Testosterone suppression in men with prostate cancer leads to an increase in arterial stiffness and hyperinsulinaemia

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ABSTRACT

The role of androgens in cardiovascular disease is uncertain. We aimed to determine the vascular effects of androgen suppression in men with prostate cancer. Arterial stiffness (or 'compliance') was measured in 16 men (71 ± 9 years, mean ± S.D.) prior to, and 3 months after, complete androgen suppression with gonadotrophin-releasing hormone analogues as treatment for prostate cancer. Fifteen control men (70 ± 7 years) also had arterial stiffness studies at baseline and 3 months later. Two measures of arterial stiffness were employed: systemic arterial compliance (SAC) was measured by simultaneous recording of aortic flow and carotid artery pressure ('area method'), and pulse wave velocities (PWVs) were recorded with the 'Complior' system. The 16 cases underwent glucose-tolerance and fasting-lipids tests on both visits.

After 3 months of testosterone suppression, there was a significant fall in SAC, which was not seen in the controls [mean change ± S.E.M., -0.26 ± 0.09 a.c.u. (arbitrary compliance unit) in the cases versus +0.06 ± 0.11 in the controls; P = 0.03]. Central, but not peripheral, PWVs tended to increase in the cases (mean change ± S.E.M. for aorto-femoral PWV, +0.5 ± 0.4 m/s for cases versus -0.3 ± 0.3 m/s for controls; P = 0.08). After testosterone suppression, fasting insulin levels increased from 6.89 ± 4.84 m-units/l to 11.34 ± 8.16 m-units/l (mean ± S.D.), total cholesterol increased from 5.32 ± 0.77 mmol/l to 5.71 ± 0.82 mmol/l and high-density lipoprotein cholesterol increased from 1.05 ± 0.24 mmol/l to 1.26 ± 0.36 mmol/l; P < 0.005 for all. No significant change occurred in body-mass index, serum glucose, low-density lipoprotein cholesterol or triacylglycerol (triglyceride) levels. Our results indicate that loss of androgens in men leads to an increase in aortic stiffness and serum insulin levels, and may therefore adversely affect cardiovascular risk.

INTRODUCTION

The measure of stiffness or ‘elasticity’ of the arterial wall is a useful surrogate marker for cardiovascular disease. Recent studies have shown increased aortic stiffness, as measured by pulse wave velocity (PWV) to be a marker of cardiovascular risk and of mortality in hypertensive patients [1,2]. Aortic stiffness, which increases with age, leads to higher systolic and lower diastolic pressures, which increases cardiac work-load and decreases cor-

Key words: androgen, arterial compliance, insulin.

Abbreviations: PWV, pulse wave velocity; DHEAS, dehydroepiandrosterone; PSA, prostate-specific antigen; HDL, high-density lipoprotein; SAC, systemic arterial compliance; SHBG, sex-hormone-binding globulin; a.c.u., arbitrary compliance unit; CI, confidence interval; ANCOVA, analysis of covariance.

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The role of androgens in cardiovascular disease in men is controversial; however, most observational studies report that high physiological androgen levels are associated with lower risk of coronary artery disease [7,8], myocardial infarction [9] and stroke [10], and low dehydroepiandrosterone (DHEAS) levels have been associated with increased aortic calcification and higher PWVs in healthy men [11]. Complete androgen removal using gonadotrophin-releasing hormone analogues is widely used as a first-line treatment for prostate cancer, and there are not many studies on the metabolic and cardiovascular consequences of this therapy. Smith et al. [12] have shown that in men this therapy results in an increase in arterial stiffness, as measured by augmentation index and time to wave reflection, although they did not study any control subjects. The purpose of this study was to compare the changes in systemic arterial compliance (SAC) and PWVs occurring in men following androgen-depletion, with that of controls studied in parallel.

