JACC Vol. 29, No. 7
June 1997:1432–6

CLINICAL STUDIES

VASCULAR FUNCTION

Arterial Reactivity Is Enhanced in Genetic Males Taking High Dose Estrogens

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Objectives. **We sought to assess whether high dose estrogen treatment is associated with enhanced arterial reactivity in genetic males.**

Background. **Although estrogens have been shown to enhance arterial reactivity in women, and are thereby thought to confer cardiovascular benefit, the vascular effects of long-term estrogen therapy in genetic males is unknown.**

Methods. **We studied the arterial physiology of 30 genetic males—15 male to female transsexuals receiving long-term high dose estrogen therapy and 15 healthy male control subjects matched for age, smoking history and vessel size. Using external vascular ultrasound, brachial artery diameter was measured at rest, after flow increase (causing endothelium-dependent dilation [EDD]) and after nitroglycerin (GTN), an endothelium-independent dilator. Blood pressure, cholesterol and testosterone levels were also measured in each subject.**

Results. **Total testosterone and free testosterone index levels were lower in the transsexuals compared with the control subjects ($p < 0.001$). In contrast, EDD was significantly higher in the transsexuals than in the control males (mean [±SD] 7.1 ± 3.1% vs. 3.2 ± 2.8%, $p = 0.001$), as was the GTN response (21.2 ± 6.7% vs. 14.6 ± 3.3%, $p = 0.002$). Total and high density lipoprotein cholesterol, blood pressure levels and baseline vessel size were similar in the two groups. On multivariate analysis, enhanced EDD was associated independently with estrogen therapy ($p = 0.02$) and with low total cholesterol ($p = 0.04$). An enhanced GTN response was also significantly associated with estrogen therapy ($p = 0.03$).**

Conclusions. **Long-term treatment with high dose estrogens is associated with enhanced arterial reactivity in genetic males, which may be due to the effects of estrogen excess or androgen deprivation, or both.**

(J Am Coll Cardiol 1997;29:1432–6)

Women of all ages have a lower risk of cardiovascular morbidity and mortality than do men (1,2). There are now over 30 epidemiologic studies supporting a beneficial effect of endogenous and administered estrogen on cardiovascular risk reduction in premenopausal and postmenopausal women (3). In particular, estrogen therapy is associated with improved arterial function in postmenopausal women (4–6). Whether long-term therapy with estrogens may confer a similar benefit in genetic males is not yet known.

Recent experimental data have suggested that the beneficial vascular effects of estrogen may occur in female but not in male animals. Hanke et al. (7) have shown an atheroprotective effect of administered estrogen on ovariectomized, cholesterol-fed female rabbits, but they were unable to demonstrate any reduction in aortic plaque size in male rabbits given identical therapy, consistent with a gender-specific effect. Furthermore, basal release of nitric oxide is higher in endothelium-intact aortic rings from female rabbits compared with male rabbits (8). Recent human data on short-term parenteral estrogen administration in men have produced conflicting results: some investigators have demonstrated that short-term intracoronary infusion of 17-beta-estradiol attenuates the constrictor response to acetylcholine in angiographically diseased coronary arteries in women but not in men (9), whereas others have found a favorable effect of estrogen administration in men, with improvement in coronary vasoreactivity (10).

No studies have yet reported the effects of long-term estrogen therapy on arterial function in men. We have therefore studied arterial endothelial and smooth muscle physiology in a group of male to female transsexuals receiving long-term high dose estrogen therapy to investigate the effects of such therapy on arterial reactivity in a male population.

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Manuscript received September 3, 1996; revised manuscript received December 11, 1996, accepted December 16, 1996.

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Published by Elsevier Science Inc.

