acute androgen effects as well as 90 days after the initiation of treatment, to assess chronic androgen effects. Measurements of total testosterone and BT were repeated 48 h, 45 days, and 90 days after initiation of treatment. Metabolic parameters were obtained at baseline and again, 48 h and 12 weeks after initiation of treatment. All patients were studied while taking their regular medications. The study was approved by the institutional ethics committee, and all patients gave informed consent to participate in the study.

## Measurement of arterial stiffness

Arterial properties were assessed according to recommended procedures (25). The determinations were made between 0800 and 1000 h at the supine position. During the examination subjects stayed with extended legs and did not talk or sleep. Room temperature was between 21 and 23 °C. All participants abstained from caffeinated beverages on the day on which the measurements were performed. Brachial blood pressure (BP) was obtained after a resting period of 15 min, using an automated BP monitor (Omron 705-CP) with the radial artery kept at the heart level during the measurement. Five measurements were averaged, enabling a determination of systolic BP (SBP), diastolic BP (DBP), mean BP, and heart rate. All measurements were performed by a single investigator (MY). Intra-observer reliability was measured by the Pearson correlation coefficient and high reliability was found for each of four parameters (C1, C2, PWV, and augmentation index (AIx) respectively) with correlation coefficients  $\geq 0.92$ .

Noninvasive assessment of arterial properties was initiated following the measurement of BP, with the following devices:

- i. The SphygmoCor system (AtCor Medical, Sydney, Australia): peripheral pressure waveforms were recorded from the radial artery at the wrist, using applanation tonometry with a high-fidelity micro-manometer (SPC-301; Millar Instruments, Houston, TX, USA). After 20 sequential waveforms had been acquired, a validated generalized transfer function was used to generate the corresponding central aortic pressure waveform (26, 27). Augmentation pressure, which reflects the increase in SBP induced by the initial recoil from the aorta and large arteries in response to the initial outflow from the left ventricle, was then derived through pulse wave analysis. The AIx (%) was defined as the augmentation pressure divided by pulse pressure (expressed in percentage). In addition, because AIx is influenced by heart rate, AIx was then normalized for a heart rate of 75 bpm (AIx@75) according to the method of Wilkinson *et al.* (28).
- ii. The Complior device (Artech-Medical, France) was used for automatic assessment of PWV, a gold standard measure of arterial stiffness. This method of PWV determination has been previously described in detail and extensively validated (29). In brief, waveforms were recorded transcutaneously over the right common carotid artery and the right radial artery, and the transit time ( $T$  time) was determined from the time delay ( $t$ ) between the appearances of the two feet of the corresponding waveforms. PWV is calculated from the measurements of pulse transit time and the distance traveled by the pulse between two recording sites as  $\text{PWV} = \text{distance (m)}/\text{transit}$

time (s), according to the foot-to-foot method (29, 30). The distance between the recording sites was measured with a tape over the surface of the body, as specified by the manufacturer, and PWV was automatically calculated by the Complior unit.

- iii. CR-2000 instrument (Hypertension Diagnostics, Eagan, MN, USA) was also used to assess systemic arterial stiffness based on the analysis of the DBP decay using a Windkessel model of the circulation (31). According to this model, the arterial tree is loaded in systole by the stroke volume and during the following diastole; the DBP contour is a function of resistance, compliance, and inertance of an isolated arterial system. The model defines two components of compliance, a proximal phase and a more distal component influencing predominantly the frequency and decay rate of the oscillatory pressure waves originating at reflecting sites in the smaller arteries. This algorithm identifies separately, through computer analysis, the total systemic large artery compliance (C1) and the small artery compliance or elasticity (C2). Pulse wave analysis was performed in duplicate, and average values were reported. The radial artery waveform was obtained with a sensor positioned over the artery and calibrated using an oscillometric method on the opposite arm. Thirty seconds of analogue waveforms were digitized at 200 samples/s. A beat-marking algorithm determined the beginning of systole, peak systole, onset of diastole, and end diastole for all beats in the 30-sec measurement period. Small artery elasticity or oscillatory components (C2 ml/mmHg $\times$ 100) and large artery elasticity or capacitive components (C1 ml/mmHg $\times$ 10) were then automatically calculated by this device.

### Biochemistry

Blood samples were collected after overnight fast. Serum glucose, lipid panel, and PSA were measured by standard automated techniques (ECLIA, Roche Diagnostics). Testosterone and BT were assayed as previously described by us (32).

