STRUCTURAL MECHANISMS OF ACUTE VENTRICULAR STRAIN SOFTENING

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ABSTRACT

Alterations in diastolic ventricular mechanics are associated with altered hemodynamic loading conditions. Mechanical preconditioning behavior suggests the myocardium may have a permanent memory of the maximum previous load consistent with strain softening property of rubber elastomers. Therefore, the focus of this work was twofold. First, we present a review of the literature with regards to the history-dependent nature of ventricular myocardium, the basis of myocardial mechanical behavior, and the mechanical property of elastomers known as strain softening. Second, we describe our effort to quantitatively demonstrate maximum-load history-dependence in myocardium at the global, local, and microstructural scales and to characterize its structural basis. We have shown that with passive inflation to new maximum pressures, LV chamber stiffness of the isolated rat heart decreased, particularly at low filling pressure. Increases in local 2-D epicardial strain were consistent with alterations in global mechanics, with the largest increases coincident with the fiber direction. Changes in epicardial sarcomere length and collagen tortuosity in softened myocardium were consistent with increases in epicardial strain. Discrepancies between endocardial sarcomere length and collagen deformation combined with transmural evidence of damage to collagen ties between interlaminar myofiber sheets, however, suggest ventricular softening involves decoupling between the myocyte matrix and extracellular collagen components. During filling, elevated interlaminar shearing between myofibril sheets and large sheet-to-sheet separation associated with overstretch in the canine heart were consistent with a loss of myocardial stiffness and damaged interlaminar collagen ties seen in the rat. Thus, strain softening, rather than viscoelastic behavior, may better explain altered diastolic mechanics associated with systolic overstretch in acute myocardial ischemia through reduced contribution of fiber-sheet architecture to passive ventricular stiffness.

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Key Words: Myocardium, mechanical properties, preconditioning, diastole, viscoelasticity

INTRODUCTION

Short- and long-term alterations in ventricular load history occur in normal physiological conditions, such as in the transition from rest to exercise [5] and the normal changes in blood pressure with aging [43]. Systolic and diastolic mechanical dysfunction occurring in a variety of pathologies are also associated with acute or chronic changes in ventricular load history.

Changes in the time-course of ventricular wall stress and strain can, in turn, affect the systolic and diastolic mechanical properties of the myocardium, both acutely and over the long term. Hence, changes in load history may not only result from pathological dysfunction, they may also contribute to it. For example, chronic hemodynamic overload can cause concentric or eccentric ventricular hypertrophy in response to altered systolic or diastolic wall stress [26]. These conditions are also associated with altered systolic [46] and diastolic myocardial mechanical properties [15] as well as geometric remodeling.

One way of classifying these and other conditions that alter mechanical load history is suggested in Table 1. Increased contractile stress with increased preload occurs almost instantaneously via the Frank-Starling mechanism, but there is also a further increase that takes place on a slower time scale [4]. Shortening-deactivation in response to transient changes in sarcomere length is another example of an acute load history-dependent property of cardiac muscle contraction [62]. Thus, cardiac function during both systole and diastole depends on the history of loading, particularly in disease conditions.

In this paper, we focus on the instantaneous reduction in resting myocardial stiffness associated with acutely elevated diastolic loading. In isolated tissues, this is often described as preconditioning behavior, though more recently, we have suggested that strain softening—also known in polymer mechanics as the Mullins effect—may be a more appropriate model [18]. There is also evidence in the intact heart that transiently increased filling pressure can soften the myocardium. Alterations in ventricular loading during cardiac surgery can have important mechanical consequences that may even be beneficial. De Hert et al [12] showed that pre-loading the LV in the arrested heart prior to removing patients from cardiopulmonary bypass resulted in a less steep end-diastolic pressure-volume relation compared with controls. As a consequence, the commonly observed delay in recovery of systolic function with bypass separation was abolished in the preload-treated patients.
### TABLE 1

<table>
<thead>
<tr>
<th>Mechanical Consequences</th>
<th>Diastole</th>
<th>Systole</th>
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<tr>
<td></td>
<td>Acute</td>
<td>Chronic</td>
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<tr>
<td>Diastole</td>
<td>CPB-Preload</td>
<td>Volume Overload</td>
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<td></td>
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<td>Hypertrophy</td>
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<td>Systole</td>
<td>Frank-Starling;</td>
<td>Pacing Asynchrony</td>
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<td></td>
<td>CPB-Preload</td>
<td>Volume Overload</td>
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<td></td>
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<td>Hypertrophy</td>
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**Softening and Overstretch in Ischemic Myocardium**

