Effect of long-term food restriction on cardiac mechanics

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Klebanov, Simon, Jeremiah T. Herlihy, and Gregory L. Freeman. Effect of long-term food restriction on cardiac mechanics. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2333–H2342, 1997.—Food restriction (FR) is the only known intervention capable of increasing mammalian life span. It not only increases longevity, but reduces the incidence of a broad spectrum of age-related pathologies, including cardiomyopathy, and retards the physiological decline associated with aging. Previous work from this laboratory has shown that long-term FR affects the contractile machinery of the heart, shifting the cardiac myosin profile from the fast, V1 isoform to the slow, V2 isoform. The aim of the present study was to determine whether FR also induces changes in cardiac mechanics. Isolated, isovolumically beating hearts were examined from four groups of rats: 1) ad libitum-fed rats killed at 10–13 mo of age, 2) FR rats offered only 60% of the calories consumed by ad libitum-fed rats and killed at the same age, 3) young ad libitum-fed rats having the same heart weights as the FR rats, and 4) ad libitum-fed rats subjected to short-term FR, i.e., for the last 3 wk of life, and also killed at 10–13 mo of age. Both short- and long-term FR profoundly and to approximately the same extent affected cardiac mechanics. Hearts from FR rats developed much higher pressures than hearts from the ad libitum-fed rats under conditions of low-calcium perfusate. This difference disappeared, however, when contractility was enhanced by either calcium or isoproterenol. FR prolonged both contraction and relaxation times. Long-term ad libitum-fed rats (adult, 10–13 mo of age) had a lower isoproterenol sensitivity than the young ad libitum-fed rats (10 wk of age). Both short- and long-term FR restored the sensitivity to isoproterenol. In summary, FR profoundly affects many aspects of cardiac mechanics, enhancing some age-related changes (prolongation of the contraction and relaxation times), attenuating another (increasing the isoproterenol sensitivity), and, finally, inducing some unique changes unrelated to age (increased pressure development under low-calcium perfusate).

isoproterenol; calcium; ventricular pressure; Langendorff preparation; diet

FOOD RESTRICTION (FR) increases the life span of rodents (27). This intervention also retards the development and severity of age-related diseases and attenuates the physiological decline associated with aging (36, 39). Despite the large literature concerning the impact of FR on a variety of systems, little is known regarding precise effects of FR on the cardiovascular system. It has been observed that baroreflex sensitivity is enhanced (19) and the incidence of cardiomyopathies is reduced (4, 41) by FR. How FR affects the intrinsic characteristics of myocardial performance remains unknown.

Extensive work has been done, on the other hand, regarding the impact of age on myocardial performance, demonstrating that cardiac muscle exhibits a variety of age-related functional alterations. These include an increase in action potential duration and propagation time (7, 8, 24, 33), lengthening of both contraction and relaxation times (6–8, 24), and a decline in the sensitivity of the aged cardiac muscle to ß-adrenergic stimulation (1, 33, 38). A number of age-associated biochemical alterations may contribute to the aging changes seen at the cellular level. In rodents, one of the most prominent biochemical changes in the aging myocardium is the shift of the myosin isoform profile from the fast, V1 isoform to the slow, V2 isoform (8, 15, 25, 34, 37). This shift is involved in and/or is associated with changes in many of the physiological parameters mentioned above.

If these changes in the mechanical performance and biochemical composition of cardiac muscle represent basic aging processes, then hearts from FR rats may exhibit a retardation in the age-associated changes. Available evidence, however, suggests that FR may actually amplify, rather than retard, some of these changes. Short-term (6 wk) FR prolongs the time to peak tension and relaxation time of papillary muscle (30). Similarly, time to peak tension and time to peak shortening are increased by FR in left ventricular columnar carnæ muscle (10). Moreover, both short- (17, 28, 29) and long-term (22, 23) FR induce a shift in the myosin isozyme distribution toward the slow V3 isoform, a shift which accentuates rather than retards age-associated changes. Not all changes induced by FR, however, enhance the aging phenotype. Short-term FR (11, 18) increased the inotropic response to ß-adrenergic stimulation in the atria. It also increased the inotropic and chronotropic responsiveness of the isolated working heart preparation to ß-adrenergic agonists (13).

