Myocardial Mechanical, Biochemical, and Structural Alterations Induced by Chronic Ethanol Ingestion in Rats

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To determine the effects of moderate ethanol consumption on the mechanical, biochemical, and structural characteristics of the heart, myocardial mechanical performance, contractile protein enzyme activity, and the number and size of myocytes were measured in male Fischer 344 rats after the ingestion of 30% oral ethanol. Papillary muscles removed from the left ventricle were greater in length, weight, and cross-sectional area than the corresponding muscles from the right side. However, no differences were found between control and ethanol-treated myocardium when either the left or right side was compared separately. Chronic ethanol ingestion resulted in an increase in resting tension in left ventricular muscles, with no alteration in peak developed tension. Moreover, time to peak tension was significantly prolonged, whereas a depression was observed in the peak rate of isometric tension development. Isotonically, left muscles from ethanol-treated rats revealed a prolongation of time to peak shortening and a marked depression in the velocity of shortening at physiological loads. No changes were noted in muscles from the right ventricle. Contractile protein enzyme activity revealed no differences in myofibrillar Mg\textsuperscript{2+}-ATPase activity in right and left ventricular myocardium between control and ethanol-treated rats in the presence of EGTA. However, at physiological activating levels of calcium, an upward shift of the myofibrillar Mg\textsuperscript{2+}-ATPase activity–calcium curve occurred in left myocardium, whereas a depression in this relation was seen in the right ventricle. As a result of chronic ethanol intake, a decrease was noted in the volume percent of myocardium occupied by myocytes, and that myocyte cell volume per nucleus was found to remain essentially constant throughout the various layers of the ventricular wall. Importantly, a 14% significant decrease in the total number of myocyte nuclei was demonstrated in the left ventricular myocardium of rats on chronic ethanol consumption. Thus, chronic but moderate alcohol ingestion resulted in depressed contractile performance, alterations in myofibrillar Mg\textsuperscript{2+}-ATPase activity, and myocyte loss. These events may serve to function as preliminary indicators of the onset of heart failure of alcoholic origin in this animal model. (Circulation Research 1992;71:346–356)

KEY WORDS • ethanol • papillary muscle • biochemistry • myocyte loss

Clinical investigations dealing with the effects of ethanol have indicated that chronic alcoholics who are asymptomatic for heart disease still display clear hemodynamic evidence of depressed pump function.\textsuperscript{1,2} This condition may reverse if early withdrawal of ethanol is instituted, but it can progress to severe left ventricular dysfunction and failure if left untreated.\textsuperscript{3} However, animal models mimicking the human condition have been difficult to obtain, complicating the analysis of the influence of ethanol on the myocardium in relation to the human disease. In this regard, experimental studies dealing with the detrimental impact of chronic ethanol ingestion have revealed a depression in ventricular dynamics.\textsuperscript{4–6} However, the mechanism responsible for this unfavorable outcome remains unclear.\textsuperscript{1–6} Recently, it has been found that chronic ethanol administration in Fischer 344 rats provoked alterations in the loading state of the myocardium, left ventricular dysfunction, and extensive cardiac chamber and wall remodeling.\textsuperscript{7} This study provides the first experimental documentation that functional and anatomic changes may be associated with multiple focal areas of tissue injury across the ventricular wall, suggesting that myocyte loss may constitute an essential component of this form of cardiomyopathy, congruent with findings in humans.\textsuperscript{1} Importantly, the interaction of damage and repair in the ventricular wall makes it difficult to establish whether the alterations in global cardiac hemodynamics are dependent on the loss of viable cells, ventricular dilation, and wall thinning or are the result of a direct action of ethanol on muscle mechanical behavior. Moreover, myocardial performance correlates with contractile protein enzyme activity,\textsuperscript{8,9} which, in an independent or combined fashion, may participate in the initiation of cardiac maladaptation.
tion in alcoholics. Therefore, to determine whether chronic consumption of moderate amounts of ethanol induces a depression of the intrinsic mechanical properties of the myocardium and associated alterations in contractile protein enzyme activity, papillary muscle performance in vitro and myofibrillar and myosin ATPase activities were determined after oral ethanol administration for a period of 8 months in Fischer 344 rats. Moreover, the changes in the number and size of myocytes were measured to establish whether myocyte loss and reactive cellular hypertrophy were implicated in the occurrence of alcoholic cardiomyopathy in this animal model. The 8-month time interval was selected to mimic the conditions previously used.7

Materials and Methods

Male Fischer rats at 4 months of age were purchased from Harlan Sprague-Dawley breeding laboratories, Indianapolis, Ind. (National Institutes of Health colony) and made alcoholic as described below. All animals were caged and fed Purina rat chow and water ad libitum.

