Profound bioenergetic abnormalities in peri-infarct myocardial regions

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Using a porcine model of postinfarction LV remodeling, we previously observed modest reductions of ATP and the phosphocreatine (PCr)-to-ATP ratio in the remote noninfarcted myocardium of animals with compensated remodeling but marked abnormalities in animals that developed overt CHF (41). The levels of mitochondrial F1F0-ATP synthase (mtATPase; the final reaction in the pathway that links carbon substrate utilization to oxidative ATP synthesis) in myocardium remote from the infarct were also decreased in animals that developed overt CHF but not in animals with compensated postinfarction LV remodeling (19). We hypothesized that the chronically elevated systolic wall stress in the border zone surrounding a myocardial infarct results in progressive abnormalities of oxidative phosphorylation and that the energetic abnormalities in this border zone can ultimately expand to involve the entire LV, thereby leading to global LV dysfunction and the development of CHF. If this is true, then during the period of compensated remodeling the border zone should demonstrate bioenergetic alterations characteristic of failing myocardium, while the remote region would show only modest alterations typical of remodeled myocardium. To evaluate this hypothesis, we compared myocardial high-energy phosphates (HEP) and PCr-to-ATP ratios in the peri-infarct border zone and remote myocardium by using 31P magnetic resonance spectroscopy (MRS) in swine 6 wk after ligation of the left anterior descending coronary artery (LAD) distal to the second diagonal. Animals developed compensated LV remodeling with a decrease of ejection fraction from 54.6 ± 5.4% to 31 ± 2.1% (MRI) 5 wk after LAD occlusion. The remote zone (RZ) myocardium demonstrated modest decreases of ATP and mtATPase components. In contrast, BZ myocardium demonstrated profound abnormalities with ATP levels decreased to 42% of normal, and phosphocreatine-to-ATP ratio (31P magnetic resonance spectroscopy) decreased from 2.06 ± 0.19 in normal hearts to 1.07 ± 0.10, with decreases in α, β, OSCP, and IF1 subunits of mtATPase, especially in the subendocardium. The reduction of myocardial creatine kinase isoform protein expression was also more severe in the BZ relative to the RZ myocardium. These abnormalities were independent of a change in mitochondrial content because the mitochondrial citrate synthase protein level was not different between the BZ and RZ. This regional heterogeneity of ATP content and expression of key enzymes in ATP production suggests that energetic insufficiency in the peri-infarct region may contribute to the transition from compensated LV remodeling to congestive heart failure.

METHODS

All experimental procedures were performed in accordance with the animal use guidelines of the University of Minnesota, and the experimental protocol was approved by the University of Minnesota Research Animal Resources Committee. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85–23, Revised 1985).

Infarct production by coronary artery ligation. Details of the animal model of postinfarction LV remodeling have been described previously (19, 41). Briefly, young Yorkshire swine (45 days old, ~10 kg) were anesthetized with pentobarbital (30 mg/kg iv), intubated, and ventilated with a respirator with supplemental oxygen. Arterial blood gases were maintained within the physiological range by adjustments of the respiratory settings and oxygen flow. A left thoracotomy was performed, and 0.5 cm of the LAD distal to the second diagonal

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branch was dissected free and totally occluded with a ligature. After coronary ligation, animals were observed in the open-chest state for 60 min. When ventricular fibrillation (VF) occurred, electrical defibrillation was performed immediately. The chest was then closed, but if the heart was dilated, the pericardium was left open. The animals were given standard postoperative care, including analgesia, until they ate normally and resumed full activity. Animals were returned to the laboratory 6 wk later for the final physiological and 31P-MRS study.

MRI protocol. MRI was performed ~5 wk after surgery on a 1.5-T clinical scanner (Siemens Sonata, Siemens Medical Systems, Isen, NJ) by using a phased-array, four-channel surface coil and ECG gating. Animals were anesthetized with 1% isoflurane and positioned in the supine position in the scanner. The protocol consisted of 1) localizing scouts to identify the long and short axis of the heart, 2) short- and long-axis cine for measurement of global cardiac function, 3) short-axis imaging with myocardial tagging in three slices for measurement of regional myocardial strain, and 4) delayed contrast enhancement for the assessment of scar size. Steady-state free precession True-FISP cine imaging used the following MR parameters: repetition time (TR) = 31 ms, echo time (TE) = 1.6 ms, flip angle = 79°, matrix size = 256 × 120, field of view = 340 mm × 265 mm, slice thickness = 6 mm (4-mm gap between slices); 16–20 phases were acquired across the cardiac cycle. Global function and regional wall thickness data were computed from the short-axis cine images by using MASS (Medis Medical Imaging Systems, Leiden, The Netherlands) for manual segmentation of the endocardial and epicardial surfaces at end diastole and end systole from base to apex.

