Mechanical Properties of Rat Cardiac Muscle during Experimental Hypertrophy

By Oscar H. L. Bing, Satoru Matsushita, Barry L. Fanburg and Herbert J. Levine

ABSTRACT

The mechanical properties of trabecular muscles from the hearts of 77 rats subjected to aortic arch constriction were compared with those from 77 unoperated and sham-operated control animals at 1, 3, 7, 14, and 28 days after operation. Significant hypertrophy, as evidenced by an increase in left ventricle to body weight ratio, was first seen at three days (P < 0.02), reached a maximum of 30 to 40% by seven days (P < 0.001), and remained relatively constant throughout the remainder of the experiment. Depression of isotonic shortening velocity and maximum isometric force of trabecular muscles from hypertrophied hearts was first seen at seven days. These changes persisted at 14 and 28 days. When alterations in muscle mechanics due to changes in muscle thickness were taken into consideration, muscles from hypertrophied hearts demonstrated a depressed maximum velocity of shortening (P < 0.001), while development of isometric tension was unaltered. The latter appeared to be maintained at least in part by a prolonged contraction time, as reflected by increases in the time to peak isometric tension (P < 0.05) and the time to peak "unloaded" isotonic shortening (P < 0.001). Resting tension was increased in trabecular muscles from hypertrophied hearts. Tissue hydroxyproline concentration was elevated with hypertrophy. The observed depression in muscle shortening velocity at light loads may be explained by altered contractile state or by increased stiffness of the parallel elastic element.

KEY WORDS

aortic constriction, isolated muscle studies, hydroxyproline, force-velocity relationships, lactic dehydrogenase

The mechanical performance of the hypertrophied heart has been of interest over the years to many investigators who have studied both the intact heart in vivo and the isolated heart muscle preparation. Although studies done in vivo may more closely approximate the situation in clinical hypertrophy, it is often difficult in these experiments to control adequately all experimental variables. A more rigid control of these variables can be obtained when using the isolated muscle preparation.

In 1961 Kerr et al. (1) reported a significant increase in the developed tension per unit dry weight of isolated hypertrophied rat papillary muscle. On the other hand, Grimm and coworkers (2) found no difference in length-tension curves between control and hypertrophied rat papillary muscles. More recently, Spann and associates (3) have studied in detail the mechanical function of papillary muscle from the hypertrophied right ventricle of the cat and found a decrease in "contractility" with no significant change in maximum isometric tension development.

We have attempted to resolve some of these apparent discrepancies in the present studies on trabecular muscles obtained from the left ventricle of the rat after aortic constriction. Our studies, which were carried out at various times up to four weeks after constriction, indicate that once hypertrophy is established, there is a decrease in maximum shortening velocity of heart muscle with no significant change in maximum development of isometric tension per unit area of muscle.
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Methods

Inbred Charles River C57 black mice weighing 180 to 220 g, were used in the present experiments. Seventy-seven animals were subjected to constriction of the aortic arch, while 77 animals served as unoperated or sham-operated controls. Operations were carried out as previously described (4) except that animals were anesthetized with 60 to 70 mg of chloral hydrate given intraperitoneally. Animals were decapitated at 1, 3, 7, 14, and 28 days after operation. The chest was opened, and the heart was removed rapidly and placed in oxygenated Krebs-Henseleit solution (5).

MUSCLE PREPARATION AND MECHANICAL MEASUREMENTS

The columnar carnea muscle of the left ventricle was dissected free, mounted between two spring clips, and placed in a chamber containing Krebs-Henseleit solution with 100 mg glucose per 100 ml. The solution was gassed with 95% O2 and 5% CO2 (pH = 7.4) and maintained at a temperature of 28°C. The lower spring clip was connected to a thin gold chain which was placed a micrometer stop for adjustment of tension for each muscle was measured at the apex of its length-tension curve. Maximum developed tension for each muscle was measured at a slightly shorter muscle length. The lever arm was made from magnesium with a ball-bearing fulcrum and a lever arm ratio of 8:1. A displacement transducer (Sanborn DC DT-050) was mounted above the lower spring clip. The upper spring clip was connected to a 0.0017-inch tungsten wire which passed through a mercury seal at the bottom of the chamber to a Statham model G7B-0.75-350 force transducer. The upper spring clip was connected to a thin gold chain which was attached to an isotonic lever arm above which was placed a micrometer stop for adjustment of muscle length. The lever arm was made from magnesium with a ball-bearing fulcrum and a lever arm ratio of 8:1. A displacement transducer (Sanborn DC DT-050) was mounted above the short end of the lever arm. The total equivalent mass of the lever arm system was 309 mg.