Of note, most of the available information comes from studies in which animals were exposed to relatively short-term FR (several weeks), such that changes in cardiac performance that mimic aging may merely represent transient responses to energy deprivation. Because the life extension produced by FR is generally proportional to the duration of the treatment (2, 9, 40), knowledge of the changes induced by long-term FR is important for our understanding of the impact of FR on longevity. The aim of the present study was to directly determine whether long-term FR enhances age-associated changes in the heart by studying various parameters of myocardial performance over a wide range of inotropic states.

MATERIALS AND METHODS

Animals: housing and diet. Specific pathogen-free, male, Fischer 344 rats were obtained from Charles River at 4 wk of
These rats were used for the size control group (see below). except that they were housed in the regular animal facilities. treated identically to the ad libitum-fed rats described above, groups. A fourth group of rats was purchased later and to 1730 h daily. The young rats were allowed to accommodate animals have been described elsewhere (21, 41). Briefly, rats age and housed in the barrier facilities at the Health Science H2334 CALORIE RESTRICTION AND CARDIAC MECHANICS included in the calculation of intraventricular volume. The from within the balloon, was usually 30–40 µl. This value was measured by water displacement after withdrawing all fluid balloon wall, the tip of the tubing and pressure transducer, (35). After the heart was mounted on the perfusion apparatus. Langendorff perfusion system. The left superior vena cava cleaned, and fed over a 1.2-mm glass tube covered with a cavae were ligated immediately, and the heart was excised submerged in oxygenated, cold (4°C) perfusate (composition given below). The severed end of the aorta was located, and the perfusate contained 1.5 mM calcium. After the stabiliza- at 37°C, and the solution was equilibrated with 100% oxygen. isoproterenol were added to the perfusate as described in the protocol (see below). The pH was adjusted to 7.4 at 37°C, and the solution was equilibrated with 100% oxygen. The perfusate was not recirculated.

Surgical preparation. Rats were anesthetized with a stand- rodent cocktail (65 mg/ml ketamine, 2 mg/ml xylazine, 2 mg/ml Lidocaine (5 µg/ml) was added to suppress ventricular ectopy. Calcium and isoproterenol were added to the perfusate as described in the experimental protocol (see below). The pH was adjusted to 7.4 at 37°C, and the solution was equilibrated with 100% oxygen. The perfusate was not recirculated.

Protocol. For mounting, instrumentation and equilibration the perfusate contained 1.5 mM calcium. After the stabiliza- period, pressure-volume curves were constructed with perfusates being changed for all hearts in the following sequence: 1) 1.5 mM, 2) 3.0 mM, 3) 1.0 mM, and 4) 0.85 mM calcium. To the fourth solution (0.85 mM calcium), increasing amounts of isoproterenol were added to yield concentrations of 5) 1 nM, 6) 3 nM, and 7) 10 nM. The volume of the balloon was increased, with increments of 20 or 40 µl, until a plateau of developed pressure (systolic minus diastolic) was reached, so that on average seven to nine measurements were taken for each pressure-volume curve with 2- to 2.5-min intervals. One pressure-volume run took ~25 min, and all pressure-volume runs were usually finished by 3.5 h after the initiation of perfusion.

Data analyses. Several parameters of cardiac mechanical performance were assessed. To characterize ventricular contractile state, developed pressure was plotted as a function of left ventricular volume. Because the hearts from different groups differed in size, the relationship between the developed pressure and the left ventricular volume normalized to left ventricular weight was also constructed. These relations were obtained for all seven perfusate compositions. Pressure-volume relations were usually concave to the abscissa (more so at higher inotropic states) and were approximated by a second-order polynomial. Because the goodness of fit was excellent (correlation coefficient >0.99), the subsequent analyses were all performed using this approximation, allowing precise matching of interpolated volumes for all hearts. Diastolic pressures, obtained by interpolation, at which the heart contracted isovolumically against the balloon. A pressure transducer (model SPR-524, Millar Instruments, Houston, TX) was placed inside the balloon. The apex of the heart was vented to allow for the drainage of left ventricular Thesbian flow and leak through the aortic valve. Initially, perfusion pressure was set at 60 mmHg and was maintained at this level by adjusting flow of a peristaltic pump (model 7518–00, Cole-Parmer Instruments, Chicago, IL) for ~20 min, the time usually required to instrument the heart. The flow was then kept constant throughout the remainder of the experiment. Pacing wires were attached within 1 mm of each other at the apex of the heart. Pacing rate was set at 300 beats/min. This value was chosen because preliminary trials showed that it was higher than the spontaneous rate generated by the maximal dose of isoproterenol. After instrumentation, the heart was allowed 10 min to stabilize so that the experiments were usually started 30 min after the attachment of the heart to the perfusion system.

