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Left Ventricular Unloading Before Reperfusion Promotes Functional Recovery After Acute Myocardial Infarction

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ABSTRACT

BACKGROUND Heart failure after an acute myocardial infarction (AMI) is a major cause of morbidity and mortality worldwide. We recently reported that activation of a transvalvular axial-flow pump in the left ventricle and delaying myocardial reperfusion, known as primary unloading, limits infarct size after AMI. The mechanisms underlying the cardioprotective benefit of primary unloading and whether the acute decrease in infarct size results in a durable reduction in LV scar and improves cardiac function remain unknown.

OBJECTIVES This study tested the importance of LV unloading before reperfusion, explored cardioprotective mechanisms, and determined the late-term impact of primary unloading on myocardial function.

METHODS Adult male swine were subjected to primary reperfusion or primary unloading after 90 min of percutaneous left anterior descending artery occlusion.

RESULTS Compared with primary reperfusion, 30 min of LV unloading was necessary and sufficient before reperfusion to limit infarct size 28 days after AMI. Compared with primary reperfusion, primary unloading increased expression of genes associated with cellular respiration and mitochondrial integrity within the infarct zone. Primary unloading for 30 min further reduced activity levels of proteases known to degrade the cardioprotective cytokine, stromal-derived factor (SDF)-1α, thereby increasing SDF-1α signaling via reperfusion injury salvage kinases, which limits apoptosis within the infarct zone. Inhibiting SDF-1α activity attenuated the cardioprotective effect of primary unloading. Twenty-eight days after AMI, primary unloading reduced LV scar size, improved cardiac function, and limited expression of biomarkers associated with heart failure and maladaptive remodeling.

CONCLUSIONS The authors report for the first time that first mechanically reducing LV work before coronary reperfusion with a transvalvular pump is necessary and sufficient to reduce infarct size and to activate a cardioprotective program that includes enhanced SDF-1α activity. Primary unloading further improved LV scar size and cardiac function 28 days after AMI. (J Am Coll Cardiol 2018;72:501–14) © 2018 Published by Elsevier on behalf of the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

BCL-2 = B-cell lymphoma-2

BCL-XL = B-cell lymphoma–extra-large

BNP = B-type natriuretic peptide

CMR = cardiac magnetic resonance

DPP = dipeptidyl peptidase

LAD = left anterior descending

LGE = late-gadolinium enhancement

LV = left ventricular

MMP = matrix metalloproteinase

MRI = magnetic resonance imaging

mRNA = messenger ribonucleic acid

P-reperfusion = primary reperfusion

P-unloading = primary unloading

PCR = polymerase chain reaction

SDF = stromal-derived factor

RISK = reperfusion injury salvage kinase

RNA = ribonucleic acid

TV = transvalvular axial-flow

Acute myocardial infarction (AMI) due to occlusion of a coronary artery is a major cause of global morbidity and mortality (1). The current paradigm for AMI therapy focuses on rapidly restoring coronary artery blood flow to re-establish myocardial oxygen supply, known as primary reperfusion (P-reperfusion). However, despite timely reperfusion, up to 25% of patients experiencing their first AMI will develop heart failure within 1 year (2). For every 5% increase in myocardial infarct size, 1-year all-cause mortality and heart failure hospitalizations increase by 20%, which imposes a significant burden on health care resources (3). For these reasons, new approaches to limit myocardial damage and subsequent ischemic heart failure remain a significant unmet need for patients with AMI.

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One explanation for these poor outcomes is that P-reperfusion paradoxically worsens myocardial damage, known as ischemia-reperfusion injury. Prior attempts to limit ischemia-reperfusion injury via vascular conditioning approaches to activate reperfusion injury salvage kinase (RISK) pathway activity or pharmacological approaches have failed to show clear clinical benefit (4,5). A critical barrier to these cardioprotective strategies is the mandate for rapid coronary reperfusion and therefore insufficient time for any therapeutic impact on myocardial injury. Improved strategies are needed to limit myocardial damage by promoting cardioprotective mechanisms before reperfusion of an infarct-related artery.