**METHODS**

**Subjects**

Men with prostate cancer (n = 16), aged 71 ± 9 years (means ± S.D.), who were about to begin androgen-suppression treatment as decided by their consultant urologist, were selected for the study (they are referred to in this article as ‘cases’). The median pre-treatment prostate-specific antigen (PSA) value of these patients was 89 ng/ml (range, 8.9–1364.0 ng/ml). Four men had been on anti-hypertensive medication for an average of 14 ± 16 years, two men had diabetes: one was managed by diet only, while the other had taken a sulphonylurea for 3 years. All were studied as out-patients, and were free from any acute illness during the study period. Immediately after baseline studies, 15 men commenced therapy with flutamide (250 mg three time daily) for 2 weeks, followed by a goserelin injection (3.6 mg) 2 weeks later. Flutamide treatment was continued for a further 2 weeks only. The patients returned 12 weeks after the first injection for repeat studies. One man chose to have an orchidectomy, which was performed the week after the baseline study. The study was repeated 14 weeks later. Apart from the above treatment, no patient commenced any new medication during the study period.

The control group was composed of 15 men, aged 70 ± 7 years: seven men had prostate cancer that did not require any treatment and eight were healthy volunteers who were recruited via a local advertisement. None were taking vasoactive medication, or had any history of hypertension, cardiac disease, diabetes or hyperlipidaemia. The healthy controls had a mean PSA level of 2.1 ± 1.9 ng/ml (mean ± S.D.). The median PSA value for the ‘cancer, no treatment’ controls was 7.8 ng/ml (range, 0.5–87.5 ng/ml). All controls returned after 14 weeks for repeat studies. None had started any new medications during this period. The study was approved by the regional ethics committee, and all patients gave informed consent.

**Study procedures**

All recordings took place in a quiet room with patients lying supine for the duration of the study. Fasting bloods were taken for lipid profile, glucose, PSA, chemical profile, testosterone, sex-hormone-binding globulin (SHBG), DHEAS and oestradiol analysis. The non-diabetic cases also had fasting insulin levels measured, after which 75 g of glucose was given orally; 2 h later, blood was taken for the measurement of repeat glucose and insulin levels. These blood parameters were also measured after 3 months in the cases. All biochemical samples were analysed by the on-site laboratory, which is a member of the appropriate U.K. National Quality Assessment Scheme. Glucose and lipid levels were determined using the AU600 Olympus Diagnostic analyser, testosterone and oestradiol were determined by radioimmunoassay using ether extraction, SHBG and DHEAS were determined by immunometric assay (Diagnostics Products Corporation Immulite, Los Angeles, CA, U.S.A., and Nichols Institute Diagnostics, San Clemente, CA, U.S.A. respectively), and insulin and PSA were determined by Abbott Axsym immunometric assay. The interassay coefficients of variation were 10% for insulin, 2.7–4.3% for high-density lipoprotein (HDL) cholesterol and 2.2–2.5% for total cholesterol. The oestradiol assay does not measure accurately at values <100 nmol/l, so these values were taken as 100 for statistical purposes.

Body-mass index and waist/hip ratio were measured on both visits. The average of three brachial blood-pressure readings was taken using an automated oscillometric machine (OMRON 705CP) after 5 min of lying supine and at 3-min intervals thereafter.

SAC was measured using the ‘area method’, which records instantaneous aortic flow and the associated driving pressure; the method has been validated and described previously [13–16]. A hand-held Doppler flow velocimeter (Huntleigh MD2) placed on the supra-ternal notch measured ascending aortic blood flow. Instantaneous aortic flow was calculated as the mean Doppler flow x left ventricular outflow tract diameter, measured using M-mode echocardiography (Aloka echocamera SSD-500). This has been shown to correlate well with readings that were obtained invasively [16]. The carotid artery pressure wave (which has been shown to be similar to the central aortic waveform [17]) was simultaneously recorded using an applanation tonometer (Millar Inc., Houston, TX, U.S.A.) that was standardized for diastolic...
and mean pressure from the Omron brachial reading. SAC was derived from the formula
\[
A_d / (R(P_e - P_a))
\]
where \(A_d\) is the area under the diastolic curve of the carotid waveform, \(R\) is the total peripheral resistance calculated as the product of mean arterial pressure and instantaneous aortic flow, \(P_e\) is the end-systolic aortic pressure and \(P_a\) is end-diastolic pressure. Arterial compliance was expressed in arbitrary compliance units (a.c.u.).