Pressure measurements. Pressure was recorded at different intraventricular volumes. The signal from the Millar pres- sure transducer was digitized using an analog-to-digital converter and an amplifier (MP 100 and DA 100, BIOPAC Systems, Goleta, CA) and visualized on a personal computer (Power Macintosh 6100/66, Apple Computer, Cupertino, CA). Data were stored on disk for off-line analyses. The recording system was calibrated to a mercury manometer. Balloons were used only if at 300-µl volume they generated pressures of <3 mmHg.

Perfusate. The perfusate was composed of (in mM) 15 glucose, 140 Na, 5 K, 1.25 Mg, 152 Cl, and 6 N-2-hydroxyethyl- liperase-N'-2-ethanesulfonic acid. Lidoaaine (5 µg/ml) was added to suppress ventricular ectopy. Calcium and isoproterenol were added to the perfusate as described in the experimental protocol (see below). The pH was adjusted to 7.4 at 37°C, and the solution was equilibrated with 100% oxygen. The perfusate was not recirculated.

Contractile performance. For each pressure-volume curve with 2- to 2.5-min intervals. One pressure-volume run took ~25 min, and all pressure-volume runs were usually finished by 3.5 h after the initiation of perfusion.
heart developed 75% of maximal developed pressure for a given perfusate composition, were compared among the groups.

At the heart rates studied, the onset of mechanical contraction was not always easily discernible from the ventricular tracings. We defined the beginning of contraction as the time at which the value for $dP/dt$ reached five times the value of the peak pressure developed for this beat. The validity of this strategy was established on records where the onset of contraction was unambiguous (data not shown).

Time to peak pressure was measured only for the highest inotropic states (3.0 mM calcium or 10 nM isoproterenol). The time required for pressure to drop from maximal to one-half of maximal, i.e., half-relaxation time, was measured for four perfusate compositions out of seven. Because time to peak pressure and half-relaxation time were affected by intraventricular volume, these parameters are reported for two volumes: the volume after the balloon was filled with 20 µl of fluid and the volume at which maximal developed pressure was reached.

Statistical analyses. Analysis of variance was used to determine significance of an effect of dietary manipulation. If repeated measurements were made at different calcium or isoproterenol concentrations in the perfusate and at different intraventricular balloon volumes, the analysis of variance for repeated measures was performed. Fisher protected least significant difference test was used for individual group comparisons.

RESULTS

Morphometry. As is seen in Fig. 1, FR decreased body weights. The FRLT rats had the lowest and ALLT rats had the highest body weight. The FRST animals had an intermediate body weight, although they were still rapidly losing weight and had not reached a steady state by the time of death (data not shown). The heart weights showed a similar pattern (Fig. 1B). By experimental design, the WC group had the same heart weight as the FRLT group. FRST decreased heart and body weights proportionally, so that the heart weight-to-body weight ratio for the FRST group was not different from that of the ALLT group (Fig. 1C). The heart weight-to-body weight ratio of the FRLT group was slightly, but statistically significantly, higher than that of the ALLT group, suggesting some degree of cardiac mass sparing with this regimen.

Pressure-volume characteristics. The isovolumic pressure-volume relation was defined for each heart under a variety of experimental conditions. Figure 2 illustrates the relations for a representative heart when the contractile performance of the heart was altered by changing either calcium or isoproterenol concentration in the perfusate. As shown in this example, each pressure-volume relation could be fitted by a quadratic equation (Fig. 2). The $R^2$ values for all hearts were generally >0.99, indicating that the fit was very good. For purposes of analyses, predicted pressure values at given volumes were calculated from the quadratic equations and were used to construct composite plots for each group and each experimental condition. In addition, because the hearts were of different sizes, the volume at which maximum pressure was developed differed, and this, by itself, influenced the slope of the pressure-volume relation. To avoid misinterpretation due to size effects, further analysis was performed after intraventricular volume was normalized to left ventricular weight.