During total coronary artery occlusion, systolic dysfunction can progress rapidly to complete loss of myofiber shortening (akinesis) and eventual systolic lengthening (dyskinesis) [19]. Systolic bulging [1, 60] or holosystolic expansion [36, 63], are common descriptions of the condition in which ischemic myocardium is subject to abnormal stress and strain during systole [20, 66].

Table 2 shows that the effects of ischemia on passive mechanics depends on several factors, such as duration of occlusion, extent of reperfusion, demand versus supply ischemia, the mathematical definition of stiffness, and species [51, 67, 70]. The specific mechanisms by which ischemia can affect diastolic ventricular mechanical properties are numerous and include decreased coronary turgor [41, 67], altered intracellular calcium loading, and ATP depletion [3].

Although the full impact of systolic overstretch on regional diastolic mechanics remains unclear, it has been associated with acute myocardial structural alterations such as degradation of extracellular collagen [8, 59, 71]. Zhao et al [71] related progressive increases in systolic bulging with histologic evidence of severe damage and loss of endomyocardial and perimysial collagen, which may result from endogenous collagenase activation and mechanical overstretch. Within two days of these structural changes, "side-to-side myocyte slippage" occurs, as quantified by a reduction in the number of myocytes in the ventricular wall [49]. In spite of this evidence, however, no clear relation has been made between overstretch of noncontracting myocardium induced by ischemia and alterations in diastolic stiffness.

To study this question further, we [17] measured epicardial strain distributions in a canine heart during baseline conditions, after 2 minutes of ischemia induced by occlusion the left-anterior descending (LAD) coronary artery, and after 30 minutes of reperfusion. End-systolic strain was computed with respect to the end-diastolic (ED) configuration at matched end-diastolic pressures (6 mmHg) for all three conditions. End-diastolic strain (Fig. 1) was computed by recording successive beats during caval occlusions to achieve a reference state at close to zero end-diastolic pressure. After 2 minutes of LAD occlusion, the ischemic region was lengthening on the epicardium during systole, and end-diastolic strain increased with respect to baseline conditions. After 30 minutes reperfusion, fiber shortening was restored to some extent in the previously ischemic zone, but the average diastolic fiber length remained elevated by 39%, with the greatest increases occurring in the region of greatest systolic dyskinesia.

Studies like this can be confounded by changes in coronary perfusion and effects such as stunning. Nevertheless, these

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### TABLE 2

<table>
<thead>
<tr>
<th>Investigator and Date</th>
<th>Change in Passive Stiffness</th>
<th>Method for quantifying stiffness</th>
<th>When stiffness assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forrester, et al (1972)</td>
<td>Decreased</td>
<td>Slope of exponential fit to P-V relation</td>
<td>40 minutes after 1 hour of ischemia</td>
</tr>
<tr>
<td>Palacios, et al (1976)</td>
<td>No change</td>
<td>Slope of pressure-circumference relations</td>
<td>At 8, 30, 60, and 120 minutes of ischemia</td>
</tr>
<tr>
<td>Pirzada, et al (1976)</td>
<td>Decreased</td>
<td>Slope of pressure-segment length relations</td>
<td>At 15 minutes to 3 hours of ischemia</td>
</tr>
<tr>
<td>Theroux, et al (1977)</td>
<td>Increased</td>
<td>Slope of pressure-segment relations</td>
<td>At 5, minutes and 2 hours of ischemia</td>
</tr>
<tr>
<td>Edwards, et al (1981)</td>
<td>Increased</td>
<td>Exponential fit of pressure-strain relations computed from segment lengths</td>
<td>At 30 seconds and 30 minutes of ischemia</td>
</tr>
<tr>
<td>Vogel, et al (1982)</td>
<td>Decreased in coronary occlusion; increased in hypoxia</td>
<td>Slope of pressure-volume relations</td>
<td>At 2 minutes global ischemia</td>
</tr>
<tr>
<td>Hess, et al (1983)</td>
<td>Increased in ischemia; decreased in reperfusion</td>
<td>Exponential fit of chamber stress-strain relations computed from ventricular axis dimensions</td>
<td>At 2 minutes of ischemia; at 1 and 10 minutes reperfusion</td>
</tr>
<tr>
<td>Visner, et al (1985)</td>
<td>Increased</td>
<td>Slope of pressure-chamber strain relations computed from ventricular axis dimensions</td>
<td>At 5 minutes of ischemia</td>
</tr>
</tbody>
</table>
Gray-scale maps of regional distribution of epicardial strain in the canine LV. The maps depict end-diastolic strain along the epicardial fiber directions at baseline, ischemia, and reperfusion conditions. The left-anterior descending artery supplies the myocardium along the left boundary of the strain maps. Average strain across the array of radiopaque beads is given for each condition. During ischemia, the apical-septal region is dyskinetic during systole, which leads to elevated end-diastolic strain consistent with a decrease in passive ventricular stiffness.