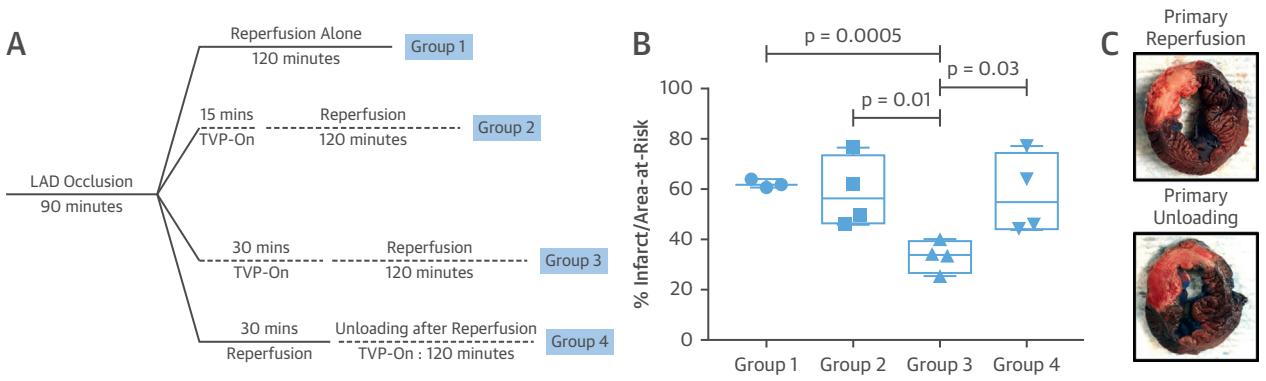
Over the past decade, percutaneously delivered transvalvular axial-flow pumps (TV-pumps) have been introduced into routine clinical practice. These devices rapidly reduce left ventricular (LV) wall stress, stroke work, and myocardial oxygen demand, while augmenting systemic mean arterial pressure without the need for surgery (6–8). We recently reported that compared with P-reperfusion, first unloading the left ventricle by using a TV-pump while delaying coronary reperfusion (primary unloading [P-unloading]) reduced myocardial infarct size by 40% to 50% (9). We further identified that P-unloading increases myocardial levels and activity of stromal-derived factor (SDF)-1α (CXCL-12), which is a constitutively and ubiquitously expressed cytokine that promotes cardioprotective signaling in AMI (10). The mechanism by which P-unloading regulates SDF-1α levels remains unknown.

In the setting of myocardial ischemia-reperfusion injury, increased expression of proteases, including matrix metalloprotease (MMP)-2 and MMP-9 and dipeptidyl peptidase-4 (DPP-4), cleave the N-terminus of SDF-1α, thereby rendering the cytokine inactive (11–14). Any remaining SDF-1α can bind to CXCR4, which promotes phosphorylation of the RISK pathway including extracellular regulated kinase (Erk), protein kinase b (Akt), and glycogen synthase kinase 3b (GSK3b). RISK activation promotes cell survival by limiting cardiomyocyte apoptosis and maintains mitochondrial integrity by preventing opening of the mitochondrial trans-permeability pore (15–17). The mechanisms underlying the cardioprotective benefit of P-unloading and whether the acute decrease in infarct size results in a durable reduction in LV scar and improvement in cardiac function remain unknown. The present study tested the importance of delayed myocardial reperfusion, explored cardioprotective mechanisms, and determined the late-term impact on myocardial function associated with P-unloading.

METHODS

EXPERIMENTAL PROTOCOL OF MYOCARDIAL INFARCTION AND MECHANICAL CIRCULATORY SUPPORT. Studies were conducted in adult, male Yorkshire swine. The Institutional Animal Care and Use Committee at Tufts Medical Center approved the study protocol. All experiments were performed according to the committee's guidelines. Animals were premedicated with Telazol (0.8 ml/kg, intramuscular; Zoetis Services LLC, Parsippany, New Jersey). General anesthesia was induced and maintained with isoflurane (1% to 2%). All animals were intubated and mechanically ventilated (Harvard Apparatus, Holliston, Massachusetts) with room air and supplemented oxygen to maintain physiological pH and oxygen saturation. Surface electrocardiography leads, an orogastric tube, peripheral 18 G venous catheters, and a rectal thermistor were placed in all animals. Heating pads were used as needed to maintain a core body temperature >99°F. Vascular access sheaths were then deployed into the right internal jugular vein (10-F), left carotid artery (7-F), and both femoral arteries (7-F) and veins (10-F). Unfractionated heparin boluses with a goal activated clotting time of 300 to 400 s, continuous lidocaine infusion (1 mg/kg), and noradrenaline (0.16 μg/min) were initiated in all animals.