Muscles placed in the chambers were stimulated 12 times per minute by parallel platinum electrodes delivering 7 m/sec square waves pulses at voltages which were 108 greater than the minimum necessary to produce maximum mechanical response. After a one-hour period of equilibration, isotonic shortening velocity was measured at increasing loads for each muscle which had been carefully stretched to the apex of its length-tension curve. In addition, a velocity measurement was made, using the lightest possible preload. This measured velocity, which was obtained at a slightly shorter muscle length, will be referred to in the report as maximum velocity (Max V). Isotonic shortening velocity was measured by drawing a tangent to the maximum slope of the displacement-time curve. Maximum developed tension for each muscle was measured at the apex of its length-tension curve. Each length of muscle was measured with a Gaertner cathometer and telescope. At the end of each experiment, the muscle between the spring clips was weighed, and the cross-sectional area was calculated, assuming cylindrical uniformity with a specific gravity of 1.000. All values were normalized for muscle length and cross-sectional area.

Each experiment was carried out with four muscles contracting simultaneously in four chambers with common temperature regulation and oxygenation. The experiment was designed so that each set of four muscles included those from an unoperated animal, a sham-operated animal, and two animals with aortic constriction. When there was no significant difference between measurements from sham-operated and unoperated animals, they will be referred to interchangeably as controls or combined to increase the sample size.

DETERMINATIONS OF HEART WEIGHTS, PROTEIN CONTENT, HYDROXYPROLINE CONTENT, LACTATE DEHYDROGENASE ACTIVITY, AND ISOENZYME PATTERNS

After removal of the trabecular muscles, the remainder of the heart was blotted and weighed. A portion of the heart was also weighed after overnight drying to a constant weight in an oven. Protein determinations were carried out by the Folin method (6) on columnae carnea muscles. The free wall of the left ventricle was divided into endomyocardial and epimyocardial slices, and hydroxyproline was measured on samples of approximately 20 mg of tissue (dry weight) according to the method of Prockop and Udenfriend (7). The assay for lactic dehydrogenase was based on the photometric changes with oxidation of DPNH at 340 nm, which is a direct measure of the reduction of pyruvate to lactate (8). The LDH isoenzymes were separated by electrophoresis at 1.5 ma for one hour with cellulose acetate strips at a buffer pH of 8.9 (9).

Results

CHANGES IN HEART SIZE AND EVALUATION FOR WATER RETENTION

At three days after aortic constriction, there was a 17.5% increase in the left ventricular weight, a 22.5% increase in the left ventricular/body weight ratio (LV/BW), and a 20% increase in the trabecular muscle cross-sectional area (Fig. 1). By seven days after operation, these values had reached their maximum of 30 to 40% above controls and remained relatively constant during the remaining 21 days of the study. All the increases were significant at and after seven days with P values <0.001.

We found little if any macroscopic evidence of pulmonary edema or hepatic congestion on
careful examination of tissues of animals with aortic constriction. The water content of hearts and lungs from these animals did not differ significantly from controls (Fig. 2). The elevated dry/wet weight ratio for lungs from unoperated animals at 14 days after operation could not be explained and appeared to be a spurious value. Trabecular muscles were individually too small to measure the dry weights accurately, but the determination of the protein content indicated that no significant water retention had taken place in these muscles. These data suggest that the group of animals with aortic constriction had left ventricular hypertrophy without congestive heart failure.

**Myocardial Mechanics**

Maximum Shortening Velocity (Max. V) and Maximum Isometric Tension of All Muscles Studied

Data are recorded in Table 1. No consistent mechanical changes were observed in trabecular muscles from animals subjected to aortic constriction at one and three days after
operation. However, at one week after operation, Max $V$ and maximum isometric tension were depressed when compared with controls. No further changes were seen at two and four weeks after operation.