Animals in the treatment group received 10% (vol/vol) ethanol in their drinking water for the first week, 20% for the next 2 weeks, and after the third week, 30% for the rest of the treatment period. This consumption of ethanol represented 30% of the total caloric intake. Since 70% of the caloric intake was received from standard Purina rat chow, it was assumed that the diet of ethanol-treated rats was nutritionally adequate. Blood samples were obtained every 2 weeks, and serum ethanol concentration was determined. After 8 months of oral ethanol ingestion, left and right papillary muscles were used for mechanical studies (see below), and the remaining ventricles from control and ethanol-treated rats were used for estimation of contractile protein enzyme activity.

An additional group of experimental and control rats, treated identically, were used for the quantitative estimation of changes in size and number of left ventricular myocytes according to the methodology described below. Separate groups of rats had to be used for these morphometric evaluations since they required perfusion fixation of the myocardium in situ.

Myocardial Mechanical Evaluation

Animals were anesthetized with ether, and the hearts were rapidly excised and placed in oxygenated Tyrode’s solution containing elevated potassium to induce diastolic arrest (mM: Na+ 151.3, Ca2+ 2.4, K+ 30.0, Mg2+ 0.5, Cl− 147.3, H2PO4 12.0, and dextrose 5.5. The right ventricular free wall and the left ventricle (plus septum) were weighed after dissection of the papillary muscles.

The left and right posterior papillary muscles were removed and suspended side by side in a muscle bath. The nontendinous end of each papillary muscle was inserted into the end of a micrometer assembly that was used to adjust external muscle length. The tendinous end of the papillary muscle was attached to a 2-cm stainless-steel lever that was connected to servo-controlled force/length transducers. The muscles were continuously perfused with normal Tyrode’s solution (4.0 mM K+). This solution was maintained at 30°C and gassed with 95% O2−5% CO2 (pH 7.2). Preparations were stimulated at 0.1 Hz by rectangular depolarizing pulses 10 msec in duration and twice the diastolic threshold in intensity.8,9

Stress–strain relations. To avoid problems due to dimensional variations between the left and right muscles, force was described in terms of the cross-sectional area involved in its production. The expression of force per unit area is tension (τ) and is expressed as millinewtons per square millimeter (1 g = 9.87 mN). As muscle is elongated, the cross-sectional area decreases so that force is generated by a smaller area than at the original length (L0), and the resultant tension is consequently higher. Thus, tension-generating ability is defined as the force-developing capacity (F) per actual instantaneous cross-sectional area (A):

$$\tau = F (\text{mN})/A_s (\text{mm}^2)$$

When a uniaxial load is applied to cardiac muscle parallel with its fibers, the tissue responds by deforming and changing its overall length. This strain (ε) is expressed as length change per original length. Thus, changes in length of a muscle from L0, which is assumed to be associated with zero stress, to a new or instantaneous length (Li) can be described by the relation that defines the change in linear strain:

$$\epsilon = \int_{L_0}^{L_i} dL/L_0 = \ln L_i/L_0 (\text{mm/mm})$$

This relation is referred to as the logarithmic or natural strain since the integral L1−L0 with respect to dLi is equal to ln L1.

Isometric characteristics. After an equilibration period of 120 minutes, during which the muscle contracted isometrically at a resting tension of approximately 9.8 mN/mm², the passive and active isometric stress–strain relations were determined by shortening muscle length in approximate 1% steps between the muscle length at which force development is maximal (Lmax) and 90% Lmax. Parameters dependent on muscle length were computed at intervals of 1% Lmax.

Isotonic characteristics. Load–velocity relations were obtained by setting muscle length at Lmax with an appropriate preload and increasing the afterload in steps from preload to the isometric level. Peak velocity of muscle shortening was measured at each afterload, and load–velocity curves were constructed. Isotonic parameters were measured at each afterload and compared at identical relative loads [(preload+isotonic afterload)/(preload+isometric developed tension)×100]. Parameters dependent on muscle load were computed at intervals of 5% relative load.

