

1 **Title: *The stronger sex, until menopause: understanding the impact of estrogen loss on***
2 ***heart function***

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31 In the first four decades of life, women are protected from cardiovascular disease (CVD)
32 compared to men, perhaps making women the stronger sex. Over the next 5-10 years,
33 however, ovarian follicle depletion initiates a gradual decrease in sex steroid hormones
34 transitioning women into perimenopause. Menopause occurs in approximately the 5th decade of
35 life and results in a near complete loss of serum estrogen (1). With menopause, the risks of
36 coronary artery disease, peripheral artery disease, aortic calcification, and stroke substantially
37 rise, and the decrease of circulating estrogen is hypothesized to adversely affect adipose
38 distribution, lipid metabolism, insulin sensitivity, and blood pressure (1). In young women with
39 either abrupt and gradual loss of estrogen due to hysterectomy, chemotherapy, and extreme
40 psychosocial stress, risk of CVD also increases (1, 2). Multiple lines of evidence suggest that
41 estrogen imparts a protective effect on the cardiovascular system at multiple scales, including
42 the endothelia, arteries, and the heart itself (1, 3, 4). However, an early clinical trial that sought
43 to diminish CVD risk in post-menopausal women by administering exogenous estrogen instead
44 increased risk of cancer, thromboembolic events, and stroke (5). To date, the protective and
45 detrimental roles of estrogen in the cardiovascular health of premenopausal women,
46 postmenopausal women, and men remain poorly understood.

47 In 2016, the National Institute of Health began requiring that biological sex be factored
48 into clinical and preclinical study designs to improve rigor and translation of basic science to
49 clinical science and care (6). Despite this, recruitment of women into clinical trials remains low
50 (7), which leaves significant knowledge gaps in the pathology and treatment of CVD in women
51 and creates disparities in prognosis compared to men. This limited knowledge has led to delays
52 in critical care for women presenting with myocardial infarction and increased mortality in aortic
53 valve replacements compared to men (8, 9). In basic science research, preclinical studies
54 persist in using predominately male animals or do not report the sex of animals within the

55 methods. Overall, the mechanisms by which female sex, estrogen, and age impact heart
56 disease are critically understudied, which confounds translation from bench to bedside.

57 The rapid report by Joll *et al.* in this issue (10) is a welcome change from business as
58 usual in cardiovascular research. To investigate the impact of estrogen loss on CVD in women,
59 the authors subjected young adult female C57BL6 mice (4 months old) to bilateral ovariectomy
60 (OVX) to induce an early menopause-like state, fed them a high cholesterol (Western) diet, and
61 aged them to 12 months. *In vivo* echocardiogram measurements were performed at 4, 9 and 12
62 months, as well as bone mass density measurements using dual x-ray absorptiometry each
63 month. At the terminal time point of 12 months, left ventricular (LV) and aortic valve (AV) tissues
64 were harvested and stained with Mason's Trichrome and Alizarin Red S to determine collagen
65 content and calcification, respectively. The authors found that bilateral OVX in combination with
66 a high fat diet and aging resulted in increased LV mass, signifying LV hypertrophy and
67 suggesting systemic hypertension. Further, no evidence of LV or AV fibrosis or calcification was
68 found. The OVX group did have a significant decrease in bone mineral density, indicating
69 osteoporosis development in agreement with prior rodent models (11). The stimulus for LV
70 hypertrophy is not elucidated by Joll *et al.* but is likely related to vascular stiffening due to aging
71 (12) and the high cholesterol diet in combination with the loss of estrogen (13). In addition, while
72 collagen accumulation was not found, collagen type and crosslinking, which play a functional
73 role in the stiffening of these tissues (14), could have been altered by OVX, the high-fat diet, or
74 aging, but were not measured. Despite these limitations, the authors are to be commended for
75 addressing the elephant in the room regarding the lack of female specific CVD research and
76 investigating the development of CVD in aging, post-menopausal women.

77 Only continual action and acknowledgement of sex differences in cardiovascular health
78 research will reduce sex-based cardiovascular health disparities. For those who take up this
79 charge, we offer a few suggested refinements to the study design used by Joll *et al.* First,

80 bilateral OVX is an overly simplified model of menopause. The loss of sex steroid hormones
81 with surgical OVX is rapid and does not mimic the gradual loss of hormones and hormone
82 receptors in the perimenopausal to menopausal transition in human women. As an alternative,
83 the 4-vinylcyclohexenediepoide (VCD) mouse model of menopause simulates ovarian failure
84 over time (15). VCD injections cause regression of small follicles and rapidly accelerate
85 depletion of the ovarian follicle reserve. During the transition to complete ovarian depletion, the
86 mice undergo a perimenopause phase similar to human women with corresponding hormonal
87 changes such as decreased estrogen, increased follicle stimulating hormone, and increased
88 luteinizing hormone. Mice receiving the VCD injection over 10-20 days begin to have extended
89 estrous cycles that taper off into a continual diestrus anovulatory phase (15). Moreover, variable
90 VCD dosing allows for manipulation of the perimenopausal phase and permits investigators to
91 optimize the perimenopause phase length to the study design. Prior research combining VCD
92 with Ang-II infusion in female C57BL6 mice showed that blood pressure increased in both the
93 perimenopausal and menopausal phase compared to intact mice with Ang-II infusion (16).
94 However, the use of VCD in physiological research is limited due to the carcinogenic and toxic
95 nature of the drug to the liver and kidneys. Other off-target effects may also limit the utility of this
96 approach for mimicking menopause in an animal model. Second, since aging is key to CVD in
97 women, performing OVX in older rodents would better recreate the effect of hormone loss on
98 the stiffened vasculature that likely exists in women in the 5th decade of life. Third, the use of
99 mice as a model of human disease is limited due to the robust compensatory mechanisms of
100 the mouse in the face of injury, disease, or genetic mutations. Using the bilateral OVX with high
101 cholesterol diet or VCD model of menopause in a larger rodent model, such as the rat, may
102 induce more substantial LV and AV remodeling, including fibrosis, closer to the human
103 condition. In combination with *in vitro* and *in silico* approaches, robust and physiologically
104 relevant *in vivo* models that recapitulate the effects of female hormone loss in conjunction with
105 aging on CVD development promise to advance equity in cardiovascular health.

106 In conclusion, Joll *et al.* provide a good first step in developing a mouse model that
107 bridges the gap between sex, hormones, and age in cardiovascular health (10). This publication
108 addresses the sorely lacking inclusion of the female sex, female sex steroid hormone effects,
109 and lifecycle in cardiovascular research. Understanding the sex-dependent and sex steroid-
110 dependent mechanisms of CVD development and progression are critical to diagnosis,
111 treatment, and prognosis of women with CVD. Moreover, uncovering the ways in which
112 estrogen protects young women's hearts, making them stronger than men's, may enable the
113 discovery of novel therapeutics for older women and men.

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116 **Conflicts of Interest**

117 No conflicts of interest to disclose.

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