preconditioning behavior in isolated cat papillary muscle. Mechanical preconditioning is also a standard protocol in biaxial testing of excised ventricular wall [35, 48, 69] and in studies of whole ventricular mechanics in isolated hearts [41, 50].

Soft tissue preconditioning behavior has commonly been associated with viscoelasticity, because preconditioning tends to reduce stiffness and decrease hysteresis. It is well established that passive myocardium exhibits viscoelasticity, i.e. stress depends on the time history of deformation with a "fading memory" [22, 34]. This property is thought to have an important impact on diastolic function in the intact heart [13, 57]. Papillary muscles creep during constant tensile loading [53] and relax following a step change in length [54]. Investigators have described the acute ventricular remodeling following acute myocardial ischemia and passive overstretch as "diastolic creep" [13, 23], implying a viscoelastic mechanism.

Vidik [65] was among the first to examine preconditioning behavior and viscoelasticity in soft connective tissues. He reported that the stress-strain curve shifted to the right with repeated loading to a given peak force, suggesting that the tissue became more compliant with successive loading. To account for this behavior in a spring-dashpot model of tendon mechanics, Vidik needed irrecoverable viscous and plastic elements. However, these modifications do not readily account for observed decreases in tissue stiffness following stretch to a new peak load.

Modeling time-history dependent mechanics in soft tissues has been largely based on the quasilinear viscoelasticity formulation [22]. Pinto and Patitucci [54] applied this model to cardiac muscle and successfully reproduced papillary muscle stress responses during rapid stretching. Huynh et al [31, 32] coupled quasilinear viscoelasticity with biphasic theory to model passive left ventricular mechanics. Multi-phasic models have also been used to explain time-dependent responses in soft tissue, such as the viscous effects due to tissue fluid movement. Yang et al [68] used nonlinear poroelasticity in a cylindrical model of the right ventricle in the stage 21 chick embryo. Simon et al [56] reviewed the use of finite element poroelastic models that couple tissue mechanics, fluid flow, and mass transport in arteries.

To date, the theories of viscoelasticity and poroelasticity have not been shown to explain experimental preconditioning behavior in soft tissues or observations of altered passive stiffness in acute myocardial ischemia. Hence, it is possible that the consequences of altered load-history actually reflect changes in the contributions of structures other than viscous elements to myocardial material properties.

Structural Consequences of Myocardial Mechanics
Elucidating the role of overstretch or preconditioning in altering passive material properties requires an understanding of how myocardial organization and microstructure contribute to the mechanics of the passive heart. Continuum models of myocardial elasticity and viscoelasticity have made advancements in integrating three-dimensional (3-D) tissue structure to describe diastolic function. Models of passive myocardial elasticity have included anisotropic, nonlinear, and nonhomogeneous constitutive properties [30, 35, 48, 55, 69]. The structural basis for these properties arises not only from the composite nature of the myocytes and surrounding collagen matrix, but on their 3-D orientation and the cell-matrix-fluid interactions as well. Theoretical models incorporating descriptions of tissue structure have demonstrated the dependency of myocardial strain and stress on local muscle fiber angle [2, 11, 27, 31], the laminar myofiber sheet architecture [10], and collagen fiber mechanics [28].

