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Oestradiol improves arterial endothelial function in healthy men receiving testosterone

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Summary

OBJECTIVE To assess prospectively the effects of low dose oestradiol on arterial endothelial and smooth muscle function in healthy men. Oestrogen use is associated with reduced cardiovascular disease in oestrogen-deficient women, however, the vascular effects of low-dose oestradiol in healthy men have not been investigated previously.

PATIENTS and DESIGN Twenty-three men (aged 32 ± 8 years) were randomized to receive depot implants of testosterone (T) alone (group 1, $n = 10$), or T with either 10 mg (group 2, $n = 7$) or 20 mg (group 3, $n = 6$) of oestradiol (E).

MEASUREMENTS Hormone levels, lipids and vascular reactivity were measured before, 1 month and 6 months after hormone implantation. Using high-resolution ultrasound, brachial artery diameter was measured at rest, during reactive hyperaemia (leading to flow-mediated dilatation, FMD, which is endothelium-dependent) and after sublingual nitroglycerin (GTN, an endothelium-independent dilator).

RESULTS Oestradiol produced a dose-dependent increase in plasma oestradiol (at 1 month 96 ± 7, 149 ± 6, 192 ± 23 pmol/l in the 3 groups, respectively, $P < 0{\cdot}001$ by ANOVA for trend). Minor side-effects (gynaecomastia, nipple tenderness) indicated that 20 mg oestradiol was the maximum tolerated dose. There was also a dose-dependent increase in FMD with oestradiol dose: at 1 month, −0·2, +0·2 and +1·8% for groups 1–3, respectively ($P = 0{\cdot}31$ by ANOVA for trend); and at 6 months, −0·8, +0·4 and +2·2% ($P = 0{\cdot}02$). The rise in oestradiol levels following treatment correlated with the improvement in FMD ($P = 0{\cdot}01$). GTN responses were similar in the 3 groups throughout the study.

CONCLUSION In healthy young men, oestradiol supplementation is associated with enhanced arterial endothelial function, a key marker of vascular health.

Much recent interest has focused on the potential cardioprotective effects of female sex steroids, especially oestrogens, which may induce protective changes in lipoprotein levels, as well as direct beneficial effects on arterial wall structure and function (Gilligan *et al*., 1994a, b). In particular, oestrogen enhances endothelial function in both premenopausal and postmenopausal women (Lieberman *et al*., 1994; Hashimoto *et al*., 1995). As endothelial dysfunction is a key event in atherogenesis (Ross, 1993; Celermajer *et al*., 1992), this beneficial vascular effect of oestrogens may contribute to its impact in reducing cardiovascular risk.

Despite these observations in women, however, it remains unclear whether oestrogen influences arterial physiology in men. In short-term studies, acute administration of supra-physiological doses of oestradiol (over minutes only) enhances endothelium-dependent dilatation in coronary arteries of women, but not of men (Gilligan *et al*., 1994b; Collins *et al*., 1995). Vascular studies of longer term oestrogen administration to healthy men are difficult, however, and therefore this question has only previously been addressed by cross-sectional, retrospective analyses of male to female transsexuals taking prolonged high dose oestrogen therapy. We have previously demonstrated that vascular reactivity was enhanced in such transsexuals compared to control males (McCrohon *et al*., 1997), a finding also documented by New *et al*. (1997). Several confounding features of these unusual populations, however, have been noted, including the androgen-lowering effects of high-dose oestrogens and possible influences of other lifestyle factors. Furthermore, the effects of lower, more physiological oestrogen doses have not been studied in males.

We have therefore studied lipid, hormone and vascular responses in a number of healthy young men enrolled in a trial involving the administration of testosterone with or without low dose oestradiol coadministration. This study was primarily designed to assess the effectiveness of these treatments for

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male contraception and provided a unique opportunity to investigate the effects of oestrogen on arterial endothelial and smooth muscle function in males, using a prospective study design.

Methods

Subjects

Male volunteers aged 18–45 years, in good health, were recruited from the general population by advertising in print and broadcast media. Subjects were excluded if baseline evaluation revealed abnormal clinical, haematological, biochemical or reproductive (semen analyses and blood hormone levels) findings.

