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Acute ascorbic acid infusion increases left ventricular diastolic function in postmenopausal women

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ABSTRACT

Objectives: We tested the hypothesis that oxidative stress contributes to reductions in left ventricular diastolic (LV) function in estrogen-deficient postmenopausal women, related in part to reduced nitric oxide (NO) bioavailability.
Study design: LV diastolic function – recorded using transthoracic echocardiography and determined as the peak early (E) to late (A) mitral inflow velocity ratio and the E to peak early (e') mitral annular velocity ratio – and brachial artery flow mediated dilation (FMD), a biomarker of NO bioavailability, were measured during acute systemic infusions of saline (control) and ascorbic acid (experimental model to decrease oxidative stress) in healthy premenopausal women (N = 14, 18–40 years) and postmenopausal women (N = 23, 45–75 years).
Results: The E/A ratio was lower (1.16[1.06–1.33] vs 1.65[1.5–2.3]; median[interquartile range]) and the E/e' ratio was elevated (8.8[7.6–9.9] vs. 6.6[5.5–7.3]) in postmenopausal compared with premenopausal women, indicating reduced LV diastolic function. E/A and E/e' were correlated with FMD ($r = 0.54$ and $r = -0.59$, respectively, both $P < 0.01$). Ascorbic acid infusion improved both FMD ($5.4 \pm 2.0\%$ to $7.8 \pm 2.6\%$) and E/e' (to 8.1[7.2–9.7], $P = 0.01$) in postmenopausal women but not in premenopausal women. Ascorbic acid did not change E/A in either group.
Conclusion: The current study provides evidence that oxidative stress contributes to reduced LV diastolic function in estrogen-deficient postmenopausal women, possibly by reducing the availability of NO.

1. Introduction

Aging is associated with an increased risk for the development of Heart failure (HF), a debilitating condition that affects nearly 6 million Americans and has been estimated to account for one-third of all disease-related mortality in American women [1,2]. Of the two phenotypes of HF, older women (>65 years) are more likely to develop HF with a preserved ejection fraction (HFpEF), characterized by impaired left ventricular (LV) diastolic function [3–7]. Although LV diastolic function declines with age, postmenopausal women experience a more rapid decline compared to age-matched men [8]. Understanding the mechanisms that contribute to the decline in LV diastolic function in postmenopausal women is important for the development of strategies to preserve cardiac function and prevent heart failure in women. The biological processes underlying the reduction in LV diastolic function in estrogen-deficient postmenopausal women are not completely understood. Estrogen-deficient postmenopausal women have a greater oxidative burden than premenopausal women [9–11]. Elevated markers of reactive oxygen species (ROS) have been reported in the failing human myocardium [12], and LV diastolic dysfunction in ovariectomized (OVX) rats is associated with elevated cardiac ROS levels [13,14]. These data suggest that oxidative stress may play a role in the reduction in LV diastolic function [14,15]. Oxidative stress could impair LV diastolic function by decreasing the bioavailability of nitric oxide (NO), a key regulator of cardiac function. Elevated levels of ROS can scavenge NO and decrease NO synthesis by suppressing the enzymatic function of nitric oxide syn-

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thase (NOS), the enzyme that catalyzes NO from L-arginine [16,17]. Whether oxidative stress is mechanistically linked to reduced LV diastolic function in postmenopausal women is unknown. Accordingly, we tested the hypothesis that oxidative stress contributes to the reduced LV diastolic function in estrogen-deficient postmenopausal women compared to premenopausal controls, related in part to reduced NO bioavailability.

2. Materials and methods

2.1. Study population

We studied 37 healthy women: 14 premenopausal (18–40 years) and 23 postmenopausal (45–75 years). Premenopausal women had regular menstrual cycles with no change in observed cycle length (21–35 days). Postmenopausal women had ≥12 months of amenorrhea. Women had not used oral contraceptives or hormone therapy for at least 6 months. Women were normotensive (resting blood pressure <140/90 mmHg), non-diabetic (fasted glucose <126 mg/dL), sedentary or recreationally active (<3 days/wk vigorous exercise), nonsmokers, and healthy as determined by medical history, physical examination, standard blood chemistries (chemistry panel, complete blood count and thyroid stimulating hormone) and electrocardiogram at rest and during incremental treadmill exercise. Additionally, women were not taking medications that could influence cardiovascular function (e.g., antihypertensive, lipid lowering medications) and had not used vitamin supplements or anti-inflammatory medications for at least 4 weeks prior to the study visit. The study was approved by the Colorado Multiple Institutional Review Board, and all participants provided a written informed consent form.