The cross-sectional areas of trabecular muscles from hypertrophied hearts were greater than those from controls (Fig. 1). To determine whether this change was responsi-

![Graph showing correlation between muscle cross-sectional area and Max $V$ and developed tension.](image)

**FIGURE 3**

Correlation of muscle cross-sectional area with Max $V$ (upper) and developed tension (lower) for all muscles studied. Each point represents a determination from a single muscle.
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>1 Day</th>
<th>3 Days</th>
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<tr>
<td></td>
<td>Control</td>
<td>Constricted</td>
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<tr>
<td>LV wt (mg) / 100 g body wt</td>
<td></td>
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<tr>
<td></td>
<td>P</td>
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<tr>
<td>LV wt (mg) / 100 g body wt</td>
<td>227 ± 3</td>
<td>203 ± 0.9</td>
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<td>Trabecular muscle cross-sectional area (mm²)</td>
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<td></td>
<td>P</td>
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<tr>
<td>Trabecular muscle cross-sectional area (mm²)</td>
<td>0.85 ± 0.05</td>
<td>0.71 ± 0.07</td>
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<td>Max V (muscle lengths/sec)</td>
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<td>P</td>
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<td>Max V (muscle lengths/sec)</td>
<td>234 ± 6</td>
<td>236 ± 9</td>
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<td>234 ± 6</td>
<td>236 ± 9</td>
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<td>Resting tension (g/mm²)</td>
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<td></td>
<td>P</td>
<td>P</td>
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<td>Resting tension (g/mm²)</td>
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<td>3.01 ± 0.10</td>
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<td>Developed tension (g/mm²)</td>
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<td></td>
<td>P</td>
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<td>Developed tension (g/mm²)</td>
<td>0.77 ± 0.08</td>
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<td>Time to peak tension (sec)</td>
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<td>P</td>
<td>P</td>
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<td>Time to peak tension (sec)</td>
<td>6.22 ± 0.43</td>
<td>7.56 ± 0.40</td>
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<tr>
<td>Time to peak shortening (sec)</td>
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<tr>
<td></td>
<td>P</td>
<td>P</td>
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<tr>
<td>Time to peak shortening (sec)</td>
<td>0.154 ± 0.003</td>
<td>0.160 ± 0.007</td>
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<tr>
<td>No. of animals</td>
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<tr>
<td>Control</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Constricted</td>
<td>9</td>
<td>13</td>
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*Values represent means plus or minus one standard error. P values listed horizontally are for differences between values for various days after operation. P values listed vertically are for differences between controls and animals with aortic constriction at any one day after operation.

Mechanical Measurements in Trabecular Muscles of Hypertrophied Hearts Matched Against Controls for Cross-sectional Area

To eliminate changes in mechanics due solely to changes in muscle thickness, muscles of similar cross-sectional area from the control and hypertrophied groups of hearts were evaluated. Data from 14 control and 19 animals with aortic constriction were summarized in Figure 4. There was a highly significant depression of Max V, while maximum isometric tension of muscles from hypertrophied hearts was unchanged from that of controls. Thus, decreases in isometric tension seen in muscles from the total group of hypertrophied hearts may be explained by the presence of thicker muscles. Decreases in Max V, on the other hand, appear to be related to some other property of the hypertrophied muscle.

Relation Between Degree of Hypertrophy with Matched Cross-sectional Areas

The Max V for muscles with the same cross-sectional area showed a high inverse correlation with the extent of hypertrophy as...
reflected by the left ventricle to body weight ratio (Fig. 5). On the other hand, no significant correlation was found between developed tension per cross-sectional area and the extent of hypertrophy.

Duration of Contraction

The duration of contraction was evaluated by measuring time to peak isometric tension and time to peak "unloaded" isotonic shortening (where "unloaded" represents the lightest possible load against which the muscle shortened). Time to peak tension of the overall group of muscles from hypertrophied hearts appeared to be slightly prolonged at 7 and 14 days after aortic constriction, although the statistical significance of this was borderline (P<0.10) (Fig. 6). At 28 days after aortic constriction, there was a 9% prolongation in the time to peak tension with a significant P value (<0.001). This variable was also prolonged for the group of muscles from hypertrophied hearts matched against controls of the same cross-sectional area (Figs. 4 and 6). There was an increase in the time to peak shortening at 7 (P<0.02), 14 (P<0.01), and 28 (P<0.001) days after the aortic constriction. A significant prolongation in time to peak shortening was also present for the muscles matched against controls for cross-sectional area.