### Statistical analyses

Continuous data such as arterial properties and age are expressed as means and s.d. The association between age, gender, and C1, C2, A1x, PWV, LDLc, HDLc, body mass index (BMI), A1C, and PWV was measured by the Pearson correlation coefficient between various parameters. Comparison between the study and control groups at baseline for all continuous parameters was performed using both Student's *t*-test for independent sample and the Mann-Whitney nonparametric test. Change over time, measured in three visits, was evaluated using a one-way ANOVA (study groups) with repeated measures, adjusted for age and BP.

The mixed model was used for the analysis to address the problem of missing measurements. All results were adjusted for age and BP. Statistical analysis was performed using the SAS for Windows, version 9.1.  $P < 0.05$  was considered to be statistically significant.

## Results

### Baseline measurements

The baseline characteristics of the 30 study participants are given in Table 2. The mean age of the hypogonadal patients at entry was  $62.5 \pm 8.3$  years, and that of the control subjects  $59.5 \pm 8.7$  years ( $P = \text{NS}$ ). As shown, BMI, LDLc, HDLc, triglycerides (TG), and FPG of the hypogonadal and control men were similar. By design, the two groups differed in their circulating testosterone and BT concentrations. In addition, hypogonadal subjects had significantly higher SBP. In the hypogonadal subjects, mean BT was  $2.01 \pm 1.04$  nmol/l and mean SBP was 137 mmHg, compared with a mean BT of  $3.85 \pm 1.04$  nmol/l and a mean SBP of 123 mmHg in the control group ( $P = 0.0001$  for BT;  $P = 0.009$  for SBP). Mean total testosterone level in the hypogonadal subjects was  $4.76 \pm 2.01$  nmol/l compared with  $10.78 \pm 6.79$  nmol/l in the control group ( $P = 0.034$ ). Of note in this cross-sectional analysis was also the negative correlation between BT and age ( $R = -0.662$ ;  $P = 0.003$ ) and testosterone and SBP ( $R = -0.474$ ;  $P = 0.05$ ).

Results of the various measurements of arterial properties in the hypogonadal men and the corresponding control group are summarized in Table 3. Arterial stiffness assessed by PWV was markedly higher in the hypogonadal men ( $9.06 \pm 2.26$  vs  $6.78 \pm 1.16$  m/s respectively). Increased PWV in the hypogonadal men was still maintained after adjustment for age and BP

**Table 2** Baseline clinical and laboratory characteristics of the hypogonadal and control groups.

|                             | Hypogonadal men (N=18) | Control men (N=12) | P value |
|-----------------------------|------------------------|--------------------|---------|
| Age                         | $62.5 \pm 8.33$        | $59.5 \pm 8.67$    | NS      |
| BMI (m <sup>2</sup> /kg)    | $27.66 \pm 3.66$       | $27.96 \pm 5.29$   | NS      |
| Weight (kg)                 | $81.24 \pm 10.56$      | $86.25 \pm 20.61$  | NS      |
| SBP (mmHg)                  | $137 \pm 14$           | $123 \pm 11$       | 0.009   |
| DBP (mmHg)                  | $79 \pm 11$            | $74 \pm 5.9$       | NS      |
| PP (mmHg)                   | 57.85                  | 48.83              | 0.002   |
| Pulse (bpm)                 | $60.7 \pm 9.3$         | $60.0 \pm 7.9$     | NS      |
| FPG (mmol/l)                | $5.51 \pm 0.79$        | $5.55 \pm 0.52$    | NS      |
| LDLc (mmol/l)               | $2.76 \pm 0.69$        | $2.95 \pm 1.11$    | NS      |
| HDLc (mmol/l)               | $1.26 \pm 0.36$        | $1.14 \pm 0.23$    | NS      |
| TG (mmol/l)                 | $1.87 \pm 0.99$        | $2.05 \pm 1.27$    | NS      |
| Total testosterone (nmol/l) | $4.76 \pm 2.01$        | $10.78 \pm 6.79$   | 0.034   |
| BT (nmol/l)                 | $2.01 \pm 1.04$        | $3.85 \pm 1.04$    | 0.0001  |

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TG, triglycerides; BT, bioavailable testosterone. Data are expressed as mean  $\pm$  s.d.

**Table 3** Arterial properties in the hypogonadal and control men (adjusted for age, SBP, and DBP).

|                  | Hypogonadal men (N=18) | Control group (N=12) | P value |
|------------------|------------------------|----------------------|---------|
| C1 (ml/mmHg×10)  | 23.23±9.12             | 19.56±4.07           | NS      |
| C2 (ml/mmHg×100) | 4.87±2.83              | 4.85±2.03            | NS      |
| Aix (%)          | 28.07±8.5              | 21.42±6.68           | 0.06    |
| PWV (m/s)        | 8.9±2.29               | 6.78±1.16            | 0.025   |

C1, capacitive; C2, oscillatory components of arterial compliance; Aix, augmentation index; PWV, pulse wave velocity. Data are expressed as mean±s.d.