FIGURE 1 Timing of Unloading Between Groups and Infarct Quantification



(A) Flowchart illustrating the effect of reperfusion alone (group 1), left ventricular unloading for 15 min (group 2) or 30 min (group 3) before reperfusion, or left ventricular unloading after reperfusion (group 4). **(B)** Infarct area as a percentage of the area at risk for each group (1-way analysis of variance = 0.017 across all 4 groups). **(C)** Representative images of infarct quantitation using triphenyltetrazolium chloride and Evan's blue counterstaining from group 1 and group 3 (n = 4 per group). **Red** staining indicates the area at risk, **white** staining indicates infarcted myocardium, and **blue** staining indicates myocardial tissue outside the area at risk. LAD = left anterior descending; TVP = transvalvular pump.

A 6-F Judkins right coronary catheter (Boston Scientific, Marlborough, Massachusetts) engaged the left coronary artery via the right femoral artery, and baseline angiograms were recorded. A 0.014-inch guidewire was delivered into the distal left anterior descending artery (LAD) and a 3.0 × 8 mm bare-metal stent (Boston Scientific) for acute studies or a 3.0 × 8 mm angioplasty balloon (Boston Scientific) for chronic studies was deployed in the mid-LAD after the first diagonal branch with angiographic confirmation of LAD occlusion. Coronary angiography also performed immediately after reperfusion and again after the end of the study protocol confirmed patency of the LAD. LAD stents were used in the acute animal study to mark the exact location for repeat balloon occlusion during Evans blue counterstaining. Animals were then euthanized with pentobarbital and phenytoin after 120 min of reperfusion.

OPTIMAL DURATION OF LV UNLOADING BEFORE REPERFUSION. To explore the optimal duration of mechanical unloading before reperfusion, adult male Yorkshire swine were divided into 4 groups (n = 4 per group) (Figure 1A). All groups underwent 90 min of LAD occlusion. In group 1, LAD occlusion followed by 120 min of reperfusion served as the control group. In groups 2 and 3, LAD occlusion was followed by insertion and activation of a TV-pump (Impella CP, Abiomed, Danvers, Massachusetts) via a 14-F sheath in the left femoral artery and maintained at maximal support (44,000 rotations/min, achieving 3.5 l/min). This action was followed by an additional 15 min (group 2) or 30 min (group 3) of occlusion, respectively, and then 120 min of reperfusion with LV unloading. In group 4, LAD occlusion was followed by reperfusion, and after 30 min of reperfusion, a TV-pump was inserted and activated for the remaining 90 min of reperfusion.

At the end of each study, animals were euthanized for determination of myocardial infarct size. Three sham-operated animals were intubated, anesthetized, and mechanically ventilated without myocardial infarction or mechanical unloading. LV tissue samples obtained from sham controls were used for tissue analysis.

FUNCTIONAL ROLE OF SDF-1α IN LV UNLOADING. To explore the functional role of SDF-1α/CXCR4 signaling or the cardioprotective effect of LV unloading, an over-the-wire coronary angioplasty balloon was used to deliver a pharmacological inhibitor of the SDF-1α receptor, CXCR4 (known as AMD3100), into the area at risk while maintaining occlusion of the LAD in a closed-chest animal model of AMI. Adult male swine were treated with intracoronary injections of either vehicle or AMD3100 (3 μg/kg/min, intracoronary over 10 min; n = 4 per group) initiated at the onset of LV unloading for 30 min before reperfusion. The dose of AMD3100 was chosen based on previous reports (15).

