Altersations in Collagen Cross-Linking Impair Myocardial Contractility in the Mouse Heart

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A number of genetic disorders in humans are associated with defects in the synthesis and metabolism of collagen, which are accompanied by multiple cardiovascular disease processes. To determine whether genetically determined cross-linking abnormalities of collagen may alter cardiac function, left ventricular papillary muscles of mice with a genetic defect in the cross-linking of collagen (Mo^Tbr) were studied in vitro. With respect to controls, increases in time to peak tension, from 102±1.4 to 125±5.4 msec (p<0.001), and time to one-half relaxation, from 76±3.0 to 98±6.1 msec (p<0.05), were measured. Moreover, resting tension at the length associated with maximum developed isometric force (L) was elevated, from 11.1±1.7 to 19.3±1.1 mN/mm² (p<0.001), and a similar difference was also seen throughout the physiological range of muscle lengths. In contrast, developed tension was depressed at 93–97% of L. Peak rate of tension rise and decay were diminished whereas time to peak rate of tension rise was prolonged. Isotonically, a decrease in the magnitude of peak shortening at L, from 4.0±0.5 to 2.0±0.2% (p<0.04), and an increase in time to peak shortening, from 100±2.3 to 129±2.8 msec (p<0.001), were seen. In addition, peak velocities of shortening and relengthening were diminished in the Mo^Tbr mouse heart. In conclusion, the impairment in collagen cross-linking alters cardiac mechanics by a reduction in force-generating ability and a prolongation of the timing parameters of the systolic and diastolic phases of contraction in vitro. (Circulation Research 1989;65:1657-1664)
progression of the disease is age dependent, and death occurs in males at approximately 6 months after birth by blood vessel rupture. The decreased mechanical strength of the vascular wall is due to the reduction in the number of cross-links in collagen and elastin fibers because there is a failure to synthesize lysine-derived aldehydes necessary in the process of the formation of cross-links. Thus, the Mo mouse exhibits characteristics similar to those found in humans with Ehlers-Danlos and Marfan syndromes and offers the opportunity of examining myocardial performance in a condition in which collagen cross-linking is impaired, mimicking the human disease. Therefore, the mechanical properties of the myocardium obtained from Mo mice were studied to determine the pathological role of collagen disorders in myocardial contractile behavior.

Materials and Methods

Study Design

Ten male Mo mice and 10 age-matched controls (N:NIH[S]) were included in this study. Animals were obtained from Dr. Douglas Grahn at the Argonne National Laboratory, Argonne, Illinois. All animals were 75-90 days of age at time of induced diastolic arrest.

Contractile Evaluation

Animals were anesthetized with ether, and hearts were rapidly excised and placed in oxygenated Tyrode's solution containing 30 mM potassium to induce diastolic arrest. The left posterior papillary muscle was removed and suspended horizontally in a myograph. The heart was weighed after dissection of the muscles to be used for the mechanical measurements.

The nontendinous end of the papillary muscle was inserted into a collet that was mounted to the rear of the galvanometer's moving iron lever was measured by a variable capacitor positioned at the front of the galvanometer’s moving iron core. Force at the tip of the lever was determined by scaling and amplifying the error signal produced because there is a failure to synthesize lysine-derived aldehydes necessary in the process of the formation of cross-links. Thus, the Mo mouse exhibits characteristics similar to those found in humans with Ehlers-Danlos and Marfan syndromes and offers the opportunity of examining myocardial performance in a condition in which collagen cross-linking is impaired, mimicking the human disease. Therefore, the mechanical properties of the myocardium obtained from Mo mice were studied to determine the pathological role of collagen disorders in myocardial contractile behavior.

Stress-Strain Relations

The active and passive stress-strain relations were obtained after an equilibrium period of 120 minutes during which the muscle contracted isometrically at a passive stress of approximately 9.8 mN/mm². The stress-strain curves were generated by reducing muscle length in 0.1-mm steps from the length associated with maximum developed isometric force (L₀) to approximately 90% of L₀ while recording active and passive forces.