Clinical exclusion criteria included: chronic medical conditions (ischaemic heart disease, hypertension, diabetes mellitus, kidney, liver, prostatic disease, infertility, or psychiatric disorders), history of thrombo-embolic disease, regular use of medications, contraindications to local minor surgery for hormone implantation (allergy to local anaesthesia, bleeding disorder including aspirin intake, keloidal proneness), contraindications to the administration of testosterone or oestradiol, history of drug/alcohol abuse or the use of anabolic steroids. For this study, 23 healthy male volunteers were recruited.

Study design

The study had a randomised, double-blinded prospective, parallel group design. The study size was determined by power calculations related to the contraceptive endpoint. The results of this part of the study have recently been published (Handelsman *et al*. 2000). Volunteers were randomised to receive testosterone (T) 600 mg alone (as four 150 mg implants) or testosterone (T) 600 mg plus oestradiol (E) 10 or 20 mg implants via subcutaneous implantation under local anaesthesia. The study commenced as a randomised comparison of T alone with T plus 10 mg oestradiol with eligible volunteers randomised in a 1 : 1 ratio to the two groups. In view of the uncertainty of the optimal oestradiol dose, an interim summary of efficacy was planned at 50% target recruitment. This interim summary maintaining masking indicated no difference between groups in the primary study endpoint (spermatogenic suppression). As a result, a third arm (T plus E 20 mg) was added and the original lower oestradiol dose arm (T plus E 10 mg) was closed. In order to maintain efficiency and balance between arms, an urn randomization scheme (Wei & Lachin, 1988) was used for volunteers entering the second half of the study. The urn randomization used a probability value of $P = 2^d/(2^d + 1)$, where d is the difference between numbers in the two arms to be balanced. The final randomization distribution was 10 men randomised to T implants only, seven received T and E 10 mg and six received T and E 20 mg.

Vascular studies were performed at baseline, one month and six months after hormone implantation. All studies were performed in the morning after an overnight fast. Vascular responses were analysed by two independent observers using high-resolution external vascular ultrasound of the right brachial artery, as described by Celermajer *et al*. (1992), using an Acuson 128XP10 mainframe and a 7·0-MHz linear array transducer. Brachial artery diameter was measured at rest, during reactive hyperaemia (leading to flow-mediated dilatation, FMD, an endothelium-dependent stimulus) and after a 400 mcg spray of sublingual nitroglycerin (GTN, an endothelium-independent dilator). Reactive hyperaemia responses were assessed following temporary (4·5 min) brachial artery occlusion with a sphygmomanometer placed below the target artery (cuff to 250 mmHg). Comparing arterial diameter measurements during reactive hyperaemia to baseline allows calculation of the values for FMD, which is predominantly due to endothelial nitric oxide release (Joannides *et al*., 1995). This method allows reliable and reproducible measurement of arterial endothelial dysfunction (Sorensen *et al*., 1995), a key marker of vascular injury (Celermajer, 1997). Operators and analysers were blinded to treatment assignment, and analysers to the stage of the experiment.

Serum hormone levels, sperm output and routine biochemistry were measured at monthly intervals throughout. T was measured by established immunoassays, free T by centrifugal ultrafiltration (AMICON) and E by radioimmunoassay. Clinical chemistry and lipids were measured by routine autoanalyser methods.

Drug description and techniques

Testosterone and oestradiol implants are fused rods composed of pure steroid crystals without excipients, shaped into small cylindrical rods (testosterone 12 mm long, 4·5 mm diameter; oestradiol 2·5 mm long, 2·25 mm diameter) [oestradiol implants supplied by Organon Pty Ltd, Sydney, Australia]. Subdermal implantation does not require sutures or antibiotics and implants are placed in the antero-lateral abdominal wall under local anaesthesia. Implants are fully biodegradable and do not require removal.

Statistical analysis

Descriptive data are expressed as mean value ± SEM. Groups were compared by ANOVA or independent sample *t*-tests, as appropriate. The effects of oestradiol (0, 10 mg and 20 mg) on lipids, hormones and vascular responses were tested by ANOVA

for trend (SPSS, version 6·0). The prospectively defined endpoint was change in FMD between baseline and 6 month visits, compared between groups. Sample size numbers were determined in a power calculation based on the contraceptive endpoint (suppression of spermatogenesis – see Handelsman *et al*. 2000); the study had relatively low power for the vascular reactivity endpoint. For example, this study had only 60% power to detect a difference of 2·5% in the treatment related change in FMD between the control and oestrogen groups at the $2P < 0{\cdot}05$ level, assuming a standard deviation of 2·5% for the baseline measurements. 'Area under curve' calculations for hormone levels were based on the monthly hormone measurements, representing the sum of all these measures divided by half the baseline plus final values. Correlation coefficients were calculated using linear regression analyses (univariate and multivariate models). Statistical significance was inferred at a two-sided P-value $< 0{\cdot}05$.