0735-1097/97/$17.00
PII S0735-1097(97)00063-6

Abbreviations and Acronyms

EDD = endothelium-dependent dilation
FTI = free testosterone index
GTN = glyceryl trinitrate (nitroglycerin)
HDL = high density lipoprotein
SHBG = sex hormone-binding globulin

Methods

Subjects. We investigated 30 adult genetic males (mean age 40 ± 8 years, range 18 to 61)—15 were consecutively studied male to female transsexuals who had been on high dose estrogen therapy for at least 6 months, recruited from specialist physicians' practices and from a transgender community support organization; and 15 were healthy male control subjects recruited from community volunteers. No subjects had a history of clinical atherosclerosis, hypertension, diabetes mellitus or hyperlipidemia, and none were taking regular cardiovascular medications. Age and tobacco smoking history were carefully matched between the groups owing to the established influence of these factors on vascular reactivity (11,12). All subjects gave written, informed consent, and the study was approved by the appropriate institutional committees on ethical practice.

The 15 healthy male to female transsexuals were 23 to 61 years old and had been receiving high dose oral estrogen therapy for an average of 5 ± 5 years (range 6 months to 21 years) (conjugated equine estrogen, 1.25 to 5 mg/day, in 11 patients; estradiol valerate by depot or orally, or both, in 3 patients; and ethinyl estradiol, 100 μg/day in 1 patient). Seven patients had also had gender reassignment surgery (involving bilateral orchidectomy), seven were taking antiandrogen agents (cyproterone acetate, 100 to 200 mg/day, in 6 patients; and spironolactone, 100 mg, in 1 patient) and four were taking progestins (medroxyprogesterone acetate, 5 mg/day), according to physician preference. The 15 healthy male control subjects were 18 to 61 years old and had never taken hormonal therapy of any kind.

Study design. Each patient had one visit to the study center, during which a history was taken, supine rest blood pressure was measured and a nonfasting blood sample was taken for estimation of total and high density lipoprotein (HDL) cholesterol (using a Hitachi 747 autoanalyzer; HDL fraction assayed after precipitation with phosphotungstate-magnesium), total testosterone (using radioimmunoassay) and sex hormone binding globulin levels (SHBG) (by immunoradiometric assay). The free testosterone index (FTI) was calculated as 100 × (testosterone/SHBG).

Arterial reactivity was measured in the right brachial artery using external vascular ultrasound, as described in detail elsewhere (13). In brief, endothelium-dependent dilation (EDD) was measured as the change in arterial diameter during a condition of reactive hyperemia, and the smooth muscle dependent response was measured as the change in diameter after a 400-μg spray of sublingual nitroglycerin (GTN). The accuracy and reproducibility of this method have recently been established (14). The degree of hyperemia is calculated from Doppler-derived flow measurements during the condition of reactive hyperemia, compared with rest flow (13). Endothelium-dependent dilation measured in this way is predominantly due to nitric oxide release by the endothelium (15), and this response in the brachial artery correlates well with coronary endothelial function (16). The ratio of endothelium-dependent compared with endothelium-independent dilation (EDD/GTN ratio) was also calculated, as described previously by others (17), to assess whether endothelial function might be enhanced in the subjects taking estrogens, even after accounting for the observed differences in smooth muscle responsiveness. Ultrasound analysis was performed in each case by two independent observers who had no knowledge of each subject's identity and the stage of each series of scans, as reported previously (13).

Statistics. Descriptive data are expressed as mean value ± SD. The two groups of subjects (transsexuals and control subjects) were compared by independent sample *t* tests. The prospectively defined primary end points of this study were EDD and GTN response. All other *t* test results were adjusted for multiple comparisons using Hochberg's modification of the Bonferroni procedure (18). The determinants of EDD and GTN-induced dilation were assessed by univariate and multivariate linear regression analyses, with age, total cholesterol, vessel size, smoking history, systolic blood pressure, degree of hyperemia and estrogen therapy being entered as the independent variables. Statistical significance was inferred at a two-sided p value <0.05.

