Endogenous Estrogens Influence Endothelial Function in Young Men

Robert Lew, Paul Komesaroff, Maro Williams, Tye Dawood, Krishnankutty Sudhir

Abstract—Males produce endogenous estrogen from testosterone via the enzyme aromatase. Previous studies have suggested a role for endogenous estrogens in cardiovascular function in men. We examined the effects of endogenous estrogen suppression via aromatase inhibition on endothelial function, systemic arterial compliance, and lipoprotein levels in healthy young men. Using a placebo-controlled double-blind randomized design, 20 healthy men, aged 18 to 32 years, were randomized to receive either the aromatase inhibitor anastrozole (1 mg) or matching placebo. Hormone, lipid levels, C-reactive protein (CRP), and homocysteine were measured. Endothelial function, determined by flow-mediated dilation of the brachial artery, and systemic arterial compliance were assessed at baseline and after 6 weeks of treatment. There was a significant decrease in 17β-estradiol concentrations with aromatase inhibition, from 85.4±4.2 to 64.3±8.1 pmol/L (mean±SD, P=0.042). Compared with baseline, a significant decrease in flow-mediated dilation was observed in subjects taking anastrozole [median, 6.1% (range, 5.2 to 13.4) to 3.5% (2.0 to 5.7), P=0.034] but not in the placebo group. No changes were observed in nitroglycerin-induced endothelium-independent dilation in either group. There was no change in systemic arterial compliance with either aromatase therapy or placebo. There were no significant changes in lipoproteins, testosterone, DHEA, CRP, or homocysteine levels in either the anastrozole or placebo group. We conclude that suppression of endogenous estrogens with an aromatase inhibitor resulted in impairment of flow-mediated dilation without significant changes in lipoproteins, homocysteine, or CRP. Our results suggest that endogenous estrogens play a direct regulatory role in endothelial function in young healthy men. (Circ Res. 2003;93:1127-1133.)

Key Words: estrogens ■ males ■ aromatase ■ endothelium ■ arterial compliance

Estrogen has been shown to induce a variety of cardiovascular effects.1 In women, estrogen therapy has been shown to lower total cholesterol,2 reduce arterial stiffness,3 and stimulate release of nitric oxide (NO).4 Estrogen also enhances endothelium-dependent vasodilation in premenopausal and postmenopausal women.5 Despite these potentially beneficial vascular effects, however, hormone replacement therapy has failed to show benefit in either primary or secondary prevention of cardiovascular disease in women.6,7

In men, estrogens are produced in significant quantities by local tissue aromatization of androgenic precursors from the testes and adrenal glands.8 It has been suggested that endogenous estrogens play a protective role in the male cardiovascular system.9 Arteries from endothelial receptor α (ERα) knockout mice show reduced basal NO release.10 Evidence for a cardioprotective role for estrogens in male humans comes from studies of a young man with an estrogen receptor mutation resulting in estrogen insensitivity. He was found to have premature coronary artery disease and endothelial dysfunction.11,12 Conversely, in elderly hypogonadal men,13 and in healthy men receiving testosterone,14 estradiol supplementation has been shown to improve endothelial function.14

The cardiovascular effects of suppression of endogenous estrogens in men have not been studied in detail. We hypothesized that pharmacological suppression of estrogens would adversely influence cardiovascular risk factors and vascular function in young men. In the present study, therefore, we examined the effects of estrogen depletion after aromatase inhibition with anastrozole on lipoproteins, sex hormones, C-reactive protein (CRP), homocysteine, vascular responses, and systemic arterial compliance in healthy young men.

Materials and Methods

Subjects
We studied 20 healthy young men. We excluded subjects who had any evidence of preexisting cardiovascular disease, current cardiovascular therapy, or renal, hepatic, respiratory, or hematological diseases. All were current nonsmokers. All subjects gave full written informed consent. The study was approved by the Alfred Hospital Human Ethics Research Committee.

Study Design
The study used a randomized, double-blind, prospective, parallel-group design. Subjects were randomly assigned to 6 weeks of

Original received May 21, 2003; revision received October 16, 2003; accepted October 16, 2003.
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© 2003 American Heart Association, Inc.
Circulation Research is available at http://www.circresaha.org DOI: 10.1161/01.RES.0000103633.57225.BC
anastrozole 1 mg (n=10) or placebo (n=10). Each subject had three visits, namely a recruitment visit and two study visits.

