Acute administration of 17β-oestradiol does not improve endothelium-dependent vasodilatation in young men

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ABSTRACT
Studies have recently demonstrated that long-term oestrogen therapy improves endothelium-dependent and endothelium-independent vasodilatation in the conductance vessels of biological males. We sought to determine if an acute single dose of oestrogen might similarly improve vasodilator function in young males. In a randomized, double-blind, placebo-controlled, crossover study, we compared the effects of 1 mg of sublingual 17β-oestradiol (E2) and placebo on endothelium-dependent and endothelium-independent vasodilatation in the brachial artery using a non-invasive ultrasound technique. We recruited 30 young males based on a power calculation. Neither acute sublingual oestrogen nor placebo affected flow-mediated vasodilatation [5.32 ± 0.78% and 5.28 ± 0.60% respectively (mean ± S.E.M.), P = 0.94]. Responses to nitroglycerine were similar after oestrogen or placebo (16.01 ± 0.86% and 15.29 ± 1.19%, P = 0.47). Basal blood flow and flow during reactive hyperaemia did not differ after oestrogen or placebo. Heart rate and blood pressure were similar during both treatment phases of the study. The absolute change in serum oestradiol levels was greater after the oestrogen treatment phase than after placebo (1509 ± 87 versus −13 ± 4 pmol/l, P < 0.0001). Despite achieving supra-physiological oestradiol levels, the acute administration of sublingual E2 does not appear to improve endothelium-dependent or endothelium-independent vasodilatation, at least acutely, in the brachial artery of young males. In keeping with our previous study, these data suggest that a period of oestrogen ‘priming’ (possibly to induce receptor-mediated nitric oxide synthesis) may be required to yield an improvement in vascular function in males.

INTRODUCTION
Observational studies strongly suggest that oestrogen replacement therapy has a cardioprotective effect in postmenopausal women [1]. However, data from a recent randomized clinical trial have questioned its utility in secondary prevention [2]. Whether oestrogen can confer a clinical benefit on males is unclear. Studies of males prescribed low to moderate doses of oestrogen for prostate cancer have reported a reduction in cardiovascular events [3,4]. A recent retrospective report on a cohort of over 800 male to female transsexuals prescribed oestrogen for the purposes of feminization has shown no increased risk of cardiovascular morbidity or mortality [5].

Studies on mechanisms via which oestrogen may exert its effect have revealed a direct influence of this hormone on vascular reactivity in both women and men [6–8]. Both clinical [8–10] and experimental studies [11] have demonstrated that oestrogen improves endothelium-
dependent vasodilatation in postmenopausal females. This benefit has been observed by a number of research groups, using a variety of techniques and oestrogen formulations studied in many vascular beds [8–10,12,13]. Some experimental studies have also suggested the involvement of non endothelium-dependent mechanisms [14–16], although the results of clinical studies do not support this [8–10,17]. Acute as well as chronic administration of oestrogen has also been shown to improve endothelium-dependent responses in female vasculature [8,12,13], but studies with acute oestrogen have yet to consistently demonstrate an involvement of endothelium-independent mechanisms [17,18]. To date, however, the few studies examining the potential effects of acute oestrogen administration on vascular function in males have yielded conflicting results [13,19–22]. We therefore sought to determine whether an acute single dose of oestrogen improves endothelium-dependent and/or endothelium-independent function in biological males. We hypothesized that sublingual oestrogen would not alter vascular function as a period of oestrogen receptor priming may be necessary. In order to best appraise the acute effects of oestrogen, the study was performed in a double-blind, randomized, placebo-controlled, crossover fashion using an appropriately calculated sample size. The dose of oestrogen chosen was equivalent to that previously shown to produce oestradiol levels corresponding to or higher than the follicular phase of the menstrual cycle, and to improve endothelium-dependent vasodilatation [22] and delay the onset of exercise-induced acute ischaemia in post-menopausal women [23–25].

