JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 5, NO. 9, 2020

EDITORIAL COMMENT

In Pursuit of Understanding the Role of Estrogens in Regulating Cardiac Structure and Function*

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Differences in the incidence and progression of cardiovascular diseases between men and women have been recognized for decades. Epidemiological observations support the concept that pre-menopausal women are largely protected from developing cardiometabolic diseases. In fact, protection also extends to pathologies that develop in organs such as kidney and liver (1). However, such protection tends to decline in post-menopausal women. A major goal in cardiovascular research is to identify and develop pharmacological strategies that can confer protection from diseases such as myocardial infarction. Many decades of work have been invested in such efforts, and multiple drug candidates were identified, developed, and evaluated in clinical trials. So far, none has been approved, and there continues to be a need to identify viable strategies that are more tailored to confer beneficial effects on the heart.

In this issue of *JACC: Basic to Translational Science,* Firth et al. (2) used a model of aortic constriction-induced heart failure (HF) in female guinea pigs to examine the putative role of estrogens in modulating cardiac function with respect to changes in cell function. The guinea pig was chosen for these studies because this species shares similar electrophysiological and Ca++ regulatory features with humans. Selected animals were ovariectomized, and a subgroup of these received 17β-estradiol supplementation, which repleted estrogen levels to significantly higher levels in the blood than those of intact animals. One hundred-fifty days post-constriction, left-ventricular (LV) structure and function were evaluated, LV myocytes were isolated from the hearts of treated and untreated ovariectomized animals, and their electrophysiology was examined. Animals with aortic constriction developed modestly enlarged LVs, cardiac hypertrophy, and a significant loss of fractional shortening relative to sham controls, which was exacerbated by ovariectomy and was reversed with 17β-estradiol supplementation. Myocytes from failing hearts had reduced calcium transient amplitude versus shams. A further 10% reduction in transient amplitude was observed in the ovariectomized group, and, when estrogen was supplemented, the size of the amplitude was restored. Total sarcoplasmic reticulum calcium content was reduced with HF and even more so with ovariectomy. Myocytes isolated from animals with aortic constriction had slower calcium transient decay versus sham. The rate of decay was further reduced in ovariectomized animal myocytes, and, when estrogen was supplemented, these myocytes had comparable rates of decay with the intact group. Spark-mediated sarcoplasmic reticulum leak was greater in ovariectomized animals compared with nonovariectomized animals, which may explain the smaller sarcoplasmic reticulum calcium content that was noted. Ovariectomy also increased the late sodium current versus intact animals with HF, an effect reversed by estrogen. Viewed together, these findings provide strong

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the view of *JACC: Basic to Translational Science* or the American College of Cardiology.

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The author attests he is in compliance with human studies committees and animal welfare regulations of the author's institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2020.08.002

evidence that the long-term absence of ovarian hormones exacerbates the decline in cardiac function during the pressure overload-induced HF and that 17β-estradiol supplementation reverses these aggravating effects

Before discussing the results of the study by Firth et al. (2), it is instructive to review what is known about estrogen biology in the heart. Three families of estrogen receptors are known. The α- and β-estrogen receptors act at the level of the cell nucleus and are known to regulate key female biological processes, such as breast tissue and uterus growth, and may also modulate tissue protection. The third family is composed of G-protein-coupled cell membrane receptors and is referred to as G-protein estrogen receptors or GPERs. A series of studies using transgenic animals suggests that although GPERs do not appear to modulate feminizing biology, they appear to act as major mediators of cardiometabolic protection (3). Thus, GPER biology may represent an area of opportunity for exploring organ-protection strategies.

However, as with most of human biology, the physiology of estrogens is complex. HF with preserved ejection fraction (HFpEF) has emerged as a disease with significantly higher prevalence (~65%) in women. Female patients with HFpEF are typically post-menopausal and present with 1 or more cardiometabolic risk factors such as hypertension and diabetes. At present, there are no therapies that appear to mitigate the progression of HFpEF; thus, there is an urgent need to understand its pathophysiology. The mechanisms underlying the higher prevalence of HFpEF in women are unknown. However, it is reasonable to hypothesize that estrogens may play a pivotal role. Of note, published literature reviews document important differences in the modulation of cardiac structure and function between men and women in response to physiological and pathological demands (4). Upon menopause, a small but significant increase in LV mass occurs in women, which correlates with body mass index. Reports indicate that aging in women is associated with the relative preservation of heart weight and myocyte number and volume, whereas in men, ~1 g of myocardium is lost per year, a loss compensated by increases in myocyte size. Post-mortem analysis indicates that apoptotic indices are 3-fold higher in men versus women in subjects free of cardiovascular disease, partly explaining the differences in myocyte loss.