Results

Baseline characteristics

The subject groups were well matched for age, smoking status, lipids, hormones, blood pressure and resting vessel size (Table 1). No subject changed their smoking habits during the course of the study. Therapeutic compliance was assured (one implant session only, at baseline) and follow-up was excellent (94% of visits). In follow-up, there were two extruded testosterone implants at week 6; one of the four T implants was extruded in one subject in the T plus E 10 mg group and similarly one of the four implants was extruded in one subject in the T plus E 20 mg group (both these subjects were included in the analyses). There were no episodes of significant bleeding or infection as a consequence of implantation. Patients receiving T alone or T plus E 10 mg experienced no adverse effects, apart from minor bruising immediately after implantation. One patient who received T plus E 20 mg experienced nipple tenderness, commencing 4 weeks postimplantation and another man experienced nipple tenderness and gynaecomastia from week 12 postimplantation. In both cases, the oestrogenic side-effects lasted for several weeks but were resolved by the end of the study.

Table 1 Baseline characteristics of the 23 subjects by assigned treatment

	T only	T plus E10mg	T plus E 20 mg
Age (years)	33·5 ± 2·5	30·4 ± 2·3	32 ± 4
Height (m)	1·78	1·79	1·80
Weight (kg)	82·5	82·0	81·1
BMI	25·9	25·8	24·9
Current or former smokers	40%	29%	67%
Pack years	4 ± 2·2	3·3 ± 2·3	8 ± 4·7
Systolic BP (mmHg)	131 ± 3·2	124 ± 6·8	120 ± 4·5
Diastolic BP (mmHg)	78 ± 3·2	70 ± 3·5	73 ± 4·5
Total cholesterol (mmol/l)	4·9 ± 0·3	4·5 ± 0·3	4·7 ± 0·3
HDL (mmol/l)	1·3 ± 0·1	1·2 ± 0·1	1·4 ± 0·2
Triglycerides (mmol/l)	1·4 ± 0·2	1·2 ± 0·3	1·6 ± 0·5
T (nmol/l)	19·0 ± 1·8	22·5 ± 2·2	21·3 ± 2·7
E (pmol/l)	92 ± 5	98 ± 6	101 ± 7

There were no significant differences in any of these measured parameters ($P > 0{\cdot}10$ for all, by one-way ANOVA). BMI, body mass index; BP, blood pressure; E, oestradiol; HDL, high density lipoproteins; T, testosterone; TG, triglycerides.

Hormone and lipid levels

Total and free testosterone remained within the normal range (total testosterone 11–35 nmol/l, free testosterone index 50–220%) throughout the study in all subjects, with no significant change over time in any group (Fig. 1). Serum oestradiol levels increased in a dose-dependent manner, with peak levels noted at 1 month postimplantation (96 ± 7, 149 ± 6, 192 ± 23 pmol/l, respectively, $P < 0{\cdot}001$ by ANOVA for trend) (Fig. 2). There was also a dose-related increase in the area under the oestrogen–time curve (523 ± 48, 784 ± 72, 929 ± 62 pmol.month/l, respectively, $P < 0{\cdot}001$). Full illustrations of the hormone levels over time from these subjects have been published in a paper describing the spermatogenic suppression of these regimes (Handelsman *et al*. 2000).

Lipid levels (total cholesterol, LDL, HDL, triglycerides) in all groups were within the normal range and did not alter

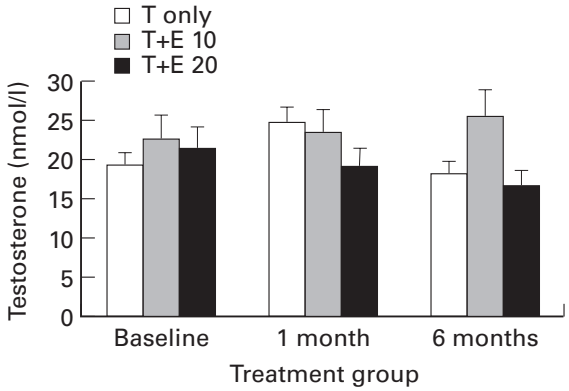

Fig. 1 Serum total testosterone levels at baseline, 1 month and 6 months postimplantation in those men receiving 0, 10 or 20 mg oestradiol with their testosterone 600 mg implants (no significant differences between groups).