The relative contribution of specific material components to myocardial elasticity, however, remains unclear. The network of collagen fibers is considered by many to be a significant determinant of diastolic ventricular stiffness [7, 28, 39]. Decreases in ventricular stiffness and alterations in geometry have been associated with inhibition of collagen cross-linking, decreased collagen content, and disruption of collagen fibers using treatments such as β-aminopro-
pionitril [37], collagenase perfusion [40], collagenolytic activation by disulfide reagents [6], and myocardial stunning [8, 71].

Horowitz et al [28] concluded from their constitutive model of the LV that collagen was the most important contributor to passive stiffness. Using a microstructural model to reproduce myocardial experimental strain, their parameter estimation implied that the mechanics of collagen uncoiling largely determined ventricular stiffness. The elastica model of perimysial collagen developed by MacKenna and others [39] also suggested that the stress arising from collagen bending and twist during passive load could contribute a significant proportion of composite tissue stress.

Recent studies [24, 25], on the other hand, have shown that the giant myofilament protein titin — which connects the myosin thick filament to the Z-band of the sarcomere and can also bind to actin — plays a dominant role in uniaxial passive tension development. Granzier and Irving [25] "functionally dissected" various components of the myocardium using K+/K+ extraction, colchicine, and trypsin digestion in trabeculae and isolated myocytes from the rat heart. Over the working range of sarcomere length (1.9–2.1 μm), titin was responsible for 70% of resting tension, while extracellular collagen and intermediate filaments contributed 20% and 10% respectively. The contribution of microtubules was considered negligible. Beyond these sarcomere lengths, the relation between titin and collagen was reversed. It has also been shown that only 40% of the I-band segment of titin is elastic, and that strain in this segment is a primary determinant of passive tension development [24].

This simple interpretation of passive stiffness, however, assumes a parallel arrangement of these structures, where each contributes linearly to total stress, analogous to the reinforcement provided by parallel fibers in composite materials in uniaxial tension along the fiber axis [9]. It is likely that this represents an oversimplification for myocardium since it ignores the possibility of nonlinear interactions between the myofilaments and cytoskeleton, and between the cell and extracellular matrix, the importance of which has been emphasized for arteries by Humphrey [29].

The biophysical basis for viscoelastic properties in the heart is also not fully understood. Viscoelasticity in the myocardium has been attributed to the extensive extracellular collagen weave surrounding myocytes as seen with scanning electron microscopy [7]. In contrast, others have shown that titin–actin binding is the most likely intracellular structure responsible for the viscoelastic response of right ventricular trabeculae [61]. The viscous properties of cardiac myocytes are known to depend on cytoskeletal microtubule density and degree of polymerization [58]. Previous investigations have demonstrated that viscoelastic behavior in the ventricle arises from movement of extracellular fluid through the connective tissue network and myocytes [64, 57]. These studies form the basis for the applying poroelastic theory to myocardial mechanics as described earlier.

Hence, myocardial elasticity, viscoelasticity, and poroelasticity apparently reflect the presence of structural networks at both cellular and intracellular scales and their interaction with the surrounding extravascular fluid. These theories, however, cannot reconcile decreases in passive stiffness with altered load-history during conditions of disease, surgical and experimental preconditioning. Thus, an alternative concept is needed to reconcile these reductions in stiffness with changes in the myocardial structural components that contribute to passive mechanics.

Mechanics of Elastomers

The field of polymer engineering offers well-developed theories for rubber elastomers that relate material structure, function, and load-history. Elastomer mechanics may provide some useful insight into myocardial load history dependence, since structural proteins derive many of their diverse mechanical properties by cross-linking and polymerization. Elastomers are characterized by viscoelastic behavior with hysteresis and the ability to experience large deformations before failure [42].

Most engineering elastomers are composites, in which the amorphous arrangement of cross-linked polymer molecules are reinforced with fillers, usually in the form of carbon black particles or high modulus fibers, to enhance stiffness and strength [52]. Similarly, the myocardium may also be viewed as a matrix of dynamically cross-linked long-chain structures (myofilaments and cytoskeletal proteins) reinforced by a surrounding fiber network (extracellular collagen). The collagen fiber itself is also a cross-linked polymer, wherein procollagen molecules form covalent bonds between reactive aldehydes groups on lysine and hydroxylsine residues [37, 47]. Thus, load history dependence and its structural basis in elastomers may suggest potential structural mechanisms similar to those associated with myocardial load history dependence.