Height and weight were measured at each visit. Each study visit involved a fasting blood sample for lipoprotein, sex hormone, CRP, and homocysteine assays, followed by assessment of flow-mediated dilation of the brachial artery and systemic arterial compliance. Each study visit was performed in the fasting state and at approximately the same time of day. Randomization was performed by a research assistant not directly involved in performing the study.

Biochemical Analysis
Fasting venous blood samples were drawn on each study day, and the following analyses were performed.

Lipoproteins
Total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides were determined enzymatically (Boehringer Mannheim/Hitachi 917 Analyser) by the Alfred Hospital Pathology Service (Melbourne, Australia).

Sex Hormones
17β-Estradiol, DHEA, sex hormone–binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone levels were measured by Dorevitch Pathology, Mayne Health (Heidelberg, Melbourne, Australia) using commercial radioimmunoassay kits.

Novel Serum-Derived Cardiovascular Risk Factors
CRP, lipoprotein(a), and homocysteine assays were performed by Dorevitch Pathology, Mayne Health using commercial kits.

Flow-Mediated Dilation of the Brachial Artery
The ultrasound method for assessing arterial physiology for endothelial-dependent and -independent dilation was performed as described and validated previously. Studies were performed in a dedicated vascular laboratory by experienced operators. The subjects rested for 5 to 10 minutes before scanning was performed. The room temperature was maintained constant (20°C to 22°C), and the lights were dimmed.

The right brachial artery diameter was measured from B-mode images (Powervision 7000) with a 7.5-Hz transducer; images were recorded onto SVHS tape (Sony SVO9500MOP) for later analysis. An ECG was obtained in the lead II position. The brachial artery was scanned 1 to 6 cm above the elbow until a clear image was obtained. A marker was defined, eg, vein, at this time and used to confirm position of the transducer during the study. (The marker was also used when measurements were made.) Ischemia was induced with the inflation of a blood pressure cuff to suprasystolic pressure, 250 mm Hg for 4.5 minutes. The brachial artery was continuously scanned during cuff deflation and for 2 minutes afterward.

Subjects were then allowed to rest for 10 minutes to allow the artery to return to a resting state. Repeat baseline measurements were then made, following which 300 μg nitroglycerin (GTN) was administered sublingually. Scans of the brachial artery were recorded for 5 minutes afterward.

An investigator, who was blinded to the subject’s status, performed the analysis. The diameter of the brachial artery was measured at the end of diastole, corresponding to the peak of the R wave on ECG. Four to five measurements were made during the three conditions: baseline, reactive hyperemia, and after GTN.

Systemic Arterial Compliance
Systemic arterial compliance (SAC) was determined by using the method of Liu et al. This method requires measurement of carotid artery pressure by applanation tonometry with use of a Millar Mikro-Tip pressure transducer (SPT-301, Millar Instruments) and measurement of volumetric aortic flow by use of a handheld continuous-wave Doppler velocimeter with a 3.5-MHz transducer (Multi-Dopplex MD1, Huntleigh Technology). Compliance is reported in arbitrary compliance units. Brachial pressure was obtained simultaneously and was used to calibrate the carotid artery pressure contour with the use of diastolic and mean pressures, from which carotid systolic pressure was derived.

Subjects rested for 10 minutes before baseline measurements were taken. Arterial compliance was obtained by the method described above, and data were analyzed on purpose-written software. Analysis was performed by a person blinded to the subjects’ status. The whole tracing was reviewed initially; the 10 best recordings were then used to obtain systemic arterial compliance.

Drug Description
Anastrozole is a novel, nonsteroidal aromatase inhibitor [1,3-benzenediacetonitrile. α,α′,α′-tetramethyl-5-(1H-1,2,4-triazol-1-yl-methyl)] that blocks the conversion of androstenedione to estrone and of testosterone to estradiol. Extensive data generated in both animals and humans indicate that this compound has no other pharmacological effects in vivo. The active drug, anastrozole, and placebo were presented in identical physical form. The subject and investigator were both unaware of the treatment.

