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Long-Term Caloric Restriction Ameliorates the Decline in Diastolic Function in Humans

Timothy E. Meyer, PHD,*† Sándor J. Kovács, PHD, MD,† Ali A. Ehsani, MD,*† Samuel Klein, MD,* John O. Holloszy, MD,* Luigi Fontana, MD, PHD*‡

St. Louis, Missouri; and Rome, Italy

OBJECTIVES We determined whether caloric restriction (CR) has cardiac-specific effects that attenuate the established aging-associated impairments in diastolic function (DF).

BACKGROUND Caloric restriction retards the aging process in small mammals; however, no information is available on the effects of long-term CR on human aging. In healthy individuals, Doppler echocardiography has established the pattern of aging-associated DF impairment, whereas little change is observed in systolic function (SF).

METHODS Diastolic function was assessed in 25 subjects (age 53 ± 12 years) practicing CR for 6.5 ± 4.6 years and 25 age- and gender-matched control subjects consuming Western diets. Diastolic function was quantified by transmitral flow, Doppler tissue imaging, and model-based image processing (MBIP) of E waves. C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta$_1$ (TGF-β_1) were also measured.

RESULTS No difference in SF was observed between groups; however, standard transmitral Doppler flow DF indexes of the CR group were similar to those of younger individuals, and MBIP-based, flow-derived DF indexes, reflecting chamber viscoelasticity and stiffness, were significantly lower than in control subjects. Blood pressure, serum CRP, TNF-α, and TGF-β_1 levels were significantly lower in the CR group (102 ± 10/61 ± 7 mm Hg, 0.3 ± 0.3 mg/l, 0.8 ± 0.5 pg/ml, 29.4 ± 6.9 ng/ml, respectively) compared with the Western diet group (131 ± 11/83 ± 6 mm Hg, 1.9 ± 2.8 mg/l, 1.5 ± 1.0 pg/ml, 35.4 ± 7.1 ng/ml, respectively).

CONCLUSIONS Caloric restriction has cardiac-specific effects that ameliorate aging-associated changes in DF. These beneficial effects on cardiac function might be mediated by the effect of CR on blood pressure, systemic inflammation, and myocardial fibrosis. (J Am Coll Cardiol 2006;47: 398–402) © 2006 by the American College of Cardiology Foundation

Normal aging causes a decline in cardiac performance, manifesting as an age-related impairment in diastolic function (DF) with little or no change in left ventricular (LV) systolic function (SF) (1–2). Specifically, LV relaxation slows, and peak early, suction-initiated LV filling decreases with advancing age, whereas the relative contribution of the atrial component of LV filling (atrial systole) increases (1–2).

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Caloric restriction (CR) slows aging and increases maximal lifespan in drosophila, *C. elegans*, mice, and rats (3). In humans, risk factors for atherosclerosis are markedly improved by long-term CR (4); however, the effect of CR on aging in humans is unknown.

The purpose of the present study was to investigate whether long-term CR with optimal nutrition is associated with an attenuation of the age-related decline in DF. To assess DF we used echocardiography and evaluated serum C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta$_1$ (TGF-β_1) concentrations.

METHODS

Study subjects. Twenty-five subjects practicing CR for 3 to 15 years (mean age 53 ± 12 years; range 35 to 82 years; 21 men, 4 women) were compared with 25 age- and gender-matched subjects eating typical Western diets (WD) using a cross-sectional design. None of the subjects had evidence of chronic disease or smoked. None of the subjects were taking lipid-lowering or antihypertensive agents or other medications. All participants were weight stable (<2 kg weight change in the preceding six months) and did not perform more than 20 min of vigorous exercise twice per week. The Human Studies Committee of Washington University School of Medicine approved the study, and all subjects gave informed consent before their participation.

From the *Division of Geriatrics and Nutritional Science, Washington University School of Medicine, St. Louis, Missouri; †Cardiovascular Biophysics Laboratory, Washington University School of Medicine, St. Louis, Missouri; and the ‡Division of Human Nutrition, Istituto Superiore di Sanità, Rome, Italy. Research supported by National Institutes of Health grants HL54179, HL04023 (Dr. Kovács), RR00036 (General Clinical Research Center), DK20579 (Diabetes Research and Training Center), and DK56351 (Clinical Nutrition Research Unit), the Whitaker Foundation (Dr. Kovács), American Heart Association (Dr. Kovács), and the Alan A. and Edith L. Wolff Charitable Trust (Dr. Kovács). Dr. Meyer was supported by Institutional National Research Service Award AG-00078.