PWV, which is inversely related to arterial compliance, was measured using the ‘Complior’ system [18], which calculates the time interval between two pulse-waves recorded simultaneously. The velocity is calculated as transit distance/transit time

Distances were measured as straight lines on the body surface. Aorto-femoral, aorto-radial and femoral-dorsalis pedis PWVs were recorded. The carotid artery pulse was used to represent the aortic pulse in all subjects and the average of ten recordings was calculated. A second method to measure PWV from the aorta to the finger was employed, which uses ECG with simultaneous pulse wave recording from a Finapres fitted on the middle phalynx of the third finger of the left hand. A custom-written program was used to calculate the time delay between the R-wave of the ECG and the second derivative of the upstroke of the digital arterial pulse wave.

Statistical methods

SPSS for Windows version 10.0 was used for statistical analysis. Unless otherwise stated, independent Student’s \(t\) test was used to compare all baseline variables between the two groups. Changes in SAC and PWV measurements were the major end-points in the study. The change from baseline was calculated in each subject, and the mean change in each group was compared, again using the independent Student’s \(t\) test. The paired \(t\) test was employed to compare the secondary endpoints of change in biochemical parameters from baseline in the cases. Appropriate non-parametric tests were used for data not fitting a normal distribution, and are stated where used. Analysis of covariance (ANCOVA) was used to adjust PWV changes for concomitant changes in blood pressure. All baseline or 3-month values are reported as means \(\pm\) S.D., numerical changes from baseline are reported as means \(\pm\) S.E.M. and non-normally distributed results are reported as medians and ranges.

RESULTS

Baseline characteristics

There was no significant difference between cases and controls in any baseline characteristics, except for a significantly higher mean waist/hip ratio, and a higher ECG–Finapres PWV in the cases (Tables 1 and 2).

Haemodynamics

After 3 months, there was a fall in blood pressure readings in both groups from baseline values [mean brachial systolic pressure changes \(\pm\) S.E.M., \(-5 \pm 3\) mmHg in the cases; \(-9 \pm 3\) in the controls; mean difference, 4; 95 % confidence interval (CI), \(-4 \pm 12\); \(P = 0.33\); Table 2]. The \(P\) value for the changes in systolic blood pressure from the baseline were 0.13 in the cases and 0.01 in the controls. Despite this drop in blood pressure, there was a fall in SAC in the cases, while it increased slightly in controls, in line with their drop in blood pressure (mean change \(\pm\) S.E.M., \(-0.26 \pm 0.09\) a.c.u. in cases versus \(+0.06 \pm 0.11\) a.c.u. in controls; mean difference, 0.8; 95 % CI, 0.003–0.61; \(P = 0.03\); Table 2). The \(P\) value for the changes in SAC from baseline were 0.01 in the cases and 0.60 in the controls.

Correspondingly, aorto-femoral PWV tended to increase in the cases, and to fall slightly in the controls, although this did not achieve statistical significance (mean change \(\pm\) S.E.M., \(+0.5 \pm 0.4\) m/s in the cases versus \(-0.3 \pm 0.3\) m/s in the controls; mean difference, 0.8; 95 % CI, \(-0.1 \pm 1.8\); \(P = 0.08\); Table 2). The \(P\) value for the changes in aorto-femoral PWV from the baseline were 0.18 in the cases and 0.26 in the controls. Adjusting the change in aorto-femoral PWV for the concomitant change in blood pressure, using ANCOVA did not alter the comparison of the two groups (\(P = 0.08\)).