At the completion of each experiment, muscle length and muscle diameter at Lmax were measured with a reticle in the eyepiece of a dissecting microscope set at a total magnification of ×30. Cross-sectional area (XS) was calculated from papillary muscle diameter (MD): XS = 3.14159 × (MD/2)². Force and the rate of force change were expressed per unit area of tissue, and velocity was expressed in muscle lengths per second.

Contractile Protein Enzyme Activity

Hearts of ethanol-treated rats were extracted and analyzed individually and simultaneously with hearts of control rats using the same reagents and incubation conditions. ATP, dithiothreitol (DTT), EDTA, EGTA,
and the proteolytic enzyme inhibitor phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma Chemical Co., St. Louis, Mo. Hearts were stored at \(-70^\circ\text{C}\) in 50% glycerol containing (mM) KCl 30, KPO4 10 (pH 7.0), DTT 2, and PMSF 0.2 before preparation of the extracts.

Myofibrils were isolated and purified with Triton X-100.10 To check the purity of the myofibrils, the modified method of Maizel11 was used. The samples were run on sodium dodecyl sulfate gradient (5–16.5%) slab gel electrophoresis in a Tris-glycine buffer system. Contaminant proteins or evidence of proteolytic breakdown were not detected in these purified myofibrillar preparations.

Cardiac myofibrillar ATPase activities were assayed in a final volume of 1 ml at 30°C in a medium containing (mM) KCl 50, ATP 2, MgCl2 2.5, imidazole 20 (pH 7.0), NaK10, and CaCl2 0.1 or EGTA 2, along with a protein concentration of 0.1–0.2 mg/ml. Myofibrillar Mg2+-Ca2+-ATPase activity will be defined as Ca2+-stimulated ATPase measured in the presence of Mg2+ (2.5 mM) and different concentrations of Ca2+. EGTA ATPase activity will be defined as basal ATPase (Mg2+-ATPase) measured in the absence of free Ca2+ but in the presence of 2.5 mM Mg2+ and 2 mM EGTA. For studying the free Ca2+-dependence of activation of myofibrillar ATPase, Ca2+-EGTA buffers were used.12 The reaction was started by addition of substrate. Protein content of the enzyme was determined by the biuret method with bovine serum albumin as a standard. Estimation of inorganic phosphate was determined by the micro method.13

Ca2+-ATPase activity of myosin in myofibrils was assayed as described previously.14 The final volume of incubation medium was 1 ml of 0.3 M KCl, 50 mM Tris chloride (pH 7.6), 10 mM CaCl2, and 5 mM ATP. The reaction was carried out at 30°C and was started by addition of substrate. The amount of enzyme added (60–75 µg) was adjusted so that only 15–20% of ATP was hydrolyzed. Results are expressed as micromolar inorganic phosphate per milligram protein per minute.

Analyses of myosin isoenzymes in myofibril preparations were done by polyacrylamide gel electrophoresis (GE-2/4, Pharmacia) using nondissociating conditions at 2°C.15,16 The running buffer contained 0.02 M Na2HPO4, 10% glycerol, and 0.01% (vol/vol) PMSF (pH 8.5) in 0.001 M EDTA.8 Cylindrical 4% polyacrylamide gels (60×6 mm) were prepared with acrylamide and N,N'-methylene-bis-acrylamide (30:8). Approximately 5 µg crude myosin extracts were layered on each gel and run at a constant voltage gradient of 14 V/cm for 20–22 hours. Gels were stained and destained,15 and densitometric scans were recorded at 595 nm on a spectrophotometer (Kontron Instruments, Milan, Italy). The relative estimate of each isoenzyme was calculated from the area under each peak.

