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Original article

Effects of calorie restriction on cardioprotection and cardiovascular health

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ABSTRACT

Multiple health benefits of calorie restriction (CR) and alternate day fasting (ADF) regimens are widely recognized. Experimental data concerning the effects of calorie restriction on cardiac health are more controversial, ranging from evidence that ADF protects heart from ischemic damage but results in developing of diastolic dysfunction, to reports that CR ameliorates the age-associated diastolic dysfunction. Here we investigated the effects of chronic CR on morphology and function of the cardiovascular system of aged rats and cardioprotective effect of CR against ischemic damage in the experimental rat model of MI. Cardiovascular fitness of 24-month old Fisher 344 rats maintained through life on *ad libitum* (AL) or CR diets was extensively evaluated via echocardiography, dobutamine stress test, pressure–volume loop analyses, pulse wave velocity measurements, and histology. Groups of 2-month old AL and 29-month old CR rats were studied for comparison. Myocardial infarction (MI) was induced by a permanent ligation of the anterior descending coronary artery in 5-month old rats maintained for 3 months on CR or AL. MI size was evaluated histologically 24 hrs following coronary ligation. Cardiac remodeling was followed-up via echocardiography. Age-associated changes in 24-month old rats consisted of 33% increase of fibrosis in the myocardium and more than 2 fold increase of the collagen in the tunica media of the aorta. There was a significant decrease in the density and total number of cardiomyocytes, while their size was increased. These morphological changes were manifested in a decline of systolic and diastolic cardiac function, increase of left ventricular and aortic stiffness, and arterio-ventricular uncoupling. Tachycardic response to dobutamine challenge was absent in the old rats. Compared to AL rats, 24-month old CR rats had reduced levels of cardiac and aortic fibrosis, increased density of cardiomyocytes that were smaller in size, attenuated diastolic dysfunction, normal systolic function and arterio-ventricular coupling. Tachycardic response to dobutamine was also intact in CR 24-month old rats and aortic stiffness was reduced. Adjustment for body weight differences through ratiometric or allometric scaling did not affect the overall pattern of differences between AL and CR rats. Attenuation of morphological and functional age-associated changes in 24-month old CR rats either was not observed at all or was smaller in 29-month old CR rats. Size of MI induced by a permanent coronary ligation as well as post-MI cardiac remodeling and function were similar in CR and AL rats. CR does not increase tolerance of myocardium to ischemic damage, but attenuates the age-associated changes in the heart and major vessels. The attenuation of age-associated changes by CR cannot be explained by the effect of lower body weight but are attributable to more intimate cellular mechanisms of CR itself. Attenuation of age-associated changes by CR waned with advancing age, and is consistent with the idea that CR postponed senescence.

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1. Introduction

Multiple health benefits of calorie restriction (CR), either through daily reduction of calorie intake (20–40% less than *ad libitum* (AL) food consumption), or via implementing an alternate day fasting regimen (ADF) have been reported in animal experiments and several human trials [1–8]. CR is the only proven way to prolong life span, at least in the rodents and lower life forms [9–12].

The salutary effects of both CR and ADF on the known risk factors for cardiovascular diseases have also been demonstrated and universally accepted [3,8,13–15]. Nevertheless, with respect to cardioprotective properties of long-term CR/ADF and their effects on general fitness of the cardiovascular system, the experimental data

Abbreviations: CR, Calorie restriction; ADF, Alternate day fasting; MI, Myocardial infarction; LV, Left ventricle; EDV, End-diastolic LV volume; ESV, End-systolic LV volume; EF, Ejection fraction; PWth, Posterior wall thickness; LVM, Left ventricular mass; LAD, Left atrial dimension; AoD, Aortic dimension; TA, Thoracic aorta; ESP, End-systolic pressure; EDP, End-diastolic pressure; PV, Pressure–volume loop analyses; HR, Heart rate; SV, Stroke volume; CO, Cardiac output; Ea, Arterial elastance; PRSW, Preload recruitable stroke work; Ees, Ventricular elastance; Eed, Myocardial stiffness.

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are not uniformly in accord. We have reported that ADF increased myocardial tolerance to ischemic damage [16]: compared with AL animals the size of myocardial infarction (MI) induced by permanent ligation of a coronary artery in rats was significantly smaller in the cohort that had been maintained on the ADF regimen during the 6 preceding months [16]. ADF rats also had reduced level of apoptosis in the peri-infarct area. Continued ADF after coronary ligation resulted in attenuation of post MI remodeling and functional decline. We have also reported, however, that long-term ADF in rats resulted in development of myocardial fibrosis associated with diastolic dysfunction and diminished cardiac reserve [17].

Our findings that chronic ADF promotes diastolic dysfunction and fibrosis contrast with results of a simultaneously published report on amelioration of age-associated diastolic dysfunction in rats maintained on chronic CR [18]. To address this apparent controversy we investigated the effects of chronic CR on morphology and function of the cardiovascular system of aged rats and cardioprotective effects of CR against ischemic damage in the experimental MI rat model.