Table 1. Baseline Characteristics of 30 Genetic Males Studied (15 male to female transsexuals and 15 male control subjects)

Characteristic	Transsexuals	Control Subjects	p Value*
Age (yr)	40 ± 11	40 ± 13	0.92
Smoking†	8/15	8/15	—
Testosterone (nmol/liter)	1.1 ± 0.9	18 ± 7	< 0.001
SHBG (nmol/liter)	110 ± 88	38 ± 17	0.07
FTI (%)	2.3 ± 2.8	51 ± 21	< 0.001
Total cholesterol (mmol/liter)	4.9 ± 0.7	5.2 ± 1.0	0.78
HDL cholesterol (mmol/liter)	1.2 ± 0.3	1.2 ± 0.4	0.92
Vessel size (mm)	4.0 ± 0.4	4.2 ± 0.4	0.61
SBP (mm Hg)	124 ± 17	121 ± 12	0.92
DBP (mm Hg)	79 ± 7	73 ± 7	0.22

*p values adjusted for multiple comparisons (see Methods). †Refers to a history of active or passive smoke exposure (see Results). Data are presented as mean value ± SD or number of subjects. DBP = diastolic blood pressure; FTI = free testosterone index; HDL = high density lipoprotein; SBP = systolic blood pressure; SHBG = sex hormone binding globulin.

Results

Baseline characteristics (Table 1). The male to female transsexuals and the control subjects were well matched for

Table 2. Arterial Study Results in 30 Genetic Males Investigated (15 male to female transsexuals and 15 male control subjects)

Variable	Transsexuals	Control Subjects	p Value
EDD (%)	7.1 ± 3.1	3.2 ± 2.8	0.001
GTN (%)	21.2 ± 6.7	14.6 ± 3	0.002
EDD/GTN	0.34	0.22	0.037
Hyperemia (%)	514 ± 145	500 ± 249	0.92

Data are presented as mean value ± SD or ratio. EDD = endothelium-dependent dilation; GTN = nitroglycerin-induced dilation.

age, smoking history, cholesterol levels, blood pressure and rest vessel size. As expected, both total testosterone and FTI levels were significantly lower in those subjects taking estrogens. Levels of SHBG tended to be higher in the transsexuals compared with the control subjects ($p = 0.07$), consistent with high dose estrogen therapy. Of the eight subjects in each group with a history of smoke exposure, five were current smokers (≥10 pack-years), two were former smokers (≥10 pack-years) and one had ongoing heavy passive smoke exposure (>1 h/day for >2 years).

Arterial reactivity studies (Table 2). The degree of reactive hyperemia was ≥500% in each group of subjects. In response to this increase in flow, EDD was significantly higher in the estrogen-treated patients compared with the control subjects (7.1 ± 3.1% vs. 3.2 ± 2.8%, $p = 0.001$). Endothelium-dependent dilation was similar in those transsexual subjects who had or had not undergone gender reassignment surgery ($p = 0.08$). In addition, the GTN-induced dilator response was also higher in the transsexual group (21.2 ± 6.7% vs. 14.6 ± 3.3%, $p = 0.002$). The EDD/GTN ratio was also significantly higher in the estrogen-treated patients (0.34 ± 0.11 vs. 0.22 ± 0.18, $p = 0.037$) (Fig. 1). Neither the EDD nor the GTN responses were significantly related to the duration of estrogen treatment in the transsexual subjects ($p > 0.25$). On multivariate analysis, enhanced EDD was significantly associated with estrogen therapy (partial $r = 0.42$, $p = 0.02$) and total cholesterol level (partial $r = -0.45$, $p = 0.04$), but not with age, blood pressure, smoking history, degree of hyperemia or vessel size ($p > 0.20$). An enhanced GTN response was also associated with estrogen therapy (partial $r = 0.44$, $p = 0.03$), but not with cholesterol levels or any of the other variables measured.