METHODS

Subjects

Thirty males (mean age±S.D.: 27±9 years) were recruited from staff in our hospital and by advertisement. The males had never taken oestrogen, androgen suppression medication, undergone orchidectomy or had a history of infertility, and all had serum testosterone levels within the normal male range. All subjects gave their written informed consent. The study was approved by the Human Research and Ethics Committee of our hospital and all procedures followed were in accordance with institutional guidelines.

Study design

All subjects were studied in the non-fasting state at the same time of day in a quiet temperature-controlled room. Subjects were asked to refrain from caffeine, smoking and alcohol for 12 h before the studies. Each subject underwent two treatment phases performed on separate days, with more than 2 days intervening to allow for a ‘washout’ period. Subjects were randomly assigned to receive 17β-oestradiol (E2; Estrace, Mead Johnson, Evansville, IN, U.S.A.) or placebo for the first treatment study and then crossed over for the subsequent study (see Figure 1). The subjects, ultrasonographer and examiners measuring the brachial artery diameter were blinded to the sequence of treatments. All measurements were performed on all subjects.

Drug protocol

A baseline measurement of flow-mediated vasodilatation (FMD) was performed at the beginning of each study. A rest period of 20 min was then observed for the vessel diameter to return to baseline. Placebo or E2 was then administered sublingually according to a randomized schedule. A further 30 min was then allowed for the peak blood levels to be achieved in accordance with previous studies [23]. FMD was then reassessed. Another 20-min rest period was then observed, followed by the administration of sublingual nitroglycerine (GTN) for assessment of endothelium-independent responses (see Figure 1).

Brachial artery ultrasound

The ultrasound studies were conducted by the same person throughout the study using a high-resolution ultrasound machine (ATL, HDI Ultramark 9) with a 7–10-MHz linear array transducer. We assessed FMD by measuring the diameter of the brachial artery before and during reactive hyperaemia (induced after deflation of a blood pressure cuff previously inflated to supra-systolic pressure for 5 min). The flow-mediated vasodilator response to reactive hyperaemia was then continuously recorded (from 30 s before cuff deflation until 5 min after deflation). Arterial flow velocity was also assessed 15 s after the cuff was released. We then assessed endothelium-independent vasodilatation via response to sublingual GTN. Brachial artery images were recorded before and for 5 min after the administration of GTN (0.6 mg) to measure maximum vasodilatation. Arterial flow velocity was also measured at 2 min 30 s. Brachial artery diameter measurements were performed by two investigators who were blinded to the treatment phases and the time points of the scans. This technique has been described in detail previously [26].

Measurement of images

Three frames for each time point in the study (see Figure 1) were grabbed on the ‘R’ wave for analysis. The observers were instructed on which portion of the vessel to measure via a diagram drawn by the ultrasonographer. Each frame was measured three times by each examiner. Hence, there were 108 measurements per study. The average of each measurement of the three frames per time
point was used to calculate FMD responses. FMD and responses to GTN were calculated as the percentage difference in diameter between baseline and after cuff inflation/deflation (hyperaemia) or after GTN administration relative to baseline.

**Reproducibility**

Coefficients of variation were used to determine reproducibility of brachial artery diameter measurements within the same observer and between observers. Within-day and between-day variability in brachial artery diameter was also assessed using these methods. Intra-observer variability for the measurement of brachial artery diameter was assessed by comparing two of the three measurements (randomly selected) for each frame. Inter-observer variability for the measurement of brachial artery diameter was assessed by comparing one (of the three) set of measurements for each observer for each frame. Within-day and between-day variability was assessed by averaging all the arterial diameter measurements of both the observers.

In this study, we found an intra-observer variability (mean ± S.D. of the difference) in measuring resting arterial diameter of 0.003 ± 0.06 mm with a coefficient of variation of 0.12%. Our inter-observer variability in measuring resting arterial diameter was 0.01 ± 0.17 mm with a coefficient of variation of 0.61%. The within-day variability in resting arterial diameter was 0.05 ± 0.13 mm with a coefficient of variation of 0.40%, and our between-day variability in measuring resting arterial diameter was 0.03 ± 0.21 mm with a coefficient of variation of 0.92%.

