Increased Diastolic Chamber Stiffness During Demand Ischemia
Response to Quick Length Change Differentiates Rigor-Activated From Calcium-Activated Tension

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Background—Increased diastolic chamber stiffness (↑DCS) during angina (demand ischemia) has been postulated to be generated by increased diastolic myocyte calcium concentration.

Methods and Results—We reproduced demand ischemia in isolated isovolumically contracting red-cell–perfused rabbit hearts by imposing pacing tachycardia during global low coronary blood flow (32% of baseline). This increased lactate production without increasing oxygen consumption and resulted in ↑DCS (isovolumic left ventricular end-diastolic pressure [LVEDP] increased 10 mm Hg, *P*<0.001, n=38). To determine the mechanism of ↑DCS, we assessed responses to a quick-stretch-release maneuver (QSR), in which the intraventricular balloon was rapidly inflated and deflated to achieve a 3% circumferential muscle fiber length change. QSR was first validated as an effective method of discriminating between calcium-driven and rigor-mediated ↑DCS. QSR imposed during demand ischemia when DCS had increased (LVEDP pretachycardia versus posttachycardia, 15±1 versus 27±2 mm Hg, *P*<0.001, n=6) reduced DCS to pretachycardia values (LVEDP post-QSR, 15±1 mm Hg, *P*<0.001), ie, elicited a response characteristic of rigor, without any component of calcium-generated tension.

Conclusions—A rigor force, possibly resulting from high-energy phosphate depletion and/or an increase in ADP, appears to be the primary mechanism underlying increased DCS in this model of global LV demand ischemia. (Circulation. 2000;101:2185-2192.)

Key Words: diastole ■ angina ■ rigor
a stable LVDP of 20 mm Hg. This relatively high LVDP was selected because (1) a high LVDP is frequent in heart failure associated with coronary artery disease; (2) the heart is functioning on the steep part of the pressure-volume curve, and any changes in diastolic chamber stiffness might be more apparent; and (3) a higher LVDP producing an increase in wall stress served to enhance the metabolic demand aspect of this model.

**Demand Ischemia**

Demand ischemia was reproduced by imposition of combined ischemia and tachycardia to increase metabolic demand ($n = 38$).

To impose ischemia, coronary blood flow was reduced to achieve a coronary perfusion pressure of 20 mm Hg, and flow was then held constant at this level. This degree of coronary perfusion pressure is comparable to the perfusion pressure distal to severe coronary stenoses in patients with angina.$^{21}$

To increase metabolic demand, the pacing rate was increased to 7 Hz, ie, tachycardia. When isovolumic LVDP had risen 10 mm Hg above the pretachycardia level, indicating a physiologically significant increase in diastolic chamber stiffness, tachycardia was terminated (pacing rate returned to 3 Hz), but reduced coronary flow (at a constant rate) continued. Then, to demonstrate reversibility of increased diastolic chamber stiffness, coronary flow was returned to baseline rates 5 minutes after tachycardia termination ($n = 7$).

Two separate control groups were studied to validate the above model of demand ischemia. Hearts in 1 group, tachycardia without ischemia, underwent 30 minutes of tachycardia (7 Hz) while coronary blood flow was allowed to increase to maintain a constant coronary perfusion pressure of 80 mm Hg, ie, increased metabolic demand without accompanying ischemia ($n = 6$). In the other group, ischemia without tachycardia, hearts underwent 30 minutes of underperfusion at a constant coronary flow rate that elicited a coronary perfusion pressure of 20 mm Hg while pacing was continued at a baseline rate of 3 Hz, ie, no increased metabolic demand was imposed ($n = 6$).

To determine whether an increase in LVDP was related to reduced ATP, ATP content in hearts subjected to demand ischemia ($n = 5$) and freeze-clamped when LVDP had increased 10 mm Hg was compared with that in hearts subjected to ischemia without tachycardia ($n = 5$) and freeze-clamped at time points matched to an equivalent duration of ischemia for each heart in the demand ischemia group.

In all experiments, hemodynamic and metabolic measurements were obtained every 5 minutes.

**Quick-Stretch-Release**

First, QSR was delivered at baseline to assess whether the maneuver itself would have any effects on function (normoxia, $n = 7$). Then, QSR was validated as an effective method of distinguishing between rigor-mediated and calcium-driven increases in diastolic chamber stiffness in the isolated heart. Rigor contracture was created by imposing sustained zero-flow ischemia after initial stabilization (zero-flow ischemia, $n = 6$). When LVDP had increased $\sim 10$ mm Hg, QSR was performed.

