Short-Term Caloric Restriction Suppresses Cardiac Oxidative Stress and Hypertrophy Caused by Chronic Pressure Overload

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ABSTRACT

Background: Caloric restriction (CR) prevents senescent changes, in which reactive oxygen species (ROS) have a critical role. Left ventricular (LV) hypertrophy is a risk factor for cardiovascular diseases. We examined whether CR alters cardiac redox state and hypertrophy from chronic pressure overload.

Methods and Results: Male c57BL6 mice were subjected to ascending aortic constriction (AAC) with ad libitum caloric intake (AL + AAC group) or 40% restricted caloric intake (CR + AAC group). CR was initiated 2 weeks before AAC and was continued for 4 weeks. Two weeks after constriction, AAC increased LV wall thickness, impaired transmitral flow velocity, and augmented myocyte hypertrophy and fibrosis, in association with enhancement of BNP and collagen III expressions in the AL + AAC group. In the AL + AAC group, oxidative stress in cardiac tissue and mitochondria were enhanced, and NADPH oxidase activity and mitochondrial ROS production were elevated. These changes were significantly attenuated in the CR + AAC group. Additionally, in antioxidant systems, myocardial glutathione peroxidase and superoxide dismutase activities were enhanced in the CR + AAC group.

Conclusions: Chronic pressure overload increased cardiac oxidative damage, in association with cardiac hypertrophy and fibrosis. Short-term CR suppressed oxidative stress and improved cardiac function, suggesting that short-term CR could be a useful strategy to prevent pressure overload−induced cardiac injury. (*J Cardiac Fail 2015;21:656−666*)

Key Words: Caloric restriction, cardiac hypertrophy, oxidative stress.

Congestive heart failure (CHF) is increasing in developed countries in association with an aging society.[1] One of the major precursors to CHF is pressure overload due to hypertension. In the initial stage, chronic pressure overload leads to cardiac hypertrophy, resulting in impairment of diastolic function. CHF associated with LV diastolic dysfunction accounts for up to 50% of the total number of cases,[2] and the prognosis of CHF associated with LV diastolic dysfunction is similar to that with systolic dysfunction.[3] However, therapeutic strategies for diastolic dysfunction rather than systolic dysfunction have not been elucidated.

Growing evidence has implied that redox-sensitive pathways have important roles in the development of cardiac hypertrophy and subsequent CHF in both experimental and clinical studies.[4,5] Oxidative stress in cardiac tissue promotes cardiomyocyte hypertrophy and apoptosis, as well as interstitial fibrosis, leading to impaired cardiac function.[5−7] As expected, antioxidants, such as N-acetyl-L-cysteine and edaravone, attenuate pressure overload−induced left ventricular hypertrophy and heart failure.[8,9] The potential sources of reactive oxygen species (ROS) production include NADPH oxidase, mitochondria, uncoupled nitric oxide synthase (NOS), and xanthine oxidase.[6,10,11] Among these ROS sources, NADPH oxidase and mitochondria are major ones in pressure-overload hypertrophy and heart failure.[10,12] On the other hand, xanthine oxidase has been reported to be activated only at the end stage of CHF in a chronic pressure overload model.[13]

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Caloric restriction (CR) is the only experimental intervention known to be able to extend life span and to reduce age-associated diseases in a variety of species.[14] In the heart, the aging process is associated with decline in left ventricular (LV) diastolic function and progress in LV hypertrophy.[15] A recent clinical study has shown that long-term CR with optimal nutrition ameliorates LV diastolic function in healthy subjects.[16] Furthermore, CR attenuates plasma malondialdehyde level in streptozotocin-induced diabetic rats.[17] However, whether CR attenuates pressure overload—induced LV hypertrophy, in association with improvement of impaired redox states, remains to be elucidated. In the present study, we therefore examined whether CR alters redox states and attenuates hypertrophy-induced heart failure in ascending aortic—constricted mice.

Methods

All procedures conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health. The protocol was approved by the Bioethics Committee of Kyoto Pharmaceutical University.