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Abbreviations and Acronyms

BMI	= body mass index
CR	= caloric restriction
CRP	= C-reactive protein
DF	= diastolic function
LV	= left ventricle/ventricular
MBIP	= model-based image processing
SF	= systolic function
TDI	= tissue Doppler imaging
TGF-β_1	= transforming growth factor-beta$_1$
TNF-α	= tumor necrosis factor-alpha
WD	= Western diet

Anthropometrics and body composition. Body weight was obtained in the morning after an overnight fast. Body fat mass was determined by dual-energy X-ray absorptiometry (DXA) (QDR 1000/w, Hologic, Waltham, Massachusetts).

Dietary assessment. Subjects recorded all food and beverages consumed and portion sizes for seven consecutive days. Food records were analyzed using the NDS-R program (version 4.03_31), which is the Nutrition Data System for research from the Nutrition Coordinating Center at the University of Minnesota (5).

Laboratory analyses. Commercial ELISA kits were used to measure serum TNF-α (Quantakine High Sensitive, R&D Systems, Minneapolis, Minnesota), high-sensitivity CRP and TGF-β_1 (American Laboratory Products Company Diagnostics, Windham, New Hampshire).

Echocardiography. A standard two-dimensional Doppler echocardiographic examination was performed with an echocardiographic imaging system (Hewlett-Packard SONOS 5500, Andover, Massachusetts) (6). Doppler transmitral flow velocity images were acquired in the apical four-chamber view according to American Society of Echocardiography criteria (6). Mitral annular velocities were recorded via pulsed wave tissue Doppler imaging (TDI) technique (7,8). For technical reasons, Doppler flow and TDI measurements were not made in all subjects.

Data analysis. Left ventricular SF was determined from fractional shortening (systolic shortening) calculated via the conventional equation (8). Standard DF indexes were measured from the transmitral Doppler E- and A-wave contours (8). Additional DF indexes were obtained by subjecting E- and A-waves to model-based image processing (MBIP) on the basis of a kinematic model of filling that was previously validated via simultaneous cardiac catheterization and echocardiography (9–11). Three mathematically independent parameters (x_o, c, k) were determined from the clinical Doppler E-wave by MBIP. The parameters account for the amplitude (load), decay rate (viscosity/relaxation), and width (average chamber stiffness) of the E-wave contour, respectively (Fig. 1) (9–11).

Statistical analysis. A Student t test was used to evaluate the significance of differences between groups when variables were normally distributed with approximately equal standard deviations. For variables not normally distributed or with unequal standard deviations, the Mann-Whitney test was used. Statistical significance was set a priori at $p < 0.05$ for all tests. All data were analyzed with a commercially available statistical package (SPSS FOR WINDOWS version 10.0, SPSS Inc., Chicago, Illinois). Data are expressed as means ± SD.

RESULTS

Study participants. The characteristics of the study subjects are shown in Table 1. Values for body mass index (BMI) in the WD group were similar to the mean range for middle-aged people in the U. S. (12). Values for body weight, BMI, and blood pressure for 18 CR subjects before they began CR are shown along with their current values in Table 2.

Nutrient intake. The WD group ate a typical WD providing approximately 2,445 ± 470 kcal/day, approximately 17% protein, approximately 52% carbohydrates, approximately 31% fat, approximately 11% saturated fat. The CR subjects ate a nutritionally balanced diet (4) (at least 100% of the Recommended daily intake for each nutrient) providing approximately 1,671 ± 294 kcal/day ($p = 0.0001$ vs. WD group), approximately 23% protein, ($p = 0.0001$ vs. WD group), approximately 49% complex carbohydrates, approximately 28% fat, approximately 6% saturated fat. Daily salt intake was lower in the CR group compared with the WD group (2.6 ± 1.3 g vs. 3.4 ± 1.0 g; $p = 0.007$).