There was no significant change in aorto-radial, femoral-dorsalis pedis or ECG–Finapres PWV readings from baseline in either the cases or controls (Table 2). On sub-group analysis, the controls with prostate cancer (\(n = 7\)) and the healthy volunteer controls (\(n = 8\)) did

<table>
<thead>
<tr>
<th>Table 1  Baseline characteristics</th>
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<tbody>
<tr>
<td>All values are mean (\pm) S.D., or number of patients. BMI, body-mass index; LDL, low-density lipoprotein.</td>
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<tr>
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<tr>
<td>Age (years)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Waist/hip ratio (m/m)</td>
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<td>Smokers: no. (pack years)</td>
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<tr>
<td>Known hypertension</td>
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<tr>
<td>Known diabetes mellitus</td>
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<tr>
<td>Total cholesterol (mmol/l)</td>
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<td>HDL–cholesterol (mmol/l)</td>
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<td>LDL–cholesterol (mmol/l)</td>
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<td>Triacylglycerols</td>
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* \(P = 0.02\); \(P > 0.10\) for all other parameters.
Table 2  Haemodynamic data
Baseline and 3-month values are means ± S.D. Changes are reported as means ± S.E.M. SBP, systolic blood pressure; DBP, diastolic blood pressure; A-F, aorto-femoral; A-R, aorto-radial; F-DP, femoral-dorsalis pedis.

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>3 months</td>
<td>Change</td>
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<tr>
<td>Heart rate</td>
<td>67 ± 6</td>
<td>68 ± 8</td>
<td>+1 ± 2</td>
</tr>
<tr>
<td>Brachial SBP (mmHg)</td>
<td>147 ± 14</td>
<td>142 ± 13</td>
<td>-5 ± 3</td>
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<tr>
<td>Brachial DBP (mmHg)</td>
<td>80 ± 10</td>
<td>77 ± 7</td>
<td>-3 ± 2</td>
</tr>
<tr>
<td>Central SBP (mmHg)</td>
<td>141 ± 13</td>
<td>137 ± 17</td>
<td>-3 ± 4</td>
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<tr>
<td>SAC (a.c.u.)</td>
<td>1.26 ± 0.58</td>
<td>1.00 ± 0.50</td>
<td>-0.26 ± 0.09</td>
</tr>
<tr>
<td>A-F PWV (m/s)</td>
<td>13.4 ± 2.7</td>
<td>14.1 ± 3.0</td>
<td>+0.5 ± 0.4</td>
</tr>
<tr>
<td>A-R PWV (m/s)</td>
<td>10.8 ± 1.7</td>
<td>11.5 ± 1.9</td>
<td>+0.6 ± 0.5</td>
</tr>
<tr>
<td>F-DP PWV (m/s)</td>
<td>10.9 ± 2.1</td>
<td>10.5 ± 2.4</td>
<td>-0.4 ± 0.5</td>
</tr>
<tr>
<td>ECG–Finapres (m/s)</td>
<td>11.7 ± 2.2</td>
<td>11.2 ± 1.7</td>
<td>-0.4 ± 0.4</td>
</tr>
</tbody>
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* Comparing ‘change from baseline’ between the two groups.
† P = 0.02 for baseline comparison; P > 0.11 for all other baseline comparisons.

not differ in any baseline or 3-month blood pressure, SAC or PWV readings.

**Insulin and lipid levels (non-diabetic cases only)**

Results of paired insulin levels from 12 out of 14 patients were available, as two individuals’ samples were damaged during centrifugation. After 3 months of androgen suppression, fasting insulin levels increased from 6.89 ± 4.84 to 11.34 ± 8.16 m-units/l (means ± S.D.) (mean change, +4.45; 95% CI, 1.94–6.96; P = 0.002; Table 3). Insulin levels that were measured 2 h post-prandially tended to rise, but not significantly, as did serum glucose. Total cholesterol levels increased from 5.32 ± 0.77 mmol/l to 5.71 ± 0.82 mmol/l (mean change, 0.39; 95% CI, 0.16–0.62; P = 0.003). HDL cholesterol increased from 1.05 ± 0.24 mmol/l to 1.26 ± 0.36 mmol/l (mean change, 0.21; 95% CI, 0.07–0.34; P = 0.005). There was no significant change in triacylglycerol or low-density lipoprotein cholesterol levels.