Quick-Stretch-Release was then performed in a model in which increased diastolic chamber stiffness was produced by a mechanism known to be generated by diastolic calcium persistence ($n = 6$). After initial stabilization, LV balloon volume was adjusted to produce an LVDP of 16\(\pm\)1 mm Hg, but thereafter volume was not changed. An intracoronary infusion of 5 mmol/L caffeine and 5 mmol/L calcium chloride was then commenced under normoxic conditions. Caffeine impairs sarcoplasmic reticular calcium reuptake and increases intracellular diastolic calcium, resulting in slowed and incomplete relaxation.

In the presence of caffeine, calcium involved in continued contractile activity is handled predominantly by the sodium-calcium exchanger.$^{19,20}$ Additional calcium loading during caffeine exposure further increased diastolic chamber stiffness. When isovolumic LVDP had increased $\sim 10$ mm Hg, QSR was performed, and then calcium and caffeine infusions were terminated.

Finally, to elucidate the mechanism of increased diastolic tension in demand ischemia, QSR was applied during the prolonged diastole immediately after tachycardia termination when LVDP had increased $\sim 10$ mm Hg during the demand ischemia protocol described above (demand ischemia, $n = 6$).

**Statistical Analysis**

Data are reported as the mean\(\pm\)SEM. Data acquired by repeated sequential measurements in individual hearts were tested by ANOVA for repeated measures. Statistical comparisons between groups were performed by 2-way ANOVA. If overall ANOVA
Results

Demand Ischemia

Hemodynamic Characteristics

In hearts subjected to demand ischemia (n=38), a decrease in coronary perfusion pressure from 80 ± 20 mm Hg (reduction in coronary blood flow from 1.05 ± 0.03 to 0.34 ± 0.01 mL · min⁻¹ · g LV wet wt⁻¹, P < 0.001) reduced LV systolic pressure (LVSP) from 123 ± 2 to 63 ± 2 mm Hg (P < 0.001) and LVEDP from 20 to 17 ± 1 mm Hg (P < 0.001), representing a loss in coronary vascular turgor (Figures 1 and 2a). Results from a typical experiment are illustrated in Figure 1. Imposition of tachycardia caused an immediate small increase in isovolumic LVEDP (incomplete relaxation), followed by a more gradual increase in LVEDP. A rise in LVEDP occurred in all hearts (mean, 15.3 ± 0.9 minutes per 10 mm Hg increase). After LVEDP had increased 10 mm Hg, tachycardia was terminated (pacing rate returned to 3 Hz), but coronary flow continued at its ischemic level. In this phase, LVEDP remained elevated by 7 ± 1 mm Hg compared with pretachycardia levels (P<0.001), representing a stable increase in diastolic chamber stiffness resulting from increased metabolic demand during low-flow perfusion.

Reversibility

In hearts undergoing reperfusion (n=7), LVEDP returned to baseline (22 ± 2 mm Hg) and LVSP recovered (88 ± 4 mm Hg) within 5 minutes, indicating the rapidly reversible nature of the ischemic increase in diastolic chamber stiffness.

Hearts subjected to tachycardia without ischemia or to ischemia without tachycardia demonstrated no significant increase in LVEDP (Figure 2a). Thus, neither tachycardia alone nor low-flow ischemia alone was sufficient, but the combination, ie, demand ischemia, resulted in increased diastolic chamber stiffness.

Metabolic Characteristics

With the onset of ischemia (demand ischemia and ischemia without tachycardia groups), hearts switched from net myocardial lactate consumption to net lactate production (Figure 2b and 2c). In ischemia without tachycardia, lactate production then remained constant. In contrast, in demand ischemia, tachycardia further increased lactate production (demand ischemia, 0.45 ± 0.03 versus ischemia without tachycardia, 0.12 ± 0.03 [μmol/L] · mL⁻¹ · min⁻¹ · g LV wet wt⁻¹, P < 0.005). Lactate production was also greater in demand ischemia than in hearts subjected to tachycardia without ischemia.

Oxygen consumption decreased in both demand ischemia and ischemia without tachycardia. Imposition of tachycardia in demand ischemia did not alter oxygen consumption. In tachycardia without ischemia oxygen, conversely, consumption increased during tachycardia. Hence, hearts in demand ischemia in which coronary flow was restricted were unable to increase oxygen consumption when energy demand was increased by tachycardia, in contrast to the group in which coronary flow was allowed to increase commensurately with increased metabolic demand.