Normal collagen content in healthy young myocardium ranges from 2% to 3%. However, myocardial collagen content increases with age, potentially reducing LV compliance. The Cardiovascular Health Study reported that in patients with HFpEF, women had significantly higher levels of the propeptide for type I collagen than men, suggesting greater levels of production of fibrillar collagen. As part of the Multi-Ethnic Study of Atherosclerosis, an evaluation of age-related myocardial fibrosis was performed in 1,231 participants (51% women, age 54 to 93 years) using magnetic resonance imaging. Results show that women had significantly greater partition coefficients, extracellular volumes, and pre-contrast T1 (parameters associated with myocardial fibrosis) than men, which increased with age, hinting at a causal role of estrogen deficiency.

In the setting of aortic stenosis, women (with similar aortic areas and gradients) demonstrate greater degrees of LV hypertrophy with increases in mass and relative wall thickness, smaller end-diastolic and systolic dimensions, and preserved LV function (shortening, ejection fraction) versus men. In animals, this pattern of LV remodeling and function is also seen in female versus male spontaneously hypertensive rats (SHRs), where greater contractile function and smaller LV volumes are noted despite similar pressures. As SHRs age, female rats have preserved LV dimensions and function, whereas male animals develop HF by 12 months. Differences in the adaptive response are also observed with volume overload caused by aortic regurgitation, in which women exhibit smaller LV end-diastolic and systolic volumes despite similar degrees of regurgitation versus men. In rat models of volume overload induced by aortocaval fistula, female hearts develop concentric hypertrophy with no loss of function and minimal dilation. In contrast, male rats have LV dilation, decreased compliance, and 10-fold higher mortality. The greatest differences were noted in the magnitude of dilation. With ischemic heart disease, men have 10-fold higher rates of apoptosis versus women in the peri-infarct region, probably accounting (at least in part) for the more aggressive course of post-infarction HF in men. In mouse models of infarction, male animals have lower indices of myocardial healing, greater LV dilation, and greater incidence of rupture. Altogether, the combination of reduced levels of chamber dilation, thicker LV walls, and myocardial fibrosis may help to explain why women at risk of developing HF preferentially develop HFpEF, leading to a greater level of impaired diastolic filling versus men. These observations extensively support the detailed examination of the role that estrogens play in modulating cardiac structure and function. Unfortunately, the body of published studies is extremely limited: in particular,

those that compare and contrast endpoints and outcomes in male versus female models. Thus, there is urgent need to further this area of research.

Bustamante et al. (5) recently evaluated the intersecting roles of aging, estrogen depletion, and excess body weight on altering cardiac structure and function. Female 18-month-old Fischer F344 rats were divided into an aged group, age + ovariectomy, and age + ovariectomy + 10% fructose in drinking water to induce weight gain. By 22 months of age, all animals developed hypertension, whereas fructose-supplemented animals further increased body weight. Echocardiography only detected mild chamber remodeling in all groups with aging. However, LV pressure-volume loop results showed significant decreases versus intact aged animals in stroke volume, stroke work, and cardiac output and increases in relaxation time with preserved ejection fraction in ovariectomized rats, thus recapitulating certain features present in patients with HFpEF. Histology indicated papillary and interstitial fibrosis with aging, which was higher in the endocardium of ovariectomy groups. Thus, the implementation of such animal models may help understand the roles that aging and estrogen depletion play in early (pre-HFpEF) development of disease.

The current study by Firth et al. (2) provides several novel insights into the role of estrogen biology in the heart. Importantly, for the majority of the endpoints measured, estrogen supplementation reversed the aggravating effects of ovariectomy on LV pressure overload-induced HF. However, given that the average estrogen levels of supplemented animals were higher than those of intact nonovariectomized controls, it remains to be determined whether these findings will be translatable to the clinical setting, wherein estrogen replacement has already been associated with increased stroke, without reducing the risk of heart attack. A limitation of the study by Firth et al. (2) is that they did not identify the mechanisms that were responsible or 17β-estradiol induced alterations excitation-contraction coupling. The authors speculate that 17β-estradiol may directly interact with estrogen receptors on the cardiac myocyte cell membrane, thereby eliciting downstream signaling cascades that modulate electromechanical coupling. Future studies will need to explore this unique regulatory biology to understand the basic physiology and pathophysiology of estrogen biology in regulating cardiac structure and function.

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KEY WORDS cardiac remodeling, estrogen, hypertrophy, ovariectomy, pressure overload