2.2. Measurements

Women were studied in the supine position following an overnight fast with proper hydration (water only). Participants were provided individualized meals based on a 3-day food intake record to ensure normal dietary patterns, including sodium intake, as described previously [18]. Meals were consumed 2-days immediately prior to any measurements. Premenopausal women were tested in the mid-follicular phase (e.g., 7–10 days after onset of menstruation) in an effort to perform measurements when estradiol was representative of average levels across the menstrual cycle. The study took place at the University of Colorado Anschutz Medical Campus Colorado Clinical Translational Sciences Institute Clinical and Translational Research Center.

2.2.1. Echocardiogram

Transthoracic echocardiographic measurements of LV diastolic function were obtained using a GE Vivid I ultrasound (GE Healthcare, Horten, Norway) using standard methods [19]. Briefly, 2 dimensional guided M-mode echocardiography was used to quantify LV structural characteristics and the Teichholz formula [20] to calculate LV volumes, ejection fraction, and fractional shortening. Pulsed-wave Doppler in the apical 4-chamber view was used to obtain mitral inflow velocities. The sample volume was placed between the mitral leaflet tips to quantify peak early filling (E) and late diastolic filling (A) velocities, E/A ratio, and deceleration time (interval from peak E to a point of intersection of the deceleration of flow with the baseline). Because mitral inflow patterns are sensitive to preload and can change dramatically with the progression of diastolic dysfunction, myocardial tissue Doppler imaging (TDI) was also performed in the apical 4 chamber view with a 2 mm sample volume at the septal and lateral mitral annulus. Septal and lateral values of peak early (e') and late (a') mitral annular velocities were calculated. The ratio between E and e' was used as the primary parameter of diastolic performance. All measurements were performed by a single trained technician and all echocardiographic images were reviewed by a board eligible cardiologist.

2.2.2. Brachial artery flow mediated dilation

Ultrasound measurements of brachial artery FMD were performed as previously described in detail by our laboratory [21,22], and according to published guidelines for assessing FMD in human participants [23]. Briefly, a pediatric cuff was placed on the upper forearm and brachial artery images were acquired ~3–6 cm above the antecubital fossa at baseline and following reactive hyperemia produced by inflating the cuff to 250 mmHg of pressure for 5 min. After the release of the arterial occlusion, the initial 10 Doppler blood flow velocity waveform envelopes were acquired and B-mode ultrasound brachial artery diameter images were measured continuously for two minutes. The dilation of the brachial artery in response to the stimulus of forearm ischemia has been shown to be dependent on the release of vasodilators, predominantly NO, from the vascular endothelium, and thus, is considered a biomarker of NO bioavailability [24]. Brachial images were analyzed for diastolic diameters using a computerized semi-automated edge-detection software that allows accurate identification and measurements of brachial artery lumen diameter (Vascular Analysis Tools v. 5.5; MIA LLC, Coralville, IA). Peripheral artery blood pressures were measured over the brachial artery using a semi-automated device (Dinamap; Johnson & Johnson, New Brunswick, NJ). All images were coded by number, blinded to menopause group and testing condition, and analyzed by the same individual. The coefficient of variation and intra-class correlation for trial-to-trial reliability measured in 10 individuals for FMD (%) were 2.2% and 0.99, respectively.

2.2.3. Body composition, physical activity, and blood sampling

Total and trunk fat percent were determined using dual energy X-ray absorptiometry (Hologic Discovery, version 12.6). Minimal waist and hip circumferences were measured and waist-to-hip ratio was calculated as previously described [21]. Leisure time physical activity was determined by the Modifiable Activity Questionnaire [25]. Fasting plasma concentrations of glucose, insulin, total cholesterol (Roche Diagnostic Systems, Indianapolis, IN), and high-density lipoprotein cholesterol (Diagnostic Chemicals Ltd, Oxford, CT) were determined using enzymatic/colorimetric methods. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation [26]. Serum concentrations of follicle-stimulating hormone (FSH), estradiol and progesterone were measured using chemiluminescence. Serum total antioxidant status (TAS), a measure of overall antioxidant defenses, was measured using an enzymatic kit (Randox Laboratories, Oceanside, CA). All blood samplings occurred on the day of vascular testing. All assays were performed at the University of Colorado Clinical Translational Research Center core laboratory.

2.3. Experimental design

To determine whether oxidative stress is mechanistically linked to the reduced LV diastolic function in estrogen-deficient postmenopausal women, we employed a common experimental model used to acutely suppress ROS as described previously by our laboratory and others [27–31]. Briefly, echocardiographic and brachial artery ultrasound measurements were obtained after 20 min of normal isovolumic saline infused systemically (control), and then repeated after 20 min of intravenous systemic infusion of a pharmacological dose of ascorbic acid. The concentration of the ascorbic acid solution prepared by the University of Colorado pharmacy was 0.06 g ascorbic acid/kg fat-free mass/100 ml of normal saline. All women received a bolus of 100 mL ascorbic acid solution given