ATP Content

We have previously reported a baseline ATP content of 18.01 ± 2.00 μmol/L ATP/g LV dry wt in this experimental preparation.17 Hearts subjected to demand ischemia (isovolumic LVEDP pretachycardia versus posttachycardia [15 ± 2 minutes], 17 ± 1 versus 26 ± 1 mm Hg, P < 0.001) had an ATP content of 9.12 ± 1.76 (μmol/L)/g LV dry wt, ie, a 50% reduction at the point at which LVEDP had increased 10 mm Hg. In ischemia without tachycardia (ischemia duration, 14 ± 3 minutes), LVEDP remained unaltered, and ATP content was 10.78 ± 0.94 (μmol/L)/g LV dry wt (P=NS versus demand ischemia). Thus, these 2 groups did not differ in end-ischemic [ATP], despite the imposition of tachycardia and the development of increased diastolic chamber stiffness in the demand ischemia group.

Quick-Stretch-Release

Groups of hearts in which QSR was performed had similar baseline hemodynamic characteristics before interventions were performed (Figures 3 through 7). QSR at baseline...
(normoxia, n=7) did not affect function (pre-QSR versus post-QSR: LVEDP, 20±1 versus 19±1 mm Hg, P=NS; LVSP, 122±3 versus 121±4 mm Hg, P=NS) (Figure 3).

With zero-flow ischemia (n=6), hearts rapidly became asystolic and LVEDP initially decreased (16±1 mm Hg) (Figure 4; Reference 21). During sustained ischemia (18±4 minutes), LVEDP progressively increased, resulting in ischemic contracture (“classic rigor”). QSR at this point instantly lysed this rigor tension (LVEDP pre-QSR versus post-QSR, 27±1 versus 17±1 mm Hg, P<0.001), ie, LV diastolic pressure decreased to precontracture values with no tension recovery.

When increased diastolic chamber stiffness occurring from increased cytosolic diastolic calcium was created by intracoronary infusion of caffeine and calcium chloride (n=6, Figure 5), LVEDP increased from a baseline of 16±1 to 28±1 mm Hg (P<0.001). QSR imposed at this point had no effect on increased diastolic tension or systolic function (pre-QSR versus post-QSR: LVEDP, 27±1 versus 26±1 mm Hg, P=NS; LVSP, 111±5 versus 109±5 mm Hg,

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**Figure 3.** QSR at baseline. Coronary artery perfusion pressure (CPP) was 85 mm Hg (normoxia). LVEDP before and immediately after QSR was 20 mm Hg, ie, unaffected. LVSP and differentiated pressure (dP/dt) increased slightly for a few cycles after QSR before returning to baseline.

**Figure 4.** QSR during classic rigor. Zero-flow ischemia (coronary perfusion pressure [CPP] of zero) resulted in contractile failure (LVSP of zero). An initial reduction in LVEDP from 20 to 17 mm Hg was followed by a progressive increase, ie, increased diastolic chamber stiffness due to rigor contracture. When LVEDP had increased from 17 to 27 mm Hg, QSR caused immediate reduction to 17 mm Hg, ie, LVEDP returned to precontracture values. Note different paper speeds. During development of contracture, speed is 0.05 mm/s.
P=NS). LVEDP, however, returned to baseline values on termination of infusion. Hence, the different responses of increased LVEDP to QSR between rigor bonds in classic rigor compared with a calcium-driven mechanism validated QSR as a method of discriminating rigor- versus calcium-mediated increases in diastolic chamber stiffness.

In demand ischemia, tachycardia was terminated after LVEDP had increased from 15±1 to 27±2 mm Hg (P<0.001, n=6). QSR subsequent to tachycardia immediately lysed increased diastolic tension (LVEDP pre-QSR versus post-QSR, 27±2 versus 15±1 mm Hg, P<0.001), ie, chamber stiffness returned to baseline (Figure 6). The decrement of LVEDP produced by QSR was identical in magnitude to the upward shift of isovolumic LVEDP sustained during pacing tachycardia. Hence, QSR during demand ischemia elicited a response similar to that with rigor contraction associated with zero-flow ischemia but unlike that with calcium-activated increased diastolic tension (Figure 7).