CHRONIC PHASE STUDY OF POST-INFARCT LV SCAR. To study the long-term effects of LV unloading on infarct size, 19 adult male Yorkshire swine were subjected to either 90 min of mid-LAD occlusion followed by immediate reperfusion (P-reperfusion) or 30 min of unloading before reperfusion

(P-unloading). Five animals died of ventricular arrhythmias during LAD occlusion before randomization or pump implantation. Of the remaining 14 animals that successfully completed the protocol, 2 animals died in the P-reperfusion group within 6 h after reperfusion due to refractory ventricular fibrillation. No animals died in the P-unloading group. In total, 7 (37%) of 19 animals died during the study protocol. The surviving 12 animals were used for analysis in the chronic study, in either the P-reperfusion group ($n = 6$), or the P-unloading group ($n = 6$) (**Figure 1A**). Animal weights were 76.7 ± 6.9 kg in the P-unloading group and 76.2 ± 2.4 kg in the P-reperfusion group ($p = 0.84$). After reperfusion, all animals were recovered and monitored for 28 days. After 28 days, animals were re-anesthetized and underwent repeat catheterization to assess infarct size according to cardiac magnetic resonance imaging (MRI) and LV hemodynamics.

PHYSIOLOGICAL CHARACTERIZATION IN VIVO 28 DAYS AFTER AMI. Changes in LV pressure and volume were assessed by using a 5-F conductance catheter system (Sigma M, CD Leycom, Hengelo, the Netherlands) deployed via the left carotid into the left ventricle. Ventricular pressure and volume were measured at 28 days after the initial infarct in the chronic-phase study by using a solid-state pressure transducer and dual-field excitation mode, respectively, as previously described (18,19). Briefly, the method measures time-varying electrical conductance across 5 to 7 ventricular blood segments delineated by selected catheter electrodes. Correct positioning of the conductance catheter along the long-axis of the left ventricle was confirmed by fluoroscopy. In previous preclinical and clinical studies, time-varying segmental conductance has been shown to reflect segmental LV volumes (20,21). Parallel conductance was assessed by injecting 20 ml of hypertonic (6%) saline into the right internal jugular vein. Absolute LV volumes were measured by subtracting parallel conductance from total conductance volumes. Stroke volume is calculated as the difference in conductance volumes at +dP/dtmax and -dP/dtmin. LV stroke work was calculated as the product of peak LV peak systolic pressure and stroke volume.

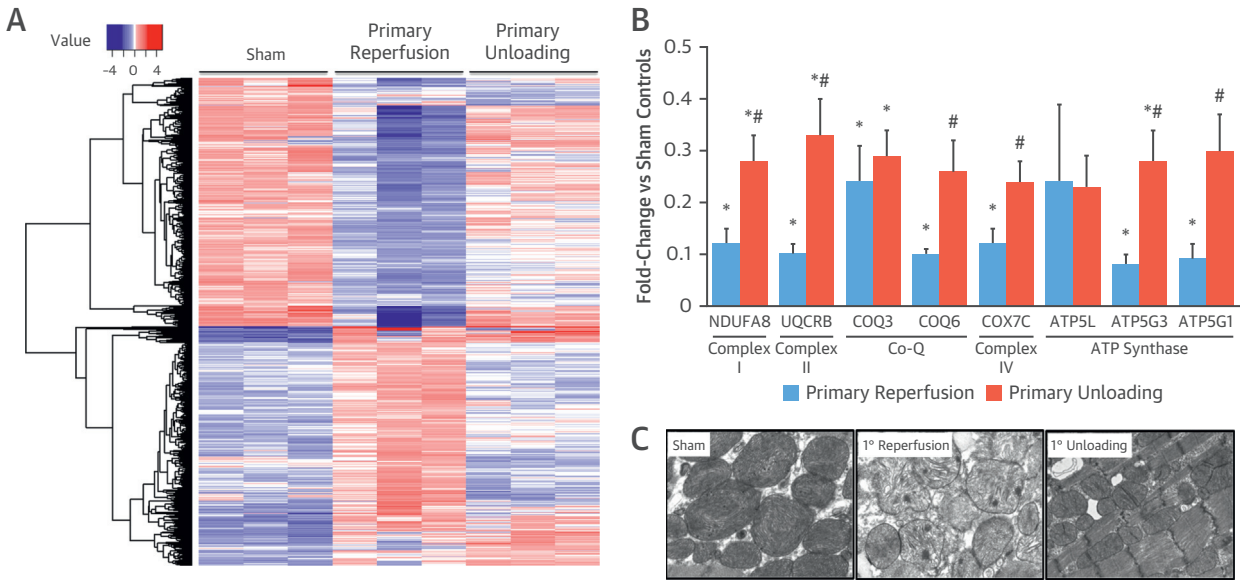
DETERMINATION OF MYOCARDIAL INFARCT SIZE. Upon completion of the acute study protocol, balloon occlusion was performed within the mid-LAD stent and Evans blue injected into both coronary vessels to delineate the area-at-risk followed by removal and sectioning of the left ventricle. Biopsy specimens were obtained from the antero-apical left ventricle distal to the site of stent deployment (infarct zone) and from the postero-basal wall (noninfarct zone) for molecular analysis; LV slices were then incubated in 1% triphenyltetrazolium chloride, as previously described. To quantify LV scar size 28 days after MI, the left ventricle was sectioned into five 1-cm slices and then incubated in triphenyltetrazolium chloride without Evans blue. LV slices were then photographed, and 3 blinded reviewers used digitized planimetry to quantify the total myocardial area, area-at-risk, and infarct zone.