Effects of altered calcium and isoproterenol concentrations on developed pressure. Pressure-normalized volume curves obtained at different calcium and isoproterenol concentrations are shown in Figs. 3 and 4, respectively. Under conditions of low-calcium perfusate, at all volumes, hearts from both FR groups developed much higher pressures than the hearts from either of the ad libitum-fed groups. Regardless of whether con-
tractile state was enhanced by addition of calcium or isoproterenol, the pressure-normalized volume relations for all groups converged. It is noteworthy that essentially the same results were obtained when the analysis was performed on the absolute volumes (data not shown). When pressures were normalized to maximal developed pressure, the normalized pressure-normalized volume relationships were superimposable for all four groups (data not shown), indicating that under a given experimental condition (calcium and isoproterenol concentrations in the perfusate), the shapes of the length-tension relation for the myocardial tissue from all four groups were similar. Thus maximal pressure was sufficient to characterize the effects of calcium and isoproterenol on contractile performance.

The effects of altered calcium and isoproterenol concentrations on maximal pressure development are shown in Fig. 5. Figure 5A shows that there were no differences in developed pressure between the FR groups and the ALLT group at the highest calcium levels. Thus FR did not alter the ability of the heart to develop pressure, despite substantial alterations in left ventricular mass. At each calcium concentration, the WC group developed lower pressures than the other three groups. It is noteworthy that both FR groups outperformed the ad libitum-fed groups at low calcium concentrations (0.85 and 1.0 mM). Figure 5B shows that 40–60% differences in developed pressures among the groups at zero isoproterenol decreased to 10–15% at the highest isoproterenol concentration (10 nM). Thus high levels of either calcium or isoproterenol eliminated or greatly reduced the difference in developed pressures between the FR and ad libitum-fed groups.

Sensitivity to isoproterenol. Our data suggest that the sensitivity of the heart to isoproterenol is affected by both age and diet. The ALLT group responded minimally to the lowest concentration (1 nM) of isoproterenol (Fig. 6). The other three groups showed signifi-
cantly greater response than the ALLT group (P < 0.05) and were not different from each other. The difference between the ALLT and WC groups suggests the presence of an age effect. Long-term and even short-term (3 wk) FR restored the sensitivity to isoproterenol in older animals.

Diastolic pressure. Figure 7 shows the impact of calcium (A) and isoproterenol (B) on diastolic pressure. An increase in either calcium or isoproterenol led to a decrease in diastolic pressure. At the lowest calcium concentration (0.85 mM), diastolic pressure tended to be lower in the FRLT group. These differences disappeared at the higher calcium concentrations. Likewise, although FRLT had a significantly lower diastolic pressure at the lowest isoproterenol concentration (1 nM), the difference among the groups disappeared at the higher concentrations.

Maximal rate of pressure rise and decline. The effects of FR on the maximal rate of pressure rise (dP/dt$_{max}$).
were similar to those exerted on the maximal developed pressure (Fig. 5). FR groups had higher dP/dt_{max} at lower contractile states, but the difference disappeared at higher contractile states.

The effects of FR on the maximal rate of pressure decline (dP/dt_{min}; Fig. 9) were also similar to those seen with maximal developed pressure (Fig. 5). The only noticeable difference was that the WC group, which developed the lowest pressure among all groups, was no longer different in terms of dP/dt_{min} from all other groups at high inotropic states (high calcium or high isoproterenol in the perfusate).

Contraction and relaxation times. Contraction time was measured for two contractile states, high calcium and high isoproterenol and two intraventricular volumes (low and high) (Fig. 10). Contraction times for the FR groups were prolonged by 5–10%. FR exerts its strongest effect on the relaxation phase, which was prolonged by up to 25%. Half-relaxation time was prolonged in both the FRST and FRLT groups (Fig. 11). The difference in the half-relaxation time was much more pronounced at higher intraventricular volumes, suggesting that in FR animals left ventricular relaxation is more sensitive to effects of loading. The half-relaxation times for the WC and ALLT groups were not different, indicating the absence of an age effect.