The tagging preparation consisted of nonselective radiofrequency pulses separated by encoding gradients for spatial modulation of magnetization (SPAMM), resulting in a tag line separation of 6 mm. Three short-axis slices were prescribed at the basal, midventricular, and apical levels identical to the cine image positions. At each slice location two sets of cine images were acquired with tag lines in orthogonal directions with the following scan parameters: TR = 6.5 ms, TE = 2.1 ms, flip angle = 14°, matrix size = 256 × 128, field of view = 320 mm × 320 mm, slice thickness = 6 mm, and a minimum of 14 cardiac phases. Tagged images were acquired for resting conditions only. Tagging data were analyzed by using the HARP analysis package (HARP version 2.0, Nael Osman, Johns Hopkins Medical School) as described elsewhere (25, 26). Two-dimensional myocardial strains were assessed offline in six circumferential myocardial segments per short-axis slice. Transmural strains were calculated between the end-diastolic and end-systolic state as the fractional change in length in the circumferential direction [systolic shortening fraction, SS% = 100 × (l_s - l_d)/l_d, where l_s = LV segment length_{diastole} (in mm), and l_d = LV segment length_{systole} (in mm)] and radial direction (systolic thickening fraction, ST%).

After acquisition of the tagged images, a bolus of 0.20 mmol/kg gadopentetate dimeglumine (Magnevist, Berlex, Monteville, NJ) was administered, and segmented inversion-recovery turbofast low-angle shot (turboFLASH) images were acquired after a 10- to 15-min delay to identify regions of myocardial scar (30). Short-axis turboFLASH imaging, from base to apex, used TR = 16 ms, TE = 4 ms, inversion time (TI) ~220 ms, flip angle = 30°, matrix size = 256 × 148, field of view = 320 mm × 185 mm, slice thickness = 6 mm (0-mm gap between slices), and two signal averages. The appropriate TI was chosen to adequately null the signal intensity of normal myocardium. Infarct size was calculated from the delayed contrast-enhanced images by using MASS to manually segment regions of nonviable tissue. Total infarct volume was calculated as the summation of the scar area from all contrast-enhanced images, and infarct size was reported in grams of infarcted myocardium (infarct volume × 1.05 g/ml) for each animal. Myocardial infarct extent was determined as the ratio of infarct mass to LV mass.

Surgical preparation. Eight animals with LV remodeling and seven size-matched normal animals were anesthetized with α-chloralose (100 mg/kg followed by a 20 mg·kg⁻¹·h⁻¹ iv), intubated, and ventilated with a respirator and supplemental oxygen. Arterial blood gases were maintained within the physiological range by adjustments of the ventilator settings and oxygen flow. A 3.0-mm-OD heparin-filled polyvinyl chloride catheter was introduced into the right femoral artery and advanced into the ascending aorta. A sternotomy was performed, and the heart was suspended in a pericardial cradle. A second heparin-filled catheter was introduced into the LV through the apical dimple and secured with a purse-string suture. A similar catheter was inserted into the left atrium through the atrial appendage. A bipolar pacing electrode was sutured onto the right atrial appendage. A 25-mm-diameter NMR surface coil was sutured onto the anterior wall of the LV adjacent to the infarct region. The pericardial cradle was then released, and the heart was allowed to assume its normal position in the chest. The surface coil leads were connected to a balanced, tuned external circuit, and the animals were positioned within the magnet.

Spatially localized 31P NMR spectroscopy technique. Spatially localized 31P NMR spectroscopy was performed using the RAPP-ISIS/FSW method (7, 10, 11, 18, 39, 40), which is the rotating-frame experiment using adiabatic plane-rotation pulses for phase modulation (RAPP)-imaging-selected in vivo spectroscopy (ISIS)/Fourier series window (FSW) method.