(8.90±2.29 vs 6.78±1.16 m/s in control group;  $P=0.025$ ). Hypogonadal men also had a borderline higher adjusted Aix (28.07±8.5 vs 21.42±6.7% in controls;  $P=0.06$ ). There was no difference between the two groups in either C1 (23.23±9.12 ml/mmHg×10 in hypogonadal men versus 19.56±4.07 ml/mmHg×10 in the control group;  $P=NS$ ) or C2 (4.87±2.83 ml/mmHg×100 in hypogonadal men versus 4.85±2.03 ml/mmHg×100 in the control group;  $P=NS$ ).

### Effects of testosterone replacement therapy

The effects of testosterone replacement therapy are shown in Table 4 and Figs 1 and 2. In hypogonadal subjects placed on testosterone replacement therapy, mean BT increased from 2.01±1.04 to 4.68±2.43 nmol/l after 48 h, 5.71±2.33 nmol/l after 6 weeks, and to 7.83±6.20 nmol/l after 3 months of treatment ( $P=0.001$ ). Total testosterone level increased from 4.76±2.01 to 17.65±7.48 nmol/l after 48 h, 19.5±14.7 nmol/l after 6 weeks, and 23.33±8.77 nmol/l after 3 months of treatment (0.0001). Testosterone and BT levels at 6 weeks and 3 months did not differ significantly. BP did not change significantly and mean levels were 137/79 mmHg at baseline, 133/78 mmHg 48 h after the initiation of treatment, and 132/78 mmHg after 3 months of treatment ( $P=NS$ ). No changes were seen in BMI,

waist circumference, TG, or FPG, but LDLc level decreased significantly, though slightly (from 2.76±0.69 to 2.65±0.41 mmol/l;  $P=0.0005$ ) after 3 months of treatment.

Some of the measures of arterial stiffness improved in patients placed on testosterone treatment. First, PWV decreased from 8.9±0.31 m/s at baseline to 8.24±0.3 m/s 48 h after initiation of treatment ( $P=0.03$ ), a decline that persisted after 3 months of treatment (8.25±0.27 m/s;  $P=0.03$ ). Secondly C1 significantly increased after 3 months of treatment from 22.5±1.22 ml/mmHg×10 at the baseline to 28.8±1.46 ml/mmHg×10 ( $P=0.03$ ). No significant changes in any of the remaining parameters of arterial function, i.e., Aix and C2, were noted. Aix decreased minimally from the baseline (27.5±1.19%) after 48 h of treatment (23.77±1.45%), but even this small and statistically insignificant decline was not sustained, such that Aix recorded after 3 months of therapy was essentially identical to the pre-treatment level (26.67±1.58%); all  $P=NS$ . C2 was also not affected by testosterone treatment (4.83±0.4, 4.26±0.41, and 4.9±0.42 ml/mmHg×100 at baseline, after 48 h, and after 3 months of the treatment respectively;  $P=NS$ ).

### Discussion

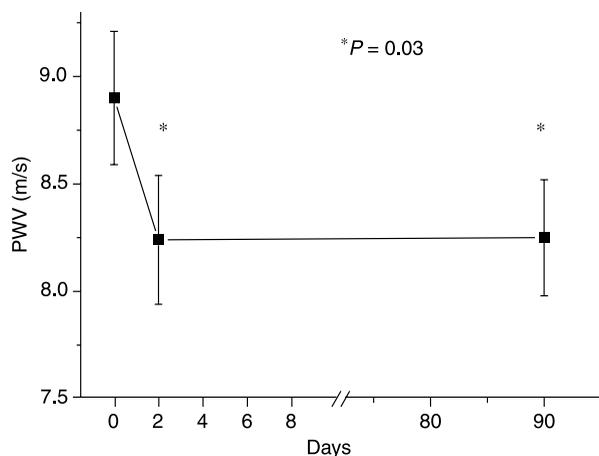
The results of the present study show that patients with acquired hypogonadism have increased arterial stiffness, as measured by PWV. This finding complements observations that GnRH analog-induced hypogonadism is associated with increased arterial stiffness as reflected by higher Aix (19) or PWV (20). In our cohort of 'spontaneous' acquired chronic hypogonadism, PWV was ~45% higher and Aix was ~33% higher in the hypogonadal group than in a matched control group, although the latter difference fell somewhat short of attaining statistical significance ( $P=0.06$ ).