To account for dimensional variations among samples, force was computed in terms of muscle cross-sectional area, that is, tension (δ), expressed in mN/mm². Since the volume of a muscle remains constant during a change of external length, the cross-sectional area is altered so that the force is generated by a different cross-sectional area than that present at the original length (L₀). Thus, the tension-generating ability is defined as the load-developing capacity (F) per actual instantaneous cross-sectional area (A) of the muscle or cell:

\[ \delta = \frac{F}{A} \]

When a uniaxial load is applied to cardiac muscle parallel to its fibers, the tissue responds by an alteration in length. This change in length from an initial value, L₀, to a new value, L₁, is described by the relation that defines the change in linear strain (ε). This is referred to as the logarithmic strain, since the integral L₁-L₀ with respect to dL is equal to ln L₁/L₀.

Twitch Kinetics

The rates of tension rise and decay were measured by an active analog differentiator to characterize the dynamics of the contractile state during an isometric event. The timing parameters of muscles stimulated at a fixed external length were obtained for the physiological range of muscle lengths by 1% increments from 90% to 100% L₀. Accordingly, time to peak isometric tension, relaxation time to 50% precontraction tension, time to peak rate of tension rise, and time to peak rate of tension decay were measured.

Load-Velocity Relations

Maximum velocities of isotonic shortening and lengthening were obtained directly at low relative loads. The total load on the muscle was increased between the preload and the isometric levels. Peak velocity was measured at each load. Load-velocity curves were constructed at L₀. To extend the load-velocity relations to lighter loads, unloaded velocities of shortening and lengthening were measured by reducing preload to near zero early in the contraction.
**Statistical Analysis**

Results are presented as mean±SE computed from the average measurements obtained from each mouse. Comparisons between control and experimental animals were performed by an unpaired two-tailed Student's t test. Values of p<0.05 were considered to be significant.

**Results**

Figure 1 shows the body weight of control and Mo** mice killed at 75–90 days of age. A 44% lower body weight was measured in experimental mice (Figure 1A). Heart weight was only 18% smaller in the Mo** mice (Figure 1B); this difference resulted in a 45% increase in the heart weight/body weight ratio (Figure 1C). All these differences were found to be statistically significant. However, they may not necessarily reflect a hypertrophic response because the relation between heart weight and body weight may not be rectilinear in nature.

Figure 2 illustrates the average length of left posterior papillary muscles (Figure 2A) and their mean cross-sectional area (Figure 2B). These two parameters of papillary muscle size were found to be comparable in control and experimental mice and indicated that the 18% difference in heart weight was not apparent at this level.

The characteristics of the isometric phase of contraction are depicted in Figures 3–8. The timing parameters of the isometric twitch demonstrated prolongations of time to peak developed tension (Figure 3A) and time to one-half relaxation (Figure 3B) in muscles removed from Mo** mice. In comparison with controls, 23% (p<0.001) and 29% (p<0.001) longer time periods were measured in the contraction and relaxation phases, respectively.

Figure 4 illustrates the isometric rates of tension rise (Figure 4A) and decay (Figure 4B), which were found to be depressed in animals with the genetic collagen disorder. The rate of tension development was 42% (p<0.001) slower in Mo** mice whereas the rate of tension fall was 54% (p<0.001) reduced in the same group. The time required to achieve these peak rates was prolonged by 38% (p<0.001) in the former case (Figure 4C) and was 19% shorter in the latter case (Figure 4D). However, the reduction in the time to peak rate of tension decay was not statistically significant. Thus, isometric contraction is delayed in onset and prolonged in relaxation in experimental animals although time to peak rate of tension rise and time to peak rate of tension fall are affected in opposite directions.

The characteristic of peak isometric developed tension throughout the physiological range of muscle lengths in control and experimental mice is shown in Figure 5. At lengths between 93% and 97% of L, statistically significantly lower values of...
active force per unit area of muscle were measured in Mo* mice. This alteration is consistent with a depressed force-generating ability of the myocardium in animals with a collagen disorder.