**Fixation Procedure and Tissue Preparation for Morphometric Evaluation**

Animals were killed by arresting the heart in diastole with 1 ml cadmium chloride (100 mM i.v.). The heart was then perfused retrogradely through the abdominal aorta with pH 7.2 phosphate buffer for 3 minutes. Perfusion pressure was adjusted to the mean arterial pressure measured in vivo. After perfusion, the coro-

Myocyte Size and Number

Ten randomly chosen tissue blocks from each left ventricle were sectioned at a thickness of 0.75 µm and stained with toluidine blue. Morphometric sampling at a magnification of >1,000 consisted of counting the number of myocyte nuclear profiles, N(n), in a measured area, A, of tissue sections in which cardiac muscle fibers were cut transversely. A square tissue area of 9,950 µm² was delineated in the microscopic field by the 42-sampling-point ocular reticle. A total of 25 such fields were evaluated in the endomyocardial and epimycocardial regions of each ventricle of each animal to determine the number of nuclear profiles per unit area of myocardium, N(n)A, and the volume fraction of myocytes, V(m)A, in the myocardium of the inner and outer layers of the wall.

Nuclear length, D(n), was determined in the endomyocardial and epimyocardial regions of each left ventricle from 37–50 measurements, each made at a magnification of ×1,250 in longitudinally oriented myocytes viewed with a microscope having an ocular micrometer accurate to 0.5 µm. Ten blocks with myofibers sectioned parallel to their length were cut, sections 2 µm in thickness were collected and stained, and seven to 10 measurements of nuclear length were recorded from each tissue section, three to five each for region of the wall.

From the estimation of N(n)A and D(n), the number of myocyte nuclei per unit volume of myocardium, N(n)V, was computed using the following equation16:

\[
N(n)V = N(n)A / D(n)
\]  

(3)

Myocyte cell volume per nucleus in the inner and outer layers of the wall of each left ventricle, V(m)V, was obtained from the volume fraction of myocytes, V(m)A, divided by the number of myocyte nuclei per unit volume of myocardium:

\[
V(m)V = V(m)A / N(n)V
\]  

(4)

The total number of myocyte nuclei in each ventricle, N(n)T, was derived from the product of N(n)V and the total ventricular volume of myocardium, V T, derived from the quotient of ventricular weight and the specific gravity of muscle tissue (1.06).16

\[
N(n)T = N(n)V \times V T
\]  

(5)

In this case, N(n)V was evaluated by averaging the values obtained in the inner and outer layers of the wall in each ventricle. A similar approach was used when regional morphometric values of volume fraction of
tissue components, numerical density of nuclei, nuclear length, and cell volume per nucleus were expressed as indicators of the entire wall. All tissue samples for these determinations were coded, and the code was broken at the end of the experiment.

**Data Collection and Analysis**

Results are presented as mean±SD and were computed from the average measurements obtained from each animal. Statistical significance between control and ethanol-treated animals was determined by a two-tailed unpaired Student's t test. Comparisons between the right and left sides of the heart in the same animal group were evaluated for statistical significance by a paired Student's t test. Values of \( p < 0.05 \) were considered to be significant. Because measurements presented could not all be obtained in every animal and because both ventricles and, at times, samples were pooled, \( n \) values for each determination are listed in the text or in the legend of each figure.

**Results**

Chronic ethanol consumption for a period of 8 months resulted in an average serum level of 30.8±16.3 mg% and was associated with a 13% decrease in overall body weight (Table 1). Left and right ventricular weights were reduced by 8% and 3%, respectively. However, the latter change was not statistically significant. Moreover, the reduction in body weight, as a result of ethanol administration, resulted in an increase in right ventricular weight/body weight ratio of 11%.

The general characteristics of left and right papillary muscles of control and ethanol-treated rats are illustrated in Table 2. No differences were observed in length, weight, and cross-sectional area of left and right papillary muscles between control and experimental animals. However, left ventricular papillary muscles from control and ethanol-treated rats were considerably longer and heavier and had a greater cross-sectional area than their respective counterparts removed from the right ventricle (Table 2). Although isometric relaxation time did not vary in either the left or

**TABLE 1. General Characteristics of Control and Experimental Rats**

<table>
<thead>
<tr>
<th></th>
<th>Control rats (( n=17 ))</th>
<th>Ethanol-treated rats (( n=22 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight (mg)</td>
<td>1,024±108</td>
<td>948±96*</td>
</tr>
<tr>
<td>LV weight (mg)</td>
<td>816±91</td>
<td>748±81*</td>
</tr>
<tr>
<td>RV weight (mg)</td>
<td>208±21</td>
<td>201±19</td>
</tr>
<tr>
<td>Heart weight/body weight (mg/g)</td>
<td>2.19±0.23</td>
<td>2.33±0.25</td>
</tr>
<tr>
<td>LV weight/body weight (mg/g)</td>
<td>1.75±0.20</td>
<td>1.84±0.21</td>
</tr>
<tr>
<td>RV weight/body weight (mg/g)</td>
<td>0.44±0.03</td>
<td>0.49±0.04*</td>
</tr>
</tbody>
</table>

LV, left ventricular; RV, right ventricular. Values are mean±SD. *\( p < 0.05 \) vs. the corresponding value obtained in control rats.