**DISCUSSION**

The role of androgens in cardiovascular disease in men has long been debated. It has been suggested that they may explain the sex difference in cardiovascular disease rates, although there is much conflicting evidence from studies. Most studies, however, seem to point to a beneficial effect of physiological levels of androgen
insulin resistance and, possibly, coronary heart disease risk [8,19–23], all through uncertain mechanisms.

In this study, androgen suppression in men resulted in a decrease in SAC, which reflects increased aortic stiffness that did not occur in a group of control men. Consistent with this fall in SAC was a trend to an increase in PWV in cases, when compared with the controls group ($P = 0.08$). This may largely be due to the small study numbers and, possibly, the short follow-up period. It may also be attributable to the fact that SAC provides an assessment of proximal aortic stiffening, whereas aorto-femoral PWV assesses stiffness from the aortic arch to the femoral artery, not including the proximal aorta. Both methods correlate well with other measures of arterial function, and with several other cardiovascular risk factors, although aorto-femoral PWV has been shown recently to predict cardiovascular mortality in hypertensive patients. No such results exist for SAC at present, but SAC has been shown to relate more closely to the presence and severity of coronary artery disease than PWV [24].

The androgen-suppressed men tended to have a smaller fall in blood pressure on their second visit (at 3 months) compared with the controls, but despite this, there was a paradoxical fall in SAC, while it increased slightly in the controls (as would be expected from the drop in blood pressure). Indeed, despite the magnitude of the decrease in blood pressure in the controls ($\pm 3\text{ mmHg}$, mean $\pm$ S.E.M.), their SAC and PWVs varied little, confirming that arterial stiffness is only partly dependent on blood pressure. Other factors, such as alterations in smooth muscle tone and/or structural alterations to the vessel wall, are potential contributors to change in arterial stiffness. It is unclear whether loss of androgens acts through such mechanisms. Animal studies show that testosterone has anti-atherogenic effects, acting through the vascular androgen receptors [25], although it is most

\begin{table}
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\begin{tabular}{lccc}
\hline
 & Baseline & 3 months & $P$ value \\
\hline
Total testosterone (nmol/l) & 19.7 $\pm$ 9.4 & 1.6 $\pm$ 0.6 & $< 0.001$ \\
SHBG (nmol/l) & 59.1 $\pm$ 24.6 & 57.6 $\pm$ 26.7 & 0.738 \\
Free testosterone index (%) & 37.2 $\pm$ 16.9 & 3.4 $\pm$ 2.1 & $< 0.001$ \\
DHEAS (nmol/l) & 3.3 $\pm$ 2.2 & 2.1 $\pm$ 1.6 & 0.002 \\
Oestradiol (nmol/l) & 171.5 $\pm$ 62.4 & 118.4 $\pm$ 55.9 & 0.009* \\
Insulin (fasting) (m-units/l) & 6.89 $\pm$ 4.84 & 11.34 $\pm$ 8.16 & 0.002 \\
Insulin (2hPP) (m-units/l) & 24.9 (3.3–161.3) & 27.1 (9.2–176.4) & 0.445 \\
Glucose (fasting) (mmol/l) & 5.6 $\pm$ 0.9 & 6.0 $\pm$ 1.2 & 0.178 \\
Total cholesterol (mmol/l) & 5.32 $\pm$ 0.77 & 5.71 $\pm$ 0.82 & 0.003 \\
HDL cholesterol (mmol/l) & 1.05 $\pm$ 0.24 & 1.26 $\pm$ 0.36 & 0.005 \\
LDL cholesterol (mmol/l) & 3.73 $\pm$ 0.79 & 3.74 $\pm$ 0.79 & 0.936 \\
Triacylglycerols (mmol/l) & 1.31 $\pm$ 0.49 & 1.50 $\pm$ 0.86 & 0.177 \\
\hline
* Values of $< 100 \text{ nmol/l}$ reported as 100 so actual fall in levels may be greater.
\end{tabular}
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unlikely that vessel wall remodelling occurred in this study due to the short follow-up period. Testosterone also has an androgen receptor-independent vasorelaxant effect on vascular smooth muscle, possibly via potassium and calcium channel inhibition, as suggested by animal studies [26,27]. This is supported by studies of intracoronary testosterone administration in men, which leads to immediate vasorelaxation [28]. Endogenous testosterone may therefore exert a continuous beneficial effect on the vessel wall. Shabsigh et al. [29] showed that within 24 h of castration, the rat displays a marked decrease in prosthetic blood flow and apoptosis of prostate vascular endothelial cells. This precedes the apoptosis of prostate epithelial cells, which indicates that testosterone is a prime regulator of prosthetic blood flow. Theoretically, the deprivation of androgens could have a similar adverse effect on the general vasculature. Oestradiol is also known to have vascular effects in men [30] and conjugated oestrogens may cause coronary artery vaso-relaxation in men [31]; however, oestradiol alone does not [32], so the fall in oestradiol levels associated with gonadotropin-releasing hormone therapy (which was underestimated in this study owing to the limitations of the assay) is unlikely to account for the increase in arterial stiffness in this study, although this possibility cannot be excluded.