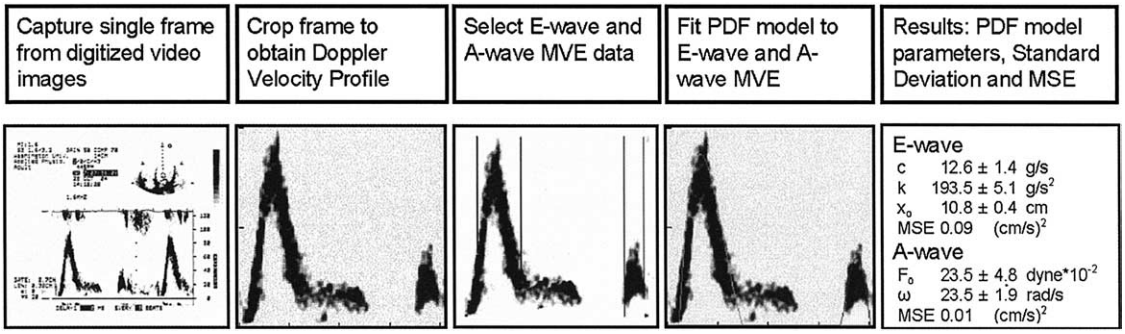

Figure 1. The model-based image processing (MBIP) method of quantitative diastolic function assessment using the Doppler image as input. MSE = mean square error; MVE = maximum velocity envelope; PDF = parameterized diastolic filling.

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Table 1. Subject Characteristics of the Study Groups

Parameter	WD (n = 25) Mean ± SD	CR (n = 25) Mean ± SD	p Value
Clinical characteristics			
Age (yrs)	53.4 ± 6.5	52.7 ± 11.9	NS*
Gender (M/F)	21/4	21/4	
Weight (kg)	84.8 ± 9.6	58.9 ± 6.2	0.0001
BMI (kg/m^2)	27.0 ± 2.1	19.7 ± 1.8	0.0001
Body fat (%)	26.0 ± 7.3	9.3 ± 7.4	0.0001*
Serum markers of inflammation			
hsCRP (mg/l)	1.9 ± 2.8 (20)	0.3 ± 0.3 (22)	0.009*
TGF-β_1 (ng/ml)	35.4 ± 7.1 (20)	29.4 ± 6.9 (22)	0.008
TNF-α (pg/ml)	1.5 ± 1.0 (22)	0.8 ± 0.5	0.002*
Resting hemodynamics			
Systolic BP (mm Hg)	131 ± 11	102 ± 10	0.0001
Diastolic BP (mm Hg)	83 ± 6	61 ± 7	0.0001
MAP (mm Hg)	99 ± 7	75 ± 8	0.0001
HR_{rest} (beats/min)	60 ± 9	56 ± 8	NS

Numbers in parentheses are the actual number of data points analyzed for specific parameter. *Mann-Whitney test was used for reported p values; otherwise *t* test was used.

BMI = body mass index; CR = caloric restriction; Diastolic BP = resting diastolic blood pressure; hsCRP = high-sensitivity C-reactive protein; HR_{rest} = resting heart rate; MAP = mean arterial pressure; Systolic BP = resting systolic blood pressure; TGF-β_1 = transforming growth factor-β_1; TNF-α = tumor necrosis factor-α; WD = western diet.

Cardiac function. No difference in LV fractional shortening was detected between the two groups (Table 3). Although E-wave peak was not different between groups, early filling fraction was greater in the CR group (Table 3). A waves of the CR group had less evidence of aging-related changes than those of the WD group. As a result, the peak E-wave to peak A-wave (E/A) ratio was greater in the CR group than in the WD group. Deceleration time was increased in the WD group, trending toward the "delayed relaxation pattern" of filling. The increased E-wave duration was solely due to increased deceleration time, because acceleration time was not different between groups.

The velocity assessed at the mitral annulus from both the lateral and septal aspects during early LV filling (E′) was significantly higher in the CR group than in the WD group (Table 3), and *c* and *k* were lower in the CR group than in the WD group (Table 3).

Table 2. Historical and Current Data of 18 CR Subjects

Parameter	Before CR Mean ± SD	CR Mean ± SD	p Value
Clinical characteristics			
Weight (kg)	71.6 ± 10.2	57.9 ± 6.1	0.0001
BMI (kg/m^2)	23.9 ± 3.2	19.7 ± 2.0	0.0001
Resting hemodynamics			
Systolic BP (mm Hg)	131 ± 17	101 ± 8	0.0001
Diastolic BP (mm Hg)	83 ± 10	61 ± 7	0.0001
MAP (mm Hg)	99 ± 11	74 ± 6	0.0001

Abbreviations as in Table 1.