**Blood sampling and assays**

Blood samples for oestradiol levels were obtained from each subject before and after each treatment phase. Serum oestradiol concentrations were measured using a high-sensitivity double-antibody radioimmunoassay (Catalogue No. KED1, Diagnostic Product Corporation, Bio-medig DPC, Doncaster, Australia). The normal range for adult males in our laboratory is 25–130 pmol/l. Blood samples for lipid profile were taken after a 10-h overnight fast to measure plasma concentrations of lipids and glucose and serum concentrations of testosterone. Concentrations of total cholesterol, high-density lipoprotein-cholesterol and triacylglycerol were measured enzymically. Low-density lipoprotein-cholesterol was calculated according to the Friedewald formula. If the triacylglycerol levels were greater than 4.5 mmol/l, low-density lipoprotein-cholesterol was not calculated.

**Statistical analysis**

Descriptive data are expressed as means ± S.D. All results are expressed as means ± S.E.M. unless otherwise stated. Statistical significance was taken at a two-tailed value of $P < 0.05$. Paired observations of responses to FMD and GTN were made using analysis of variance and Student’s $t$-test.
Calculation of sample size

A sample-size calculation was performed based on a 90% power to detect a difference of 3–6% in FMD at a \( P \) value of \(<0.01\). This was based on FMD responses and the standard deviations to oestrogen therapy reported in previous papers using the same ultrasound technique \([6,22,27,28]\). From this we estimated that a sample of between 17 and 29 subjects was required. Thirty subjects were therefore recruited.

RESULTS

The clinical and morphometric characteristics of the group are described in Table 1. There was no difference in heart rate and mean arterial blood pressure responses between the two treatment studies (oestrogen versus placebo).

Table 1  Clinical characteristics
Values are expressed as means ± S.D. HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

<table>
<thead>
<tr>
<th>Characteristics (n = 30)</th>
<th>Mean ± S.D.</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>27 ± 9</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>23 ± 6</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.82 ± 0.22</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.8 ± 1.3</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.28 ± 0.57</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>1.2 ± 0.9</td>
</tr>
</tbody>
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Vasodilator responses

There were no differences in baseline arterial diameter on either day before administration of oestrogen or placebo \([4.68 ± 0.10 \text{ mm} \text{ and } 4.65 ± 0.10 \text{ mm} \text{ respectively (mean ± S.E.M.), } P = 0.44\]. Furthermore, baseline arterial diameter did not significantly alter after the administration of oestrogen or placebo \([4.74 ± 0.10 \text{ mm} \text{ and } 4.73 ± 0.10 \text{ mm} \text{ respectively, } P = 0.93\) or before the administration of GTN \([4.72 ± 0.10 \text{ mm} \text{ and } 4.71 ± 0.10 \text{ mm} \text{ respectively, } P = 0.85\].

FMD was similar on each day before the administration of oestrogen or placebo \([5.95 ± 0.67\% \text{ and } 5.31 ± 0.57\% \text{ respectively, } P = 0.31; \text{ mean difference } = 0.006 (95\% \text{ confidence interval: } −0.006–0.019); \text{ see Figure 2}\]. Similarly, there was no difference in FMD responses after the administration of either oestrogen or placebo \([5.32 ± 0.78\% \text{ and } 5.28 ± 0.60\% \text{ respectively, } P = 0.94; \text{ mean difference } = 0.004 (95\% \text{ confidence interval: } −0.011–0.012); \text{ see Figure 2}\]. Vasodilatation in response to GTN did not differ after the administration of oestrogen or placebo \([16.01 ± 0.86\% \text{ and } 15.29 ± 1.19\% \text{ respectively, } P = 0.47; \text{ mean difference } = 0.007 (95\% \text{ confidence interval: } −0.013–0.027); \text{ see Figure 3}\].

