JACC Vol. 29, No. 7
June 1997:1437–44

Long-Term Estrogen Therapy Improves Vascular Function in Male to Female Transsexuals

GISHEL NEW, MBBS, KATRINA L. TIMMINS, BSc(Hons), STEPHEN J. DUFFY, MBBS, BINH T. TRAN, BSc(Hons), RICHARD C. O'BRIEN, MBBS, PhD, RICHARD W. HARPER, MBBS, FACC, IAN T. MEREDITH, MBBS, PhD, FACC

Melbourne, Australia

Objectives. **This study sought to examine the effects of long-term estrogen therapy on vascular function in male to female transsexuals and to compare the findings with those observed in men and premenopausal women.**

Background. **Gender differences in coronary artery disease have largely been attributed to the beneficial effects of estrogen on vascular function and plasma lipids in women. However, the effects of estrogen on the male vasculature have not been widely studied.**

Methods. **We compared the effects of estrogen on vascular function in 14 male to female transsexuals, 14 age-matched men and 15 premenopausal women. Flow-mediated vasodilation and response to nitroglycerin were assessed in the brachial artery using noninvasive ultrasound.**

Results. **Flow-mediated vasodilation was similar in transsexuals and women but greater than that in men ([mean ± SE] 11.5 ± 1.3% and 9.4 ± 1.1% vs. 5.2 ± 1.0% respectively, $p < 0.005$). Responses to nitroglycerin were also greater in transsexuals and women than in men (21.6 ± 1.7% and 21.0 ± 0.9% vs. 14.5 ± 1.2%, respectively, $p = 0.0005$). These differences persisted even after adjusting for vessel size. Despite similar total cholesterol levels, transsexuals had high density lipoprotein cholesterol levels similar to those in women and greater than those observed in men (1.76 ± 0.12 and 1.82 ± 0.11 mmol/liter vs. 1.35 ± 0.07 mmol/liter, respectively, $p < 0.005$). Moreover, triglyceride levels were greater in transsexuals than in men and women, and low density lipoprotein cholesterol (LDL-C) particle size was smaller (25.7 ± 0.2 nm vs. 26.2 ± 0.1 and 26.6 ± 0.1 nm, respectively, $p = 0.0001$). Serum testosterone (an index of estrogen therapy in transsexuals) was markedly suppressed in transsexuals and similar to that in women. Univariate analysis revealed that there was a strong inverse correlation between serum testosterone and flow-mediated vasodilation ($r_s = -0.48$, $p < 0.005$). Multivariate analysis revealed that the best combination of predictors of flow-mediated vasodilation was serum testosterone, vessel size and LDL-C ($R^2 = 0.3$, $p < 0.005$).**

Conclusions. **Long-term estrogen therapy appears to improve vascular function in male to female transsexuals and occurs despite higher triglyceride levels and the presence of small, dense LDL-C. The beneficial effects of estrogen are not gender specific or solely mediated through endothelium-derived nitric oxide.**

(J Am Coll Cardiol 1997;29:1437–44)

Gender differences in coronary artery disease (CAD) have been known for many years (1). The differential risk of CAD between men and premenopausal women has largely been explained in terms of the beneficial effects of endogenous estrogen and is supported by the fact that postmenopausal women taking hormone replacement therapy (HRT) appear to have a significantly lower risk of CAD than their untreated counterparts. This benefit is possibly even greater in women with proven CAD (2).

The mechanisms by which estrogen exerts its cardioprotective effects are probably multifactorial. The benefits may in part be due to favorable alterations in the lipid profile. Studies in postmenopausal women have shown that estrogen replacement therapy reduces low density lipoprotein cholesterol (LDL-C) and raises high density lipoprotein cholesterol (HDL-C). However, only a fraction of the beneficial effects can be explained by these changes (3). Another postulated mechanism is that estrogen exerts a direct effect on the vascular endothelium and smooth muscle. Several workers have shown (4,5) that short-term administration of estrogen in postmenopausal women improves endothelium-dependent vasodilation in both the coronary and peripheral circulations. Estrogen may also alter basal blood flow in these beds (4). Although the mechanism is unclear, experimental studies (6) suggest that the improvement in vasodilator effects of estrogen may be partly mediated through the L-arginine–nitric oxide (NO) pathway.

The effects of estrogens in men are less clear. Early studies suggested that men prescribed high dose estrogen had an

From the Cardiovascular Centre, Cardiology Unit, Monash Medical Centre and Department of Medicine, Monash University, Melbourne, Australia. This study was supported by Cardiovascular Research Centre funds. Dr. New is supported by a Monash University Graduate Scholarship. Dr. Duffy is supported by a Postgraduate Medical Research Scholarship from the National Health and Medical Research Council of Australia, Canberra.

Manuscript received November 11, 1996; revised manuscript received January 7, 1997, accepted March 4, 1997.

Address for correspondence: Dr. Ian T. Meredith, Cardiovascular Research Centre, Cardiology Unit, Monash Medical Centre, 246 Clayton Road, Clayton, Melbourne, Australia, 3168. E-mail: ian.meredith@med.monash.edu.au.

0735-1097/97/$17.00
PII S0735-1097(97)00080-6

Abbreviations and Acronyms

CAD = coronary artery disease
ECG = electrocardiogram, electrocardiographic
FMD = flow-mediated vasodilation
GTN = nitroglycerin
HDL-C = high density lipoprotein cholesterol
HRT = hormone replacement therapy
LDL-C = low density lipoprotein cholesterol
NO = nitric oxide
NOS = nitric oxide synthase
TG = triglycerides

increased cardiovascular morbidity and mortality (7,8). However, low to moderate doses appear to have a beneficial effect on mortality (9,10). Interestingly, both clinical and experimental studies (7,11,12) indicate that estrogen has a favorable effect on plasma lipoproteins in men similar to that observed in postmenopausal women taking HRT.

