Aging Is Associated With Endothelial Dysfunction in Healthy Men Years Before the Age-Related Decline in Women

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Objectives. This study assessed whether aging is associated with progressive endothelial dysfunction, whether the pattern of any age-related decline in vascular health is different in men and women and whether any gender difference is consistent with known changes in hormonal status.

Background. Coronary and cerebrovascular disease are much less common in young and middle-aged women compared with men, although the gender difference in death from atherosclerosis is less marked after the menopause. Endothelial dysfunction is an early event in atherogenesis and is important in dynamic plaque stenosis in later life. The effect of aging on endothelial function in men and women, however, is not well known.

Methods. We used high resolution ultrasound to study endothelium-dependent and endothelium-independent vascular responses. Brachial artery physiology was investigated in 238 subjects (103 men, 135 women; mean [±SD] age 38 ± 17 years, range 15 to 72) with no known risk factors for atherosclerosis. The responses to reactive hyperemia (flow-mediated dilation, which is endothelium dependent) and to glyceryl trinitrate (an endothelium-independent dilator) were assessed for all the subjects and then for men and women separately.

Results. On multivariate analysis for the whole group, reduced flow-mediated dilation was related to older age (r = -0.34, p < 0.0001). In men, flow-mediated dilation was preserved in subjects aged ≤50 years but declined thereafter at 0.21% /year. In women, flow-mediated dilation was stable until the early 50s, after which it declined at 0.49% /year (p = 0.002 compared with men). In contrast, there was no significant change in the glyceryl trinitrate response with aging in either gender.

Conclusions. Aging is associated with progressive endothelial dysfunction in normal humans, and this appears to occur earlier in men than in women. In women, however, a steep decline commences at around the time of the menopause. This is consistent with a protective effect of estrogen on the arterial wall.

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Life expectancy is greater for women than for men, in large part because of their lower incidence of cardiovascular death in middle age (1). Male gender remains a cardiovascular risk factor, even after controlling for other influences, such as cigarette smoking and hypertension (2,3). Many investigators have suggested that the basis for the protection of young and middle-aged women from atherosclerosis is their relative excess of estrogenic hormones (4–6).

Endothelial dysfunction is an important early event in experimental models of atherosclerosis (7). It has recently been demonstrated in asymptomatic children and young adults with vascular risk factors (8) and may contribute to morbidity and mortality by disturbing arterial physiology in adults with established coronary stenoses (9,10). Previous studies have shown that increasing age is associated with coronary endothelial dysfunction but have only examined small numbers of subjects, most of whom had multiple vascular risk factors and were undergoing catheterization for suspected coronary artery disease (11,12). Using a noninvasive method, we examined endothelial function and its relation to age in a large number of healthy men and women who did not have these potentially confounding influences.

Our observations suggest that there is a difference in the pattern of age-related vascular injury in men and women, with a steep decline in endothelial function occurring in women after the menopause. This supports the concept of a hormonal influence on vascular disease and risk.

Methods

Subjects. We studied 238 subjects (103 men, 135 women; mean [±SD] age 38 ± 17 years, range 15 to 72) recruited from hospital staff, families, friends and community volun-
teers over a 2-year period. Subjects were included only if they were symptomatic, lifelong nonsmokers and had no family history of premature vascular disease. None were known to have hypertension, diabetes or hyperlipidemia. Because cholesterol levels increase with age in a normal population, we included all subjects with total cholesterol below the 90th centile for their age and gender (13). No subjects were taking regular medications. Thirty-six of the women had not had a menstrual period for >1 year; their average age at menopause was 51 ± 1 years. None of these postmenopausal women was taking hormone replacement therapy. All studies were approved by the local ethics committee.