**Discussion**

The acute and reversible decrease in diastolic distensibility during angina due to sustained actin-myosin interaction during diastole may be related to myocardial ATP depletion (with a concomitant increase in ADP) resulting in rigor, to diastolic persistence of an increased intracellular calcium concentration, or to a combination of the 2. In the present

![Figure 5](image-url) QSR during a calcium-driven increase in diastolic chamber stiffness. Caffeine and calcium (Ca++) during normoxic perfusion (coronary perfusion pressure [CPP] 80 mm Hg) increased contractile function (LVSP increased from 110 to 120 mm Hg and isovolumic LVEDP from 12 to 25 mm Hg). LVEDP was unaffected by QSR but immediately decreased to 13 mm Hg when infusions were terminated.

![Figure 6](image-url) QSR during demand ischemia. Ischemia (coronary perfusion pressure [CPP] 20 mm Hg) decreased LVSP to 61 mm Hg and LVEDP to 14 mm Hg. Tachycardia increased isovolumic LVEDP from 14 to 26 mm Hg, ie, increased diastolic chamber stiffness. QSR imposed after tachycardia immediately reduced LVEDP to 14 mm Hg, ie, to precontracture values.
study using a model simulating some of the features of demand ischemia during angina, responses to quick length changes support a rigor mechanism, without involvement of a calcium-driven tension.

In this model, we reproduced reversible diastolic dysfunction with demand ischemia: isovolumic LVEDP increased with combined low-flow ischemia and tachycardia but not with either ischemia or tachycardia alone and was characterized by increased lactate production with an inability to increase oxygen consumption (Figures 1 and 2). Here, the globally ischemic LV served to model the regionally ischemic region in a patient with angina or a large-animal model with single coronary artery stenosis. This, by imposing homogeneous conditions throughout the LV, facilitated determination of mechanisms underlying diastolic dysfunction. However, reduction of coronary artery perfusion pressure before tachycardia, with a marked global reduction in coronary artery flow, resulted in profound contractile dysfunction. Systolic dysfunction of this degree does not usually accompany regional ischemia or angina unless there is also severe global ischemia, eg, left main or 3-vessel disease, severe aortic stenosis, or systemic hypotension and tachycardia. Hence, our model does not simulate all the hemodynamic features observed during regional demand ischemia in humans or large-animal models (in which resting coronary flow in a stenotic segment and contractile function may remain unchanged), and this limitation prevents direct extrapolation of our results to clinical angina.

Quick-Stretch-Release

The QSR maneuver, in which a sudden increment (1% to 10%) in length is followed by a rapid return to baseline length, has been used to distinguish between calcium-activated and rigor tension in unstimulated skeletal16 and papillary15 muscle. After QSR, rigor bonds are characterized by tension lysis and by failure of immediate tension redevelopment, so that poststretch tension remains markedly reduced relative to the prestretch level. In contrast, QSR imposed on muscle with continuous calcium-activated cross-bridge cycling is followed by incomplete lysis and by a rapid redevelopment of tension to its prestretch value.

Here, QSR was similarly applied in the actively contracting isolated heart without deleterious effects (Figure 3). During tonic contracture due to classic ischemic rigor, QSR caused immediate, complete, and sustained lysis of diastolic tension (Figure 4), but when an increase in diastolic chamber stiffness was driven by diastolic persistence of calcium, QSR failed to alter diastolic tension (Figure 5).

Thus, we could examine effects of QSR under specific conditions in which diastolic dysfunction occurred in contracting hearts. We hypothesized that in demand ischemia, if increased LVEDP were produced by persistent cross-bridge cycling, equivalent to a state of sustained partial systole due to diastolic persistence of increased calcium, then QSR would cause no significant lysis of diastolic tension. Conversely, if rigor force were responsible, QSR should effectively lyse this tension. If a combination of these mechanisms were operative, then an intermediate response would be predicted. In our model, QSR produced complete lysis of increased diastolic tension resulting from demand ischemia, a behavior typical of rigor without any component of a calcium-driven tension (Figures 6 and 7).