**TABLE 2. Muscle Characteristics and Isometric Kinetic Performance**

<table>
<thead>
<tr>
<th></th>
<th>Control rats (( n=10 ))</th>
<th>Ethanol-treated rats (( n=15 ))</th>
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</thead>
<tbody>
<tr>
<td>Left posterior papillary muscles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle length (mm)</td>
<td>8.39±0.80</td>
<td>8.97±0.57</td>
</tr>
<tr>
<td>Muscle weight (mg)</td>
<td>10.34±3.72</td>
<td>13.17±3.06</td>
</tr>
<tr>
<td>Cross-sectional area (mm²)</td>
<td>1.04±0.29</td>
<td>1.37±0.39</td>
</tr>
<tr>
<td>Time to peak tension (msec)</td>
<td>176±8.1</td>
<td>190±11.6*</td>
</tr>
<tr>
<td>Time to half relaxation (msec)</td>
<td>163±15.7</td>
<td>165±16.7</td>
</tr>
<tr>
<td>Peak rate of tension rise (mN/mm²/sec)</td>
<td>716±91</td>
<td>548±72*</td>
</tr>
<tr>
<td>Peak rate of tension decay (mN/mm²/sec)</td>
<td>284±37</td>
<td>273±40</td>
</tr>
<tr>
<td>Time to peak rate of tension rise (msec)</td>
<td>92±2.5</td>
<td>100±5.0*</td>
</tr>
<tr>
<td>Time to peak rate of tension decay (msec)</td>
<td>136±22.8</td>
<td>137±20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control rats (( n=10 ))</th>
<th>Ethanol-treated rats (( n=15 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right posterior papillary muscles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle length (mm)</td>
<td>5.17±0.78†</td>
<td>5.16±0.98†</td>
</tr>
<tr>
<td>Muscle weight (mg)</td>
<td>2.92±1.03†</td>
<td>3.93±1.20†</td>
</tr>
<tr>
<td>Cross-sectional area (mm²)</td>
<td>0.53±0.10†</td>
<td>0.71±0.21†</td>
</tr>
<tr>
<td>Time to peak tension (msec)</td>
<td>162±8.8†</td>
<td>167±12.3†</td>
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<tr>
<td>Time to half relaxation (msec)</td>
<td>135±18.6†</td>
<td>118±14.6†</td>
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<tr>
<td>Peak rate of tension rise (mN/mm²/sec)</td>
<td>776±103†</td>
<td>703±139†</td>
</tr>
<tr>
<td>Peak rate of tension decay (mN/mm²/sec)</td>
<td>355±66†</td>
<td>373±95†</td>
</tr>
<tr>
<td>Time to peak rate of tension rise (msec)</td>
<td>78.5±15.5</td>
<td>81.7±11.5</td>
</tr>
<tr>
<td>Time to peak rate of tension decay (msec)</td>
<td>108±14.5</td>
<td>109±12.8</td>
</tr>
</tbody>
</table>

Values are mean±SD. *\( p < 0.05 \) vs. the corresponding value obtained in control rats. †\( p < 0.05 \) vs. the corresponding value obtained in left ventricular muscles.
right muscle after ethanol consumption, left myocardium displayed a prolonged relaxation time in comparison with its right ventricular counterpart (Table 2). The rate of force development was significantly depressed with ethanol in the left muscle, whereas no change between groups was seen in the right side. Values in the right muscle for both groups were elevated when compared with the corresponding measurements in the left myocardium. The peak rate of force decay in right and left ventricular myocardium was not significantly different between control and ethanol-treated rats. However, this mechanical property was greater in the right myocardium than in the left myocardium in the presence and absence of ethanol. A significant prolongation in time to peak rate of tension rise was noted in the left myocardium of ethanol-treated rats, whereas no change was seen in this parameter in the right ventricular myocardium. Similarly, no change in time to peak tension decay was observed between groups and sides of the heart (Table 2).