**Study design.** Total plasma cholesterol and rest supine blood pressure were measured in every subject. All then had a noninvasive ultrasound study of the right brachial artery to test endothelial and smooth muscle function, as previously described (8). Arterial diameter was measured at rest, during reactive hyperemia, again at rest and after administration of sublingual glyceryl trinitrate, using a standard 7.0-MHz linear array transducer and 128 XP/10 system (Acuson). Previous investigators have demonstrated that conduit artery dilation in response to flow increase is endothelium dependent (14,15), whereas the dilator response to glyceryl trinitrate is endothelium independent (9,16). Hyperemic microvascular responses are also endothelium independent (17).

Each subject lay at rest for at least 10 min before the first rest scan was obtained. Increased flow was then induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 300 mm Hg, followed by deflation after 4.5 min. The second scan was taken for 30 s before and 90 s after cuff deflation. Fifteen minutes was then allowed for vessel recovery, and a further rest scan was then recorded. Sublingual glyceryl trinitrate spray (400 µg) was then administered, and 3 to 4 min later the last scan was done. Seven subjects declined sublingual glyceryl trinitrate. Doppler-derived flow measurements were obtained during the first rest scan and again during the 1st 15 s of reactive hyperemia. The electrocardiogram (ECG) was monitored continuously throughout the study.

**Data analysis.** All scans were recorded on super VHS videotape for later analysis. Vessel diameter was measured by two independent observers unaware of the clinical details and stage of the experiment, as described previously (8). The mean vessel diameter and percent dilation for each patient were taken as the average of the measurements of the two observers.

**Statistics.** The distributions of flow-mediated dilation, glyceryl trinitrate response, age, cholesterol, vessel size and hyperemia were summarized by sample means and standard deviations, and pairwise relations were expressed by Pearson correlation coefficients. Treating flow-mediated dilation and glyceryl trinitrate dilation as response variables and age, cholesterol and vessel size as independent variables, we investigated their simultaneous relation by multiple regression analyses, from which we obtained partial correlation coefficients. Men and women were then analyzed separately.

For flow-mediated dilation, a multiple regression was carried out with a "vessel size" term plus two "age" terms, with coefficients depending on whether the subject's age was above or below a specified change point \( t_{\text{max}} \). Thus, for age below the change point \( t \), the regression model is Flow-mediated dilation = \( a + b(\text{Size} - \text{Mean size}) + c(\text{Age} - t) \), where \( \text{Mean size} \) is the mean vessel size, whereas for age above the change point, the model is Flow-mediated dilation = \( a + b(\text{Size} - \text{Mean size}) + d(\text{Age} - t) \). The "most likely" change point \( t_{\text{max}} \) produced the minimal residual standard deviation \( \sigma_{\text{max}} \) over a range of possible change points from 30 to 65 years. To summarize the support for each possible change point \( t \), the "profile likelihood" \( \sigma_{\text{max}}^2/\sigma_{t}^2 \) was plotted, which has maximal 1 at \( t = t_{\text{max}} \), as derived from a likelihood ratio test procedure (18). To validate the two-straight-line model compared with a smooth curve, a quadratic function in age was also fitted to the data; the calculated residual standard deviation was smaller for the two-straight-line model. Men and women were compared with respect to the gradient of adjusted flow-mediated dilation with age, both before and after their estimated change point, using a Z test based on their estimated standard errors. For glyceryl trinitrate, the regression coefficient \( b \) arose from a simple linear regression on vessel size; that is, Glyceryl trinitrate = \( a + b(\text{Size} - \text{Mean size}) \).

For each subject, flow-mediated and glyceryl trinitrate-induced dilation were adjusted for vessel size by subtracting \( b(\text{Size} - \text{Mean size}) \), and these adjusted values were then plotted against age.

**Results**

The age distribution of the subjects and their vascular responses to increased flow and to glyceryl trinitrate are shown in Figures 1 and 2. Scans from all 238 subjects were of sufficient quality for analysis, and no plaques were observed in any subject. Total plasma cholesterol was 191 ± 39 mg/dl (range 101 to 299), and there was a positive correlation between age and cholesterol level (\( r = 0.58, p < 0.01 \)). No subjects were hypertensive (blood pressure <160/90 mm Hg in all). In response to an increase in flow of 487 ± 172% after cuff deflation, there was an increase in brachial artery diameter of 7.9 ± 3.9% (range 1% to 18%). There was no correlation between age and degree of reactive hyperemia (\( r = -0.06, p = 0.49 \)) or between flow-mediated dilation and hyperemia (\( r = 0.09, p = 0.16 \)). In response to glyceryl trinitrate, arterial diameter increased by 19.4 ± 5.8% (range 4% to 38%).