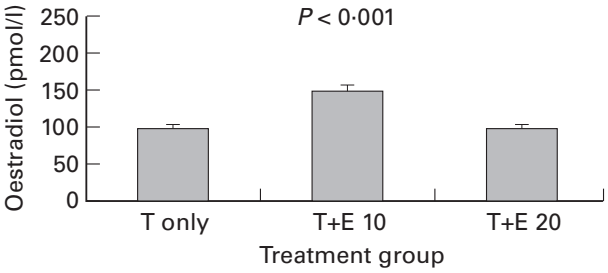

Fig. 2 Peak oestradiol levels (1 month after implantation) in those men receiving 0, 10 or 20 mg oestradiol with their testosterone 600 mg implants ($P < 0{\cdot}001$).

significantly in any group throughout the 6-month period of observation ($P > 0{\cdot}10$).

Vascular reactivity

The resting vessel size was similar in the three groups at baseline; 3·9, 3·9 and 4·0 mm, respectively, and did not change significantly throughout the study. The flow mediated dilatation response was also similar in the three groups at baseline; 5·1, 3·9 and 2·3%, respectively ($P = 0{\cdot}16$). The mean change in FMD from baseline was greater in those receiving more E; at 1 month; $-0{\cdot}2$, $+0{\cdot}2$ and $+1{\cdot}8\%$ ($P = 0{\cdot}31$, ANOVA for trend); this was statistically significant at 6 months; $-0{\cdot}8$, $+0{\cdot}4$ and $+2{\cdot}2\%$ ($P = 0{\cdot}02$) for groups 1–3, respectively (Fig. 3). The absolute values for FMD at 6 months, however, were not significantly different between the three groups of subjects ($P > 0{\cdot}2$).

On univariate analysis, the change in FMD at 6 months correlated significantly with both the peak oestradiol levels recorded and with oestradiol increase over the entire 6-month period of observation (area under the curve) ($P = 0{\cdot}01$)

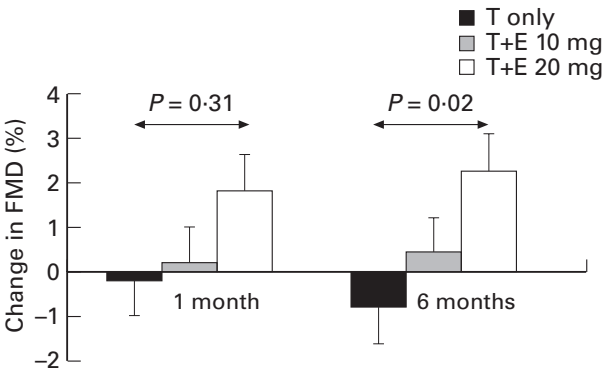

Fig. 3 Change in flow-mediated dilatation (compared to baseline values) at 1 and 6 months after implantation, according to the oestradiol dose given.

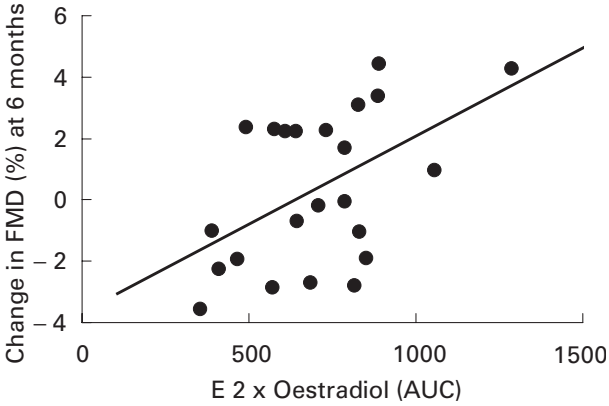

Fig. 4 The relationship between overall 6 month oestradiol levels (AUC, area under the curve) and the change in flow-mediated dilatation at 6 months, in 23 healthy men. $r = 0{\cdot}50$; $P = 0{\cdot}01$.