Statistics
Data are expressed as mean±SEM. Statistical analysis was performed using the Sigmastat 2.0 (Jandel Corporation) software program. The differences between groups with respect to clinical characteristics, flow-mediated dilation, GTN-induced dilation, and SAC at baseline and the effects of treatment (anastrozole versus placebo) on flow-mediated dilation, GTN, and SAC were analyzed using the unpaired t test or the Mann-Whitney U test in the case of nonparametric data. Statistical significance was set at P<0.05.

Results
There were no significant adverse events reported. All subjects tolerated the procedures well. There was one withdrawal from the treatment (anastrozole) group after the first study visit for unspecified reasons.

Demographics
The average age in the anastrozole and placebo groups were 22.6±1.3 years (mean±SEM) and 21.0±0.8 years (P=NS). There was no statistical difference in the baseline characteristics between the anastrozole group and placebo group in terms of age, weight, body mass index, and systolic and diastolic blood pressure at baseline.

There was no difference between the anastrozole and placebo group at baseline in terms of serum estradiol, testosterone, sex hormone–binding globulin, CRP, lipoprotein(a), homocysteine, total cholesterol, HDL cholesterol, LDL cholesterol, or triglycerides.

Effect of Anastrozole on Hormone Levels
After 6 weeks of anastrozole treatment, the estradiol level decreased significantly (85.4±4.2 to 64.3±8.1 pmol/L, P=0.042). There was no significant change in the estradiol level in the placebo group (Table 1). There were no significant changes in the testosterone, androstenedione, or sex hormone–binding globulin levels (Table 1).

Serum LH levels did not change with 6 weeks of anastrozole therapy. The FSH level did increase in the anastrozole group from 3.4±0.4 U/L at baseline to 4.5±0.6 U/L, P<0.001, consistent with a decrease in estradiol levels.

Effect of Anastrozole on Lipid Levels
Lipoprotein levels (total cholesterol, HDL, LDL, and triglycerides) at baseline were in the low range for the general population. There were no significant changes in the total
Effect of Anastrozole on Flow-Mediated Dilation of the Brachial Artery
The resting brachial artery diameter was 4.7 and 4.8 mm in the anastrozole and placebo group, respectively, at baseline. There was no significant difference in baseline brachial artery diameter between the two groups and between the first and second visits.

Flow-mediated dilation response at baseline was similar in the two groups (9.4 ± 2.2% and 7.7 ± 1.0%, P = 0.45). The flow-mediated dilation response in the anastrozole group was significantly impaired on the second visit (9.4 ± 2.2% versus 4.7 ± 1.3%, P = 0.034) (Figure 1). There was no significant change in the flow-mediated dilation response in the placebo group (7.7 ± 1.0% versus 7.9 ± 0.63%, P = 0.91) (Figure 1). The GTN response (endothelium-independent, smooth muscle–dependent) was similar in the two groups at baseline; there was no significant change in GTN response after the 6-week treatment period with either anastrozole or placebo (Figure 2).

Effect of Anastrozole on Arterial Mechanics
There was no significant effect of either anastrozole or placebo on any of the novel serum cardiovascular risk markers. Our results suggest that endogenous estrogens are important in endothelial function in young healthy men.

Data are mean ± SEM. There were no significant changes in the cholesterol, HDL, LDL, or triglyceride levels after 6 weeks of anastrozole or placebo treatment. There was no significant effect of either anastrozole or placebo on any of the novel serum cardiovascular risk markers. *n = 8 for hs-CRP.
individual with an estrogen receptor mutation and two men with aromatase deficiency has provided insights into the role of endogenous estrogens in males. The individual reported to have a disruptive mutation in the estrogen receptor gene, and thus estrogen insensitivity, was shown to have premature coronary artery disease. His serum estradiol and estrone, serum FSH, and luteinizing hormone concentrations were elevated; serum testosterone concentrations were normal. Electron beam computed tomography scanning showed premature calcification of a coronary artery. Furthermore, brachial artery studies showed absence of flow-mediated dilation in response to ischemic cuff occlusion despite preserved nitroglycerin and estradiol-induced vasodilation.