The long-term effects of estrogen on vascular function in men has not been studied in detail. Long-term studies of estrogen in men are logistically difficult, except in specific populations, such as male to female transsexuals or men with prostatic cancer. However, studies in the latter group are often compounded by advancing age and the presence of comorbid illnesses. Male to female transsexuals, prescribed estrogen for the purpose of feminization, provide a unique opportunity to study the long-term effects of estrogen on the male vasculature.

Therefore, the aim of the present study was to examine the effects of long-term estrogen therapy on vascular function in male to female transsexuals and to compare the findings with those observed in men and premenopausal women.

Methods

Subjects. Fourteen male to female transsexuals, 14 age-matched healthy men and 15 age-matched healthy premenopausal women between 21 and 57 years old were recruited (Table 1). Subjects were enrolled from October 31, 1995 to October 10, 1996. The transsexuals were recruited from the Gender Dysphoria Clinic of the Department of Psychological Medicine at Monash Medical Centre. The transsexuals had been taking estrogen (ethinyl estradiol [10] or conjugated equine estrogen—Premarin [4]) for at least 5 months (61 ± 70 months, mean ± SD). The doses prescribed were approximately two to three times the conventional oral contraceptive and comparable to high dose HRT. The mean oral dose of ethinyl estradiol was 118 ± 60 μg, and the mean oral dose of Premarin was 1.03 ± 0.35 mg. The doses of estrogen prescribed were sufficient to produce "medical orchidectomy," thereby reducing serum testosterone levels to within the normal female range. Three transsexuals had also undergone surgical orchidectomy as part of their gender reassignment program; 11 transsexuals were taking spironolactone; and 2 were also taking cyproterone acetate for hirsutism. One transsexual had multiple coronary risk factors (hypercholesterolemia, non–insulin-dependent diabetes mellitus and a family history of CAD); one had a family history of CAD; and another had hypercholesterolemia with a LDL-C level of 4.5 mmol/liter. Three other transsexuals were smokers.

Table 1. Baseline Characteristics

	TX (n = 14) (mean ± SD)	Men (n = 14) (mean ± SD)	Women (n = 15) (mean ± SD)
Age (yr)	41 ± 9	41 ± 7	40 ± 8
BMI (kg/m^2)	23 ± 3	25 ± 4	24 ± 5
W/H	0.86 ± 0.07	0.87 ± 0.05	0.76 ± 0.06*
SBP (mm Hg)	126 ± 15	123 ± 9	116 ± 12
DBP (mm Hg)	75 ± 11	74 ± 5	71 ± 11
No. of CRFs	0.57 ± 0.85†	0	0
Testosterone (nmol/liter)	1.8 ± 1.3	17.2 ± 5.4*	1.9 ± 0.5

*$p = 0.0001$. †$p < 0.005$. BMI = body mass index; CRFs = coronary risk factors; DBP = diastolic blood pressure; SBP = systolic blood pressure; W/H = waist/hip ratio; TX = transsexuals.

The men and women were recruited by advertisement. Male control subjects had never taken estrogen, spironolactone or cyproterone acetate or undergone orchidectomy or had a history of infertility. All had serum testosterone levels within the normal male range. Female control subjects were premenopausal, had regular menses, were not taking hormonal contraception and were studied during the follicular or luteal phase of their menstrual cycle to maximize the effects of estrogen, as described in previous studies (13). Control subjects were excluded if they had any coronary risk factors, including a total cholesterol or LDL-C level greater than the 75th percentile for their age and gender based on the Risk Factor Prevalence Study (14). All subjects gave written informed consent. The study was approved by the Human Research and Ethics Committee of Monash Medical Centre, and all procedures followed were in accordance with institutional guidelines.

Study design. All subjects were screened with a medical history, examination and electrocardiogram (ECG). Blood samples were taken after a 10-h overnight fast to measure plasma concentrations of lipids, glucose and serum concentrations of testosterone and estradiol in women. All specimens were measured within 48 h of blood sampling. Total cholesterol, HDL-C and triglyceride (TG) concentrations were measured enzymatically. LDL-C was calculated according to the Friedewald formula. If the TG levels were >4.5 mmol/liter, LDL-C was not calculated. LDL-C particle size was measured using 3% to 13% gradient gel electrophoresis (Gradipore, New South Wales, Australia). Serum testosterone levels were used as an index of estrogen therapy in the transsexuals and were measured by radioimmunoassay. Serum estradiol levels were not measured in the transsexual group because ethinyl estradiol and conjugated equine estrogen (the agents used by the transsexuals for feminization) have low cross reactivity with the radioimmunoassay for estradiol.

Brachial artery ultrasound. Brachial ultrasound studies were performed in the morning in a quiet, temperature-controlled laboratory. Blood pressure was monitored in the left arm at 2-min intervals by an automated blood pressure recorder (Critikon, Dinamap 845 XT BP monitor). ECG leads were attached to the ultrasound recorder for on-line continuous heart rate monitoring. The ultrasound studies were conducted by the same examiner throughout the study. All patients rested in the supine position for at least 10 min before the study. Studies of the right brachial artery were performed using a high resolution ultrasound machine (ATL, HDI Ultramark 9) with a 7- to 10-MHz linear array transducer. Longitudinal images of the brachial artery were obtained proximal to the antecubital fossa wherever the best ultrasound image could be obtained. Operating variables of the machine were kept constant during each study. Transmit focus zones were set approximately to the depths of the anterior and posterior vessel walls. Images were magnified, whereas depth and gain settings were set to optimize the image of the vessel wall, in particular, the media–adventitia interface ("m" line), as previously described (15). When an adequate transducer position was obtained, the skin was marked and the arm kept in a constant position throughout the study. A still photograph of the baseline image in addition to anatomic markers, such as side branches, were used to aid the examiner in maintaining a constant transducer position throughout the study.