In contrast to the observation in active tension development (Figure 5), passive stress along the physiological range of muscle lengths was found to be consistently increased in muscles obtained from Mo* mice (Figure 6). This elevation in passive tension was present throughout, suggesting an abnormality in the diastolic properties of the isovolumic phase of the cardiac cycle.

Figure 7 depicts the alterations in mechanical performance found in the isometric phase of contraction in which both developed and passive tension as well as the timing parameters for these events have been observed to be impaired in the presence of altered collagen cross-linking. It should be apparent that the kinetic performance of diseased muscle is prolonged (Figure 7A) and the rates of tension change are depressed as well (Figure 7B).

The mechanical characteristics of afterloaded isotonic contractions from control and experimental mice are presented in Figures 8–10. As shown in Figures 8A and 8B, the extent of shortening from L was reduced in diseased muscles whereas the time required to attain the maximal degree of isotonic muscle shortening was prolonged. Specifically, peak shortening was decreased by 50% (p<0.001), and time to peak shortening was 29% (p<0.001) longer. Moreover, time to peak velocity of shortening (Figure 8C) was increased by 26% (p<0.01). Time to peak velocity of relengthening, however, was essentially maintained (Figure 8D).

The inverse relations between isotonic load and speed of muscle shortening and relengthening are illustrated in Figures 9A and 9B. Peak velocity of shortening at different loads within the physiological range was depressed in Mo* mice; the alteration was more impaired at 0.1–0.3×total load (Figure 9A). Peak velocity of relengthening was affected in a similar manner, and the alteration in this parameter also appeared to be greater at lower loads (Figure 9B).

By comparing Figures 9A and 9B, it should be apparent that the velocities of shortening and relengthening are identical in control mice, but this does not apply to Mo* animals. In mice with collagen disorder, the velocity of shortening was consistently greater than that of relengthening from 10% to 70% relative load. These differences were all found to be statistically significant (p<0.05).

Figure 10 illustrates in a schematic form the effects of the lack of collagen cross-linking on the isotonic shortening phase of muscle contraction. Despite an increase in time to peak shortening in Mo* mice (Figure 10A), the depressed velocity of shortening...
Discussion

The results of the present study indicate that a genetically determined alteration in collagen metabolism is associated with an impairment in myocardial dynamics of the Mo* mouse heart. These abnormalities affected both the systolic and diastolic phases of the contraction process as follows: active tension was found to be diminished whereas passive tension was increased. The reduced capacity to generate active stress was apparent only at muscle lengths between 93% and 97% of L and was lower but not statistically significantly different at the other muscle lengths within the physiological range. On the other hand, the increase in passive stress was seen at all muscle lengths examined. Moreover, the timing parameters of the isometric twitch were prolonged in association with depressed rates of tension rise and decay. Isotonically, peak shortening and relengthening velocities were dimin-
lished, and the kinetic parameters of these events were augmented.

The findings summarized above, which describe the characteristics of the mechanical performance of the heart in a condition of impaired collagen cross-linking, are consistent with the depressed contractile behavior commonly seen in pressure overload hypertrophy as well as in the reactive growth response of the aging heart. However, the inability to develop adequate force at physiological muscle lengths demonstrates that a much greater impairment of cardiac dynamics occurs in the Mo*/ mouse. The small size of the animals does not allow the collection of measurements of ventricular function to support the concept that an elevated afterload on the heart is present in this animal model. On the other hand, a significant increase in the heart weight/body weight ratio seems to indicate that cardiac hypertrophy has occurred and suggests an abnormal loading state on the myocardium. This is further supported by the observation that active stress is impaired after severe myocardial dysfunction and overt ventricular failure. However, because of these limitations, a direct causal relation between the impairment in connective tissue cross-linking and the depressed muscle contractile behavior cannot be postulated. Moreover, alterations in the uptake and release of calcium within the cells may interfere with active force development independently from the collagen abnormality.