There are several differences, however, between our study and these earlier reports. First, Smith *et al.* (19) as

**Table 4** The effect of testosterone replacement therapy on clinical and biochemical characteristics in hypogonadal men.

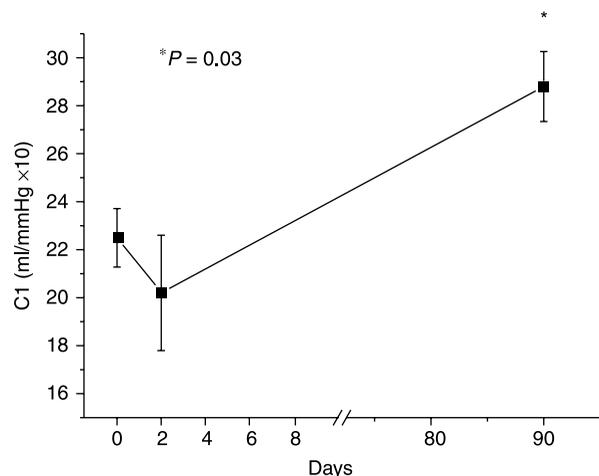
|                              | Baseline   | 48 h       | 6 weeks    | 12 weeks   | P value            |
|------------------------------|------------|------------|------------|------------|--------------------|
| Age                          | 62.5±8.3   |            |            |            |                    |
| BMI (m <sup>2</sup> /kg)     | 27.66±3.6  | 28.18±3.3  | 28.2±2.9   | 28.35±3.3  | NS                 |
| Weight (kg)                  | 81.24±10.6 | 82.67±10.0 | 82.78±9.8  | 83.20±9.9  | NS                 |
| Waist circumference (cm)     | 97.7±9.4   | 98.7±9.5   | 98.55±9.1  | 98.6±9.5   | NS                 |
| BP (mmHg)                    | 137/79     | 133/78     | 131/77     | 132/78     | NS                 |
| PP (mmHg)                    | 57.85      | 54.68      | 55.2       | 54.10      | NS                 |
| Heart rate (bpm)             | 60.7±9.3   | 59.7±9.2   | 59.5±8     | 59.2±8.2   | NS                 |
| LDLc (mmol/l)                | 2.76±0.69  | 2.73±0.87  |            | 2.65±0.41  | 0.0005 (1, 2≠3)    |
| TG (mmol/l)                  | 1.87±0.99  | 1.75±0.89  |            | 1.16±0.67  | NS                 |
| Total testosterone (nmol/l)  | 10.79±6.8  | 17.65±7.48 | 19.52±14.7 | 23.33±8.77 | 0.0001 (1≠2, 3, 4) |
| BT (nmol/l)                  | 2.01±1.04  | 4.68±2.43  | 5.71±2.33  | 7.83±6.2   | 0.001 (1≠2, 3, 4)  |
| Daily testosterone dose (mg) | 53.3±12.9  | 67.9±24.9  |            | 69.6±28.1  | 0.024              |
| Hematocrit %                 | 41.35±2.5  | 43.5±4.0   |            | 41.75±1.06 | NS                 |
| PSA (μg/l)                   | 0.87±0.9   | 1.27±1.05  |            | 0.35±0.2   | NS                 |

Data are expressed as mean±s.d.



**Figure 1** The effect of transdermal testosterone treatment on PWV in hypogonadal men. Pre-treatment values are shown as 'day 0', whereas on-treatment measurements recorded 48 h and 3 months after initiation of treatment are shown as days 2 and 90. Results are given as mean  $\pm$  S.E.M.

well as Dockery *et al.* (20) studied GnRH-induced hypogonadism, a rather acute form of hypoandrogenicity unaccompanied by other pituitary abnormalities. Second, BP was considerably higher, and indeed, clearly within the hypertensive range in the cohorts of prostate cancer patients assessed in the former studies, but not in our patients (e.g., 152/90 in reference (18) versus 137/79 mmHg in our patients). Because BP *per se* affects AIx (33) the results of the two studies are not necessarily comparable. Of potential importance is also the fact that our patients were, on the average,  $\sim$ 5 years younger than the subjects evaluated by Smith *et al.* (19) and 8–10 years younger than in the report by Dockery and associates (20).



**Figure 2** Effect of transdermal testosterone treatment on C1 in hypogonadal men. Pre-treatment values are shown as 'day 0', whereas on-treatment measurements recorded 48 h and 3 months after initiation of treatment are shown as days 2 and 90. Results are given as mean  $\pm$  S.E.M.

