Chronic Alternate Day Fasting Results in Impaired Diastolic Compliance and Diminished Systolic Reserve in Rats

Supporting information

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Methods

Echocardiography

Echocardiography (Sonos 5500, a 12 MHz transducer) was conducted under light sodium pentobarbital anesthesia (30 mg/kg, i.p.) as described previously (7, 30, 31). Briefly, parasternal long axis views were obtained and recorded to ensure that the mitral and aortic valves, as well as the apex, were visualized. Short axis views were recorded at the mid-papillary muscle level. End-diastolic and end-systolic left ventricular (LV) areas were calculated from endocardial area tracings in 2D mode (short and long axis views) on digital images captured on cineloop, using the leading edge method. End-diastolic volume (EDV) and end-systolic volume (ESV) were calculated by a Modified Simpson's method. Ejection fraction (EF) was then derived as EF = (EDV - ESV)/EDV x100. Stroke volume (SV) was calculated as EDV - ESV and Cardiac output (CO) - as SV x Heart Rate (HR). LV posterior wall thickness (PWth) was measured from M-mode at LV long-axis tracings. LV mass (LVM) was estimated from M-mode tracing of LV in long axis. Left atrial dimension (LAD) and aortic dimension (AoD) were measured from long axis M-mode tracings of basal aorta and left atrium at end-diastole, as recommended by the American Society of Echocardiography, and LAD/AoD was used to normalize an LA size. All reported indices of echocardiography were presented as is (Figure 2) or normalized for body weight (BW) either ratiometrically or allometrically (11, 12). All measurements were averaged over three to five consecutive cardiac cycles and made by a single observer blind to the identity of the tracings. Reproducibility of measurements of given observer had been tested on the subgroup of animals. Variability of result did not exceed 5%.

Hemodynamic Measurements

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), intubated and ventilated. A bilateral thoracotomy was performed in the sixth intercostal space. A 1.4F-

combined micromanometer/conductance catheter (Millar Instruments Inc., Houston, TX) was inserted into the LV from the apex. LV end-diastolic pressure (EDP), EDV, ESV, SV, +dP/dt, -dP/dt, isovolumic relaxation time (τ) and arterial elastance (Ea) were determined in 10-20 digitally averaged cardiac cycles. LV end-systolic elastance (Ees), preload recruitable stroke work (PRSW) and end-diastolic stiffness (Eed) were measured using a gradual preload reduction technique. Arterio-ventricular (AV) coupling was calculated as Ea/Ees. All hemodynamic measurements were reported either as is or scaled for a body weight differences ratiometrically or allometrically

Dobutamine Stress Test

After completion of hemodynamic evaluation the left femoral vein was cannulated and infusion of Dobutamine, a predominantly β_1 AR agonist, in concentration of 50 μg/ml of normal saline was started. The infusion rate was regulated by an infusion pump, which was set at consecutive regimens of 100, 200, 300, and 400 μL/kg/min. Each infusion regimen was continued for 5 minutes. During each consecutive infusion regimen each animal had received 0.5, 1.0, 1.5, and 2.0 ml/kg of saline containing 25, 50, 75, and 100 μg/kg of dobutamine respectively. Therefore, the test actually consisted of two combined stresses: a volume load combined with inotropic stimulation, i.e., a stress similar to that induced by a dynamic exercise. Hemodynamic indices at steady state were collected prior to drug infusion and at the end of each infusion regimen (a total of five measurements).