Detailed experiments documenting voxel profiles, voxel volumes and spatial resolution attained by this method have been published previously (18). In this application of RAPP-ISIS/FSW, the signal origin was first restricted to a 12 × 12 mm² two-dimensional column perpendicular to the LV wall. The signal was later localized into three well-resolved and five partially resolved layers along the column and, hence, across the LV wall. Localization along the column was based on B1 phase encoding and employed a 9-term Fourier series window as previously described (18). Whole wall spectra were obtained with the ISIS technique, defining a column 12 × 12 mm² perpendicular to the heart wall. The calibration of spectroscopic parameters was facilitated by placing a polyethylene capillary filled with 15 μl of 3 M phosphonoacetic acid into the inner diameter of the surface coil. This phosphonoacetic acid standard was used only for calculating the 90° pulse length of the RAPP-ISIS method (10, 11, 18). The position of the voxels relative to the coil was set according to the B1 strength at the coil center, which was experimentally determined in each case by measuring the 90° pulse length for the phosphonoacetic acid standard contained in the reference capillary at the coil center. NMR data acquisition was gated to the cardiac and respiratory cycles by using the cardiac cycle as the master clock to drive both the respirator and the spectrometer as previously described (40). The surface coil was constructed from a single-turn copper wire 25 mm in diameter with each side of the coil leads soldered to a 33-pF capacitor. Complete transmural data sets were obtained in 10-min time blocks by using a TR of 6–7 s to allow for full relaxation for ATP and PCr, and ~95% relaxation of the PCr resonance (18, 40).

The ratios of PCr to ATP were calculated for each transmurally differentiated spectra set as previously described (7, 10, 18, 39, 40). All resonance intensities were quantified by using integration routines provided by the SISCO software.

Calculation of myocardial free ADP level. Myocardial free ADP levels were calculated from the creatine kinase (CK) equilibrium expression (17), where $K_\text{eq} = 1.66 \times 10^7$ and cytosolic pH $7.1$: [ADP] = ([ATP] × [Cr_{free}])/([PCr] × [H^+] × K_\text{eq})$, where [Cr_{free}] is the concentration of free creatine. PCr and ATP values obtained from the spectra were calibrated from chemically determined ATP levels measured from the biopsy. [Cr_{free}] was calculated by subtracting the PCr values from total creatine measured in the biopsy.

1H NMR spectroscopy technique. We have recently reported the 1H NMR methods in detail (3, 23). In brief, radiofrequency transmission and signal detection were performed with the dual-tuned 28-mm-diameter surface coil. A single-pulse-collection sequence with a frequency-selective Gaussian excitation pulse (1 ms) was used to selectively excite the N-δ proton resonance signal of the proximal...
histidine in deoxymyoglobin (Mb-δ). This technique provided sufficient water suppression because of the large chemical shift difference between water and Mb-δ (>14 kHz). The NMR signal was optimized by adjusting the radiofrequency pulse power by using the water signal as a reference. A short TR (25 ms) was used due to the short longitudinal relaxation time (T1) of Mb-δ. Each spectrum was acquired in 5 min (10,000 free induction decays). Although the short T1 of Mb-δ and fast acquisition prevent gating to the cardiac cycle, the signal loss due to motion is negligible compared with the inherently broad line width of the Mb-δ peak.

**Hemodynamic measurements.** Aortic and LV pressures were measured using Spectromed TNF-R pressure transducers positioned at midchest level (39, 40, 41). All data were recorded on an eight-channel Coulbourn R14–28 direct-writing recorder.

**Myocardial blood flow measurements.** Myocardial blood flow was measured by using radionuclide-labeled microspheres (15-μm diameter) as previously described (39, 40, 41).

**Mitochondrial ATPase subunit levels.** Protein levels of F1F0-ATPase complex α-, β-, γ, OSCP, and IF1 subunits (EC number 3.6.3.14) were examined as previously described (1, 19, 32). Briefly, frozen heart samples (~100 mg) were ground in a liquid nitrogen-cooled mortar and added to 1 ml of ice-cold buffer (0.25 M sucrose, 50 mM Tris·HCl, 10 mM sodium azide, pH 8.5). The samples were homogenized and incubated for 1 h at room temperature (RT). Samples were centrifuged at 10,000 g for 10 min at RT. The supernatants were analyzed by Western blot, and protein concentrations were determined by a modified Lowry analysis (Sigma, Protein Assay Kit). Antibodies against specific subunits of the mtATPase (7B3 anti-α-subunit, 19D3 anti-β-subunit, 2B1B1 anti-OSC subunit, and 2H10 anti IF1 subunit) were produced as previously described (19).

**Myocardial CK isozyme and citrate synthase measurements.** Specimens from three hearts of each group were used to examine myocardial protein expression levels of CK isozymes by Western blot as previously described (37). A parallel Western blot analysis was performed. The protein extracts were run on 12% SDS-PAGE gels for 3 h at 180 V by using the Protean Electrophoresis apparatus (Bio-Rad). Commercially prepared molecular weight standards and purified proteins [CK-M and CK-mitochondrial isoform (CK-Mito); Aalto] were run as controls. The protein subunits were transferred for 1 h at 250 mA in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol). Goat polyclonal antibodies specific to CK-M and CK-Mito (Santa Cruz Biotechnology, Santa Cruz, CA) were sequentially directed against their respective protein subunits bound to the membrane. Anti-porcine citrate synthase monoclonal antibody (US Biological, Swampscott, MA) was directed against its protein bound to the membrane. Densitometry of the film allowed for relative quantitation of the protein subunits and citrate synthase.