Experimental Animals and Protocols

Male c57BL6 mice (6 weeks of age) were housed in individual cages and fed a standard rodent diet ad libitum for 2 weeks. The average caloric intake was measured from daily food intake for these 2 weeks. Then, mice at 8 weeks of age were randomly divided into 3 groups: (1) ascending aortic constriction (AAC) with ad libitum intake (AL + AAC) group, (2) AAC with caloric restriction (CR + AAC) group, and (3) ad libitum intake without AAC (AL) group. AL + AAC and AL mice continued to be fed ad libitum, whereas CR + AAC mice were fed with 60% of the average caloric intake for 4 weeks (2 weeks before AAC and 2 weeks after AAC). CR diets were enriched in vitamins and minerals such that restricted animals were not nutrient-deficient. At 10 weeks of age, AL + AAC and CR + AAC mice were anesthetized with an intraperitoneal injection of sodium pentobarbital (35 mg/kg). Under controlled ventilation, pressure overload was produced by means of AAC as previously described.[18] The AL group were treated similarly, except that the suture around the ascending aorta was not tied.

LV Echocardiographic Studies

Transthoracic echocardiography was performed with a 15-MHz sector scan probe (Sonos 5500; Phillips Medical Systems Japan, Tokyo, Japan) under light anesthesia with the animals breathing spontaneously. Before and 2 weeks after AAC, 2-dimensionally guided M-mode recordings were obtained from the short-axis view at the level of mitral tip to determine diastolic interventricular septum wall thickness, diastolic posterior wall thickness, and LV end-diastolic (LVEDd) and end-systolic (LVEDs) diameters. Fractional shortening (FS) was calculated as $100 \times ((\text{LVEDd} - \text{LVEDs})/\text{LVEDd})$. LV filling was assessed by means of transmitral Doppler echocardiography with the use of the apical 4-chamber view. In addition, to confirm pressure gradient across the constricted ascending aorta, peak flow velocity of ascending aorta was measured at 3 days after AAC. Three to 5 beats were averaged for each measurement.

Sample Collection and Histologic Analysis

After echocardiography, blood samples were collected and the mice were killed. The heart was excised and weighed. The left ventricle was separated from the right ventricle, weighed, and cut transversely at midlevel. Formalin-fixed paraffin-embedded tissue samples were cut into 4-μm sections and hematoxylin-eosin (HE) and Masson trichrome stainings were performed on 2—3 sections in each paraffin block. The size of cardiac myocytes was assessed by means of cross-sectional area measurement with the use of HE-stained sections. Interstitial fibrosis was analyzed semiquantitatively with the use of Masson trichrome—stained sections. Five independent fields of myocardium from each mouse were photographed with an optical microscope system (Olympus IX71). Then myocyte cross-sectional area and the percentage area of interstitial fibrosis were calculated. To measure cross-sectional myocyte area, suitable area of the section was defined as the one with circular capillary profiles and myofiber shapes (indicative of a true transverse section). Then, the circumferences of 200 myocytes were traced and counted according to computerized pixels. Interstitial fibrosis was quantified by counting the blue pixel content in a computer-assisted image analysis program, and calculated as the sum of fibrosis area divided by the sum of all connective issue and muscle area in the field.

Measurement of Blood Glucose and Plasma Insulin Levels

Blood glucose and plasma insulin levels were determined with commercially available kits (Glutestace, Sanwakagaku Co, Nagoya, Japan; Mouse Insulin ELISA Kit (AKRIN-011), Shibayagi, Gunma, Japan).

Cardiac Gene Expression

Total RNA was isolated from left ventricles with the use of Isogen II reagent (Nippon Gene, Tokyo, Japan) and stored at −80°C. Total RNA was reverse transcribed with the use of Takara Reverse Transcription Regent kit (Takara Bio, Shiga, Japan). Gene expressions of brain natriuretic peptide (BNP), a marker of cardiac hypertrophy, collagen type III, a major component of cardiac fibrosis, and tumor necrosis factor (TNF) α, a marker of myocardial inflammation, were evaluated by means of real-time quantitative polymerase chain reaction with the use of Sybr Green (Thermal Cycler Dice Real Time System; Takara Bio). The gene-specific primers were: for BNP, forward primer 5′-GAGGTCACTCCTATCCTCTGG-3′ and reverse primer 5′-GCCATTTCCTCCGACTTTTCTC-3′; for collagen type III, 5′-TGACTGTCCCACGTAAGCAC-3′ and 5′-GAGGGCCATAG CTGAACTGA-3′; and for TNF-α, 5′-CCACGTCGTAGCAAA CCACC-3′ and 5′-GCAGCCTTGTCCCTTGAAGA-3′. Quantified mRNA expressions were normalized to corresponding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control.

Detection of 8-Hydroxydeoxyguanosine

The histological examination of 8-hydroxydeoxyguanosine (8OHdG), a biomarker of oxidative stress, was performed by means of immunostaining. Formalin-fixed paraffin-embedded samples (n = 7) were cut into 4-μm specimens. Two sections from each sample were deparaffinized and placed in an inhibition