2. Methods

2.1. Animals and experimental design

Two-month old male Fisher344 rats, obtained from Charles River Laboratories (Wilmington, MA), were placed in the NIA vivarium and housed and studied in conformance with the NIH Guide for the Care and Use of Laboratory Animals, Manual 3040-2 (1996), with Institutional Animal Care and Use Committee approval. The diagram of experiments is presented in the supplement Fig. 1. Upon arrival all animals were divided into a control group fed *ad libitum* NIH-07 (7022) diet (AL) and a calorie restricted (CR) group, whose daily calorie intake was reduced by 40%. Both groups had unlimited access to water. Forty-five animals from each diet group were set aside for longevity study and were euthanized when found in the moribund condition during routine daily inspection. The rest of the rats were placed into different experiments at different time points. (1) After 3 months on their respective, CR or AL diets, 29 rats from each diet group (5-month old at the time) were randomly selected and subjected to a coronary ligation to study the cardioprotective effects of CR against ischemic damage. (2) At 24 months of age (after 22 months on respective diets) 12 rats from CR (24CR group) and 21 rats from AL (24AL group) were subjected to extensive evaluation of cardiovascular structure and function via echocardiography, dobutamine stress test, hemodynamic assessment using pressure–volume loop analyses, measurement of pulse-wave velocity and histological evaluation of the heart. Additional groups, ten 2-month old AL rats (2AL group) and fifteen 29-month old CR (29CR group) rats (27 months on CR) were subjected to the same vigorous evaluation.

2.2. Echocardiography (Echo)

All rats were subjected to Echo before invasive measurements (pressure–volume loop analyses) or surgery, as previously described [17]. Briefly, under Isoflurane anesthesia (2% in oxygen) via face mask, a 12-MHz transducer (HP Sonos 5500, Hewlett-Packard Inc) was used to obtain 2D images of the left ventricle (LV) at long and short axes and 4 chamber views. End-diastolic and end-systolic LV areas were calculated from endocardial area tracings in the 2D mode (short and long axis views) on digital images captured on cineloops, 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 × 100. Stroke volume (SV) was calculated as EDV − ESV and Cardiac output (CO) as SV × Heart Rate (HR). LV posterior wall thickness (PWth) was measured from the M-mode LV long-axis tracings. LV mass (LVM) was estimated from the 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 LAD. Thoracic aorta (TA) was scanned just above the diaphragm using a 30 MHz transducer (707B, Visual Sonics, Toronto, Canada). 2D images and M-mode tracings were obtained in long and short axis views. TA lumen diameter at maximum (TA max) and minimum (TA min) dimensions were measured off-line from M-mode tracings during each cardiac cycle using NIH imaging software. TA lumen fraction expansion was calculated as (TA max − TA min)/TA min. All reported echocardiographic indices are presented as absolute (Fig. 2) or normalized for body weight (BW), either ratiometrically or allometrically [17,19,20]. 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 a subgroup of animals and variability did not exceed 5%.

Following a baseline Echo, 24-month old rats were injected i.p. with 10 μg/kg of Dobutamine and the Echo was repeated 5 minutes later.

2.3. Hemodynamics

Prior to euthanasia pressure–volume analyses were performed as previously described [17]. Briefly, under Isoflurane anesthesia (2% in oxygen), rats were intubated and mechanically ventilated. A bilateral thoracotomy in 4th and 5th intercostal space was performed. After opening the pericardium, a 2F 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 (tau) and arterial elastance (Ea) were determined in 10–20 digitally averaged cardiac cycles while the ventilator was stopped. LV end-systolic elastance (Ees), preload recruitable stroke work (PRSW) and end-diastolic stiffness (Eed) were measured using a graded preload reduction technique. Arterio-ventricular (AV) coupling was calculated as Ea/Ees. The test was concluded by advancing the catheter into thoracic aorta to measure arterial blood pressure. All hemodynamic measurements for 24-month old groups were reported either as is or scaled for body weight differences ratiometrically or allometrically [17,19,20].

2.4. Pulse wave velocity (PWV)

PWV was measured in 12 rats from the 24CR group and 11 from the 24AL. Under Isoflurane anesthesia (2% in oxygen), rats were intubated and ventilated. Left femoral artery was isolated, ligated, and a 1F conductance catheter was inserted and advanced to the thoracic aorta (approximately 100 mm). After recording of several pressure waves and corresponding ECG, the catheter was withdrawn for exactly 50 mm and data recording was repeated. Using the R wave of the ECG as a time marker, the average time between R waves and starting points of five corresponding pressure waves at thoracic and abdominal sections of aorta were measured. The transit time of the pressure wave from upper thoracic aorta to lower abdominal aorta was calculated as the time difference between two measurements. Since the distance between two points of measurement was known (exactly 50 mm), the PWV was calculated as 50 mm/difference between transit times.

2.5. Gross pathology and histological assessment

Histological staining and analyses were performed as described previously [17]. 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