Table 3. Systolic and Diastolic Function Indexes of the Study Groups

Parameter	WD (n = 25) Mean ± SD	CR (n = 22) Mean ± SD	p Value
Systolic function			
Fractional shortening (%)	36 ± 6	34 ± 6 (23)	NS
Diastolic function			
E_{peak} (cm/s)	64.3 ± 12.6	70.8 ± 13.4	NS
A_{peak} (cm/s)	53.0 ± 10.2	45.7 ± 9.0	0.011
E/A	1.24 ± 0.28	1.61 ± 0.44	0.001*
AT (ms)	92.6 ± 8.8	86.0 ± 15.0	NS
Atrial filling fraction	0.35 ± 0.05	0.28 ± 0.06	0.0001
Early filling fraction	0.65 ± 0.05	0.72 ± 0.06	0.0001
DT (ms)	192.6 ± 32.5	173.8 ± 34.0	0.012
IVRT (ms)	81.5 ± 12.6 (24)	76.5 ± 11.4	NS*
E_{dur} (ms)	287.5 ± 27.4	259.9 ± 34.6	0.001
TDI			
E'_{Septal} (cm/s)	9.7 ± 1.9 (23)	11.8 ± 3.3 (12)	0.02
A'_{Septal} (cm/s)	10.0 ± 1.7 (23)	10.3 ± 2.4 (12)	NS
$E'_{Lateral}$ (cm/s)	10.2 ± 2.8 (23)	14.3 ± 3.0 (12)	0.001*
$A'_{Lateral}$ (cm/s)	10.0 ± 1.7 (23)	10.1 ± 2.1 (12)	NS
MBIP-derived parameters			
x_o (m)	0.09 ± 0.02	0.10 ± 0.02	NS
c (g/s)	19.6 ± 3.6	14.9 ± 5.0	0.001
k (g/s^2)	218.9 ± 44.6	180.1 ± 41.6	0.003
kx_o (g cm/s^2)	19.6 ± 4.7	17.7 ± 4.1	NS

Numbers in parentheses are the actual number of data points analyzed for specific parameter. *Mann-Whitney test was used for reported p values; otherwise *t* test was used.

A_{peak} = peak A-wave velocity; $A'_{Lateral}$ = peak A′ on lateral wall; A'_{Septal} = peak A′ on septal wall; AT = E-wave acceleration time; *c* = damping constant; DT = E-wave deceleration time; E/A ratio = ratio of early transmitral flow velocity to atrial flow velocity; E_{dur} = duration of the E-wave; E_{peak} = peak E-wave velocity; $E'_{Lateral}$ = peak A′ on lateral wall; E'_{Septal} = peak E′ on septal wall; EF = ejection fraction; IVRT = isovolumetric relaxation time; *k* = left ventricular chamber stiffness parameter; kx_o = peak atrio-ventricular pressure gradient for E-wave generation; MBIP = model-based image processing; TDI = tissue Doppler imaging; x_o = load, related to VTI of E-wave; other abbreviations as in Table 1.

Blood analyses. Serum CRP, TNF-α, and TGF-β_1 concentrations were lower in the CR group than in the WD group ($p < 0.05$) (Table 1).

DISCUSSION

Nothing is known regarding the effects of long-term CR with optimal nutrition on cardiac function in non-obese humans. In this study, we assessed heart function in subjects practicing CR for 3 to 15 years. Our data show that long-term CR with optimal nutrition improves DF relative to healthy, age- and gender-matched control subjects eating a standard WD.