The experimental testing of elastomers is known to require repeated loading to a given peak load before a stable mechanical response is achieved [34, 44, 52], similar to preconditioning behavior in soft tissues. Preconditioning in elastomers is generally referred to as strain softening, or the Mullins effect after rubber scientist L. Mullins. He is attributed with first describing this property in detail in 1947 [44] for rubber vulcanizates, natural rubbers that have been reinforced with filler particles such as carbon black and cross-linked by heating.

Mullins demonstrated that the stiffness of rubbers declines after a new maximum load is experienced for the first time. Once the load exceeded levels previously experienced, the stiffness converged to that of the material as if it was being loaded for the first time. Later, it was shown by others that filler particles were not a prerequisite for the Mullins effect to occur [34]. The effect is apparently greatest in filled vulcanizates, where much of the stiffness the fillers impart is lost with stretching. Mullins and Tobin [45] modeled this phenomenon by describing the filled rubber as a two-phase composite consisting of a hard and soft phase. The process of stretching converts the hard phase to the soft phase, where most of the specimen deformation takes place. With loading to new maximum deformation, more of the hard phase is progressively converted.

Expanding on the earlier work of Mullins, Johnson and Beatty [34] developed the hard/soft phase model of the Mullins effect into a method for predicting uniaxial stress for any strain history. Their model was based on the concept of maximum-deformation history-dependent strain amplification as originally posed by Mullins. The strain amplification is derived from the assumption that local strain in the soft phase is greater than the macroscopic strain because of the presence of the hard phase. With the progressive conversion of hard phase to soft, deformation of the soft phase approaches that of the composite. By empirically determining the relation between the strain amplification factor and maximum load, this model reproduced the stress-strain relation in response to a stretch history of progressively increasing maximum load for a variety of polymer materials such as buta-n, neoprene, and silicon rubber. Additionally, these investigators showed that the strain amplification model also explained the decrease in frequency of the transverse vibrations of a rubber string with progressive strain softening.

Johnson and Beatty attempted to explain the structural mechanism of the Mullins effect by suggesting that the hard phase might be interpreted as clusters of molecular chains held together by short chain clusters. As the material is deformed, chain segments are pulled from the clusters of chain molecules and hard regions are made soft through separation of chain segments from these clusters. Separation of chain segments within the rubber matrix may involve permanent disruption of intermolecular cross-links, whereas cross-linking in tissues is dynamic and hence the Mullins effect is likely to be temporary. Scission and reformation of cross-links in elastomers has long been recognized as the structural basis for their history-dependent properties [14].

Thus, the strain softening theory of load history-dependent properties of elastomers may provide a framework for understanding preconditioning behavior in resting myocardium, and polymer mechanics may offer insight into the structural basis of myocardial...
preconditioning behavior. This information may in turn reveal how systolic overstretch during acute ischemia initiates structural alterations that lead to diastolic dysfunction.

In the following sections, we describe recent studies from our laboratory on the mechanisms of preconditioning behavior in passive ventricular myocardium. Because passive material properties reflect the composite structure of myocardium, these acute mechanical changes are likely to be the consequences of specific alterations in the tissue architecture. This structural damage may have direct significance to ventricular dilatation and remodeling in diseases. Hence the goal of this work is to test the hypothesis that preconditioning behavior is the result of strain softening both in the whole ventricle and at the local tissue level, and to identify candidate microstructural mechanisms of this response.

Strain Softening in Global Ventricular Mechanics
We have investigated whether strain softening (or the Mullins effect) may explain the reduced left ventricular stiffness previously associated with the strain-history dependent preconditioning phenomenon [18]. Passive pressure-volume relations were measured in the isolated, arrested rat heart during LV balloon inflation and deflation cycles. With inflation to a new higher maximum pressure, the pressure-volume (P-V) relation became less stiff without a significant change in unloaded ventricular volume. Figure 2 depicts typical P-V relations for a loading protocol in which the maximum pressure was increased from 20 mmHg to 30 mmHg. The altered shape of the inflation segment with the new maximum pressure was particularly evident in the low (diastolic) pressure range.

