Incidence of cardiovascular disease is consistently greater in men than in women during the first five decades of life.1 Because this gender disparity begins early in life, it is likely that a combination of genetic, environmental (possibly intrauterine) factors, and actions of sex-specific hormones contribute to the disease process (see Nagel and vom Saal).2 However, most research has focused on understanding how sex steroid hormones, estrogens and androgens, affect vascular function in men and women.

Historically, an “estrogen protection” hypothesis, where estrogens limit development of atherosclerosis, has been tested, although an “androgen detrimental” hypothesis is also plausible, but less studied. Although these hypotheses provide a starting point for design of experiments to study effects of hormones on vascular function, review of available data suggests a more complex interaction among the sex steroids than this simple dichotomy.3 Therefore, new approaches are needed to dissect mechanisms of how sex steroids interact in development of vascular disease in both sexes.

The study by Villablanca et al, reported in this issue, uses an approach of examining the contribution of estrogen receptor alpha (ESR1) in development of early atheroma in male mice.4 The design is based on two published lines of research: one which forms the basis of the estrogen “protection” hypothesis, and the other which suggests that disruption and/or polymorphisms in ESR1 are associated with accelerated atherosclerosis and cardiovascular events in men.

The estrogen protection hypothesis arose predominately from large cohort studies, where perimenopausal women treated with estrogen or estrogen-progestin for relief of menopausal symptoms had reduced cardiovascular disease compared with untreated women.5,6 From experimental studies, estrogen treatment of ovariectomized animals affected several processes that would promote repair of damaged vascular endothelium, reduce adhesion of aggregating platelets and leukocytes, and cause vasodilatation and thus limit development of occlusive vascular lesions (see Mendelsohn and Karas7 for review). In addition, estrogen affects lipid metabolism such that estrogen treatment increases serum concentrations of high-density lipoproteins while decreasing low-density lipoproteins. Estrogen also reduces lipid uptake by macrophages and their adhesion to endothelial cells, which should limit formation of fatty streaks in the arterial wall.

In males with normal testicular function, endogenous estrogen is produced enzymatically from testosterone by aromatase in many tissues, including the arterial wall. Theoretically, then, some vascular effects of testosterone could be mediated indirectly through conversion to 17β-estradiol.3 Indeed, inhibition of aromatase in men decreases circulating levels of 17β-estradiol.8 Estrogen effects are mediated through two receptors, estrogen receptor α (ESR1) and β (ESR2). These receptors, located on separate somatic chromosomes 6 and 14, respectively, are present in men and women. Little is known about how expression of these receptors is regulated in vascular tissue of men and women. However, ESR2 as measured by mRNA is the predominant subtype found in human blood vessels collected as surgical waste, and there is a suggestion that the ratio of ESR1:ESR2 may be greater in blood vessels from males compared with females.9

The second line of evidence supporting the design of the studies from Villablanca et al comes from observations that loss of ESR1 in men is associated with loss of flow-mediated dilation of the brachial artery.10 The fact that this flow-mediated dilatation is diminished in men treated with aromatase inhibitors8 suggests a link between the physiological response and testosterone-derived estrogen activation of ESR1. Polymorphisms in ESR1 in men are also associated with accelerated atherosclerosis and increased risk for myocardial infarction.11–13 Therefore, it is attractive to propose that in men, estrogen produced through local aromatization of testosterone would also provide “protection” against development of cardiovascular disease through mechanisms involving ESR1.

To test this hypothesis requires reliable and characterized animal models. To that end, Villablanca and colleagues9 sought to characterize atheroma in male mice lacking ESR1 receptors. In their experiments, disruption of ESR1 was performed in mice of a mixed genetic background (129/J and C57BL/J6). Animals were fed a high fat diet. At time points up to six months, aortae were removed for histological quantification of number and extent of atheromatosus lesions. Lesions were characterized by deposits of intracellular fat and large numbers of foam cells but were not considered complex lesions nor did they contain fibrotic caps or calcification. The rate of development and number of fatty lesions were greater in wild-type compared with ESR1 knockout animals. This was unexpected given the association in humans between the disruption and polymorphisms of ESR1 and extent of cardiovascular disease in men.

Discrepancies between observations in experimental animals as models for disease and disease in humans must be
reconciled. One consideration is that the genetic manipulation of ESR1 results in a variant gene product that might have some biological activity. There are over 300 single nucleotide polymorphisms in the human estrogen receptor public database. Relative prevalence of a particular genetic variant while showing association to disease progression in humans may not exist in isolation of other genetic variants.