Blood flow responses

There was no difference in baseline blood flow before the administration of oestrogen or placebo \([240 ± 22 \text{ ml/min} \text{ and } 260 ± 31 \text{ ml/min} \text{ respectively, } P = 0.46\]. There was also no difference in baseline blood flow 30 min after administration of oestrogen or placebo \([252 ± 24 \text{ ml/min} \text{ versus } 225 ± 22 \text{ ml/min respectively, } P = 0.17\]. The per-

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**Figure 2**  Percentage change in arterial diameter before and after the administration of placebo or oestrogen in response to reactive hyperaemia (flow-mediated endothelium-dependent vasodilatation)

Neither placebo nor oestrogen had an effect on vasodilatation \((P \text{ not significant})\). Circles represent individual data points; circles with a bar represent means ± S.E.M.
percentage change in flow (hyperaemia) was also the same before (504 ± 36 % and 527 ± 43 % respectively, \( P = 0.62 \)) and after administration of either oestrogen or placebo (521 ± 46 % and 577 ± 47 % respectively, \( P = 0.19 \)). There was no difference in blood flow before and during GTN administration for either treatment phase.

**Oestradiol levels**

The mean (±S.E.M.) serum oestradiol level before oestrogen administration was 61 ± 6 pmol/l and before placebo was 80 ± 9 pmol/l. The serum oestradiol level was 1570 ± 87 pmol/l after oestrogen administration and 67 ± 7 pmol/l after placebo administration. The absolute change in oestradiol level was significantly greater after oestrogen administration compared with after placebo (1509 ± 87 pmol/l and −13 ± 4 pmol/l respectively, \( P < 0.0001 \); see Figure 4). Similarly, the percentage change was significantly higher after oestrogen compared with after placebo (3285 ± 435 % and −14 ± 5 % respectively, \( P < 0.0001 \); see Figure 4).

**DISCUSSION**

This study sought to determine whether the acute administration of oestrogen could improve vascular function in the brachial artery of biological males. Despite 1 mg of sublingual E₂ increasing serum oestradiol to supra-physiological levels, which have previously been shown to attenuate exercise-induced ischaemia in post-menopausal women [23], we were unable to demonstrate an improvement in flow-mediated endothelium-dependent vasodilatation or GTN-induced endothelium-independent vasodilatation compared with placebo.

Previous clinical studies have given conflicting results regarding the acute effects of oestrogen supplementation on vascular reactivity in males. While Blumenthal et al. [19] showed an improvement in endothelium-dependent vasodilatation in the coronary circulation of males with intravenous conjugated equine oestrogen, and Reis et al. [20,21] demonstrated acute attenuation of abnormal coronary vasoconstriction, others have shown differing results. Collins et al. [13] were unable to demonstrate an acute improvement in endothelium-dependent vasodilatation in men after intracoronary E₂, and Kawano et al. [22] were also unable to demonstrate an improvement in FMD with transdermal E₂. Our findings therefore differ from those of Blumenthal et al. and Reis et al. but agree with those of Collins et al. and Kawano et al.

These findings also contrast with previous observations [6,27] with respect to long-term administration of oestrogen therapy in males. It is possible that the lack of effect with an acute single dose of E₂ may be related to the presence of fewer oestrogen receptors and hence an obligatory requirement for oestrogen ‘priming’ in males. This effect may depend on dose, exposure time and
formulation of the oestrogen used. In postmenopausal women, the improvement in oestrogen-mediated endothelium-dependent vasodilatation has been shown to be nitric-oxide-dependent [29] and may be mediated via the increased production of constitutively derived nitric oxide synthase [30]. Experimental studies have demonstrated the reduced basal release and/or activity of nitric oxide and the delayed induction of nitric oxide synthase with oestrogen supplementation in the male compared with the female vasculature, suggesting that a longer exposure period or a higher dose of oestrogen may be necessary in males before the full effect is achieved [31–34]. Oestrogen stimulates its own receptor formation in females. It is possible that oestrogen may take longer to stimulate its own receptors in men. However, recent experimental studies have shown that even acute oestrogen supplementation can induce rapid genomic signalling to increase endothelium-derived nitric oxide synthase [35] which may be dependent on oestrogen receptors [36,37]. Another possible explanation for our negative results compared with those from previous reports in postmenopausal females is that men have higher circulating levels of testosterone compared with females, and this may attenuate the potential benefit of oestrogen administered to males. Interestingly, however, the few reports on the effects of testosterone on vascular reactivity in both men and women have in fact demonstrated a beneficial effect on endothelium-dependent vasodilatation [38,39].