Subcellular Mechanisms of Increased Diastolic Tension

The subcellular mechanisms underlying increased chamber stiffness in demand ischemia have received relatively little study. One report proposed a mechanism of increased diastolic myocyte calcium concentration based on the observation that exposure to caffeine during the last 30 seconds of pacing tachycardia exacerbated the degree of increased diastolic chamber stiffness sustained during demand ischemia.6 However, this conclusion is confounded because caffeine itself may have contributed importantly to cytosolic calcium overload. For example, increased diastolic chamber stiffness can occur in normoxic hearts on exposure to caffeine: in our experimental model (Figure 5), caffeine increased LVEDP, which was further exaggerated by superimposed calcium loading. Thus, the observation that caffeine augmented an increase in ischemic diastolic stiffness does not prove that the initial ischemia-induced increase in stiffness itself was calcium-driven.

In contrast, many previous studies have investigated the mechanism of ischemic contracture in a variety of models, eg, hearts in situ or subjected to hypoxia or zero-flow ischemia, or isolated muscle strips or myocytes subjected to metabolic inhibition. Under these conditions, an increase in diastolic calcium level has been widely reported,7,8 consistent with a calcium-driven mechanism for the contracture. This remains an appealing explanation, although no cause-and-effect relationship has been definitively established. Others report no correlation between increased myocyte calcium and increased diastolic tension9,22 and favor an alternative mechanism of rigor.5–11,23 These models, however, comprise a heterogeneous group of ischemic states, and their results may not be readily extrapolated to the demand ischemia of clinical angina.
Our result of diastolic tension lysis by QSR during demand ischemia supports a rigor mechanism secondary to ATP depletion as the basis of increased diastolic chamber stiffness. However, we could not demonstrate a lower average tissue [ATP] in hearts subjected to demand ischemia (in which an increase in diastolic chamber stiffness occurred) compared with hearts subjected to similar ischemia but without tachycardia, in which no increase in diastolic tension occurred. In both groups, [ATP] decreased by only 50%. Thus, we could not correlate the increase in ischemic diastolic tension with total tissue ATP depletion.

However, these ATP measurements do not rule out rigor tension as the mechanism for the increase in ischemic diastolic chamber stiffness. Rigor tension may be generated in the presence of only modest reductions in [ATP] when [ADP] increases and may be correlated with increased diastolic stiffness. We cannot be certain, however, that [ADP] increased in this demand ischemia model, because it did not increase significantly in other studies of low-flow ischemia from our laboratory. Nevertheless, even severe ATP depletion occurring in only a small group of myocytes would be undetected by measurements of total tissue [ATP]. During demand ischemia, a population of more severely energy-deprived myocytes vulnerable to rigor may be interspersed among normally contracting cells. Experiments in isolated myocytes have consistently demonstrated inexcitability and contractile failure at the time of rigor shortening. Thus, in the isolated heart undergoing demand ischemia, the continued development of phasic contractile force when diastolic pressure is elevated is consistent with the idea that some myocytes are not in a rigor state and are capable of contracting, whereas others are in a rigor state and are inexitable. In isolated hearts, cell-by-cell electron microscopy revealed a highly heterogeneous distribution of development of ischemic contracture when diastolic chamber stiffness had increased during low-flow ischemia. The extent of diastolic chamber stiffness increase may be related to the number of myocytes in rigor, which may progressively increase with continued demand ischemia (Figure 1). Contracture may be reversible, as demonstrated here when the supply-demand mismatch was corrected and as observed during reoxygenation of anoxic myocytes.

Characteristics of the Model

Our experimental preparation confers many advantages for modeling demand ischemia. The right ventricle is decompressed and the pericardium freed, which eliminates interactions with the LV. The heart is subjected to global underperfusion, which prevents the confounding mechanical influence of dysynchronous contraction of ischemic and nonischemic segments associated with regional ischemia. The isovolumic preparation allows the use of QSR as an investigative tool. Use of a red-cell perfusate at 37°C containing glucose (5.5 mmol/L) and free fatty acid at a normal ratio of FFA to albumin provides normal levels of the major myocardial substrates and ensures a normal rate of oxygen delivery at physiological coronary flow rates. Elucidation of diastolic dysfunction during demand ischemia in isolated heart appears to require a critical interplay and relationship between coronary flow, energy supply, and energy demand. For example, we have found it impossible to reproduce demand ischemia–induced increases in LVEDP in hearts perfused with crystalloid solutions or at temperatures <37°C.

In summary, in this model of demand ischemia in the isolated heart in which we simulated features of diastolic anginal physiology, responses to quick length changes supported a mechanism of a reversible rigor-like tension underlying increased diastolic chamber stiffness and not a calcium-driven force.

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References


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