**DISCUSSION**

This is the first study to examine the effects of long-term FR on cardiac mechanics, and the results show that FR substantially changes mechanical performance. The most pronounced effect was that under the low-calcium perfusate condition, the hearts from both long-term and short-term FR rats (the FRLT and FRST groups, respectively) could develop much higher pressures than the hearts from both young and adult ad libitum-fed rats (WC and ALLT groups, respectively). In addition, we confirmed that both contraction and relaxation times are prolonged by short-term FR and extended this observation to long-term FR. The results of the present study also agree with previous reports in that β-adrenergic sensitivity was increased by short-term FR. We showed for the first time that the sensitiv-
ity was similarly increased by long-term FR. Although our oldest animals were mature adults, further studies will be needed in senescent rats to see if these changes are persistent, possibly participating in the life-prolonging effects of FR.

Low-calcium perfusate tolerance. The hearts of FR animals developed much higher pressures at 0.85 mM calcium than those of ad libitum-fed rats (Figs. 3 and 5). This observation agrees with previous findings (32) that short-term FR elicited a similar increase in inotropic state at low calcium. The differences in pressure development between the groups disappeared or were greatly decreased at high inotropic states regardless of whether calcium or isoproterenol was used to enhance contractility (Figs. 3–5). This suggests that although the contractile machinery is capable of developing similar maximal pressures, contractile performance is regulated differently among the groups.

We can only speculate as to which aspect of the excitation-contraction process participates in this phenomenon. It is possible that FR led to a higher calcium transient in the face of low perfusate calcium. If this results from either an increase in transsarcolemmal calcium flux or more sensitive calcium-triggered calcium release, it is difficult to explain why the differential behavior of the FR animals is not present at high calcium also. Alternatively, it is possible that calcium binding to regulatory proteins differed among the groups so that calcium was bound more avidly in the FR groups. This is consistent with longer relaxation times (Fig. 11); the calcium binding would be saturated at high calcium concentration, such that FR and ad libitum-fed groups would behave similarly under those conditions. Further studies will be needed to specifically define the mechanism of this observation.

Pressure-volume relations. Pressure-volume relations were examined for the four experimental groups (Figs. 3 and 4). Despite differences among the groups in the maximal developed pressure and in the volume at which maximal pressure was achieved, pressure-volume relations for a particular perfusate composition were practically superimposable when normalized to the maximal developed pressure and left ventricular weight. This suggests that cardiac dimensions, such as the unstressed left ventricular chamber volume and left ventricular weight, changed proportionally.

Fig. 9. Maximal rate of relaxation, dP/dt. Effects of calcium (A) and isoproterenol (B) on dP/dt are shown. At low inotropic states (low calcium and low isoproterenol concentrations), hearts from both food-restricted groups had much higher dP/dt than hearts from both ad libitum-fed groups. Group descriptions and animal numbers correspond to those shown in Fig. 1. Because isoproterenol concentrations were tested in presence of low calcium (0.85 mM), left sets of columns in A and B are identical. Groups that do not share a common symbol are statistically different (P < 0.05).

Fig. 10. Contraction times. Contraction time was measured for 2 highest inotropic states [3.0 mM calcium (A) and 10 nM isoproterenol (B) in perfusate] at low (left set of columns) and high (right set of columns) intraventricular volumes. Both food-restricted groups had longer contraction times than both ad libitum-fed groups. Group descriptions and animal numbers correspond to those shown in Fig. 1. Groups that do not share a common symbol are statistically different (P < 0.05).
The age-related differences in inotropic response to isoproterenol with a smaller increase in pressure than the older ad libitum-fed group (ALLT, 10–13 mo old) responded to the lowest concentration of heart (1, 33, 38). The older ad libitum-fed group was much more pronounced at high volumes (Figs. 10 and 11), indicating that left ventricular relaxation for the FR groups was more sensitive to changes in the volume. This observation suggests that some changes in load-dependent relaxation may be induced by FR. Direct experiments will be needed to more fully clarify this observation.