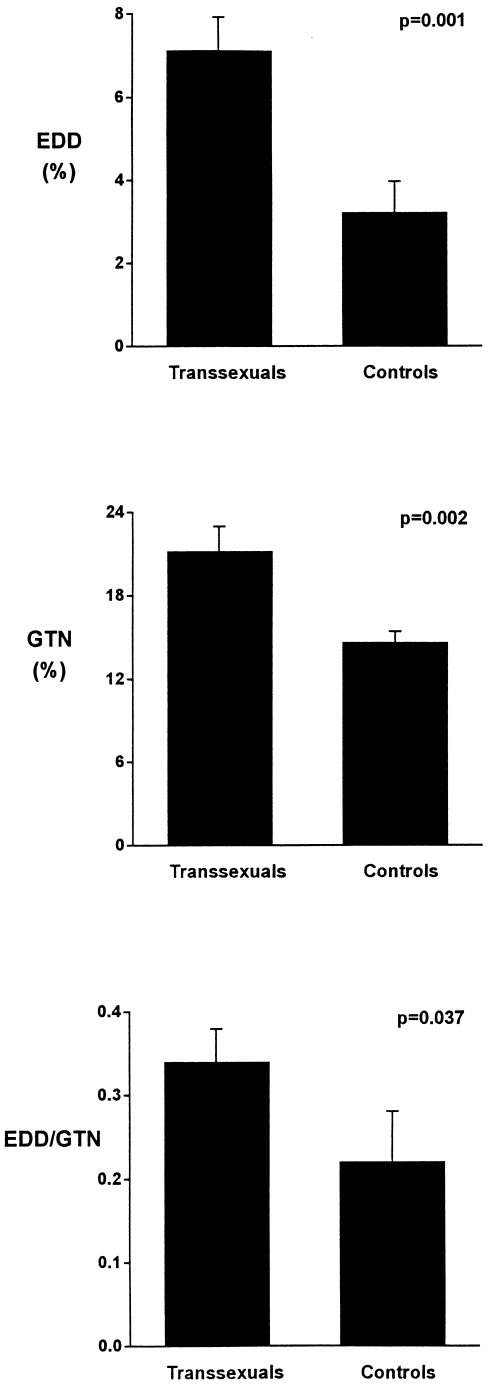

Figure 1. Endothelium-dependent dilation (EDD), dilator response to nitroglycerin (GTN) and the EDD/GTN ratio (mean ± SEM) were all significantly higher in the transsexual subjects compared with the control subjects.

Discussion

In this study, we have found evidence of enhanced endothelial function (suggested by the higher EDD/GTN ratio) in genetic males taking high dose estrogen therapy. In addition, smooth muscle dilator responses to both endothelium-derived nitric oxide (as tested by EDD) (15) and to an exogenous source of nitric oxide (as tested by the GTN response) were also enhanced in the subjects taking estrogens. Because abnormalities in the vasodilator responses of arterial endothelial and smooth muscle cells are important both in the early stages of atherosclerosis (13,19) and in determining the dynamic behavior of advanced atherosclerotic plaques (20), these estrogen-related changes in arterial reactivity may be of pathophysiologic benefit in these subjects. Caution must be applied to this interpretation, however, as previous data demonstrate that high dose estrogens may be prothrombotic in elderly men (21).

As female gender is associated with decreased cardiovascu-

lar risk throughout life, especially before menopause, estrogen has been proposed as a protective factor. This hypothesis has been supported by numerous epidemiologic and experimental studies (3–6). Such benefit may be due to estrogen's effects on lipids (22,23) and/or to direct effects on arterial function (4–6). Although estrogens may be "cardioprotective" in females, information on any similar benefit on arterial physiology in men has previously been unavailable, as there are relatively few men receiving estrogen therapy.

