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EDITORIAL COMMENT

Sex Hormones as Novel Risk Biomarkers for Atherosclerosis in Peripheral Vascular Disease*

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Atherosclerosis respects no vascular territory, and its complications can be acute and devastating, as exemplified by myocardial infarction, stroke, or sudden death. Peripheral arterial disease (PAD) is a harbinger of generalized atherosclerosis, and its relentless progression is associated with profound disability, peripheral limb loss, and strong association with coronary and carotid diseases. Risk factors or biomarkers for atherosclerosis are useful for risk stratification, primary and secondary prevention, and to establish targets for treatment. Traditional risk biomarkers for atherosclerosis are well known, including smoking, hypertension, diabetes, lipid abnormalities, and family history, among others.

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While general risk biomarkers are well known, there are some regional vascular territory differences in the relative strengths of association of these markers. For example, in peripheral vascular disease, smoking has a particular potent effect on disease severity and progression, while hypertension has less of an impact on outcome. However, robust risk markers for PAD are not commonly available.

Sex Hormones as Risk Markers for PAD

In this issue of the *Journal*, Tivesten et al. (1) demonstrated in a cross-sectional study the association between endogenous circulating sex hormone levels and ankle-brachial index (ABI) in 3,014 elderly men who were enrolled in the Swedish arm of the MrOS (Osteoporotic Fractures in Men) study, with an average age 75.4 years. The ABI reflects the state of arterial perfusion in the lower limb and, when measured carefully, is a useful index of peripheral vascular health. Serum-free testosterone was positively associated with ABI, suggesting a vascular protective link, whereas free estradiol was negatively associated with ABI.

This is certainly the largest cohort to date linking sex hormone levels with PAD in the elderly men who are at greatest risk for the latter. While the interaction of sex hormones and atherosclerosis remains complex and at times controversial, this finding is consistent with the overall accumulated data to date on testosterone and estrogen's relationship with vascular atherosclerosis (Table 1).

Sex hormone imbalance in elderly men is coming into prominence. Hypogonadism in aging men is increasingly recognized as a prevalent condition: 20% of men older than 60 years, 30% of those older than 70 years, and 50% those older than 80 years of age. The hypogonadal state in men has been found to be associated with an increased cardiovascular risk (2). In contrast, in a placebo-controlled study of elderly men with chronic stable angina, daily supplementation with a low-dose testosterone for several weeks led to symptomatic improvement (3). The findings of this current study can be viewed in light of these earlier observations.

The relationship between endogenous estrogen and acceleration of atherosclerosis is also being increasingly recognized. Testosterone is converted to estradiol by aromatase in tissues of men, albeit the conversion rate is slow, around 0.2%. Ultimately, 80% of plasma estradiol in men originates from aromatization of testosterone and androstenedione, and no more than 20% is secreted by the testes. However, the cumulative plasma estradiol levels in some elderly men are higher than corresponding levels in postmenopausal women. Serum estradiol levels in older men have been positively correlated to the burden of coronary heart disease (4) and carotid artery disease (5).

Controversies Regarding Hormones and Atherosclerosis

However, the relationship between hormones and atherosclerosis is not without its controversies. The observation that coronary disease develops in women on the average of 10 years later in life compared with men and the increase in incidence of coronary disease after menopause led to a hypothesis that estrogen protects against atherosclerotic complications in women. However, disappointingly, the results from large randomized trials of estrogen replacement therapy in postmenopausal women, such as the Women's Health Initiative, failed to confirm any cardiovascular benefit of estrogen both for primary and secondary prevention (6).

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Table 1 Comparison of the Effects of Androgens and Estrogens on Atherosclerotic Coronary and Peripheral Artery Disease

	Androgens			Estrogens	
	High Endogenous Levels	Exogenous Administration	Low Endogenous Levels	High Endogenous Levels	Exogenous Administration
Coronary artery system		↓ men (3,14)	↑ men (4,12)	↑ men (2,4)	↑ women (6)
Peripheral arterial system					
Carotid artery			↑ men (4)	↑ men (5)	↑ women (6)
Lower extremity	↓ men (1)			↑ men (1)	

↓ = *decreased* risk of atherosclerotic complications; ↑ = *increased* risk of atherosclerotic complications.

To extend these observations on estrogen further, application of estrogens to men, for example as a component of the chemotherapy for prostate cancer, has also been associated with serious side effects of deep venous thrombosis and increases in cardiovascular-related death.