Alternatively, the hypothesis that effects on the vasculature are “protective” and mediated solely through conversion of testosterone to estrogen and ESR1-mediated mechanisms may be naïve and should be revised. Aromatase was observed by immunohistochemistry in the endothelium, media, and adventia of the aortic wall suggesting that enzymatic conversion of testosterone to 17β-estradiol could occur within the aorta. However, androgen receptors are present in vascular tissue and nonaromatizable androgens initiate vascular effects. Therefore, given the potential for stoichiometric interactions of testosterone with aromatase and androgen receptors, it is unlikely that all physiologically relevant testosterone would be completely converted to estradiol independent of activation of androgen receptors (Figure). Indeed, sex-specific differences in vascular function are still observed in male pigs where estrogen levels exceed those of females by ~10-fold. In contrast to estrogen receptors, the gene for the androgen receptor is on the X chromosome. It is unknown at this time whether genetic variation in this receptor show sex-related associations with cardio-vascular disease.

In the study by Villablanca et al, castration (which reduced both systemic testosterone and estradiol exposure) reduced the average area of the atheroma lesion by over 90%, but a difference in atheroma between wild-type and ESR1 knockout mice was still present although not statistically significant. Whether all of testosterone’s effects are mediated by aromatization cannot be answered by this study because the effect of castration was not evaluated with concomitant inhibition of aromatase. Furthermore, whether the effect of castration could be reversed by selective replacement of testosterone was not confirmed, leaving open the possibility that another testicular secretion could contribute to vascular effects. Therefore, it is possible that vascular actions of testosterone are mediated directly through stimulation of androgen receptors as well as indirectly through conversion to 17β-estradiol and stimulation of ESR2.

It should be recognized that cross talk exists between the steroid receptors. For example, estrogen modulates androgen receptor expression as well as transcriptional activity. In addition, nonaromatizable androgens downregulate expression of estrogen receptors. Thus, ESR1 knockout mice may have compensatory changes in androgenic pathways that could affect the phenotype.

Factors affecting quantity and distribution of vascular androgen receptors are unknown. Although activation of androgen receptors can initiate both nongenomic and genomic effects in vascular tissue, it is not known how the androgen receptor regulates the synthesis or activity of endothelial nitric oxide synthase (eNOS). In contrast, regulation and activation of eNOS by estrogen receptors is well established.

The animal model studied by the Villablanca group may be useful in understanding hormonal interactions in early atheroma as defined by adhesion of macrophages and subendothelial accumulation of fat and foam cells. These responses are consistent with effects of nonaromatizable testosterone and androgen receptor blockade on increased expression of vascular cell adhesion molecule (VCAM)-1 in cultured endothelial cells and enhanced apoptosis of endothelial cells. Effects of androgens on lipogenesis are beginning to be defined, and there is evidence that sterol regulatory element–binding proteins are key mediators of lipogenic effects of both androgens and estrogens in a variety of tissues. Studies taking advantage of androgen receptor antagonists, aromatase inhibitors, castration, and/or replacement of testosterone or estrogen to castrated animals could be designed to investigate the stages of atheroma development.

Finally, vascular effects of ESR2 in ESR1 knockout mice should be considered. ESR2 inhibits proliferation of vascular smooth muscle. Responses regulated by ESR1 and 2 may be opposite and differentially regulated in adipose and endothelial cells.
lial cells. Therefore, experiments are also needed to understand the contribution of ESR2 in atheroma development in both male and female animals. Atherosclerosis in humans is a multifactorial disease process which includes lipid accumulation, remodeling of the vascular wall, infection, and calcification. In the era of sex-based medicine and genomics it is important to define similarities and differences in these processes as regulated by sex steroids in males and females. Therefore, studies in experimental animals that relate genetic sex, hormonal status, and receptor polymorphisms to vascular healing after mechanical injury14 or lipid accumulation, as in the article by Villablanca et al, provide important tools to understand effects of hormones on various stages of the disease process. However, each method provides an incomplete picture of disease progression in humans, and discovery of novel therapeutics will be hampered by generalization of hormonal functions as being "beneficial," "protective," or "detrimental" when sex- and cell-specific actions of hormones remain incompletely understood. Further investigation is needed to understand actions of testosterone on the vascular wall, contributions of androgen and estrogen receptor polymorphisms to vascular function, and better definition of responses mediated by ESR2 in various stages of the atherogenic process.

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