Resting Length-Tension Relationships

In these experiments the resting tension was measured at a muscle length that resulted in peak isometric tension. There was a statistically significant increase in resting tension in muscles from hypertrophied hearts at three days after aortic constriction (Table 1), and this variable remained elevated at all subsequent times except at two weeks when no difference from controls was found.

### BIOCHEMICAL DETERMINATIONS

#### Evolution for Chronic Hypoxia of Muscle

It has been suggested that hypoxia is present in hypotrophied heart muscle and...
Summary of findings from control and hypertrophied hearts with muscles of similar cross-sectional area (A). In rats with hypertrophy, left ventricular/body weight (LV/BW), time to peak shortening (TPS), and time to peak tension (TPT) are increased; Max V is depressed, and maximum developed isometric tension (DT) is similar in both groups.

May even be the initiating stimulus for hypertrophy (10). Heart muscle from rats subjected to chronic hypoxia have increased mechanical tolerance to hypoxia in isolated muscle studies (11). As noted by Mager and coworkers, there is also a shift in the LDH subunit distribution toward the "M" type (12).

It was considered that chronic hypoxia may be present in hypertrophied rat heart muscle and could play a role in the mechanical changes observed in our studies. To investigate this possibility, trabecular muscles from the left ventricles of animals four weeks after aortic constriction were subjected to a hypoxic environment, and the time course of changes in developed tension was compared with that of trabecular muscles from control animals.

As demonstrated in Figure 7, no enhanced ability of hypertrophied muscles to tolerate hypoxia was observed. Hearts obtained from rats four weeks after aortic constriction were...
RAT CARDIAC MUSCLE IN EXPERIMENTAL HYPERTROPHY

![Graph correlation of left ventricular-body weight ratio with Max V (upper) and developed tension (lower) to trabecular muscles of similar cross-sectional area. Each point represents a determination from a single muscle.](image)

Correlation of left ventricular-body weight ratio with Max V (upper) and developed tension (lower) to trabecular muscles of similar cross-sectional area. Each point represents a determination from a single muscle.

![Graph effect of hypertrophy on the time to peak isometric tension and time to peak "unloaded" isotonic shortening. Each bar graph indicates the percent change from controls.](image)

Effect of hypertrophy on the time to peak isometric tension and time to peak "unloaded" isotonic shortening. Each bar graph indicates the percent change from controls.

Also examined for tissue LDH concentration and subunit distribution. In these experiments no increase in the LDH concentration or shift in the electrophoretically assayed isoenzyme distribution was noted. Thus, these studies provide no evidence for chronic tissue hypoxia in the hypertrophied hearts.

**Hydroxyproline**

The left ventricle was analyzed for hydroxyproline in control and hypertrophied hearts. As shown in Table 2, there was an increase in the hydroxyproline concentration in hearts from animals with aortic constriction. The increase in concentration was greater in endomyocardial than in epimyocardial samples. The relationship between tissue endomyocardial hydroxyproline concentration and the...
Effect of hypoxia on developed tension of trabecular muscles from unoperated and sham-operated animals and those subjected to aortic constriction. Experiments were carried out so that muscles from each of the four groups indicated by symbols were studied simultaneously in a common gassed medium. There were ten muscles in each group. At zero time, all muscles were exposed to 95% Ne and 5% CO₂. Changes are expressed as percent of tension at zero time, and standard errors are indicated by brackets.

Max V of trabecular muscles is demonstrated in Figure 8. Although there appeared to be a significant over-all inverse correlation between these measurements ($r = 0.48$, $P < 0.001$), there were a number of trabecular muscles with a depressed Max V from hypertrophied hearts whose hydroxyproline concentrations were within the control range. Conversely, there were several hypertrophied hearts with elevated hydroxyproline concentrations but with Max V within the control range.