CARDIAC MRI WITH LATE-GADOLINIUM ENHANCEMENT. Animals in the chronic-phase study underwent a cardiac MRI with late-gadolinium enhancement (LGE) 28 days after the initial infarct using a Philips Achieva 1.5-T scanner (Philips Healthcare, Best, the Netherlands). Steady-state free precession breath-hold cine images were obtained in 3 long-axis planes and sequential short-axis slices from the atrioventricular ring to the apex. LV and right ventricular volume, mass, and ejection fraction were measured by using standard volumetric techniques and analyzed with commercially available software (QMASS version 7.4, Medis Medical Imaging Systems, Leiden, the Netherlands) by a blinded observer experienced in cardiac magnetic resonance (CMR) analysis. LGE images were acquired 10 to 15 min after intravenous administration of 0.2 mmol/kg gadolinium-diethylenetriamine penta-acetic acid with breath-hold 2-dimensional, phase-sensitive inversion recovery sequences in identical places as in cine images. LGE regions were defined by using full width at one-half maximum (>50% of maximum myocardial signal intensity) with manual adjustment when needed. Areas with LGE were summed to generate a total volume of LGE and are expressed as a proportion of total LV myocardium (%LGE).

MICROARRAY ANALYSIS. Whole-transcriptome expression analysis was performed on ribonucleic acid (RNA) isolated from the infarct zone after the acute phase protocol using Porcine 1.0 ST microarrays. (The Online Appendix presents details.) All raw and processed data from this microarray analysis can be accessed under the Gene Expression Omnibus accession number GSE108644. Quantitative polymerase chain reaction (PCR) and Western blot analysis confirmed expression of significantly regulated genes and their activation in altered pathways.

ELECTRON MICROSCOPY. LV tissue samples were obtained from the center of the infarct zone, washed and fixed with 3% glutaraldehyde in phosphate buffer, and then embedded in epoxy resin. Electron micrographs were acquired and analyzed for cardiomyocyte injury, including mitochondrial swelling and integrity.

FIGURE 2 Microarray Analysis and Preservation of Mitochondrial Integrity With LV Unloading