On multivariate analysis of all subjects, flow-mediated dilation was independently related to age (\( r = -0.34, p < 0.0001 \)) and vessel size (\( r = -0.35, p < 0.0001 \)) but not to the cholesterol level (\( r = -0.09, p = 0.14 \)). In contrast, although the glyceryl trinitrate response was also related to vessel size.
Figure 1. Aging and flow-mediated dilation (FMD) in 103 men (left) and 135 women (right). Top, Individual data points for percent flow-mediated dilation adjusted for vessel size. Bottom, Likelihood of a change point existing at any given age; this is highest for men at age 41 years and for women at 53 years. In women, there is a steeper decline in flow-mediated dilation after their change point.

(r = −0.45, p < 0.0001), there was no relation with age (r = −0.10, p = 0.18) (Table 1).

Gender difference. In men, flow-mediated dilation (adjusted for vessel size) did not decline until the end of the fourth decade. Change-point analysis suggested that an age-related decline occurred subsequently, from ~41 years of age, after which flow-mediated dilation decreased by 0.21%/year. In contrast, flow-mediated dilation was preserved in women until the early 50s (most likely change point at age 53 years), after which it declined significantly faster than in men (0.49%/year, p = 0.002). There was a very narrow range in the age at menopause in this study, and separate analysis of years since menopause versus flow-mediated dilation in women yielded a similar slope of decline. In contrast, there was no age-related decline in glyceryl trinitrate-induced dilation in either gender.

Discussion

This study showed that aging is associated with progressive loss of flow-mediated dilation in the systemic arteries of healthy subjects with no risk factors for vascular disease. In contrast, responses to glyceryl trinitrate are preserved into old age, and this suggests that aging leads to endothelial dysfunction. Because there is no age-related change in the hyperemic flow response, it is unlikely that progressive proximal atherosclerosis could explain the arterial vasodilator abnormalities observed in this study.

We also observed that the pattern of age-related changes in arterial physiology is different in men and women. In men, endothelial responses decline gradually after the fourth decade, whereas in women, vascular physiology remains healthy for a further 10 years. Subsequently, the rate of decline is faster in women, so that endothelial dysfunction is present in almost all subjects by 65 years of age. This pattern of vascular damage parallels the known difference between the genders in cardiovascular morbidity and mortality in middle and old age (1,19), where men are at much higher risk from age 40 to 50 years, but women “catch up” in later life.

The process of atherosclerosis does not appear to differ
between men and women, and the risk factors known to predispose to vascular disease affect both genders equally (20). We have previously shown that this noninvasive method can detect flow-mediated dilation in healthy subjects and that risk factors such as hypercholesterolemia (21) and cigarette smoking (22) are associated with abnormal endothelial physiology in both male and female children and young adults. Aging is associated with an increasing prevalence of diabetes, hypertension and hypercholesterolemia, and in the previous studies of both Vita et al. (11) and Yasue et al. (12), in which coronary endothelial dysfunction was found in most subjects >30 years of age, risk factors may have had an important confounding effect.

Study limitations. In this study, by excluding all subjects who had ever smoked or who had known hyperlipidemia, hypertension, diabetes or a positive family history, we investigated the effects of aging per se on vasomotor responses and compared endothelial function in male and female subjects. Previous studies enrolled too few patients to examine any gender differences in the effects of aging on endothelial function (11,12). The method we used to study endothelial and smooth muscle function is noninvasive and well tolerated, allowing investigation of a large number of subjects with neither risk factors nor symptoms. The accuracy of 7.0-MHz ultrasound is sufficient to allow detection of small diameter differences (as little as 0.1 mm), and the reproducibility of the technique has been established previously (8,22,23). Although only superficial systemic arteries can be studied, atherosclerosis is a diffuse process, and there is a correlation between the presence of disease in different large arteries in the same subject (24). Recently two groups have reported that brachial artery vasomotor responses are well correlated with coronary endothelial physiology and coronary atherosclerosis (25,26).