**Discussion**

Our finding of a depressed maximum shortening velocity of the hypertrophied rat left ventricular trabecular muscle is consonant with the studies of Spann et al. (3) on the hypertrophied right ventricular papillary muscle of the cat. As in the studies with the cat and those of Grimm et al. (2) with the rat, we found no alteration in peak isometric tension normalized for cross-sectional area. This finding might appear at variance with that of Kerr and coworkers (1) who reported that developed tension per unit muscle weight was increased in the hypertrophied heart. Normalization of tension on a muscle weight basis, however, is misleading since a short muscle with a given cross-sectional area would be credited with more tension developed than a longer one. The finding that maximum shortening velocity is depressed in hypertrophied heart muscle suggests at first that the contrac-
tile state of the muscle is likewise depressed. It should be made clear, however, that the velocities measured in these experiments are those of muscle and not of its contractile element. In the present study two properties of hypertrophied muscle influence the interpretation of the observed depressed shortening velocities. The first is the finding that the resting tension of hypertrophied muscle is increased. In a three-component model of heart muscle, an observed decrease in muscle shortening velocity may be due to an increase in the stiffness of the parallel elastic component without a change in contractile element velocity (13). Since the resting tension of hypertrophied muscle was increased, the depressed muscle velocities observed in this study might be explained on this basis.

Secondly, depressed muscle velocities during early contraction might be due to changes in the time courses of the active state. Thus, delayed onset of the active state would be manifest as a depression of early, lightly loaded shortening velocities. A study of normal and hypertrophied preparations by quick release experiments could better resolve the question of inconstant active state intensity.

The unchanged isometric tension development in the presence of a depression in velocity of contraction can be explained most readily by a prolongation in the duration of contraction. Our studies, contrary to those of Spann et al. (3), show clearly that time to peak isometric tension and time to peak isotonic shortening are prolonged during experimental hypertrophy. Similar changes have been reported to take place in the rat heart during aging (14).

The findings emphasize the potential error of comparing muscles that have different cross-sectional areas. The inverse relationship between muscle thickness and tension development per unit cross-sectional area has been demonstrated previously (15). The reason for this relationship is not clear, but it may be due to hypoxia of the core of the muscle preparation since the relationship does not hold for very thin strips of muscle. In our experiments the selection of muscles of similar cross-sectional areas obviated the problem of varying muscle thickness.

Contrary to previous reports (2, 3), a significantly elevated resting tension per unit cross-sectional area was found in muscles from hypertrophied hearts in the present study. The measurement of resting tension at a length where peak developed tension is achieved (Lmax) is subject to a sizable potential error since the resting tension curve is rising steeply and Lmax is not always clearly defined. In addition, stress relaxation takes place, resulting in progressively lower values of resting tension. In these experiments the muscle was stretched no further than the peak of the active tension curve. Resting tension was measured at a similar time after stretch in all experiments. Thus, the degree of stress relaxation in all muscles should have been similar. In spite of some variation of the data, a significant elevation of resting tension was generally seen in hypertrophied trabecular muscles. It is possible that an increase in connective tissue, indicated by the increase in hydroxyproline concentration of hypertrophied hearts, could be responsible for the elevated resting tension seen in hypertrophied muscle.

A period of increased tension per unit cross-sectional area of muscle is present after aortic constriction prior to the load normalizing process of hypertrophy. It is of interest that changes in muscle mechanics did not occur during this period but only after cardiac hypertrophy was established. Indeed, a good correlation between Max V and the degree of hypertrophy was noted (Fig. 5). These findings suggest that factors related to hypertrophy itself rather than the immediate effects of an increased workload are somehow responsible for the decreases in muscle shortening velocity.

As reported by others (16, 17), an increase in hydroxyproline concentration in hypertrophied hearts was noted in this study. Although there appeared to be an inverse correlation between hydroxyproline concentration and Max V, there were many hyper-
trophy which has been produced by other means, such as increased volume work. This possibility has received recent support from Turina and coworkers (20) who found that left ventricular hypertrophy associated with chronic volume load in intact dogs did not alter the force-velocity relationship.

Finally, in the intact heart a disproportionate increase in the amount of hypertrophy may compensate for the decrease in shortening velocity and enable the hypertrophied heart to function in an apparently normal fashion.

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References


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