The technique for assessing endothelium-dependent and -independent vasodilation has been described in detail elsewhere (15). In short, we assessed flow-mediated, endothelium-dependent vasodilation by measuring the arterial diameter of the brachial artery before and during reactive hyperemia (induced after deflation of a blood pressure cuff previously inflated to suprasystolic pressure for 5 min). We then assessed endothelium-independent vasodilation in response to sublingual nitroglycerin (GTN). Arterial flow velocity was measured with a pulsed Doppler signal at a 70° angle to the vessel with the range gate (1.5 mm) in the center of the vessel. The flow-mediated vasodilator response to reactive hyperemia was then continuously recorded (from 30 s before cuff deflation and until 5 min after deflation). Arterial flow velocity was also assessed 15 s after the cuff was released. Rest baseline diameter and flow were then reassessed 20 min later. Nitroglycerin (0.4 mg) was then administered sublingually, and the brachial artery image was recorded for 5 min to measure maximal vasodilation. Arterial flow velocity was again measured at 2 min 30 s.

Data analysis. Maximal arterial diameter after reactive hyperemia was assessed 45 to 90 s after cuff deflation and 3 to 5 min after GTN administration, in accordance with previously described methods (15). The ultrasound images were recorded on Super-VHS videotape for subsequent analysis. Images were digitized using a frame grabber (Scion LG-3) and then stored on a Macintosh computer (Centris 650). All images were grabbed on the R wave (end-diastole) of the continuous ECG recording. Subsequent analysis was performed on the same computer using a modified version of the public domain NIH Image Program, originally developed at the U.S. National Institutes of Health. The diameter of the vessel was measured by two investigators (K.L.J., B.T.T.) who had no knowledge the scan sequence and gender of the subjects. Arterial diameter was measured from a fixed anatomic marker in all scans of the same patient. Arterial diameter was measured from the anterior "m" line (media–adventitia interface) to the media–lumen interface on the posterior wall. Approximately 20-mm straight segments of the artery were measured automatically, and the average diameter was calculated. Two comparable and representative frames were analyzed. Each frame was measured three times, and all six measurements were then averaged. The percent change in the brachial artery diameter was then calculated in response to reactive hyperemia (flow-mediated vasodilation [FMD]) and GTN. FMD was also adjusted for vessel size (by dividing by baseline diameter) because previous studies (15,16) have shown a strong negative correlation between vessel diameter and FMD ($r = -0.81$) and GTN ($r = -0.80$). If there were >3% discrepancy between the two investigators' results, the diameters were remeasured. Blood flow was calculated by multiplying the velocity–time integral of the Doppler flow signal by heart rate and the cross-sectional area of the vessel. Flow velocity was measured from the center of the vessel, which may overestimate absolute values; however, relative values before and after cuff inflation are accurate (15). Hyperemia (percent increase in flow) was calculated by subtracting the maximal flow recorded ~15 s after cuff deflation from the baseline flow divided by the baseline flow (% Hyperemia = [{Fp − Fb}/Fb∗100], where Fp = peak flow; and Fb = baseline flow).

Statistical analysis. Descriptive data are expressed as mean value ± SD and results as mean value ± SE. Statistical significance was taken as $p < 0.05$. Between-group comparisons were made using one-way analysis of variance with Bonferroni correction for multiple comparisons. Logarithmic transformation of serum testosterone and triglycerides were used in the analysis of variance. Univariate analysis using a Spearman rank correlation was used to determine the relation between the dependent variables (responses to FMD and GTN) and independent variables (age, vessel size, body mass index, waist/hip ratio, total cholesterol, HDL-C, LDL-C, LDL-C particle size, TG, testosterone and systolic and diastolic blood pressures). Multivariate analysis was performed using multiple linear regression to determine the best combination of predictor variables.

Results

The clinical and morphometric characteristics of the subjects are described in Table 1. There were no significant differences in age, body mass index or systolic and diastolic blood pressures. Waist/hip ratio was less in women than in transsexuals and men ([mean ± SD] 0.76 ± 0.06 vs. 0.85 ± 0.07 and 0.88 ± 0.05, respectively, $p = 0.0001$). The average number of risk factors was greater in transsexuals than in the men and women ($p < 0.005$, for transsexuals vs. men and women). Serum testosterone levels were similar in transsexuals

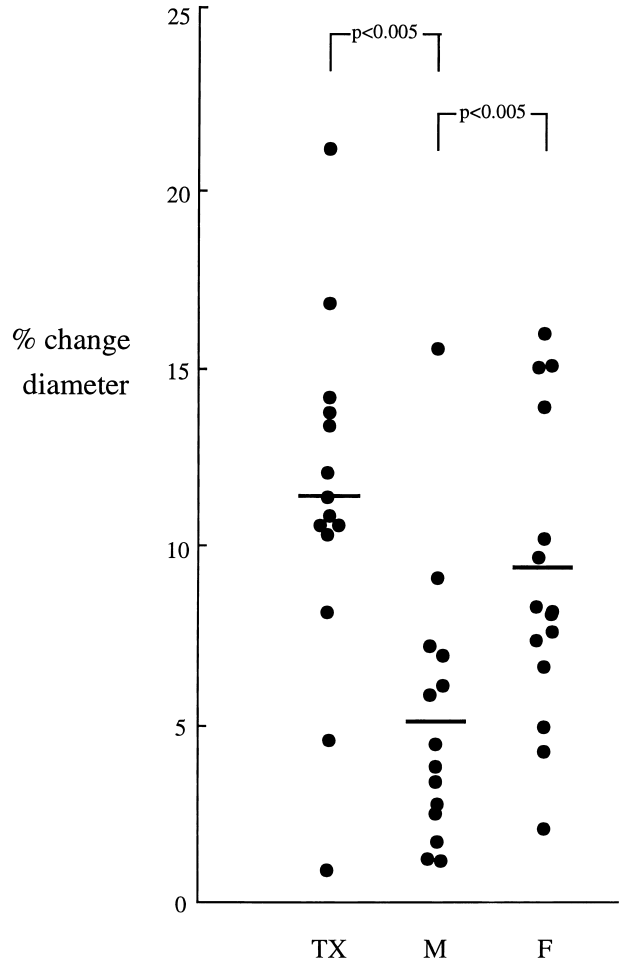


Figure 1. Flow-mediated vasodilation in male to female transsexuals (TX), men (M) and premenopausal women (F). **Horizontal lines** = group mean values; **circles** = individual subjects.