Contraction and relaxation times. Our observations (Figs. 10 and 11) are in agreement with previous studies which demonstrated that FR prolonged both the contraction (10, 30) and relaxation (30, 31) phases of the cardiac cycle. Several biochemical changes may be responsible for the prolongation of contraction duration. One of the most important determinants of the rate of contraction is the cardiac myosin composition. Both short-term (17, 28, 29) and long-term (22, 23) FR shift the myosin composition from the V$_2$ to the V$_3$ isoform. This shift may in part explain prolongation of the time to peak tension.

Both time to peak tension and half-relaxation time were affected by the intraventricular volume (Figs. 10 and 11). With an increase in the volume, both time parameters increased. Analysis of variance for repeated measures showed that in addition to the effect of diet on half-relaxation time, an interaction between diet and the volume was also present. The difference in the half-relaxation time between the FR and ad libitum-fed groups was much more pronounced at high volumes (Figs. 10 and 11), indicating that left ventricular relaxation for the FR groups was more sensitive to changes in the volume. This observation suggests that some changes in load-dependent relaxation may be induced by FR. Direct experiments will be needed to more fully clarify this observation.

Short- vs. long-term FR. We included the FRST group to provide data on the rapidity with which diet induces changes in cardiac mechanics and energetics. Prior observations on the time course of myocardial adaptation to dietary manipulations are conflicting. Although it is known that myosin isoyme redistribution occurs within 3 wk of the onset of FR (28, 29), mechanical changes may occur for up to 6 wk after the onset of FR (10). In addition, protein-calorie malnutrition enhances certain indexes of cardiac efficiency within 2 wk of treatment (12, 13), and the whole body basal metabolic rate declines very quickly after the initiation of FR (14, 16, 20, 26), suggesting that the myocardial basal metabolic rate may also decrease. Thus the time course of various adaptations may differ. In the present study, all effects were already present by 3 wk after the onset of FR. The magnitude of these effects was not different between short- and long-term FR, indicating that transformation of the heart in response to FR is a relatively rapid and long-lasting process.

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Limitations of the study. This study must be interpreted in light of experimental limitations. First, the number of interventions necessitated that the experiments run for ~3.5 h. This is a relatively long time for the isolated heart preparation, especially in view of the fact that we used crystalloid perfusion. It was notable that we used coronary perfusion pressures of 60 mmHg, which tended to minimize edema. In addition, we found less than a 15% decline in peak systolic pressure within the first 2 h of the study (before isoproterenol infusion), suggesting our preparations were stable, and there was uniform and strong response to the β-agonist. Thus we are comfortable that decay of the preparation did not substantially affect our data.

Second, we did not perform testing over a complete dose range of isoproterenol in our hearts. As such, the maximal response to this agonist was not defined in each heart. We feel, nonetheless, that the conclusions on sensitivity to the lowest concentration of isoproterenol are valid. Significant differences were not present in response to 20 nM isoproterenol. Also, when the response to 1 nM isoproterenol is expressed as a percent of the response to 10 nM, the difference between the groups persists. The response of the ALLT group was approximately one-half of those of all three other groups. It is, thus, likely that the greater response of the young and FR hearts to the low concentration of isoproterenol signifies greater sensitivity to the inotropic agent.

Third, the use of isolated hearts entails many inherent weaknesses. Clearly, studies in intact, conscious animals would be preferable. Whether loss of nervous system control, alterations in temperature, use of artificial pacing, and other aspects of our procedure altered our results is not known. Because, however, each group was treated in an identical fashion, the differences we found between groups are likely to represent real effects of FR on cardiac mechanics.

Finally, it should be pointed out that because the hearts of the different groups were of different size, the question of how to properly normalize the data from the mechanical studies is important. For the assessment of calcium and isoproterenol response, we normalized the abscissa to the mass of the left ventricle, following the technique proposed by Suga et al. (34a). Because the dietary intervention we used has been previously shown to substantially affect our data.

We thank Emilio Garcia and Danny Escobedo for excellent technical support. This work was supported by National Institute on Aging Grants AG-11088, T32-AG-00205, and K07-AG-00469 and by the Research Service of the Department of Veterans Affairs. Address for reprint requests: G. L. Freeman, Medicine/Cardiology, 7703 Floyd Curl Dr., San Antonio, TX 78284.

Received 16 January 1997; accepted in final form 27 June 1997.

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