Gross Pathology and Histological Assessment

Histological staining and analyses were performed as described previously (7). Briefly, the hearts and lungs were removed and weighed (wet weight). Hearts were further cut into two pieces through the short axis. The basal half was fast frozen and stored, and the apical half was used for histological analysis. Myocardial tissue sections were subjected to Masson's trichrome and

hematoxylin-eosin staining. Myocyte cell size and density were measured in H&E-stained sections. Only myocytes which nuclei were clearly identified were counted. Myocyte diameter was measured as the shortest distance across the nucleus in transverse cell sections. Diameters of 100 myocytes from 5 randomly selected microscope fields (x200 magnification) from the LV posterior wall were averaged to represent the myocyte diameter of a given specimen. Myocyte density was calculated from the same area in the same fashion. Myocardial tissue fibrosis was measured in Masson's trichrome-stained sections and was expressed as a fraction of a microscopic field (x100 magnification) of the LV posterior wall. An average of 5 randomly selected fields represented results of a given specimen. Person assessing histological slides was blinded to a grouping.

Scaling for Body Weight Differences

The effect of the body weight differences between groups on cardiovascular variables was assessed using linear regressions which was calculated for each parameter of interest against body weight among all animals of both groups at given time point. If resulting linear regression did not differ from zero, the body weight differences could not affect a parameter of interest and any adjustment to differences in body weight was not necessary. On the contrary, if linear regression was significantly different from zero, the body weight differences might affect the results of measurement and scaling was needed. We used two consecutive approaches to adjust for body weight differences. At first the ratiometric scaling was tried, i.e., a variable of interest was divided by a body weight. A new derived variable was again plotted against body weight. If the resulting slope was not different from zero, the scaling was achieved and the effect of body weight was excluded from any differences between groups. If the slope was still statistically different from zero after ratiometric scaling, the more sophisticated allometric scaling procedure was applied. In this procedure the parameter of interest was divided by a body weight in the

power, which was defined as a slope of the unadjusted variable against body weight. This procedure certainly excluded any effect of body weight. All echocardiographic and hemodynamic variables were reported before and after scaling for body weight differences.

Results

Figure 1 represents the changes in early LV filling (E wave) and late filling during systole (A wave). E wave is consistently reduced with time in ADF rats. At 6 mo it is significantly lower than at the baseine and at the AL animals at the same time.

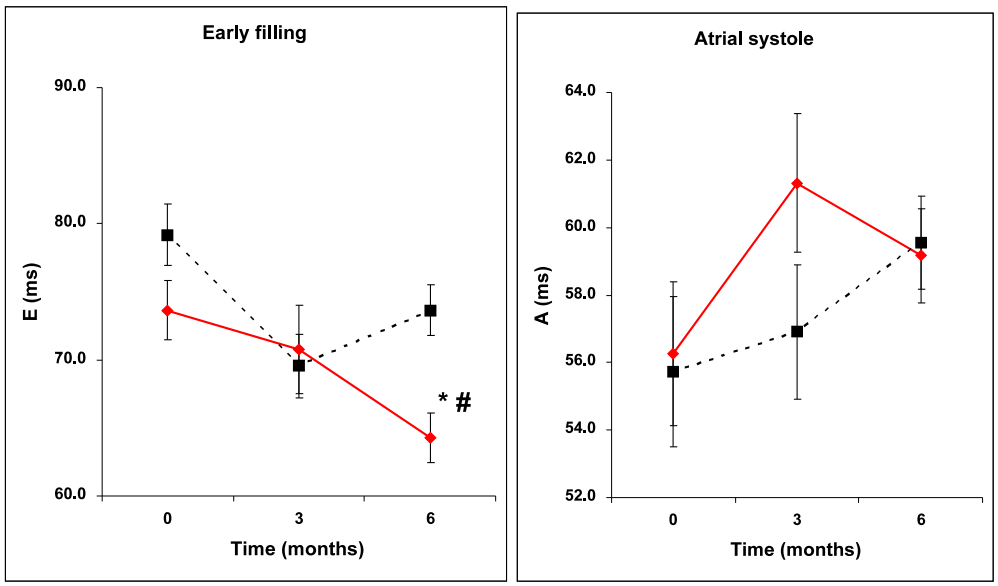

Figure 1. Doppler–measured mitral valve flow velocity. Early (left) and late (right) LV filling in ADF and AL rats. * -p<0.05 vs other group. # - p<0.05 vs baseline.