and women but lower than those observed in men (1.8 ± 1.3 and 1.9 ± 0.5 nmol/liter vs. 17.2 ± 5.4 nmol/liter, respectively, p = 0.0001 for transsexuals and women vs. men).

Flow-mediated vasodilation. The percent change in brachial arterial diameter after reactive hyperemia was similar in transsexuals and women but greater than that observed in men ([mean ± SE] 11.5 ± 1.3% and 9.4 ± 1.1% vs. 5.2 ± 1.0%, respectively, p < 0.005 for transsexuals and women vs. men) (Fig. 1, Table 2). Baseline brachial artery diameter was similar in transsexuals and women but less than that observed in men (4.0 ± 0.1 and 3.6 ± 0.1 mm vs. 4.9 ± 0.2 mm, respectively, p = 0.0001 for transsexuals and women vs. men). After adjusting responses for brachial artery diameter, FMD remained similar in transsexuals and women but greater than that in men (2.9 ± 0.4% and 2.7 ± 0.4% vs. 1.2 ± 0.3%, respectively, p = 0.001 for transsexuals and women vs. men) (Table 2).

Rest blood flow was similar in transsexuals and men but greater than that in women (134 ± 14 and 161 ± 21 ml/min vs. 90 ± 8 ml/min, respectively, p < 0.01 for men vs. women). There was no difference in hyperemic flow between the three groups.

Table 2. Vascular Variables

	TX (n = 14) (mean ± SE)	Men (n = 14) (mean ± SE)	Women (n = 15) (mean ± SE)
FMD (%)	11.5 ± 1.3	5.2 ± 1.0*	9.4 ± 1.1
Baseline vessel size (mm)	4.1 ± 0.1	4.9 ± 0.2†	3.6 ± 0.2
aFMD (%/mm)	2.9 ± 0.4	1.2 ± 0.3‡	2.7 ± 0.4
Baseline flow (ml/min)	134 ± 14	161 ± 21	90 ± 8§
Hyperemia (% inc in flow)	687 ± 113	564 ± 90	740 ± 103
GTN (%)	21.6 ± 1.7	14.5 ± 1.2‖	21.0 ± 0.9
aGTN (%/mm)	5.5 ± 0.6	3.1 ± 0.4†	6.0 ± 0.4

*p < 0.005. †p = 0.0001. ‡p < 0.001. §p < 0.01. ‖p = 0.0005. aFMD = flow-mediated dilation adjusted for vessel size (percent flow-mediated vasodilation divided by baseline diameter [mm]); aGTN = nitroglycerin-induced vasodilation adjusted for vessel size (percent nitroglycerin-induced vasodilation divided by baseline diameter [mm]); FMD = flow-mediated vasodilation; GTN = nitroglycerin-induced vasodilation; inc = increase; TX = transsexuals.

GTN-induced vasodilation. Vasodilation in response to sublingual GTN was 33% greater in transsexuals and women than men (21.6 ± 1.7% and 21.0 ± 0.9% vs. 14.5 ± 1.2%, respectively, p = 0.0005 for transsexuals and women vs. men) (Fig. 2, Table 2). Similarly, after adjustment for brachial artery diameter, GTN-induced vasodilation remained greater in transsexuals and women than in men (5.5 ± 0.6% and 6.0 ± 0.4% vs. 3.1 ± 0.4%, respectively, p = 0.0001 for transsexuals and women vs. men) (Table 2).

Lipid profiles. Total cholesterol levels were similar in all groups ([mean ± SE] 5.5 ± 0.4, 5.1 ± 0.2 and 5.0 ± 0.1 mmol/liter for transsexuals, men and women, respectively) (Table 3). HDL-C levels were also similar in transsexuals and women and approximately 23% higher than that observed in men (1.76 ± 0.12 and 1.82 ± 0.11 mmol/liter vs. 1.35 ± 0.07 mmol/liter, respectively, p < 0.005 for transsexuals and women vs. men). Plasma LDL-C levels were similar in transsexuals and women, but lower than those observed in men (2.6 ± 0.3 and 2.8 ± 0.2 mmol/liter vs. 3.3 ± 0.2 mmol/liter, respectively, p < 0.05 for transsexuals vs. men). TG levels were substantially higher in transsexuals than in women and men (2.3 ± 0.4 mmol/liter vs. 0.8 ± 0.1 and 0.9 ± 0.1 mmol/liter, respectively, p = 0.0001 for transsexuals vs. women and men). LDL-C particle size was smaller in transsexuals than in women and

Table 3. Lipid Profiles

	TX (n = 14) (mean ± SE)	Men (n = 14) (mean ± SE)	Women (n = 15) (mean ± SE)
TC (mmol/liter)	5.5 ± 0.4	5.1 ± 0.2	5.0 ± 0.1
HDL-C (mmol/liter)	1.76 ± 0.12	1.35 ± 0.07*	1.82 ± 0.11
LDL-C (mmol/liter)	2.6 ± 0.3	3.3 ± 0.2†	2.9 ± 0.2
TG (mmol/liter)	2.3 ± 0.4‡	0.9 ± 0.1	0.8 ± 0.1
LDL-C particle size (nm)	25.7 ± 0.2‡	26.2 ± 0.1	26.6 ± 0.1

*p < 0.01. †p < 0.05. ‡p = 0.0001. HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; TC = total cholesterol; TG = triglycerides; TX = transsexuals.