Contrary to expectation, the passive stress-strain relation was shifted upward and to the left in Mo*/ mice whereas the impaired cross-linking of collagen would have suggested a decreased passive stress at each muscle length. In this regard, the administration of an external lathyrogen in pressure-overloaded hearts was capable of restoring normal myocardial compliance. The finding in the current

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**Figure 8.** Bar graphs showing changes in peak shortening (panel A), time to peak shortening (panel B), time to peak velocity of shortening (panel C), and time to peak velocity of relengthening (panel D) in control (open bar) and Mo*/ (hatched bar) mice. Results are presented as mean±SE. *Significantly different (p<0.05) from the corresponding value in control mice.

**Figure 9.** Graphs showing load-velocity relations at a bath Ca concentration of 2.4 mM. Values were obtained from a series of afterloaded isotonic contractions at an initial muscle length at which peak isometric active tension was obtained (L). Load is expressed as relative load [(total isotonic load/total isometric load)×100]. Velocity is expressed as muscle length per second, calculated as (mm/sec)/L. Panel A: Peak velocity of muscle shortening. Panel B: Peak velocity of muscle relengthening. Results are presented as mean±SE. *Significantly different (p<0.05) from the corresponding value in control mice.
Panel A: Representative muscle length change from left ventricular posterior papillary muscles removed from control and Mo<sup>1</sup> mice. Muscle length is plotted against time. Mechanical parameters used to describe the velocity of isotonic contractions are indicated by arrows pointing to the control trace. PS, peak shortening; TPS, time to peak shortening. Panel B: Representative speed of muscle shortening and relengthening during isotonic contractions from an initial muscle length at which peak isometric active tension was obtained. Bath Ca<sup>2+</sup> concentration was 2.4 mM. Relative loads [total isometric load/total isometric load × 100] were identical. The genetical defect in the latter was expected to generate a reduced stiffness of cardiac muscle in the Mo<sup>1</sup> mouse. This was the case at muscle lengths greater than 98% L. This phenomenon can be assumed to be a compensatory adaptive process that would tend to counteract the depressed force-developing capacity by allowing shifts along the Starling curve at smaller changes in loading states.

Mechanical characteristics of isotonic contraction showed that velocities of shortening and relengthening were impaired in mice with inhibited cross-linking of collagen. However, the latter parameter was altered to a greater degree than the former one; this alteration resulted in an increase of the ratio of velocity of shortening to velocity of relengthening. Such an imbalance appears to suggest that the elastic recoil properties of the myocardium are affected under this condition. The storage and/or dissipation of energy by the extracellular connective tissue compartment during contraction may become altered in Mo<sup>1</sup> mice causing a reduction in the velocity of muscle relengthening. It follows that abnormalities in the rapid filling phase of the heart may be present in vivo and contribute to the occurrence of myocardial dysfunction. Similar findings have been described in hypertensive hypertrophy and aging although the velocity of shortening and the velocity of relengthening have been observed to be equally depressed.

An assumption made in the present investigation is that the localized damage produced by clamping one muscle end is similar in both animal groups. Direct evaluation of sarcomere dynamics would solve this potential complexity inherent in in vitro studies of muscle mechanics, but posterior papillary muscles in the mouse are generally too large to be analyzed in this manner. The mechanics of an undamaged region of the muscle may yield results quantitatively different from those obtained in the entire muscle although the timing parameters are not qualitatively affected. In this regard, no noticeable differences were found between the information obtained from the entire muscle itself and its inner portion; this finding suggests that the observations made in the present investigation were not significantly influenced by the potential contribution of muscle damage produced in the positioning of the tissue samples in the muscle bath for mechanical estimation.

In conclusion, changes in the collagen cross-linking are associated with alterations in cardiac...
Acknowledgment

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References

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Key Words: collagen cross-linking • papillary muscle • mechanics • mouse
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