The discovery of several individuals with aromatase deficiency, two of whom are male, has also provided additional evidence of the role of estrogens in males. Of interest, the phenotype of the individuals with aromatase deficiency is similar to that found in the man described above with the estrogen receptor mutation, especially in regards to poor bone mineralization and tall stature. One of the individuals with aromatase deficiency had elevated LDL, triglycerides, and insulin levels, indicative of insulin resistance. Analysis of the plasma hormone levels of one of the subjects with aromatase deficiency revealed undetectable estrogens and very high circulating androgens. Circulating FSH and LH were also elevated, indicative of an important role of estrogens in the negative feedback regulation of gonadotrophins in males as in females. To date, no cardiovascular studies have been performed on these individuals with aromatase deficiency. However, there is evidence of impaired endothelium-dependent vasodilation in the aorta of aromatase knockout mice, suggesting a role for endogenous estrogens in the regulation of endothelial function in this model.

In the present study, the hormone changes with a decrease in estradiol level and increase in FSH were expected; these patterns are similar to individuals with aromatase deficiency, except not as severe. There was no statistically significant change in the LH level ($P=0.20$) in our subjects treated with aromatase inhibition. The testosterone levels did not increase either; this might have been related to the magnitude of the estradiol decrease (24.7%). Although there was a significant decrease in estradiol with anastrozole in our subjects, estradiol was still present in the circulation, in contrast to the undetectable levels in the two subjects with aromatase deficiency who had very high androgen levels.

Low doses of estrogen have also been shown to have a beneficial effect on the male cardiovascular system. In a group of hypogonadal men, estradiol valerate 1 mg was shown to increase HDL, enhance basal NO release, and attenuate vasoconstrictor response to norepinephrine and angiotensin II in the forearm arteries. Supplementation of estradiol in this group of men with a very low basal estradiol level (estradiol <30 pmol/L) resulted in improved basal NO release.

Cross-sectional studies on the effects of estrogen supplementation in male-to-female transsexuals have shown an improvement in arterial reactivity. These subjects were generally exposed to a combination of various preparations of estrogens and progestins. One study in healthy male subjects who took testosterone (600 mg) with or without estradiol (10 or 20 mg) showed an estrogen-related, dose-dependent increase in endothelium-dependent dilation of the brachial artery. From these studies and the present study, there is evidence that physiological and pharmacological levels of estrogen induce beneficial effects on the endothelium in men.
In contrast, the Coronary Drug Project, an investigation conducted 25 years ago into the cardiovascular effects of estrogen administration in men after myocardial infarction, showed an excess of deaths and recurrent infarction in the treatment group. This trial, which used high doses of conjugated equine estrogens, was subsequently abandoned, and the subject has not been studied in detail since.28 There has been increased knowledge on the role of estrogens since the coronary drug project was abandoned, especially the potential adverse effects with high doses. Other studies have also failed to yield positive results. A short-term study on the role of estrogen supplementation on acetylcholine-induced vasoconstriction of the coronary arteries failed to show any effect in men but did show improvement in women.29 Kawano et al30 also showed a gender difference in improvement in flow-mediated dilation with estrogen supplementation, with an improvement in women but no change in men.

Both forms of the estrogen receptor, ERα and ERβ,31 have been found in endothelial cells. Estrogens have the ability to modulate endothelial function via classical receptor activation and gene transcription as well as via more rapid non-genomic mechanisms, such as mitogen-activated protein kinase–dependent endothelial NO synthase activation.32 Physiological levels of estrogen have recently been shown to downregulate ERα levels in ovine endothelial cells, but with prolonged exposure to estradiol, there was upregulation of the number of ERα but not ERβ.33 Whether a decrease in physiological levels of estradiol, via the partial inhibition of aromatase, resulted in changes in endothelial ER density in our subjects is unclear.

There were no changes in large artery mechanics after treatment with anastrozole. The effect of estrogen on systemic arterial compliance is perhaps minimal in men. In male to female transsexuals, treatment with estrogens has consistently shown an improvement in endothelial function, as measured by flow-mediated dilation.26 But in the same cohort of subjects taking long-term estrogens, no effect on systemic arterial compliance was found.34 Changes in arterial compliance may not be central to the vascular effects of estrogen on vascular function, at least in males. It is possible that the lack of effects of estrogen on arterial compliance in men may relate to the duration of therapy, although short-term fluctuations in estrogen levels during the menstrual cycle have been shown to induce changes in arterial compliance in premenopausal women.35

There were no effects on cholesterol, HDL, LDL, and triglycerides after 6 weeks of anastrozole therapy. In women, the effects of estrogens on lipoproteins are well-known. However, the role of physiological levels of estrogens on lipoproteins in males is not fully understood. Both men with aromatase deficiency had elevated cholesterol, LDL, and triglycerides (ie, higher than the recommended range).23,36 The man with an estrogen receptor mutation had total cholesterol level below the 5th percentile, and both the LDL and HDL cholesterol levels were at the 10th percentile for a man of the patient’s age.12 It is thus difficult to draw conclusions on the effect of endogenous estrogens on cholesterol in males.