Possible indirect mechanisms of action include effects on insulin and lipid pathways, which are both independently related to arterial stiffness [33,34]. Two prospective studies found that low serum-testosterone levels were an independent predictor of the development of non-insulin-dependent diabetes in men [22,35]. We observed a 63% increase in fasting serum insulin (a useful surrogate marker of fall in insulin sensitivity [36]) after 3 months of androgen suppression, a finding that is in agreement with two previous studies [12,37]. It is unclear why this occurs, but testosterone can modulate hepatic and lipoprotein lipases in visceral adipose tissue, which consequently affects insulin clearance [38]. There was no change in body-mass index or waist/hip ratios in our study subjects, but this does not exclude an increase in visceral adiposity. We also observed an increase in total cholesterol levels after androgen suppression; however, this was probably due to the significant increase in HDL cholesterol, which is in agreement with previous studies [23,37]. It is not clear whether these metabolic changes persist beyond 3 months, as these studies have also been of similar short duration. The rise in insulin levels might have been expected to cause a decrease in HDL levels, so clearly other factors which we have not investigated, are involved in the complex relationship between androgens, metabolic cardiac risk factors and arterial haemodynamics.

There are a number of limitations to this study. First, the numbers used were small, and the follow-up period was short at just 3 months, both of which are likely to account for the lack of a significant change in PWV, as mentioned earlier. A further study of longer duration is planned to investigate if the observed changes in both arterial stiffness and metabolic parameters are maintained over time. There are obvious limitations in using men with prostate cancer for studies of arterial compliance, namely the presence of the cancer itself, which might directly or indirectly affect arterial compliance. A placebo group could not be employed, so we included controls with prostate cancer who had localized disease that did not require treatment (and therefore had normal androgen levels). These controls did not show any difference in baseline or 3-month arterial compliance indices or blood pressure readings, compared with the healthy volunteer controls. The cases, however, tended to be less healthy than the controls: more advanced prostate cancer, slightly older, higher waist/hip ratio, slightly higher baseline systolic blood pressure and lower arterial compliance readings, and five cases were either diabetic or known hypertensives. The cases, however, acting as their own controls did show a significant deterioration in SAC from baseline, whereas the controls did not. A study of androgen suppression effects on arterial stiffness in healthy men is impractical, but is likely to give the same result; however, given the current uncertainty on how best to treat early prostate cancer, it is important to know of potential adverse cardiovascular effects of this widely used therapy in an age group at relatively high risk of cardiovascular disease. Many men continue this treatment for several years, and there is a reported increased incidence of cardiovascular deaths in men with prostate cancer, for unclear reasons [39].

In summary, we have demonstrated that testosterone suppression therapy in men with prostate cancer leads to an increase in arterial stiffness and a rise in serum insulin, which may consequently increase cardiovascular risk profile. This may be of importance in the unanswered question of the risks and benefits of treating early prostate cancer. It may also have implications for the potential role of testosterone replacement therapy in elderly men.

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Androgens and arterial stiffness

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