(Fig. 4). On multivariate analysis, the change in FMD at 6 months was independently related to oestradiol levels (either peak oestradiol or area under the oestradiol–time curve, $P < 0{\cdot}001$), but not to subject age, cholesterol level, blood pressure, smoking status or vessel size. GTN responses (endothelium-independent, smooth muscle dependent responses) were similar in the three groups at baseline (14·2, 14·5, 18·7%, respectively, $P = 0{\cdot}08$). The mean change in response to GTN did not differ significantly between the groups at 1 month; 0·0, $+0{\cdot}1$ and $-1{\cdot}2\%$ ($P = 0{\cdot}88$) or at 6 months; $+1{\cdot}3$, $+2{\cdot}8$ and $+0{\cdot}3\%$ ($P = 0{\cdot}40$) for groups 1–3, respectively.

Discussion

In healthy young males, low dose oestradiol implants significantly enhanced arterial endothelium-dependent dilatation, in a dose-dependent manner, without altering vessel size or smooth muscle dependent responses. This is consistent with enhanced nitric oxide release by the vessel wall, with potentially beneficial antiatherogenic effects (Cooke & Tsao, 1994). The improvement in endothelial function observed in these men correlated with the oestradiol levels achieved after hormone treatment.

Oestrogens in men

Previously, the question of oestrogen's arterial effects in men has been investigated in experimental animals, studies of the short-term effects of oestrogens in humans, and retrospective cross-sectional analyses of male to female transsexuals. These previous studies have provided conflicting results. Animal studies by Hanke *et al*. (1996) found that aortic plaque size was reduced in cholesterol-fed ovariectomised female rabbits given

oestrogen, but not in castrated male rabbits on identical therapy. The vascular effects of ultra short-term parenteral oestrogen administration in coronary disease patients have been investigated, with Collins *et al*. (1995) finding that 17-β-oestradiol attenuated acetylcholine-induced coronary arterial constriction in females, but not in males. Similarly, the acute sublingual administration of 17-β-oestradiol did not improve endothelium-dependent vasodilatation in young men, despite achieving supraphysiological oestradiol levels (New *et al*., 1999). By contrast, the recent report of improved endothelial function in men following the cessation of testolactone (an aromatase inhibitor), which inhibits the conversion of testosterone to oestradiol in tissues, suggests that there may be beneficial effects of oestradiol on male endothelial function (Komesaroff *et al*., 1999).

Recently, two cross-sectional studies of the effects of long-term high-dose oral oestrogens in genetic males (male to female transsexuals) have shown enhanced arterial reactivity, compared with age-matched male controls (McCrohon *et al*., 1997; New *et al*., 1997). These studies suggested that oestradiol might have beneficial vascular effects in human males. However, there were several potential confounding variables in the studies of these unusual populations, including variation in the castrative therapy used, the dose and route of oestrogen administration, the use of progestins, the high percentage of smokers and an older population. The current study of healthy young men investigated the effects of oestradiol together with testosterone, in order to avoid the hypogonadal effects of using oestrogen alone. Nevertheless, this prospective study of oestrogens in men provides unique data on the effects of lower dose, medium term oestrogen supplementation and support an oestrogen-related enhancement in nitric oxide-mediated arterial dilatation.

Mechanisms of action

The observed benefit of oestrogen in this setting may be due to receptor-mediated or receptor-independent mechanisms. Oestrogen receptors have been identified in human endothelial (Venkov *et al*., 1996) and smooth muscle cells (Karas *et al*., 1994). Stimulation of the endothelial receptor produces enhanced NO production via upregulation of NO synthase activity (Hayashi *et al*., 1995), and indeed Sudhir *et al*. (1997) have recently reported the case of a man with endothelial dysfunction secondary to a disruptive mutation of the oestrogen receptor gene. As endothelial cell oestrogen receptor expression in male cells may be enhanced by long-term oestrogen exposure (Rabelo & Tata, 1993; Sato *et al*., 1994), it is possible that vascular responses to oestrogen may therefore be improved in men following chronic oestrogen administration, via a receptor-mediated mechanism. This also may explain a slower time course of oestrogen-related vascular improvement in men (over 6 months) compared to studies showing more rapid effects in women (over 4–8 weeks) (Rajkumar *et al*., 1997).