Figure 1A illustrates that a significant elevation in resting tension occurred in left muscles from experimental animals, whereas right muscles were not affected by ethanol. Resting tension in right myocardium was consistently higher than that in left myocardium. Finally, no difference in isometric developed tension was noted between groups and the different sides of the heart (Figure 1B).

Isotonic performance. The mechanical ability of papillary muscles to shorten under identical loads is shown in Figure 2. Peak shortening at afterloads in the physiological range was diminished in left papillary muscles from ethanol-treated rats. However, these differences did not reach statistical significance (Figure 2A). On the other hand, right ventricular muscles shortened less than left muscles in control and experimental rats (Figure 2B). As illustrated in Figure 3, time to peak shortening was significantly prolonged in the left muscle, remaining essentially constant in right muscles after ethanol administration. Moreover, this parameter in control right muscles was found to be greater than in the corresponding left muscles, whereas ethanol produced the opposite effect.

The inverse relation between speed of muscle shortening and afterload is illustrated in the load–velocity curves shown in Figure 4. Maximal velocity of shortening throughout the physiological range of loads was reduced as a result of ethanol in left papillary muscles only. However, shortening velocity was consistently higher in the left than in the right myocardium for both groups. Changes in the maximal velocity of muscle lengthening (Figure 5) throughout the range of physiological loads showed a pattern substantially identical to that described for velocity of shortening in Figure 4.
Contractile Protein Enzyme Activity

Activity of myofibrillar Mg\(^{2+}\)-ATPase in left and right ventricles of controls and experimental animals is illustrated in Figure 6. Although no differences were found in right or left ventricular myocardium between groups in the presence of EGTA, opposite changes were seen in the two ventricles as a result of ethanol consumption at physiological calcium levels. Specifically, at concentrations of calcium greater than pCa 7, an upward shift of the curve occurred in the left myocardium with ethanol, whereas a downward shift developed in the right myocardium. These differences in the curves were statistically significant. However, alcohol abolished the difference in these curves between the two ventricles.

Figure 7 documents the effects of ethanol on myofibrillar Mg\(^{2+}\)-ATPase activity in the presence of EGTA and at maximum calcium concentration, depicting the variability in the collected results. Animals maintained on alcohol demonstrated an elevated myofibrillar Mg\(^{2+}\)-ATPase activity at peak calcium concentration in the left ventricle and a decreased myofibrillar Mg\(^{2+}\)-ATPase activity in the right ventricle. Alcohol eliminated the difference between the left and right myocardium in this biochemical parameter. Myosin Ca\(^{2+}\)-ATPase and the relative amount of the V\(_1\) myosin isoform in both ventricles are presented in Figures 8 and 9. Alcohol had no effect in either case.

Morphometric Analysis

Quantitative analysis of left ventricular myocardium performed in a subset group of animals in which the tissue was fixed by perfusion of the coronary vasculature revealed significant changes in tissue composition and cell number as a result of alcohol ingestion. The volume percent of myocardium occupied by myocytes decreased from 82.4±1.92% to 77.5±1.18% in the outer region of the wall and from 79.2±2.05% to 77.8±3.29% in the inner layer of the ventricle. It should be pointed out, however, that the 6% reduction in the epimyocardium was statistically significant (p<0.001), whereas the smaller change in the endomyocardium did not reach statistical significance. When these regional results were combined to yield an average value across the wall, this tissue parameter was found to be reduced by 4% (p<0.005) from a control value of 80.8±1.59% to an experimental value of 77.7±1.80%.
By using the morphometric approach described in "Materials and Methods," the changes in myocyte cell volume per nucleus and in the total number of myocyte nuclei in the ventricular myocardium were obtained, and these results are illustrated in Figure 10. Myocyte cell volume per nucleus remained essentially constant in the epimyocardium and endomyocardium and, as an average, across the wall after alcohol administration. However, a 14% statistically significant decrease ($p<0.01$) in the total number of myocyte nuclei in the left ventricular myocardium was demonstrated in ethanol-treated rats.