Mechanism of benefit. In this study, the enhanced arterial reactivity observed in the male to female transsexuals may be due to estrogen therapy itself. Estrogen receptors are expressed in endothelial (24) and smooth muscle cells (25). In women, although some estrogen-related benefit is mediated by favorable changes in the lipid profile (26), both short- (4,5,27) and long-term estrogen therapy (6,28) are associated with improved arterial function. Benefits at both an endothelial and smooth muscle level have been demonstrated (4,5). Recent experimental findings have suggested, however, that similar benefits may not occur in men, at least in the context of short-term parenteral estrogen therapy in men undergoing coronary angiography. Collins et al. (9) have shown that short-term intracoronary administration of estradiol in humans converted acetylcholine-induced arterial vasoconstriction to relaxation in the atherosclerotic arteries of women but not of men. Similar gender-specific effects of estrogen have been reported in ovariectomized rabbits (7). Reis et al. (10), however, have recently demonstrated an improvement in vasoreactivity with short-term estrogen administration in men with angiographically smooth coronary arteries.

In this current study, by contrast, beneficial vascular effects of estrogens were observed in asymptomatic men without signs or symptoms of coronary artery disease who were taking long-term hormonal therapy. The mechanism of improved EDD may be due to either estrogen-related enhancement of endothelial nitric oxide production (29) or to antioxidant effects of estrogens (30), or both, with consequently decreased levels of oxygen-derived free radicals in the vessel wall and thus enhanced nitric oxide bioavailability. It is also possible that some estrogen-related benefits may occur at the smooth muscle level. Estrogen's effects may also be mediated through nonestrogen receptor pathways; for example, estrogen may lower cholesterol levels by stimulating low density lipoprotein cholesterol clearance by the liver, partially by means of the asialoglycoprotein receptor on hepatocytes, rather than by specific estrogen receptors (31).

The observed improvement in arterial reactivity in our estrogen-treated patients may also be related to androgen deprivation to castrate levels, rather than to direct estrogen effects. Androgen deprivation in this context is primarily due to feedback inhibition of testosterone production through the hypothalamus and pituitary, or it may be due to bilateral orchidectomy in some subjects. The additional role of antiandrogen treatment in the setting of castrate testosterone levels is probably negligible. We have recently found that androgen deprivation to castrate levels is also associated with significantly enhanced EDD in older men being treated for prostate cancer, compared with control subjects (32). Because these older men with androgen deprivation had no enhancement in GTN response, it is possible that the beneficial effect on endothelium-independent dilation seen in the transsexual group in the current study may be more likely related to a direct smooth muscle effect of high dose estrogen. Similar effects of supraphysiologic doses of estrogen at the smooth muscle level have also been demonstrated in other investigations (4,33).

In this study, hormone-related changes in total and HDL cholesterol were not observed—that is, these lipid levels were similar in the transsexuals and control subjects. Therefore, major estrogen-related alterations in the lipid profile were unlikely to have contributed to the observed vascular reactivity differences between the transsexual and control subjects. There was, however, an inverse relation between total cholesterol level and EDD when all subjects were considered together, as we have previously found (34).

Study limitations. A limitation of this study is its cross-sectional, nonrandomized nature. Therefore, although subjects were closely matched for known vascular risk factors, such as age and cigarette smoking, it is possible that unmeasured differences between the groups may have been present. The fact that over half of the control subjects had a history of significant smoke exposure, and several were >45 years old, accounts for the relatively low EDD observed in the control group (11,12). A prospective study of the effects of long-term estrogen therapy in men would be difficult to conduct owing to the limited access to transsexuals who have not already received treatment and to the sensitivities in recruiting and investigating men during their transition from the male to female phenotype. Another limitation is the complex hormonal effects involved in transsexuality, and therefore uncertainty remains as to the relative contribution of estrogen excess versus androgen deprivation in producing the observed changes in vascular function.

Conclusions. This study demonstrates that arterial endothelial and smooth muscle cell dilator responses are significantly enhanced in male to female transsexuals compared with healthy men. These data are consistent with a beneficial effect of estrogen therapy in the arteries of genetic male subjects.

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