There were also no changes in hs-CRP, homocysteine, or lipoprotein(a) after anastrozole therapy for 6 weeks in the present study. Estrogen therapy in the form of hormone replacement therapy has been shown previously to increase CRP37; the effect of estrogen withdrawal is unknown in women and men. Homocysteine levels rise in postmenopausal women,38 and hormone replacement therapy results in a decrease in homocysteine levels.19 Low-dose estrogen therapy, 0.5 to 2.0 mg of oral 17β estradiol, has also been shown to reduce homocysteine levels in elderly men.40 Menopause is associated with a 10% to 30% increase in plasma levels of lipoprotein(a), which falls with estrogen therapy41,42; one trial in elderly men did not show any changes in lipoprotein(a) with oral estradiol supplementation.40 Physiological levels of estradiol may have no role in influencing the levels of these novel cardiovascular risk markers in men; alternatively, in our study, the magnitude of change in the estradiol levels after aromatase inhibition may have been too small to effect a noticeable change.

In the present study, we have shown that partial inhibition of aromatase, resulting in a 25% decrease in plasma 17β estradiol, produced significant impairment in brachial artery flow-mediated dilation in healthy young men. It has been estimated that in males the testes can account for 15% of circulating estrogens,43 and estrogen production, both intra-testicular and extragonadal, is of physiological significance throughout adult life. Vascular expression of aromatase22 suggests a local paracrine role for estrogen in blood vessels. The contribution of local versus systemic aromatase suppression in the present study to impairment of endothelial function is unclear; it is possible that the degree of vascular aromatase inhibition exceeded that reflected by the 25% decrease in plasma estradiol concentrations.

### Table 3. Systemic Arterial Compliance and Blood Pressure Before and After Either Anastrozole or Placebo Treatment

<table>
<thead>
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<th>Anastrozole (n=9)</th>
<th>Placebo (n=10)</th>
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<tbody>
<tr>
<td>Systemic arterial compliance, ACU</td>
<td>0.69±0.02</td>
<td>0.69±0.02</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>114±4</td>
<td>117±4</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>69±4</td>
<td>63±2</td>
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<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 Weeks</th>
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<th>Baseline</th>
<th>6 Weeks</th>
<th></th>
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</thead>
<tbody>
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<td>Systemic arterial compliance, ACU</td>
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<td>0.664</td>
<td>0.02</td>
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<td>0.71±0.02</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>114±4</td>
<td>117±4</td>
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<td>0.822</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>69±4</td>
<td>63±2</td>
<td>0.254</td>
<td>67±3</td>
<td>64±3</td>
<td>0.513</td>
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Data are mean±SEM. ACU indicates arbitrary compliance units. There was no significant change in compliance or blood pressure after 6 weeks of either anastrozole or placebo therapy.
This study is limited by the small number of subjects studied and the short time period of follow-up (6 weeks) after administration of the aromatase inhibitor anastrozole. It is unclear whether the impaired endothelial function observed would persist with more prolonged suppression of aromatase and thus sustained estrogen deficiency. The clinical implications of our study remain to be determined. Although it is unlikely that estrogen therapy in its present forms would be indicated for improvement of endothelial function in men, our study confirms a role for estrogen in vascular function in men.

In conclusion, partial suppression of endogenous estrogen with an aromatase inhibitor, anastrozole, results in impairment of flow-mediated dilation unrelated to changes in plasma lipoproteins, CRP, or homocysteine. Endogenous physiological levels of estrogen thus seem to have a potentially important role in the male cardiovascular system.

Acknowledgments
R.L. was supported by a Postgraduate Medical Research Scholarship from the National Heart Foundation of Australia.

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Circ Res. 2003;93:1127-1133; originally published online October 30, 2003;
doi: 10.1161/01.RES.0000103633.57225.BC
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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