**Study protocol.** Ventilation rate, volume, and inspired oxygen content were adjusted to maintain physiological values for arterial PO2, PCO2, and pH. Aortic and LV pressures were monitored continuously throughout the study. Myocardial blood flow and hemodynamic measurements were acquired simultaneously with the 1H and 31P MR spectra. After spectra were obtained in the peri-infarct zone, myocardial biopsy was performed. The protein extracts were run on 12% SDS-PAGE gels for 3 h at 180 V by using the Protean Electrophoresis apparatus. The proteins were transferred for 1 h at 250 mA in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol). Goat polyclonal antibodies specific to CK-M and CK-Mito (Santa Cruz Biotechnology, Santa Cruz, CA) were sequentially directed against their respective protein subunits bound to the membrane. Anti-porcine citrate synthase monoclonal antibody (US Biological, Swampscott, MA) was directed against its protein bound to the membrane. Densitometry of the film allowed for relative quantitation of the protein subunits and citrate synthase.

**Data analysis.** Hemodynamic data were measured from the strip-chart recordings. The transmural blood flow distribution was determined from the microsphere measurements. Data were analyzed with one-way ANOVA for repeated measurements. A value of $P < 0.05$ was considered significant. When significant results were found, individual comparisons were made by using the Scheffé method.

**RESULTS**

**Animal model and anatomic data.** Six of the 14 pigs with LAD ligation died within the first 24 h after coronary occlusion. The anatomic data from the remaining eight infarct pigs and seven normal pigs are summarized in Table 1. The LV weight-to-body weight ratio and right ventricular weight-to-body weight ratio were 37% and 24% greater in hearts with postinfarction LV remodeling than hearts of size-matched normal swine, respectively ($P < 0.05$, Table 1). The distal LAD occlusion resulted in a 14% of LV mass damage. None of the eight pigs with LV remodeling developed overt evidence of clinical end-stage heart failure. Collagen contents in regions of infarct, border zone, and remote zone were evaluated by an

Table 1. Anatomic data

<table>
<thead>
<tr>
<th>Infarct Size, (SSA/LVSA)</th>
<th>LV Wt, g</th>
<th>RV Wt, g</th>
<th>LV Wt/Body Wt, g/kg</th>
<th>RV Wt/Body Wt, g/kg</th>
<th>Control (n = 7)</th>
<th>LVR (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45±5</td>
<td>118±16</td>
<td>40±3</td>
<td>2.63±0.14</td>
<td>0.91±0.10</td>
<td>0.14±0.02</td>
</tr>
<tr>
<td></td>
<td>44±8</td>
<td>146±19*</td>
<td>51±6</td>
<td>3.32±0.34*</td>
<td>1.13±0.16*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = $ no. of pigs. LV, left ventricle; RV, right ventricle; LVR, LV remodeling; SSA, scar surface area; LVSA, LV surface area.

$p < 0.05$ vs. control.
image analysis system (NIH image J program). In the infarct zone, the collagen content was \(78 \pm 8\%\) in volume, whereas in the border zone and the remote zone, collagen was virtually undetectable with values of \(0.8 \pm 0.1\%\) and \(0\%\) for border zone and remote zone myocardium, respectively. The border zone tissue sample was taken beginning 3 mm away from the margin of the mature yellowish LV scar. There was no structural difference between the remote zone and border zone myocardium by conventional pathological evaluation. Collagen occupied most of the area in the LV scar 6 wk after myocardial infarction.

**Hemodynamic data and LV function.** Hemodynamic data are shown in Table 2. In hearts with postinfarction LV remodeling, hemodynamic variables were not significantly different compared with the normal control group (Table 2). LV end-diastolic pressure was not significantly increased in the postinfarct hearts, indicating compensated LV remodeling. The MRI cine experimental data demonstrated LV dilatation with moderate dysfunction as indicated by a decrease of LV ejection fraction from baseline, 55\% to 31\%, 5 wk after LAD occlusion (Table 2, \(P < 0.05\)).