Aging and the cardiovascular system. Aging is associated with a number of anatomic and hemodynamic changes in the vascular system of all subjects, including collagen degeneration, loss of elastin, increased intima-media thickness of the arteries and reduced vascular compliance (27,28). Despite these age-related changes, the arterial response to glyceryl trinitrate was preserved in our study. This is consistent with other studies that have shown that pharmacologic doses of exogenous nitrovasodilators remain effective in older adults (29,30) and that these drugs are still therapeutically effective in geriatric patients (16). In contrast, we observed an age-related decline in endothelial function in both men and women. Previous investigators have shown that the dilator capacity of both large and small coronary vessels in response to acetylcholine, an endothelium-dependent vasodilator, also decreases with increasing age in subjects with chest pain but angiographically smooth coronary arteries (11,12,31).

The mechanism whereby aging is associated with impaired endothelial function is not known. Possibilities include an age-related decrease in release of endothelium-derived relaxing factor(s), or their increased catabolism in the vessel wall, or increased release of constricting factors (31). In vitro aging is associated with increased monocyte-endothelial cell adherence, which may result from age-related increases in peroxidative stress (32). Our data suggest that women may be protected from these age-related changes for a decade longer than men.

Gender differences. This gender difference in the pattern of age-related decline in endothelial function is best explained by the influence of hormonal factors. Estrogens may protect women from atherogenesis (33,34). After the menopause, decreased estrogen levels are associated with a more atherogenic lipid profile (19,35); however, the effects of estrogen on plasma lipids can explain only a small part of the beneficial effects on the risk of vascular disease (36). Estrogens are vasoactive hormones and have direct effects on the arterial wall (37,38). In experimental animals, surgically induced menopause is associated with a loss of endothelium-dependent coronary vasorelaxation (39). In postmenopausal animals, estrogen replacement may restore normal endothelial function (6). Intravenous estrogen acutely improves endothelium-dependent vasorelaxation in postmenopausal women with coronary artery disease (40), and sublingual estrogen has a beneficial effect on myocardial ischemia in similar patients (41). In our study, the age at which endothelial dysfunction was seen in women was around the time of the menopause. These data therefore suggest, but do not prove, that estrogens may protect against endothelial injury.

Injury of the endothelium is important both because it is a key early event in atherogenesis and because it may
increase risk in subjects with established atherosclerosis by predisposing to vasoconstriction and thrombosis (9). The recent demonstration that L-arginine, the precursor of endothelium-derived relaxing factor, not only protects the endothelium but also retards atherosclerosis in the cholesterol-fed rabbit suggests that endothelial dysfunction may be intimately involved in the pathogenesis of atheroma (42).

Clinical implications. Cardiovascular disease is the most common cause of death in the United States, and the known gender difference in life expectancy is largely related to the increased risk of coronary and carotid atherosclerotic complications in middle-aged men. Our data suggest that aging per se, irrespective of exposure to known risk factors, may be associated with earlier physiologic abnormalities in the systemic arteries of men compared with women. This is consistent with observations that both old age and male gender are independent risk factors for cardiovascular mortality and that estrogens may protect women from the age-related decline in vascular health. The association between menopause and the subsequent change in endothelial function that we have observed is provocative, but prospective studies will be required to see whether hormone replacement therapy prevents or retards the age-related changes in arterial physiology in postmenopausal women. Many such women are potentially candidates for hormone replacement therapy, not just for well-being and prevention for osteoporosis but also for protection of the arterial wall and reduction of vascular risk. The availability of a noninvasive method to assess vascular health will allow investigation of the appropriate type and timing of such hormonal therapy, both in individual subjects and in clinical trials.

References

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