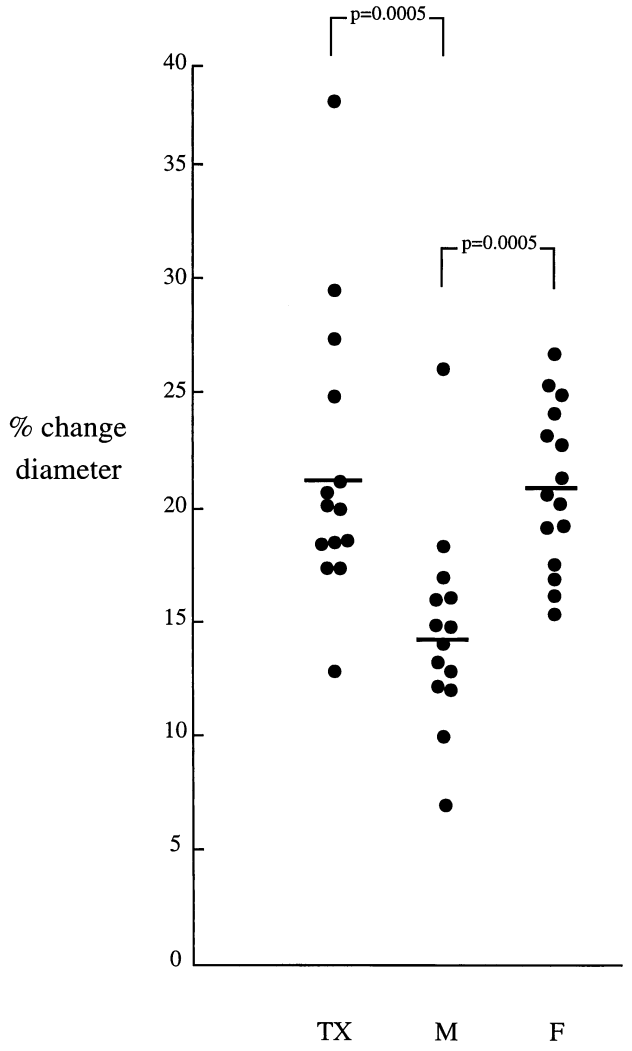


Figure 2. Nitroglycerin-induced vasodilation in male to female transsexuals (TX), men (M) and premenopausal women (F). Symbols as in Figure 1.

men (25.7 ± 0.2 nm vs. 26.6 ± 0.1 and 26.2 ± 0.1 nm, respectively, $p = 0.0001$ for transsexuals vs. women and men).

Regression analysis. Univariate analysis revealed that the best single predictor of FMD was serum testosterone—our index of estrogen therapy ($r_s = -0.48$, $p < 0.005$). Other predictors were HDL-C ($r_s = 0.42$, $p < 0.01$) (Fig. 3), vessel size ($r_s = -0.38$, $p < 0.01$), waist/hip ratio ($r_s = -0.36$, $p < 0.02$) and LDL-C ($r_s = -0.34$, $p < 0.05$) (Fig. 4). Multiple regression analysis revealed that the best combination of predictors of FMD was serum testosterone, vessel size and LDL-C ($R^2 = 0.3$, $p < 0.005$).

With respect to GTN-mediated vasodilation, univariate analysis revealed the best single predictor to be vessel size ($r_s = -0.46$, $p < 0.001$). Other predictors were serum testosterone ($r_s = -0.43$, $p < 0.01$) and waist/hip ratio ($r_s = -0.31$, $p < 0.05$). On multivariate analysis, the best combination of predictors of response to GTN was vessel size, serum testosterone and LDL-C ($R^2 = 0.36$, $p < 0.001$).