**Regional LV wall thickness and contractile function by MRI.** The regional LV wall thickness data are summarized in Table 3. The LV systolic shortening fraction measured by tagged MRI was \(-2 \pm 3\%\) and \(16 \pm 5\%\) in the border zone (\(P < 0.001\)). These data are in agreement with more traditional measurements of systolic wall thickening fraction measured with ultrasonic crystal transducers (40a). Regional LV contractile function calculated in segments 1–6 are representing the anterior papillary-, anterior-, septal-, posterior-, posterior papillary- and lateral-LV segments, according to the coronary perfusion pattern. The LAD ligation used in this study resulted in a thin-walled infarct involving the anterior wall (segment 2), while the septal and anterior papillary segments (segments 1 and 3, respectively) represent border zone. The remaining segments (segments 4–6) represent the remote zone perfused by the left circumflex coronary artery. Border zone systolic shortening fraction was significantly decreased compared with the remote zone in hearts with postinfarction LV remodeling.

**Myocardial blood flow.** Regional myocardial blood flow data from the anterior and posterior LV wall are summarized in Table 4. Mean and regional myocardial blood flows in the border zone and remote zone of the remodeled hearts were not significantly different from blood flows in normal hearts (Table 4).

**Transmural HEP and Pi levels.** Typical transmurally differentiated \(^{31}\)P NMR spectra obtained from the peri-infarct zone and the remote zone of one heart with LV remodeling are shown in Fig. 1A. Myocardial HEP and Pi levels in both areas are summarized in Table 5. The subepicardial voxel (Epi) was positioned over the outer edge of the LV wall, while the voxel most distant from the coil (subendocardial voxel, Endo) was positioned over the subendocardium. Spectra from the remote zone were characterized by high PCr and ATP levels (Fig. 1A). The PCr-to-ATP ratio was markedly lower in the peri-infarct zone. The PCr-to-ATP ratio was most severely decreased in the inner layer of the peri-infarct region, being only 46\% of that in normal hearts (Fig. 1A and Table 5).

In principle, the deeper voxels (i.e., more distant from the outer LV wall) contain contributions from LV cavity blood because of partial volume effects (i.e., they can be occupied both by LV wall and LV chamber), recognizable by the presence of 2,3-diphosphoglycerate (2,3-DPG) resonances in the \(\sim 3\)-parts per million (ppm) region of the spectra (Fig. 1B). The presence of both blood and cardiac muscle in the same voxel has the potential to distort ATP levels and PCr-to-ATP ratio measurements because blood contains ATP but not PCr. The ATP contribution from blood to the subendocardial spectrum PCr-to-ATP ratio has been previously examined (38) and found to be trivial because 1) the blood ATP concentration is much lower than in the cardiac muscle, and 2) the motion of the blood within the heart makes the small resonance peaks broader and therefore within the noise level. In the present study, the contribution of blood in the NMR region of interest may be greater because of the thinner wall in the peri-infarct area. To assess this possibility, the blood ATP contribution was examined with a phantom filled with fresh heparinized blood. Prominent resonance peaks of 2,3-DPG appear at \(\sim 3\) ppm with phosphodiester (PDE) at \(\sim 1\) ppm. No ATP resonance was detected. These data demonstrate that the contribution of LV cavity blood ATP to the subendocardial PCr-to-ATP ratio is negligible.

**Myocardial oxygenation evaluated by \(^1\)H MR spectroscopy.** No deoxymyoglobin resonance peak was detected either in normal hearts or in the peri-infarct or remote regions of hearts with postinfarction LV remodeling (spectra not shown). This implies that the myocardium was essentially fully oxygenated and that the abnormalities in oxidative phosphorylation observed in hearts with LV remodeling were not the result of myocardial ischemia or oxygen insufficiency. On the basis of the signal-to-noise ratio of Mb-\(\delta\) during partial and complete LAD occlusions (3), the resonance peak should be recognized when there is \(>10\%\) myoglobin desaturation (3, 23).
Biopsy data and myocardial free ADP concentration. Myocardial biopsy data are summarized in Table 6. ATP levels measured by HPLC were decreased in swine with LV remodeling compared with normal swine, and this abnormality was most severe in the peri-infarct region (P < 0.05, Table 6). Total creatine content was decreased in the peri-infarct region of hearts with LV remodeling. Free ADP tended to be increased in the remote region of the post-infarct hearts, and this increase was significant in the peri-infarct region.

Mitochondrial levels of mtATPase subunits. Typical Western blots for α-, β-, OSCP, and IF1 subunits of mtATPase and β-actin are summarized in Table 7. Protein levels of mitochondrial α-, β-, OSCP, and IF1 subunits of mtATPase were decreased in the subendocardium of the peri-infarct region (P < 0.05) but not in remote zone of hearts with LV remodeling (Table 7).