Potential Biological Mechanisms

Atherosclerosis, from the initial macrophage infiltration into the subintima of the blood vessel wall, to the eventual plaque rupture, is a process driven by inflammation. Estrogen in the appropriate context can act as a proinflammatory agent.

Exposure of mononuclear inflammatory cells to estradiol increases the production of interferon-γ, which is a major inflammatory cytokine that predisposes to atherosclerosis, and in turn decreases the production of anti-inflammatory cytokines (7). Furthermore, cultured human endothelial cells, when exposed to estradiol, are predisposed to death via apoptosis through the Fas/FasL pathway (8). Exogenous administration of estrogen increases plasma and tissue levels of matrix metalloproteinases, with the latter contributing to the thinning of the fibrous cap of atherosclerotic plaque, facilitating rupture. Interestingly, male estrogen receptor-α knockout mice exhibit decreased susceptibility to atherosclerosis (9).

Testosterone appears to have the opposite effect to estrogen in terms of atherogenesis. Testosterone supplementation directly protects castrated cholesterol-fed male rabbits from atherosclerosis (10). Androgens, including testosterone, have been shown to suppress the activity of proinflammatory cytokines while enhancing the activities of anti-inflammatory factors. Testosterone also inhibits inflammatory cell adhesion to vascular endothelium, the earliest step in atherosclerosis, through down-regulation of adhesion molecules on endothelial cells. Testosterone suppresses expression of vascular cell adhesion molecule-1 by its derivative, 5α-dihydrotestosterone (11).

Testosterone and PAD: Association or Causal?

In a cross-sectional study such as MrOS, the positive association between ABI and serum-free testosterone levels may be directly causal, or associated with each other due to other intermediary factors. While the foregoing biological mechanisms may provide a direct causal relationship between estradiol with proatherosclerosis and androgens with antiatherosclerosis effects, sex hormones may also act indirectly through other vascular risk factors.

Specifically, low testosterone levels in men are associated with insulin resistance, an atherogenic lipid profile, and the risk of cardiovascular diseases (2,12). Because androgens are important regulators of the total fat mass and its distribution, hypogonadism in men is also associated with obesity. Conversely, androgen replacement therapy in elderly men has beneficial effects on the profile of several cardiovascular risk factors, including lipid profiles and decreased fat mass (2,13).

Basic laboratory investigations also support these clinical observations. Testosterone deficiency by castration enhances atherosclerosis in male mice, which is exhibited by an unfavorable profile in low-density lipoprotein metabolism. Knockout of androgen receptor gene in male mice shows reduced insulin sensitivity and impaired glucose tolerance associated with obesity (14).

Implications for Clinical Practice

The study by Tivesten et al. (1) therefore represents the largest study to date linking ABI positively to serum-free testosterone and negatively to estrodiol. While this is an association found in a cross-sectional study, the findings are consistent with other studies linking the same hormone markers for atherosclerosis. There are also fundamental biological mechanisms explaining these associations.

However, for the markers to be useful clinically, the results will need to be replicated in other cohorts. The markers should also be relatively stable in the serum, and have significant independent contributions to prognosis from other existing known markers, such as low-density lipoproteins, smoking status, fasting plasma glucose, and others.

However, to be a true *risk factor* rather than just a biomarker for atherosclerosis or peripheral vascular disease, one would need to complete the loop by showing that therapeutic interventions aimed at, for example, increasing testosterone levels, truly can alter vascular outcomes. Realizing the challenges in the hormone replacement therapy in postmenopausal women, this will be a significant undertaking.

However, realizing that estrogen may be proinflammatory and accelerates atherosclerosis, whenever estrogen ad-

ministration is contemplated, a thorough search for underlying risk factors for vascular disease should be done for the patient. This would apply to both women in the perimenopause setting and men undergoing treatment for prostate cancer. If very high vascular risk exists for the patient, the estrogen exposure should be carefully evaluated in terms of risk versus benefit, accompanied by aggressive management of the known existing risk factors for vascular disease.

An intriguing question remains as to whether we should consider testosterone replacement therapy in elderly men with low levels of intrinsic testosterone and multiple risk factors for atherosclerosis. The purpose is to attenuate the progression of atherosclerosis, but this treatment may have additional benefit for improving quality of life and other risk factors as well. These types of questions are triggered by epidemiologic study such as that by Tivesten et al. (1), but can only be clarified in time through fundamental investigations, and carefully designed and conducted clinical trials.

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