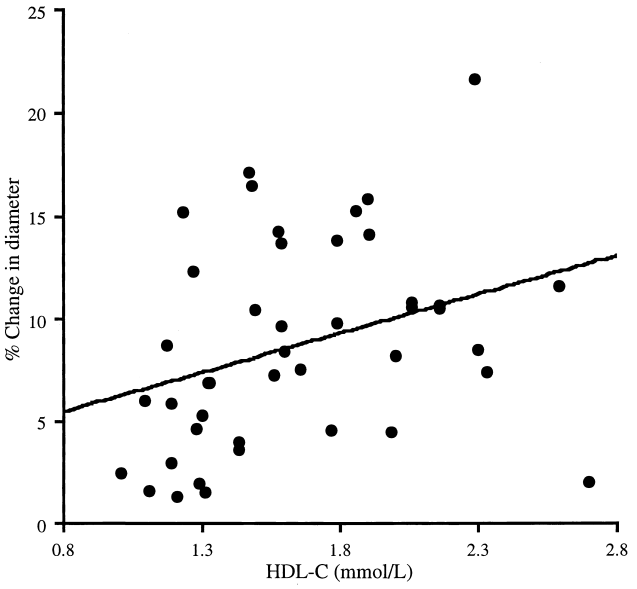


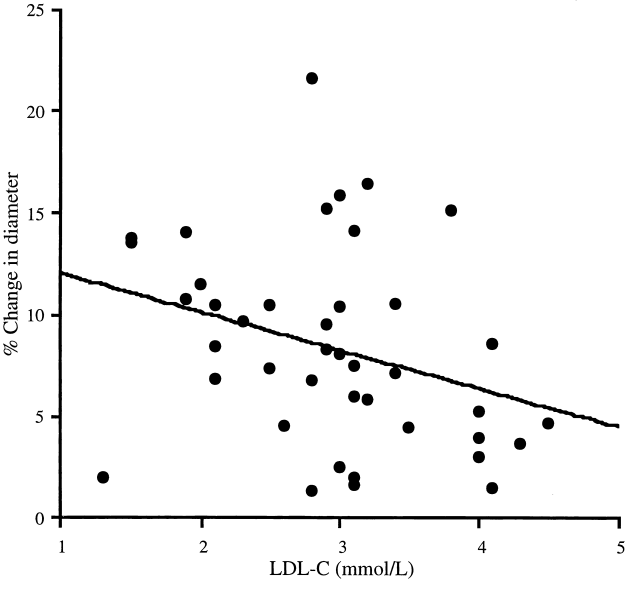
Figure 3. Relation between FMD and HDL-C in all groups ($r_s = 0.42$, $p < 0.01$). **Diagonal line** = linear relation; **circles** = individual subjects.

The magnitude of the FMD and GTN responses in the transsexual group did not correlate with duration, dose or formulation of estrogen therapy. Moreover, FMD and GTN responses were not related to the presence of coronary risk factors, use of spironolactone or gender reassignment surgery in the transsexual group.

Discussion

Previous studies in women have demonstrated (13,17,18) that estrogen appears to improve endothelium-dependent as

Figure 4. Relation between FMD and LDL-C in all groups ($r_s = -0.34$, $p < 0.05$). Symbols as in Figure 3.



well as -independent vasodilation. To our knowledge, this study is the first to report that long-term estrogen therapy enhances vascular function in men. This finding indicates that the effects of long-term estrogen therapy on the vasculature are not gender specific. Moreover, because we demonstrated both endothelium-dependent and -independent effects of estrogen, the improvement in vasodilation cannot be solely attributed to endothelium-dependent NO-mediated mechanisms.

Using a well validated, noninvasive ultrasound technique, we demonstrated that long-term administration of estrogen can enhance endothelium-dependent and -independent vasodilation in the conduit arteries of men. Specifically, we were able to show that both flow-mediated vasodilation, a process known to be largely NO dependent in humans (19), and GTN-induced vasodilation are enhanced in male to female transsexuals compared with age-matched men. The vascular responses in the transsexuals are strikingly similar to those observed in age-matched premenopausal women.

The observation that estrogen improved both endothelium-dependent and -independent vasodilation is consistent with the findings of Hashimoto et al. (13). They observed an increase in both endothelium-dependent and -independent vasodilation in women during the follicular and luteal phases of their menstrual cycle (when serum estrogen levels are high) (13). However, Lieberman et al. (5) found that estrogen replacement therapy improved endothelium-dependent vasodilation only. More recent studies (20–22) suggest that the effects of estrogen are unlikely to be solely mediated through endothelium-dependent NO.

Effects on endothelium-dependent vasodilation. The mechanisms underlying estrogen's capacity to improve vascular function in men were not examined in the present study. Several experimental and clinical studies in women (6,17) have proposed that estrogen enhances endothelium-dependent NO-mediated vasodilation. How this might occur remains unclear. The possibilities include 1) an alteration in receptor or postreceptor signaling; 2) augmentation of endothelial NO synthesis; or 3) a reduction in NO sequestration by oxygen-derived free radicals. Studies by Weiner et al. (23) in a pig model suggest that estrogen does indeed augment NO synthase (NOS) activity. Interestingly, Weiner et al. also found that estrogen induced calcium-dependent NOS activity in both female and male tissues, although a longer duration of treatment was necessary to induce NOS in male tissue. The delay in response in male tissue presumably is due to the need for estrogen receptor priming.

Estrogen may also enhance endothelium-dependent vasodilation by preventing NO sequestration by oxygen-derived free radicals. NO readily combines with such oxygen-derived free radicals, which results in a loss of its biologic activity. Estrogen may act to directly inhibit or scavenge such oxygen-derived free radicals, thereby preserving released NO. The beneficial effects of estrogen in preserving and restoring endothelium-dependent vasodilation in experimental models of hypercholesterolemia may in part be explained by a free radical scavenging effect (24).

Effects on endothelium-independent vasodilation. As alluded to earlier, the augmented GTN-induced vasodilation observed in transsexuals in the present study indicates that the effect of estrogen on vascular function is not solely confined to an endothelium-dependent NO-mediated mechanism. Augmented GTN-induced vasodilation with estrogen has been observed in other studies (in women) (13,18) and may reflect an increased responsiveness of the NO–guanylate cyclase pathway at the level of the smooth muscle cell. Alternatively, the effects of estrogen may be through other pathways that regulate vascular smooth muscle tone (20–22). Modification of other intrinsic vasodilator pathways or inhibition of the prevailing vasoconstrictor forces may be involved. A recent study (20) using explanted precontracted coronary arteries, demonstrated estradiol-induced coronary relaxation with and without intact endothelium and after NO and prostaglandin inhibition. Estrogen has also been shown to have calcium antagonistic properties in the coronary arteries (22) and to inhibit endothelin-1–induced vasoconstriction through inhibition of calcium influx (25). Moreover Polderman et al. (26) have shown that estrogen therapy reduces endothelin-1 levels in transsexuals. Estrogen increases potassium conductance in vascular smooth muscle, a shift that may favour vasodilation (27). In addition, estrogen can modulate adrenergic responses in many vascular beds; however, the specificity of its effect on endothelium or smooth muscle cells has not yet been established (28).