Myocardial CK isoform protein expression and mitochondrial density. Figure 2 illustrates Western blots examining the mitochondrial citrate synthase protein level, and CK isoform proteins in the border zone and remote zone. Myocardial CK-M and CK-mito isoform protein was decreased significantly, and this was most severe in the peri-infarc border zone relative to the remote zone (Fig. 2). This change did not result from a change in mitochondrial density because the mitochondrial citrate synthase protein level was not different between the border zone and remote zone (Fig. 2). The results also demonstrate that citrate synthase protein expressions were not significantly different between border zone and remote zone myocardium or between normal hearts and hearts with post-infarct LV remodeling (Fig. 2).

DISCUSSION

This study is the first demonstration, to the best of our knowledge, that abnormalities of ATP-producing enzymes and HEP content are expressed nonuniformly in the postinfarct dysfunctional LV. Both the alterations in myocardial HEP and mtATPase were substantially more severe in the peri-infarct region than in the remote zone of hearts with compensated postinfarct LV remodeling. These abnormalities mirror the contractile dysfunction of the border zone myocardium and may play a role in the eventual transition to heart failure.

Animal model. In the current study, permanent occlusion of the mid-LAD was used to produce myocardial infarct involving the LV anterior wall and apex. Because of the paucity of coronary collateral vessels in swine (36), the near absence of blood flow in the infarct region resulted in development of a thin-walled apical aneurysm. Interestingly, the LAD occlusion model used in this study resulted in a higher mortality (43% within the first 24 h) than the proximal left circumflex coronary (LCx) occlusion model that we have previously (<10% mortality) despite similar infarct size in the two models (~25% of LV mass) (41). The incidence of acute VF was similar after LAD and LCx occlusion (~80% of individuals within the 1st hour after coronary occlusion) but was promptly reversed by electrical defibrillation in nearly all animals with LCx ligation, while only ~60% of animals responded when VF occurred after LAD ligation. The mechanism for this difference in acute arrhythmic mortality is uncertain but might be related to ischemic injury to the His-Purkinje system during LAD occlusion, which does not occur with LCx occlusion. In our previous studies LCx coronary occlusion resulted in remodeling of the noninfarcted myocardium with LV dilatation, elongation, and hypertrophy of individual myocytes (19, 41), and alterations in myocardial HEP content, with ~30% of animals developing evidence of overt CHF (19, 41). In contrast, none of the pigs that survived the LAD occlusion in the present study developed clinical evidence of CHF during the 6-wk observation period. To examine the degree of LV dysfunction produced by distal LAD occlusion, MRI was carried out before and ~5 wk after distal LAD occlusion. MRI cine demonstrated significant LV dilatation with a decrease of LV ejection fraction from the preocclusion baseline (P < 0.05; Table 2), indicating LV dysfunction consistent with compensated postinfarct remodeling.

Myocardial perfusion and function in the peri-infarct region. Several investigators have reported that the zone of dysfunctional myocardium produced by coronary artery occlusion extends beyond the infarct region to include a variable boundary of adjacent normal appearing tissue (2, 12). In a canine model of acute LCx coronary artery occlusion, we found a lateral region of depressed systolic wall thickening that extended an average distance of 9.6 mm beyond the infarct border (12). Systolic dysfunction in this peri-infarct zone might be the result of 1) an intermediate degree of hypoperfusion, 2) increased local systolic wall stress, or 3) intrinsic abnormalities of myocardial contractile function. Early investigators suggested that a gradient of collateral blood flow might exist at the border of an infarct, with graded hypoperfusion extending out from the central region of infarct. Subsequent reports demonstrated that coronary microvessels function essentially as end vessels with sharp boundaries between adjacent vascular beds but that intermediate levels of mean blood flow can exist as the result of admixture of peninsulas of ischemic tissue intermingled with regions of normally perfused myocardium (27). However, Murdock et al. (24) demonstrated that the boundary of the intermingled coronary microvessels is no more than 3
results in an abnormal radius of curvature of the border region that acts to amplify wall stresses. Using echo contrast imaging to delineate the infarct perfusion boundary following acute coronary occlusion in an ovine model, Jackson et al. (14) observed that radial displacement of the infarct region during systole forced the adjacent myocardium to curve outward, thereby decreasing the local radius of curvature and more than doubling computed systolic wall stress in the border region. Several investigators have studied changes in wall stress in the setting of chronic infarction. Finite element analysis of magnetic resonance images from the LV of sheep studied 10–22 wk after producing an apical infarct demonstrated increases of wall stress in the peri-infarct region at end-diastole that persisted throughout systole, supporting the contention that increased wall stresses contribute to abnormal function in the border zone (2, 35). Guccione et al. (9) used a three-dimensional model to assess the mechanism for systolic dysfunction of the border zone in sheep 10 wk after coronary occlusion to produce an apical aneurysm. They found that the depressed systolic function in the border zone could not be fully explained by increased wall stress and changes in material properties of the infarcted tissue but required a primary abnormality of contractile performance of the border zone myocardium. This appeared to be a large effect, requiring ~50% decrease in intrinsic contractility to fully explain the abnormal function in the border zone (9).