![Figure 2](image)

Left ventricular pressure-volume relations for three cycles for a typical rat heart as the maximum cycle pressure was increased from 30 to 60 mmHg. A change in the shape of the ascending P-V relation consistent with an increase in chamber compliance occurs after the LV experienced a peak cycle pressure (P_{max}) of 60 mmHg for the first time.

To further examine the dependence of increased global compliance on the history of maximum loading, we used three distinct loading protocols in which the pattern of maximum cycle pressure, P_{max}, was varied. When P_{max} was sequentially increased from 10 to 20, 30, 60, 90, and 120 mmHg, the pressure-volume relation became progressively less stiff (Protocol A, Fig 3). When the loading sequence was changed by including three extra loading cycles to P_{max} = 60 mmHg immediately after the cycles to P_{max} = 10 mmHg, the P-V relations for P_{max} = 20 and 30 mmHg fell on the same curve as that of the early P_{max} = 60 mmHg (Protocol B, Fig 3). Finally, when three extra cycles were included to P_{max} = 120 mmHg, all the P-V relations beyond P_{max} = 10 mmHg fell on the same curve (Protocol C, Fig 3). Thus, we found that the decrease in ventricular stiffness seen in the altered P-V relations depended significantly both on the on the history (p<0.05) and magnitude (p<0.01) of maximum previous pressure.

![Figure 3](image)

Pressure-volume relations for a typical heart in protocols A, B, and C. Only the preconditioned ascending limb for each unique maximum pressure is shown. (A) With each new maximum pressure in protocol A, the pressure-volume relation becomes less stiff. (B) The pressure-volume relations in protocol B for maximum pressures of 20 and 30 mmHg fall on the same curve as that of the early maximum pressure of 60 mmHg. The relations become less stiff after the maximum pressure exceeded 60 mmHg. (C) Similarly, all the pressure-volume relations following the early inflation to 120 mmHg lie on the same curve. Note that the volume at zero pressure changed very little. (Reproduced by permission of the American Society of Mechanical Engineers).

To determine if this result could be explained by myocardial viscoelastic behavior, we developed a quasilinear viscoelastic model based on the ventricular pressure-relaxation response to produce nonlinear pressure-volume relations with hysteresis. This model was unable to show any significant change in ventricular stiffness with new maximum pressure. We then incorporated a strain softening theory proposed by Johnson and Beatty [34] into the model by modifying the elastic response with a volume-amplification factor that depended on the maximum previous pressure. This model more accurately reproduced the experimentally observed behavior (Fig. 4). Thus, the preconditioning behavior of the myocardium is better explained by strain softening rather than viscoelasticity and may be due to damage to elastic components, rather than the effects of viscous tissue elements.
(A) Pressure-volume relations based on the viscoelastic model for three loading cycles. Although the relations exhibit hysteresis, their shape did not change following loading to the new maximum pressure to 60 mmHg. (B) Pressure-volume relations of the viscoelastic model incorporating strain softening theory for the same loading history. Similar to experimental results (Fig. 1), the hysteresis increased in the pressure-volume loop during the initial loading to 60 mmHg, and subsequent loading demonstrated an increase in chamber compliance. (Reproduced by permission of the American Society of Mechanical Engineers).

**FIGURE 4**

**Strain Softening in Regional Myocardial Tissue Mechanics**

The corresponding changes in local myocardial mechanics and structure with overstretch, however, were later elucidated by Emery, et al [16]. We measured two-dimensional strain on the left ventricular (LV) epicardium in isolated arrested rat hearts sequentially inflated to increasing peak cavity pressures of 10, 30, and 120 mmHg. Strains at matched LV pressures increased significantly (p < 0.002) as the maximum pressure previously experienced by the LV increased (Fig. 5), but could not be explained by the small changes in the unloaded geometry. Compared with \( P_{\text{max}} = 10 \) mmHg, relative increases in fiber strain for \( P_{\text{max}} = 30 \) and 120 mmHg (100 and 149%, respectively) were significantly greater (p<0.001) than the corresponding increases in cross-fiber (51 and 78%) and fiber shear (57 and 86%) strains.

Using prolate spheroidal finite element model of the rat LV which reliably reproduced experimental strains, progressive decreases in epicardial biaxial wall stiffness up to 87% were found with increasing \( P_{\text{max}} \), but were not different in the fiber and cross-fiber directions.