It has been shown in mice that indexes of DF are beneficially affected by CR (13). Interestingly, the DF changes observed in our CR subjects were very similar to those found by Taffet et al. (13) in lifelong CR mice. Systolic function assessed by fractional shortening changes relatively little with normal aging (1–2). Both groups in our study had normal SF; however, DF was significantly better in the CR than in the WD group and similar to that of

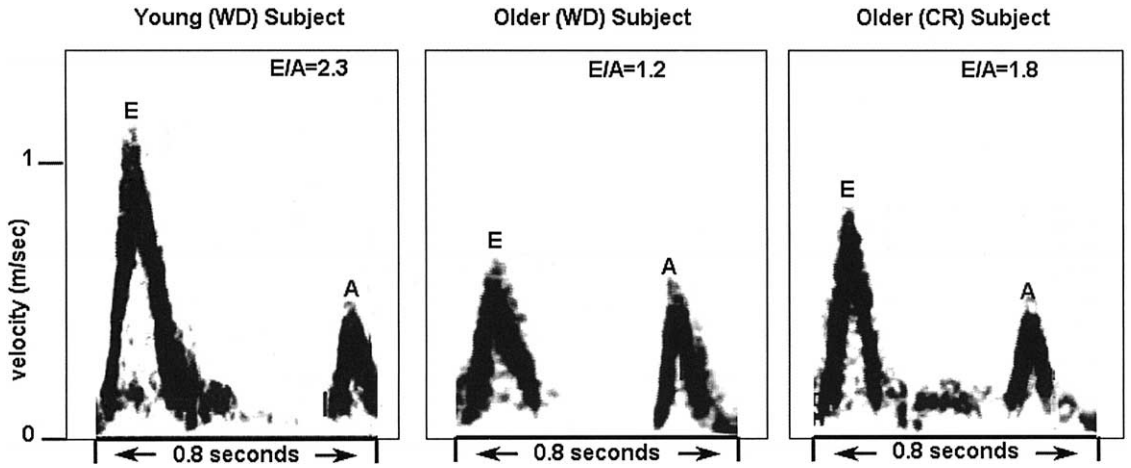

Figure 2. Doppler mitral inflow patterns in a typical young individual **(left panel)**, age-matched comparison subject **(middle panel)**, and representative caloric restriction (CR) subject **(right panel)**. WD = Western diet.

younger individuals (Fig. 2). The CR group had lower A_{peak} velocities, higher early filling fraction, and higher E/A, demonstrating better DF. Consequently, lower A_{peak} values could mitigate against left atrial hypertrophy. Moreover, TDI-derived E′, which normally decreases with aging, was higher in the CR subjects, suggesting improved longitudinal function compared with the WD group. Importantly, E′ has also been shown to be negatively associated with both diastolic blood pressure and BMI (14).

Further evidence of better DF in the CR subjects is provided by the MBIP-derived LV chamber stiffness parameter *k*, which was significantly lower in the CR subjects compared with the WD group. The viscoelastic chamber constant *c* was also lower in the CR group, demonstrating a decreased impediment to filling. Because E_{peak} was not different between groups, the WD group must expend more energy to achieve the same peak velocity during early filling.

Data from rat studies show that collagen content increases in the aging heart and correlates with increasing myocardial stiffness, whereas CR results in less accumulation of cardiac connective tissue (15). Transforming growth factor-β_1, an anti-inflammatory cytokine, plays a major role in regulating myocardial extracellular matrix protein deposition and degradation (16). A sustained increase of TGF-β_1 facilitates the development of fibrosis (16). Brooks et al. (17) demonstrated that TGF-β_1-deficient mice survived longer and exhibited less myocardial fibrosis and lower myocardial stiffness compared with age-matched control subjects. Furthermore, overexpression of TNF-α leads to upregulated expression and release of TGF-β_1, which in turn increases myocardial collagen content (18). Our findings demonstrate that systemic inflammation was markedly reduced in CR compared with WD subjects, as reflected by low serum concentrations of CRP and TNF-α. These findings taken in concert suggest that CR has a salutary effect on DF by lowering systolic blood pressure and decreasing systemic inflammation and myocardial fibrosis.

Study limitations. Because our study was not randomized, our cross-sectional study design does not allow us to assign causation, although the observed significant differences on the basis of cross-sectional data are an important first-step in elucidating the effects of long-term CR in humans. Another minor limitation is that some echocardiographic parameters were not obtained in all subjects for technical reasons.

Conclusions. We conclude that CR has cardiac-specific effects that ameliorate aging-associated changes in DF. These beneficial effects on cardiac function might be mediated by the effect of CR on blood pressure, systemic inflammation, and myocardial fibrosis.

Reprint requests and correspondence: Dr. Luigi Fontana, Division of Geriatrics, Washington University School of Medicine, 4566 Scott Avenue, Box 8113, St. Louis, Missouri 63110. E-mail: lfontana@im.wustl.edu.

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