The border zone surrounding a region of infarct is of interest because of evidence that progressive dysfunction of the peri-infarct myocardium over time may contribute to the transition from compensated remodeling to CHF. Using an ovine model of apical infarct in which LV geometry was assessed over an 8-wk period after infarction, Jackson et al. (13, 14) found a fully perfused border zone surrounding the infarct that progressively expanded to involve additional contiguous myocardium. Early after infarct, this border region was narrow with moderately impaired systolic function. However, the border zone gradually expanded with progressive contractile dysfunction.

### Table 5. Myocardial PCr-to-ATP ratio data

<table>
<thead>
<tr>
<th></th>
<th>Epi</th>
<th>Mid</th>
<th>Endo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.12±0.25</td>
<td>2.08±0.16</td>
<td>1.98±0.16</td>
</tr>
<tr>
<td>LVR 8)</td>
<td>2.01±0.15</td>
<td>1.82±0.13†</td>
<td>1.74±0.10†</td>
</tr>
<tr>
<td>RZ</td>
<td>1.25±0.11*</td>
<td>1.16±0.09*</td>
<td>0.81±0.09*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of pigs; PCr, Phosphocreatine. *P < 0.01 vs. RZ; †P < 0.05 vs. control.

### Table 6. Biopsy and myocardial free ADP concentration data

<table>
<thead>
<tr>
<th></th>
<th>[ATP], μmol/g dry wt</th>
<th>[Creatine], μmol/g dry wt</th>
<th>[ADP]free, μmol/g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.8±1.2</td>
<td>130.8±7.9</td>
<td>0.30±0.07</td>
</tr>
<tr>
<td>LVR 8)</td>
<td>17.8±1.3*</td>
<td>114.4±9.3</td>
<td>0.39±0.08</td>
</tr>
<tr>
<td>RZ</td>
<td>10.1±0.8†</td>
<td>85.3±5.1†</td>
<td>0.55±0.07†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of pigs. *P < 0.05 vs. control; †P < 0.05 vs. RZ.
Histological examination of myocardium from the border zone demonstrated myocyte hypertrophy and vacuolization with interstitial fibrosis, suggesting a myopathic process. Although the mechanisms for this progressive abnormality are uncertain, the present study suggests that abnormalities of energy production in the border zone myocardium may contribute to ongoing myocyte dysfunction.

**Myocardial ATP concentration.** In agreement with previous reports from hearts with compensated postinfarct remodeling, ATP concentrations in myocardium remote from the infarct were decreased by ~25% in the present study (Table 6) (19, 29, 41). However, ATP content was decreased to less than half normal in the peri-infarct border zone. Although an increase in collagen content in the peri-infarct region could have contributed to this change, we found no significant increase of border zone collagen in the present study, implying a decrease of ATP within the myocytes. The mechanism for decreased ATP in the border zone is uncertain but may result, at least in part, from the increased ADP levels in the border zone myocardium. Increases of free ADP can activate adenylate kinase, which catalyzes transfer of a phosphoryl group between two molecules of ADP to form one molecule each of AMP and ATP (21). AMP can be acted on by 5'-nucleotidase to produce adenosine. Unlike the nucleotides, adenosine can be transported into the interstitial space where it is further degraded to inosine and hypoxanthine and can leave the heart through the coronary circulation (21, 31). Loss of adenosine can result in depletion of ATP because de novo resynthesis of adenine nucleotides is a slow and energy costly process. This concept is supported by the recent report of Gourine et al. (8) in vivo mtATPase activity is likely to be less than its Vmax when stimulated to their maximal achievable oxygen consumption (23). Although it is uncertain whether the observed decrease in mtATPase subunit expression could directly impair contractile performance in the border zone, a reduction in mtATPase activity could require an elevation in cytosolic ADP levels to maintain a given rate of ATP synthesis. Such an increase in myocardial free ADP has the potential to decrease myocardial contractile performance (33, 34, 39). It is important to note that the reductions of these key enzymes in ATP production did not appear to result from decreased mitochondria density because the mitochondrial enzyme citrate synthase was not different between border zone and remote myocardium.