Thus, although passive ventricular overloading causes direction-dependent increases in epicardial strain, these changes are the consequence of local myocardial softening that is actually independent of direction, at least within the plane of the epicardium. However, this leaves open the possibility that mechanical alterations are anisotropic in planes transverse to the epicardium. One structure that is observed in transverse planes is the laminar sheet structure that organizes myofibrils into layers about 4 cells thick [38]. Therefore, we designed experiments to investigate the hypothesis that these or other structures are involved in myocardial softening.

**FIGURE 5**

Epicardial pressure-strain relations (mean + SE) referred to local fiber and cross-fiber axes in the isolated rat heart for maximum previous left ventricular pressures of 10, 30, and 120 mmHg. A: fiber strain (\( E_f \)); B: cross-fiber strain (\( E_{cc} \)); C: fiber shear strain (\( E_{sf} \)). Data from third loading cycle for each maximum pressure. Pressure-strain relations were significantly altered by the effect of \( P_{\text{max}} (P<0.02) \), but the relative increases in \( E_{cc} \) were significantly greater in the other two components (P<0.001). (Reproduced by permission of the American Physiological Society).

**Structural Consequences of Myocardial Strain Softening**

The manner in which tissue structural components such as myofibrils or extracellular collagen change with myocardial strain softening were investigated by Emery [17]. Quantitative histological methods in light and scanning electron microscopy revealed significant differences in tissue morphology in the unloaded and loaded (10 mmHg) rat left ventricle after softening the LV with passive inflation to 120 mmHg. In the loaded state, mean sarcomere length (SL) was 3.8%, 8.1%, and 11.3% longer in softed tissue than control (P < 0.05) at the epicardial, midwall, and endocardial locations, respectively (Fig. 6). Although softening increased the transmural SL gradient, there were no differences in residual strain as determined by measuring the opening angle of a LV ring (P > 0.6). Softening also did not alter mean unloaded perimyocardial collagen tortuosity (1.143 in control vs. 1.130 in softed; P > 0.4), but did lower the mean tortuosity through the wall with load (e.g., 1.11 vs. 1.05 at midwall; P = 0.06).

The product of SL and tortuosity provided a measure of the coupling between cellular and extracellular matrix deformation. The endocardial region in softed tissue, where inflation strains are highest, was the only location where the mean SL-clotortuosity product increased with load. Histological estimates of myocyte and
collagen "strain" also showed substantial differences between SL and tortuosity deformation at the endocardium.

In longitudinal-radial views from scanning electron microscopy, subjective scores for the integrity of the interlaminar collagen ties connecting sheets of myocytes were significantly lower (P < 0.05) and observations of disrupted ties were more frequent in softened tissue compared with control (Fig 7). Thus, myocardial strain softening, previously observed in myocardium at the global and tissue scales, is manifest as a reduction in stiffness at the microstructural level. Furthermore, it is associated with endocardial mechanical decoupling between the myocyte and perimyselial collagen deformation and may result from stretch-dependent damage to interlaminar collagen ties linking sheets of myocytes.

![Graph showing SL (μm) at different locations](image)

**Transmural Location**

**FIGURE 6**

Sarcomere length distribution (mean + SE) at three midwall locations for control (P<sub>max</sub>=10 mmHg) and softened (P<sub>max</sub>=120 mmHg) myocardial tissue for LV fixation pressure (P<sub>fix</sub>) of 10 mmHg. Also shown is SL for control tissue for fixation pressure of 0 mmHg, corresponding to unloaded reference state.

**Load History Dependence In 3-D Myocardial Mechanics**

While observed decreases in epicardial biaxial stiffness were associated with myocardial overstretch, the microstructural evidence suggests that softening at the epicardium may partially reflect changes with load in the mechanics of deeper layers. In particular, the damage of interlaminar collagen ties we observed [17] suggested that shearing between the laminar sheets may be elevated in overstretched myocardium. Changes in global LV mechanics during passive inflation to new maximum loads may reflect altered transmural deformation of the ventricular wall. Therefore, to relate transmural deformation to elastic structures during passive LV inflation, we [17] measured the pressure-volume relation and three-dimensional (3-D) finite strains in the LV free wall of a canine heart with respect local myocyte fiber and sheet orientations measured by others [10, 38].