Receptor-independent pathways include possible antioxidant, nongenomic and androgen-suppressing effects of exogenous oestrogens. Oestrogens are known to have potent antioxidant potential, as measured by fatty acid and sterol oxidation (Subbiah *et al*., 1993), and this may improve redox balance in the vessel wall and thereby enhance endothelium-dependent dilatation by improving local bioavailability of NO (Laursen *et al*., 1997). Recently, Komesaroff *et al*. (1998) have demonstrated that oestrogen has a rapid-onset and rapid-offset effect on cutaneous endothelial function in men, consistent with a nongenomic dilator mechanism. Oestrogens administered acutely in men have also been demonstrated to favourably modulate coronary vasomotor responses to cold pressor and acteylcholine testing (Blumenthal *et al*., 1997; Reis *et al*., 1998). Finally, oestrogen may act by suppressing androgen production via the hypothalamic–pituitary axis.

We have previously demonstrated that complete androgen withdrawal is associated with enhanced endothelial function in older men (Herman *et al*., 1997). In the current study, however, there was no significant fall in testosterone levels during oestrogen treatment, and therefore this mechanism of oestrogen action is unlikely to have caused the oestrogen-related improvement in vascular reactivity. As the lipid profiles were not altered by any treatment throughout the 6-month period, this is also unlikely to have contributed to the enhanced FMD in the oestradiol groups in this study.

Clinical implications

Oestrogens cannot be recommended for use in men, although the demonstration that oestrogen may enhance endothelial function in males raises the possibility that selective oestrogen response modification might be beneficial. Potential adverse effects of parenteral oestrogen administration in men include the local complications of subcutaneous implantation and the systemic effects of exogenous oestrogen. In this study, 20 mg oestradiol resulted in dose-limiting side-effects, indicating that higher levels of oestradiol might not be tolerated satisfactorily. Concerns regarding an increase in thrombotic events with higher dose supplemental oral oestrogen administration were raised by the results of the Veterans Administration Cooperative Urological Research Group (VACURG), which compared various endocrine therapies for prostatic carcinoma. This study was terminated prematurely due to high dose oral oestrogens, including Diethylstilbestrol, increasing thrombotic deaths (Byar & Corle, 1988). The association of cardiovascular mortality with oestrogen administration was dose-related. The

oral administration of the lower dose of 2·5 mg conjugated oestrogens daily for an average of 56 months, in 1101 men who had recovered from myocardial infarction in The Coronary Drug Project, did not reveal a statistically significant difference in mortality, when compared to placebo (The Coronary Drug Project, 1973). The area of sex steroid interactions with vascular biology in men might therefore be interesting for further prospective study.

Study limitations

This was a relatively small study, but provided an opportunity to investigate the effects of oestrogen in healthy men prospectively. Although powered for a contraceptive endpoint with low power for showing a beneficial effect on endothelial function, the vascular effects of oestrogen were sufficiently marked and consistent to allow the demonstration of a significant improvement in endothelial-dependent dilatation. It is unlikely that the exclusion criteria for the contraceptive study, such as prostatic disease and infertility, would have biased the vascular reactivity endpoints measured in this study. Although the three groups of randomised men were closely matched for age, lipids and blood pressure, it is possible that unmeasured differences may have been present between the groups. The baseline FMD tended to be nonsignificantly lower in the high dose oestradiol group, which may have been due to a nonsignificantly higher proportion of smokers in this group. These subjects demonstrated the greatest improvement in FMD over time. Although this may be due to a 'regression to the mean', the dose dependency of the oestradiol effect on vascular reactivity (see Fig. 3) strongly suggests that our observations reflect a true biological effect.

Measurement of arterial endothelial function has recently become established as an important method for the detection of early arterial abnormalities in humans (Celermajer, 1997). We have previously reported the reliability and reproducibility of this technique for accurate measurement of vascular reactivity (Sorensen *et al*., 1995). Although we tested brachial artery responses, brachial FMD measurement reflects predominantly nitric oxide release (Joannides *et al*., 1995) and correlates significantly with coronary endothelial function (Anderson *et al*., 1995) and coronary atherosclerosis (Neunteufl *et al*., 1997). Recently, preliminary data have linked endothelial responses to clinically relevant cardiovascular outcomes (Neunteufl *et al*., 1999; Al Suwadi *et al*., 1999).

Conclusions

These data demonstrate that low-dose oestradiol supplementation enhances arterial endothelial function in healthy young men, in a dose-dependent manner.

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