The mechanism for the observed regional differences in expression of the mtATPase is uncertain. Both generalized neurohormonal activation and local activation of paracrine and autocrine mechanisms contribute to LV remodeling after myocardial infarction. Generalized neurohormonal activation would not be expected to cause regionally heterogeneous changes in bioenergetic characteristics, but paracrine or autocrine mechanisms could do so if there were local differences in the levels of activation of these systems. Biomechanical strain triggers stretch-activated signaling pathways in cardiomyocytes that lead to induction of a program of genes that can culminate in either myocyte hypertrophy or apoptosis (20). The concept that stretch-activated signaling pathways can be turned

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**Table 7. Densitometric intensities for protein bands from Western blots of α-, β-, OSCP, and IF1 ATPase subunits normalized to β-actin (ratios)**

| Subunits | Control | LVR | Ctrl | | | | | |
|----------|---------|-----|------|-----|-----|-----|-----|-----|-----|
| Endo     | Mid     | Epi | BZ   | Mid | Epi | Ctrl | Mid | Epi |
| α        | 1.81±0.14 | 1.95±0.16 | 1.95±0.19 | 0.29±0.09† | 0.91±0.15* | 0.86±0.18* | 1.04±0.15† | 1.20±0.18* | 1.22±0.18* |
| β        | 0.82±0.09 | 0.91±0.08 | 0.79±0.08 | 0.43±0.09† | 0.63±0.14* | 0.65±0.09 | 0.57±0.06† | 0.68±0.08 | 0.79±0.12 |
| OSCP     | 1.41±0.18 | 1.53±0.28 | 1.49±0.14 | 0.72±0.09† | 1.05±0.23 | 1.10±0.18 | 1.28±0.14† | 1.35±0.15 | 1.54±0.21 |
| IF1      | 1.16±0.09 | 1.31±0.13 | 1.41±0.11 | 0.28±0.09† | 0.66±0.03* | 0.61±0.05* | 0.69±0.06† | 0.79±0.11* | 0.72±0.10* |

Values are means ± SE; control, n = 7; LVR, n = 8. *P < 0.05 vs. corresponding control; †P < 0.05 vs. corresponding perisar area (BZ); ‡P < 0.05 vs. corresponding Epi.
on regionally in vivo is supported by studies in rats 6 wk postinfarction where substantial differences in mRNA levels for ANP, endothelin-1, and IGF-1 were found between border zone and remote myocardium (4, 20), with highest expression in areas subjected to high mechanical stresses. The ability of these local changes to produce functional responses is supported by an ovine model of postinfarct remodeling in which myocyte hypertrophy was greater in the border zone than in the remote myocardium (16). However, no reports are available examining the effect of stretch on mitochondrial function.

**Myocardial CK isoform protein expression.** Similar to the ATPase subunit expression as discussed above, the reduction in expression of myocardial CK-M and CK-mito isoform protein was also significantly more severe in border zone than the remote zone myocardium (Fig. 2, each $P < 0.05$) that was independent from changes in mitochondrial density, suggesting
a regional myocyte overstretch or oxidative stress (e.g., wall stress) induced protein expression changes.

Limitations. Inclusion of scar tissue within the samples for HEP and Western analysis could result in a decrease of muscle-related components. In the present study, hearts were examined 6 wk after LAD ligation. Because of the paucity of collateral vessels in swine, the apical infarct had evolved into a well-defined aneurysm with a distinct demarcation between infarcted and normal tissue. Biopsies were taken at least 3 mm away from the border of the infarct to avoid contamination with scar tissue. Furthermore, the collagen content of the border zone was not increased relative to the remote myocardium. This is in agreement with our previous study using a similar porcine model (35) in which collagen content was examined in multiple pathological sections extending from the scar area through the border zone, and into the remote myocardium. In that study we found that collagen content of sections taken 3 mm away from the infarct border were indistinguishable from sections from the remote region. NMR measurements are even less likely to be affected because scar tissue does not contain significant HEP. Furthermore, the pulse sequence used in the present study confined the sample volume to a discrete column perpendicular to the surface coil, thereby minimizing sampling from regions lateral to the region of interest.

In conclusion, steady-state levels of myocardial ATP and mitochondrial ATPase subunits are heterogeneously expressed in postinfarct LV, with values markedly lower in the peri-infarct region than in remote myocardium. The abnormalities in the peri-infarct myocardium did not result from alterations of blood flow but mirror the depression of systolic function in this region. This regional heterogeneity of mtATPase expression and ATP content suggests that energy insufficiency in the peri-infarct region may contribute to the transition from compensated LV remodeling to congestive heart failure.

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