As P<sub>max</sub> was increased from 5 to 120 mmHg, there was a progressive increase in the resting volume of the canine heart. However, this increase in cavity size did not fully account for the increase in LV volume at a pressure of 5 mmHg (from 26 ml to 39 ml). The strain component representing shear deformation between laminar sheets in a direction perpendicular to the myofiber direction showed large increases with P<sub>max</sub> of up to 120% and 74% at the subepicardium and midwall locations, respectively, during passive inflation. The normal strain between sheets at the subendocardium increased by over 380% as the maximum pressure was increased. In the unloaded state only the normal strain along the myofiber direction showed progressive increase in permanent strain with increasing overstretch.

Since changes in pressure-volume and pressure-strain relations cannot be fully accounted by the permanent increase in unloaded cavity size, the canine myocardium demonstrated marked global and transmural myocardial softening. Furthermore, the alterations in wall strains appear consistent with a loss of stiffness between laminar sheets. This behavior is also consistent with the hypothesis that collagen ties are damaged during progressive ventricular overstretch.

![SEM images](image)

**FIGURE 7**

SEM images of the longitudinal-radial plane from control (A: x750) and softened (B: x1000) myocardium fixed at 10 mmHg LV pressure. Collagen ties or "struts" connect sheets of myocytes. Disrupted ties (denoted by arrows) were more prevalent in tissue from softened group.

**CONCLUSIONS**

It is clear that myocardial mechanical properties depend on the history of maximum previous load at three scales: the whole ventricle, the local tissue, and myocardial microstructure. Myocardial strain softening is an important consequence of acute multiaxial overstretch of the laminar sheet architecture in the ventricular wall. As outlined in the schematic in Fig. 8, softening is manifested in altered deformation of both the cellular and extracellular matrix in response to load. In particular, it may reflect a loss of myocardial stiffness between laminar sheets consistent with the proposed mechanism in which overstretch disrupts interlaminar collagen ties. Thus, large passive strains in the noncontracting ventricular wall at the onset of acute regional ischemia may contribute to diastolic dysfunction and have important consequences for the subsequent remodeling processes via myocardial strain softening.
Schematic and representation of proposed 3-D microstructure in control and softened myocardium for unloaded and loaded conditions. Schematic is a synthesis from histological data in the rat heart and from 3-D strains measured in the canine heart. With inflation to high maximum loads, interlaminar collagen ties between sheets of myocytes are likely to be disrupted. Upon inflation, interlaminar shearing between myocyte sheets in softened tissue may then be greater than in control tissue.

While our quasilinear viscoelastic model of ventricular filling represented the first attempt to incorporate a theoretical description of strain softening in myocardial mechanics, a model that describes the dependency of 3-D anisotropic material properties on maximum-load history would be more informative. One approach could formulate the material constants in a constitutive law to depend upon the maximum local strain. The question still remains, however, as to which strain component would be most appropriate. This work suggests that sheet shearing strain or sheet-to-sheet normal strain may be likely candidates.

Application of other histological methods to study softened myocardium may verify the decoupling of cellular and extracellular deformation and damage of interlaminar ties with overstretched. It remains a distinct possibility that intracellular structures such as the titin-myosin complex or intramolecular collagen crosslinks [47] may also be disrupted and contribute to the loss of myocardial stiffness. Techniques such as immunohistochemical epitope tagging of titin domains [25] could address the former, while cyanogen bromide digestion of salt-extracted collagen fragments [33] may be appropriate for studying the latter.

Future efforts to relate myocardial softening with systolic overstretch during acute ischemia should measure 3-D local transmural strains. The trends in transmural deformation with overstretch suggest that ventricular softening may not actually be evident in epicardial surface strains in the dog. Thus, an approach similar to that of Villarreal et al [66], in which diastolic filling strains during venae cavae occlusion were determined from columns of beads placed in ischemic and non-ischemic sites, would likely be more informative. After brief episodes of myocardial ischemia, changes in diastolic transmural strains with respect to fiber and sheet orientation could then be correlated with the magnitude of end-systolic strains as well as histological evidence of tissue damage.

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REFERENCES

18. Emery, JL, JH Omens, and AD McCulloch. Strain softening in


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