Importantly, in this study we did not observe any effect of acute E_2 on endothelium-independent (GTN-induced) vasodilatation, but others have postulated that non-endothelial [14–16,40], although not necessarily non-nitric-oxide, mechanisms [41] may be involved in oestrogen’s acute vasodilator effects. Freay et al. [42] have also demonstrated an acute relaxing effect of E_2 in male and female rat aortic rings that is both endothelium-independent and oestrogen-receptor-independent. They have shown this effect to be mediated via inhibition of Ca^{2+} entry. Our current findings on the effects of acute E_2 on endothelium-independent vasodilatation are consistent with several previous studies in both men and women [10,13,19] but differ from our findings of long-term oestrogen therapy in the conduit vessel of male to female transsexuals [6].

**Limitations**

Despite the double-blind, randomized, placebo-controlled, crossover design, an appropriate sample size and reproducibility, we cannot completely exclude a small benefit from acute oestrogen administration on endothelial function in young males. A minor improvement (< 3%) is plausible, but much larger numbers of subjects would be required to demonstrate this. Importantly, only small numbers were required to demonstrate a significant benefit with chronic oestrogen therapy in males in two previous independent studies [6,27].

We chose to use sublingual E_2 as it can rapidly produce supra-physiological serum levels of oestradiol, thereby avoiding the need for intravenous infusion. Although adequate levels of oestradiol were achieved in our study, these were short lived and induced by a synthetic formulation of oestrogen. It is possible that the use of ‘natural’ conjugated oestrogens or a longer ‘exposure’ time would have induced a different result and may explain the results of previous studies [19,21].

Other factors could also affect our results. Our studies were performed in the non-fasting state. Studies have recently shown differences in FMD responses associated with acute changes in glucose [43] and triacylglycerol levels [44], which are known to fluctuate with time and type of food ingestion. These effects, however, should have influenced both arms of the study equally. Finally, in our study we only examined healthy young males. It is possible that acute oestrogen might improve FMD in men with baseline impaired vasodilatation such as may occur as a result of age, coronary artery disease or the presence of risk factors for vascular disease. This issue needs to be addressed by further studies.

**Clinical implications**

Improvement in endothelial dysfunction has been proposed as an important factor in various acute coronary syndromes such as vasospasm or unstable angina [45]. Although the recent HERS trial [2] did not demonstrate a benefit of hormone replacement therapy on long-term clinical outcomes in women with coronary artery disease, an acute dose of oestrogen may potentially have a therapeutic role in alleviating ischaemia, perhaps by acutely reversing this endothelial dysfunction. This has been demonstrated in some [23–25], but not all [46], studies of postmenopausal women with proven coronary disease and ischaemia. However, despite the favourable experimental data [45,47], other current clinical evidence [48] and the results of our study suggest that this is unlikely to occur in males.

**CONCLUSIONS**

The present study demonstrates that acute oestrogen supplementation does not appear to alter endothelium-dependent or endothelium-independent vasodilator function in young males. This contrasts with our previous findings on the effects of chronic oestrogen supplementation in biological males. These data suggest that a period of oestrogen ‘priming’ may be necessary to achieve vasodilator effects in males.
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