**Discussion**

The results of the present investigation indicate that chronic ingestion of moderate amounts of ethanol was associated with alterations in cardiac mechanical performance, abnormalities in contractile protein enzyme activity, changes in the volume composition of the ventricular tissue, and myocyte cell loss. In addition, distinct differences were found between the two ventricles, suggesting that prolonged ethanol consumption may affect the left more than the right ventricle. Thus, sustained alcohol intake induced a depression in myocardial contractility and biochemical and structural defects that together may contribute to the development of ventricular dysfunction in vivo in this animal model.

**Mechanical Effects of Ethanol**

Data in the current study demonstrate that chronic ethanol consumption produced changes in isometric contractions characterized as an increase in resting tension, prolongation of contraction duration, and a decrease in the speed of isometric tension development. Isotonically, the speeds of muscle shortening and lengthening were significantly lower in ethanol-treated rats despite a prolongation of isotonic shortening time. Importantly, these mechanical abnormalities were restricted to the left myocardium, whereas the right myocardium showed no significant alterations in mechanical behavior. These observations in vitro can be expected to affect myocardial relaxation in vivo, leading to impairment in diastolic function. Thus, the current results are consistent with the hemodynamic changes previously found in Fischer 344 rats 8 months after oral ethanol administration.

Differences in the functional properties of left and right myocardium in the absence of ethanol are in agreement with recent investigations in which the contractile behavior of cardiac tissue has been examined biventricularly in various strains of rats. However,
Although prolonged ethanol ingestion has been found to delay the onset of myocardial function in alcohol-exposed large animals,18–20 it cannot be excluded that similar delayed behavior will be observed in alcohol-exposed myocytes.21,22 The differential adaptation of the two ventricles to moderate but prolonged ethanol ingestion was not examined. The current results cannot exclude the possibility that similar diets, for longer periods of time and/or with alcohol representing a larger fraction of caloric intake, may alter the mechanical properties of right myocardium.

Alterations in the passive properties of the left papillary muscle seen here with alcohol may be due to an increase in myocardial fibrosis7 and/or an excess of activating calcium at the myofilaments during the diastolic phase of the cardiac cycle. This increase in crossbridge activity may result from either inadequate uptake of calcium during relaxation and/or alterations in the sensitivity of the myofilaments to free ionized calcium.10 This latter phenomenon may have its biochemical correlate in the increased activation seen in myofibrillar ATPase activity at physiological levels of calcium. On the other hand, a prolongation of contraction duration in and of itself during the isometric and isotonic phases of the cardiac cycle can cause an elevation of end-diastolic pressure that is due to activation of the systolic phase of contraction before the completion of isovolumic relaxation of the previous beat.

The changes in contractility found with alcohol tend to mimic the alterations repeatedly documented in pressure-overload hypertrophy of different etiology in rats14,23 as well as in genetically determined cardiomyopathies in rodents23 and hamsters.26 Since the loading characteristics of the myocardium were not comparable in all these conditions, it is difficult to identify a common etiologic mechanism. However, myocyte cell loss, as discussed below, has been found to be a consistent phenomenon in most of these pathological states. Loss of contractile cells would produce an eleva-

![Figure 7](http://circres.ahajournals.org/content/353/Fig7.png)

Figure 7. Bar graphs showing changes in the myofibrillar ATPase activity in the left (control, n=5; alcoholic, n=6; panel A), right (control, n=5; alcoholic, n=5; panel B), and alcoholic (left, n=8; right, n=5; panel C) myocardium in the presence of 2.5 mM EGTA and pCa 4.5 for control and ethanol-treated (alcoholic) hearts. Pi, inorganic phosphate. *Significantly different (p<0.05) from the corresponding result in control muscles.

![Figure 8](http://circres.ahajournals.org/content/353/Fig8.png)

Figure 8. Bar graphs showing myosin Ca2+-ATPase activity in the left (control, n=4; alcoholic, n=8; panel A) and right (control, n=5; alcoholic, n=5; panel B) myocardium in control and ethanol-treated (alcoholic) rats after 8 months of chronic ethanol consumption.
tion in systolic and diastolic stress on the remaining cells even in the absence of a hemodynamic overload. Importantly, such a relation has been found in the aging myocardium of Fischer 344 rats. 27

Consistent with the findings in the current investigation, previous studies comparing the mechanical performance of normal myocardium have shown that the timing parameters of muscle contraction were prolonged in the left myocardium when compared with the right myocardium. 18-20,28,29 In contrast to the current and previous 18-20 observations, speed of isotonic shortening was reduced in left muscle compared with the right muscle. 28,29 This discrepancy is difficult to explain. However, species 28 and strain 29 differences and methodological variations in terms of bath temperature, rate of stimulation, electrolyte concentrations, and in vitro loading characteristics may have contributed to the variability in this relation.