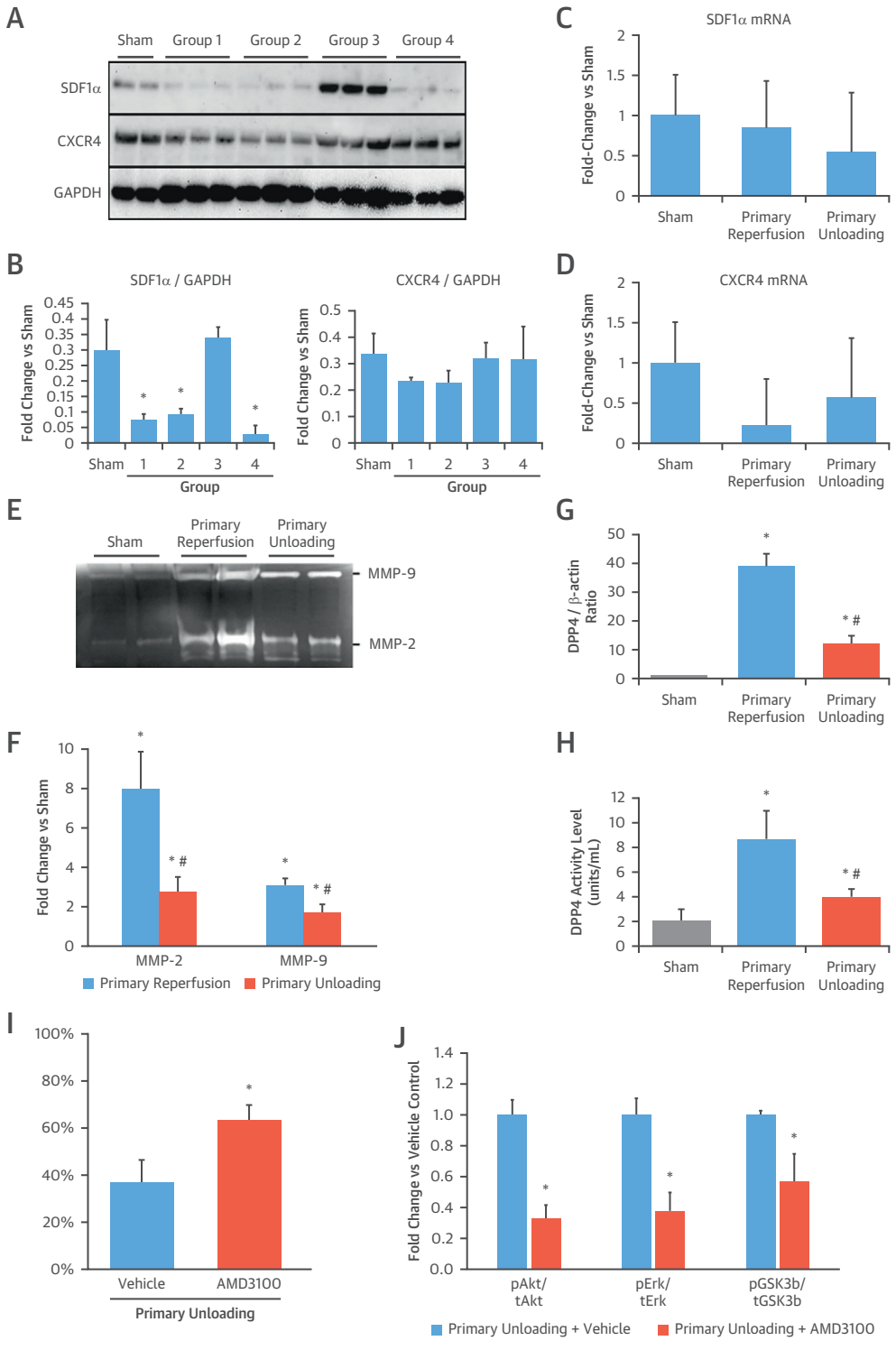
(A) Genomic heat map illustrating the shift in gene expression among sham-operated controls, reperfusion alone (group 1), and left ventricular (LV) unloading for 30 min before reperfusion (group 3) (n = 3 per group). **(B)** Graph illustrating relative messenger ribonucleic acid levels of representative genes from key components of the electron transport chain from within the infarct zone of group 1 **(blue)** or group 3 **(orange)**. **(C)** Representative transmission electron micrographs of cardiomyocyte mitochondria from sham controls and from within the infarct zone of group 1 and group 3. *p < 0.05 versus sham control; #p < 0.05 versus primary reperfusion.

QUANTIFICATION OF SDF-1α AND CXCR4 LEVELS. Total protein was extracted from tissue homogenates, isolated as previously described (22-24). SDF-1α protein levels were quantified in LV tissue isolated from sham-operated animals and infarct zones using Western blot analysis and an enzyme-linked immunosorbent assay. Circulating serum levels of SDF-1α were quantified by using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota). CXCR4 levels in LV tissue isolated from sham-operated animals and infarct zones were quantified by Western blot analysis (Abcam, Cambridge, United Kingdom). Immunoblot analysis was then performed as previously described.