As has previously been argued, distinctions should also be made between the mechanisms underlying the short- and long-term effects of estrogen. Although we observed that vascular function is enhanced in men receiving long-term estrogen therapy, recent reports on the short-term effects of estrogen on the male vasculature are contradictory. Blumenthal et al. (29) found that intracoronary Premarin enhanced the endothelial-dependent vasodilator response in men with mildly atheromatous coronary arteries. However, Collins et al. (30) were unable to demonstrate augmented endothelium-dependent vasodilation after intracoronary 17-beta-estradiol in a similar group of men. Our data, indicating a beneficial effect of long-term estrogen therapy, may reflect the need for estrogen receptor priming or other longer term genomic responses.

Effects on lipoproteins. In the present study we observed that transsexuals prescribed estrogen therapy had a 23% higher HDL-C level and an 23% lower LDL-C level than age-matched men. Although the HDL-C and LDL-C levels were comparable with those observed in women, transsexuals had a substantially higher TG level and smaller LDL particle size. Similar changes in lipid profiles have been reported (12,31) in transsexuals prescribed Premarin (both with and without castration) and in men treated with estrogen for prostate cancer.

Previous studies have shown (32) that various lipid subfractions are important determinants of the integrity of vascular endothelium. Furthermore, aggressive modification of plasma lipids can improve endothelial vasodilator function in humans

(33). One interesting finding of our study was that vascular function was enhanced in transsexuals despite the presence of smaller, dense LDL-C particles. Small dense LDL-C has been found to be associated with premature CAD in humans (34). The finding that vascular function was enhanced in the presence of smaller LDL-C particles suggests that estrogen acts through more complex mechanisms than lipid profile modifications alone.

Effects on male vasculature. There is a paucity of studies examining the effects of estrogen on cardiovascular disease in men. Some studies (9,10) have suggested that low dose estrogen reduces cardiovascular morbidity and mortality in men after myocardial infarction. Others have reported (7,8,35) an increased incidence of cardiovascular events, particularly for higher doses of estrogen. These findings have led to the view that estrogen may have a deleterious effect on the male vasculature. Not surprisingly, there are few recent studies and limited data on the long-term effects of estrogen in adult men. Most data have been derived from studies of men with prostate cancer receiving estrogen for the purpose of androgen suppression. Interpretation of the findings from this cohort is often confounded by advanced age, comorbid illness and the presence of established coronary heart disease. Male to female transsexuals therefore provide a valuable insight into the long-term effects of estrogen therapy in men. Our study cohort was relatively young (mean age 41 years) and by and large free of coronary risk factors or established vascular disease.

Limitations of the study. An important limitation of the present study is that it is a cross-sectional comparison of a small number of subjects and does not provide an insight into the vascular function in the transsexual group before commencing estrogen therapy. However, longitudinal studies are difficult because male to female transsexuals invariably commence estrogen therapy before their presentation to a gender dysphoria clinic.

Another limitation of our study was the lack of direct and quantitative measurement of ethinyl estradiol and Premarin. These forms of estrogen do not completely cross react with the antibodies in the available radioimmunoassays for estradiol. We therefore relied on the suppression of serum testosterone, which is conventionally used as an index of estrogen therapy in male to female transsexuals.

We were also unable to determine whether the improvement in vascular function in transsexuals is due to estrogen itself or to the relative testosterone deficiency created by estrogen therapy and other forms of androgen suppression used in these subjects. It is possible but as yet unresolved that the improvement in vascular function is due to androgen suppression. A recent abstract by Herman et al. (36) is consistent with this hypothesis. However, several recent studies have demonstrated (37–39) that the short-term administration of testosterone improves both endothelium-dependent and -independent vasodilation in the coronary and forearm circulations that is gender independent. Studies in hypogonadal men before and after treatment will ultimately provide an important insight into this question.

Conclusions. Gender differences in CAD have traditionally been attributed to the beneficial effects of estrogen on plasma lipids and vascular function in women. Whether this benefit can be conferred on the male population is unclear. We therefore studied the effects of long-term estrogen therapy on vascular function in male to female transsexuals—a cohort that provides a unparalleled insight into the effects of the female sex hormones on male vasculature. We found that the beneficial effects of estrogen are not gender specific or solely mediated through NO-mediated mechanisms. Improved vascular function in men taking estrogen may in part be explained through alterations in the lipid profile, but other factors are likely to be involved.

Clearly, more studies into the short- and long-term effects of estrogen in men are required, in particular, studies into the mechanisms by which estrogen may exert its effects.

We gratefully acknowledge the tireless assistance of Karen L. Berry BSc(Hons), Annette M. Bond, BA and Anthony N. Hunter, MBBS.

References

1. Castelli WP. Epidemiology of coronary heart disease: the Framingham Study. Am J Med 1984;76:4–12.
2. Sullivan JM, Vander Zwaag R, Hughes JP, et al. Estrogen replacement and coronary artery disease: effect on survival in postmenopausal women. Arch Intern Med 1996;150:2557–62.
3. Barrett-Connor E, Bush TL. Estrogen and coronary heart disease in women. JAMA 1991;265:1861–7.
4. Reis SE, Gloth ST, Blumenthal RS, et al. Ethinyl estradiol acutely attenuates abnormal coronary vasomotor responses to acetylcholine in postmenopausal women. Circulation 1994;89:52–60.
5. Lieberman EH, Gerhard MD, Uehata A, et al. Estrogen improves endothelium-dependent, flow-mediated vasodilation in post-menopausal women. Ann Intern Med 1994;121:936–41.
6. Van Buren GA, Yang D, Clark KE. Estrogen-induced uterine vasodilation is antagonized by L-nitroarginine methyl ester, an inhibitor of nitric oxide synthesis. Am J Obstet Gynecol 1992;167:828–33.
7. The Coronary Drug Project Research Group. The Coronary Drug Project. JAMA 1973;226:652–7.
8. Robinson RW, Higano N, Cohen WD. Long-term effects of high dosage estrogen therapy in men with coronary heart disease. J Chronic Dis 1963;16:155–61.
9. Stamler J, Katz LN, Pick R, et al. Effects of long-term estrogen therapy on serum cholesterol-lipid-lipoprotein levels and on mortality in middle-aged men with previous myocardial infarction. Circulation 1960;22:658.
10. Marmorston J, Moore FJ, Kuzma OT, et al. Effects of Premarin on survival in men with myocardial infarction. Proc Soc Exp Biol 1960;105:618.
11. Pyndath TI and Chanapai S. Elevation of serum HDL and HDL cholesterol in cholesterol-fed male rabbits treated with estrogen. Atherosclerosis 1981; 38:255–65.
12. Damewood MD, Bellantoni JJ, Bachorik PS, et al. Exogenous estrogen effect on lipid/lipoprotein cholesterol in transsexual males. J Endocrinol Invest 1989;12:449–54.
13. Hashimoto M, Akishita M, Eto M, et al. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. Circulation 1995;92:3431–5.
14. Risk Factor Prevalence Study Management Committee. Risk Factor Prevalence Study: Survey No. 3 1989. Canberra: National Heart Foundation of Australia and Australian Institute of Health, 1990.
15. Celemajer DS, Sorenson KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111–5.