A recent report by Webb *et al.* indicated that oral testosterone therapy administered to men with coronary artery disease and low testosterone levels can reduce AIx (34). Again, the subjects studied in that report were rather different from our hypogonadal cohort. First, the degree of testosterone deficiency if any, was relatively minor, such that baseline testosterone and BT levels were more than twofold higher than in our hypogonadal patients. Second, AIx was extremely high in Webb's study, presumably reflecting a very advanced form of arterial stiffening in patients with well-established atherosclerosis as reflected by the presence of clinical coronary disease. Despite these differences in patient characteristics and the specifics of the experimental outcome, these studies, including our own, collectively lend support to the possibility that testosterone has a favorable effect on large artery stiffness.

Several lines of evidence support the concept that circulating testosterone affects arterial properties. There is an apparent inverse correlation between serum testosterone and BP (35). Furthermore, in a cross-sectional study of 55 older men, Dockery *et al.* reported that PWV was inversely related to circulating free testosterone index (36). In the Baltimore Longitudinal Study of Aging, an arterial stiffness index calculated from peak systolic and end diastolic diameters of the common carotid artery and simultaneous brachial artery BP correlated negatively with serum testosterone, after adjustment for confounding factors such as age, pulse pressure, FPG, and BMI (37). The finding in that study that serum testosterone obtained 5 years prior to the assessment of arterial properties predicted the arterial stiffness index, suggested a cause and effect relationship between low testosterone and the evolution of subsequent increased arterial wall rigidity.

Despite this apparent overall agreement that low serum testosterone is linked to increased arterial stiffness, the effect of testosterone replacement therapy on arterial properties remains a surprisingly understudied area. Circumstantial evidence suggests that the overall vascular effects of androgen replacement therapy are complex. Many of the study protocols of testosterone replacement therapy relied on testosterone depot injections, which are inherently linked to supraphysiological serum testosterone levels during significant parts of the treatment cycle. Likewise, observations in experimental models that testosterone replacement may increase BP by such mechanisms as increased oxidative stress, enhancement of tubular sodium and water reabsorption, activation of the renal renin-angiotensin system and endothelin, and reduced nitric oxide availability (38) could potentially reflect, at least in part, pharmacological rather than physiological effects of testosterone.

To our knowledge, this is the first report of how testosterone replacement therapy, aimed at attaining physiological and relatively stable concentrations of

serum testosterone through the use of transdermal testosterone preparation, acutely and chronically affects noninvasively determined arterial properties in clearly hypogonadal men. A unique aspect of the present report is also the fact that arterial properties were examined by several independent techniques, albeit in a small number of subjects. We observed a rapid but partial decline in PWV, such that the lowered PWV after 48 h and 3 months of treatment was still above that seen in control eugonadal men. At least the early favorable effect on PWV likely reflects functional adaptation of the large arteries to testosterone. Of interest is the observation that C1, another measure of arterial stiffness, which was not impaired at the outset of the study, improved significantly by 3 months of therapy, thus indicating increased compliance of the conduit arteries with normalization of serum testosterone. This delayed effect may reflect structural changes in the large arteries, in addition to the rapid functional effects detected by the early reduction in PWV. Notably, AIX was unaffected by treatment regardless of the assessment phase. We are presently unable to explain why these three measures of arterial stiffness, which presumably reflect predominantly larger artery status, show a differential pattern both in the baseline analysis and in their response to testosterone treatment. The possibility that, in the hypogonadal men in whom testosterone replacement therapy had been withdrawn prior to the initiation of this study, longer withdrawal period might have allowed the detection of better agreement among the various parameters cannot be excluded. However, a recent report indicated a poor correlation between PWV and C1 and recommended, for that reason, the use of a combination of techniques (39) rather than reliance on one method.

In conclusion, acquired hypogonadism in men is associated with increased arterial stiffness as reflected in increased PWV and, possibly, in increased AIX. Transdermal testosterone replacement exerted a rapid, though partial favorable effect on PWV. Large artery compliance as measured by C1 improved after 3 months, suggesting that chronic testosterone replacement therapy is required to modify some arterial properties. Because initial rapid effects are more likely to reflect functional changes, whereas more prolonged treatment can potentially drive structural effects, these results raise the possibility that testosterone can precipitate both functional and structural arterial responses. Whether or not longer term treatment will induce further changes in arterial stiffness, perhaps through direct changes in arterial structure or remodeling of body composition, remains to be determined in future studies.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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