Biochemical Abnormalities

Differences in calcium responsiveness of cardiac myofibrillar Mg\(^{2+}\)-ATPase activity occurred biventricularly
as a result of chronic ethanol intake. In the presence of EGTA, this enzyme activity remained essentially constant in alcoholic rats, whereas with increasing calcium concentration an upward shift of the dose–response curve was seen in the left ventricle. In contrast, a downward shift developed in the right ventricle. On the other hand, myosin Ca\(^{2+}\)-ATPase activity and myosin isoenzyme distribution did not change after chronic ethanol administration. These biochemical defects may explain, at least in part, the alterations found in the mechanical properties of left and right ventricular myocardium in alcoholic rats. The elevated myofibrillar Mg\(^{2+}\)-ATPase activity in the left ventricle of alcoholic animals at physiological calcium concentrations was associated with an increase in resting tension in the corresponding muscle preparation. However, it is questionable whether a relation exists between these biochemical and functional adaptations, since resting tension was greater in the right than in the left myocardium of alcoholic rats, whereas myofibrillar ATPase activity was comparable in the two ventricles. 

Equally difficult to interpret are the results at saturating free calcium ion concentrations (pCa \(\geq\) 6). Ethanol consumption was associated with an increased enzymatic activity in the left ventricle and a decreased activity in the right ventricle. Since this portion of the dose–response curve most likely reflects the systolic phase of the cardiac cycle, a relation could exist with developed tension measured isometrically in vitro. In this regard, developed tension was slightly enhanced in the left myocardium in spite of a significant loss of myocytes, which would tend to have the opposite effect. In contrast, reduction in peak isometric force in right muscles correlated with reduction in myofibrillar Mg\(^{2+}\)-ATPase activity in this side of the heart. Finally, preservation of myosin ATPase activity and isoenzyme distribution with alcohol suggests that abnormalities in the troponin tropomyosin complex\(^{10}\) may have occurred, with moderate but sustained ethanol consumption accounting for the changes in myofibrillar Mg\(^{2+}\)-ATPase activity discussed above. A similar dissociation between myofibrillar ATPase and myosin ATPase in pathological states has been previously shown in human dilated cardiomyopathy\(^{30}\) and in nonocclusive coronary artery constrictions in rats.\(^{31}\)

**Myocyte Size and Number**

Present results indicate that a significant loss of myocytes occurred after prolonged ethanol consumption. However, average myocyte cell volume per nucleus remained essentially constant. Although the mechanism responsible for myocyte loss is difficult to identify, this phenomenon appears to be a consistent pattern in cardiomyopathies of different etiologies.\(^{16,27,32}\) Long-term hypertensive hypertrophy,\(^{32}\) aging in humans\(^ {39}\) and in animal models,\(^ {16}\) diabetes and hypertension combined,\(^ {34}\) and ischemic cardiomyopathy associated with nonocclusive coronary artery constriction\(^ {31}\) show scattered loss of myocytes across the ventricular wall. In contrast to the observation here, the unaffected cells in all the other conditions hypertrophy in an attempt to compensate for the loss of mass and function. Therefore, alcoholic cardiomyopathy in rats is typically characterized by the absence of reactive hypertrophy at the cellular level in spite of depressed global and myocardial contractile performance. The extensive chamber remodeling taking place after ethanol leads to a significant elevation in ventricular loading\(^7\) without a growth response of viable myocytes. This impaired capacity of myocytes to expand and meet the augmentation in diastolic stress\(^ {35}\) may find its basis in the effects of alcohol on the phosphoinositol pathway inhibiting cell growth.\(^ {35}\) In a similar manner, moderate but chronic ethanol ingestion may alter membrane permeability\(^ {35}\) and, consequently, cell viability, engendering myocyte lytic necrosis. The possibility of alcohol exerting a toxic impact on myocytes is also supported by the finding that ethanol intake vasodilates the coronary circulation,\(^ {13}\) reducing the potential of vasoconstrictor myocardial lesions.

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**References**

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