16. Celermajer DS, Sorenson KE, Georgakopoulos D, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation 1993; 88(Pt 1):2149–55.
17. Tagawa H, Tagawa T, Shimokawa H. Estrogen acutely improves nitric-oxide mediated and non-nitric oxide mediated endothelium-dependent vasodilation in postmenopausal women [abstract]. Circulation 1995;92 Suppl I:I-68.
18. Gilligan DM, Badar DM, Panza JA, et al. Acute vascular effects of estrogen in postmenopausal women. Circulation 1994;90:786–91.
19. Joannides R, Haefeli WE, Linder L, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation 1995;91:1314–9.
20. Chester AH, Jiang C, Borland JA, et al. Estrogen relaxes human epicardial coronary arteries through non-endothelium-dependent mechanisms. Coronary Art Dis 1995;6:417–22.
21. Sudhir K, Chou TM, Mullen WL, et al. Mechanism of estrogen-induced vasodilation: in vivo studies in canine coronary conductance and resistance arteries. J Am Coll Cardiol 1995;26:807–14.
22. Jiang C, Poole-Wilson PA, Sarrel PM, et al. Effect of 17β-estradiol on contraction, Ca^{2+} current and intracellular free Ca^{2+} in guinea-pig isolated cardiac myocytes. Br J Pharmacol 1992;106:739–45.
23. Weiner CP, Lizasoain I, Baylis SA, et al. Induction of calcium-dependent nitric oxide synthases by sex hormones. Proc Natl Acad Sci USA 1994;91: 5212–6.
24. Keaney JF, Shwaery GT, Xu A, et al. 17β-estradiol preserves endothelial vasodilator function and limits low-density lipoprotein oxidation in hypercholesterolemic swine. Circulation 1994;89:2251–9.
25. Jiang C, Sarrel PM, Poole-Wilson PA, et al. Acute effect of 17β-estradiol on rabbit coronary artery responses to endothelin-1. Am J Physiol 1992;263: H271–5.
26. Polderman KH, Stehouwer CDA, Van Kamp GJ, et al. Influence of sex hormones on plasma endothelin levels. Ann Intern Med 1993;118:429–32.
27. Harder DR, Coulson PB. Estrogen receptors and effects of estrogen on membrane electrical properties of coronary vascular smooth muscle. J Cell Physiol 1979;100:375–82.
28. White MM, Zamudio S, Stevens T, et al. Estrogen, progesterone, and vascular reactivity: potential cellular mechanisms. Endocrinol Rev 1995;16: 739–51.
29. Blumenthal RS, Gloth ST, Reis SE, et al. Acute administration of estrogen improves coronary vasomotor response to acetylcholine in men [abstract]. J Am Coll Cardiol 1996;27 Suppl A:287A.
30. Collins P, Rosano MC, Sarrel PM, et al. 17b-estradiol attenuates acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease. Circulation 1995;92:24–30.
31. Moorjani S, Dupont A, Labrie F, et al. Changes in plasma lipoproteins during various androgen suppression therapies in men with prostatic carcinoma: effects of orchidectomy, estrogen, and combination treatment with lutenizing hormone-releasing hormone agonist and flutamide. J Clin Endocrinol Metab 1988;66:314–22.
32. Kuhn FE, Mohler ER, Satler LF, et al. Effects of high-density lipoprotein on acetylcholine-induced coronary vasoreactivity. Am J Cardiol 1991;68:1425–30.
33. Anderson TJ, Meredith IT, Yeung AC, et al. The effects of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. N Engl J Med 1995;332:488–93.
34. Crouse JR, Parks JS, Schey HM, et al. Studies of low-density lipoprotein molecular weight in human beings with coronary artery disease. J Lipid Res 1985;26:566–74.
35. Blackard CE, Doe RP, Mellinger GT, et al. Incidence of cardiovascular disease and death in patients receiving diethylstillboestrol for carcinoma of the prostate. Cancer 1970;26:249–56.
36. Herman M, Adams MR, Mc Credie RJ, et al. Androgen deprivation is associated with improved endothelial function in adult men [abstract]. Circulation 1996;94 Suppl I:I-278.
37. Chou TM, Sudhir K, Hutchison SJ, et al. Testosterone induces dilation of canine coronary conductance and resistance arteries in vivo. Circulation 1996;94:2614–9.
38. Yue P, Chatterjee K, Beale C, et al. Testosterone relaxes rabbit coronary arteries and aorta. Circulation 1995;91:1154–60.
39. Costarella CE, Stallone JN, Rutecki GW, et al. Testosterone causes direct relaxation of rat thoracic aorta. J Pharmacol Exp Ther 1996;277:34–9.