QUANTIFICATION OF MMP-2, MMP-9, AND DPP-4 LEVELS AND ACTIVITY. MMP-2 and MMP-9 activities in homogenates of heart tissues were determined by zymography as previously described (25). Briefly, gelatin zymography was performed with sodium dodecyl sulfate polyacrylamide gel electrophoresis gels containing 1 mg/ml of porcine gelatin. Samples were prepared under nonreducing conditions. Gel electrophoresis was performed at 150 V for 1 h. After electrophoresis, the gel was washed in 2.5% Triton X-100 solution with gentle agitation for 6 h at room temperature, followed by replacement with developing buffer containing 50 mM Tris-HCl (pH 7.5), 0.2 M NaCl, 5 mM $CaCl_2$, and 0.2% Brij-35. The gel was agitated at room temperature for 30 min, placed into fresh developing buffer, and incubated at 37°C overnight. The following morning, gels were stained with 0.5% Coomassie Brilliant Blue R-250 in 40% methanol and 10% acetic acid for 2 to 4 h and destained in 40% methanol and 10% acetic acid at room temperature. Gelatinolytic bands were quantified by scanning densitometry with ImageJ software (National Institutes of Health, Bethesda, Maryland). DPP-4 protein levels were quantified by immunoassay, and activity levels were measured by using a commercially available activity assay kit (MilliporeSigma, Burlington, Massachusetts).

QUANTIFICATION OF APOPTOTIC SIGNALING PATHWAYS. Immunoblot analysis was performed by using antibodies against porcine B-cell lymphoma (BCL)-2 (Cell Signaling Technology, Danvers, Massachusetts), BAX (Cell Signaling Technology), B-cell lymphoma-extra-large (BCL-XL) (Cell Signaling Technology), caspase-3 (Cell Signaling Technology), and glyceraldehyde-3-phosphate dehydrogenase. Expression of apoptosis regulatory protein levels were normalized to both total protein levels and glyceraldehyde-3-phosphate dehydrogenase.

FIGURE 3 SDF-1a Levels Are Up-Regulated and Proteolytic Degradation of SDF-1a Is Down-regulated in LV Unloading



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TUNEL staining was performed by using 10-μm thick sections obtained from the peri-infarct zone fixed in 4% paraformaldehyde/phosphate-buffered saline for 20 min. Slides were permeabilized on ice with 0.1% Triton X-100 in 0.1% sodium citrate, and sections were labeled in the dark at 37°C for 60 min. Slides were rinsed with phosphate-buffered saline, and nuclei were labeled with ProLong Gold Antifade with DAPI (Life Technologies, Grand Island, New York). Images were acquired by using an Eclipse E800 fluorescence microscope (Nikon Corporation, Tokyo, Japan) and Openlab version 5 software (Perkin Elmer, Waltham, Massachusetts). TUNEL-positive cells were counted at 10× magnification by an investigator blinded to experimental group and are expressed as a percentage of all nuclei.

REAL-TIME QUANTITATIVE PCR. For all cell-based real-time PCR experiments, total RNA was extracted directly with Trizol (Thermo Fisher Scientific, Waltham, Massachusetts) and converted to complementary deoxyribonucleic acid with a High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). For all real-time PCR experiments, samples were quantified in triplicate by using 40 cycles performed at 94°C for 30 s, 60°C for 45 s, and 72°C for 45 s with an ABI Prism 7900 Sequence Detection System (Thermo Fisher Scientific) using appropriate primers (Online Appendix).

STATISTICAL ANALYSIS. Results are presented as mean ± SD. An unpaired Student's *t*-test or one-way analysis of variance was used to compare continuous variables between groups. All data within groups over time were analyzed by using nonparametric 2-way repeated measures analysis of variance. Simple linear regression analysis was used to evaluate for a correlation between 2 parameters. All statistical analyses were performed with GraphPad Prism (GraphPad Software, La Jolla, California). An alpha-level of $p < 0.05$ was considered to indicate a significant effect or between-group difference.

RESULTS

LV UNLOADING FOR 30 MIN BEFORE REPERFUSION REDUCES ACUTE INFARCT SIZE COMPARED WITH REPERFUSION ALONE. LV unloading for 30 min before reperfusion reduced myocardial infarct size compared with reperfusion alone (33.3 ± 5% vs. 62.2 ± 1.7% infarct/area-at-risk, group 3 vs. group 1, respectively; $p < 0.01$) (Figure 1). LV unloading followed by rapid reperfusion within 15 min (group 2) or after reperfusion (group 4) failed to reduce myocardial infarct size compared to P-reperfusion alone.

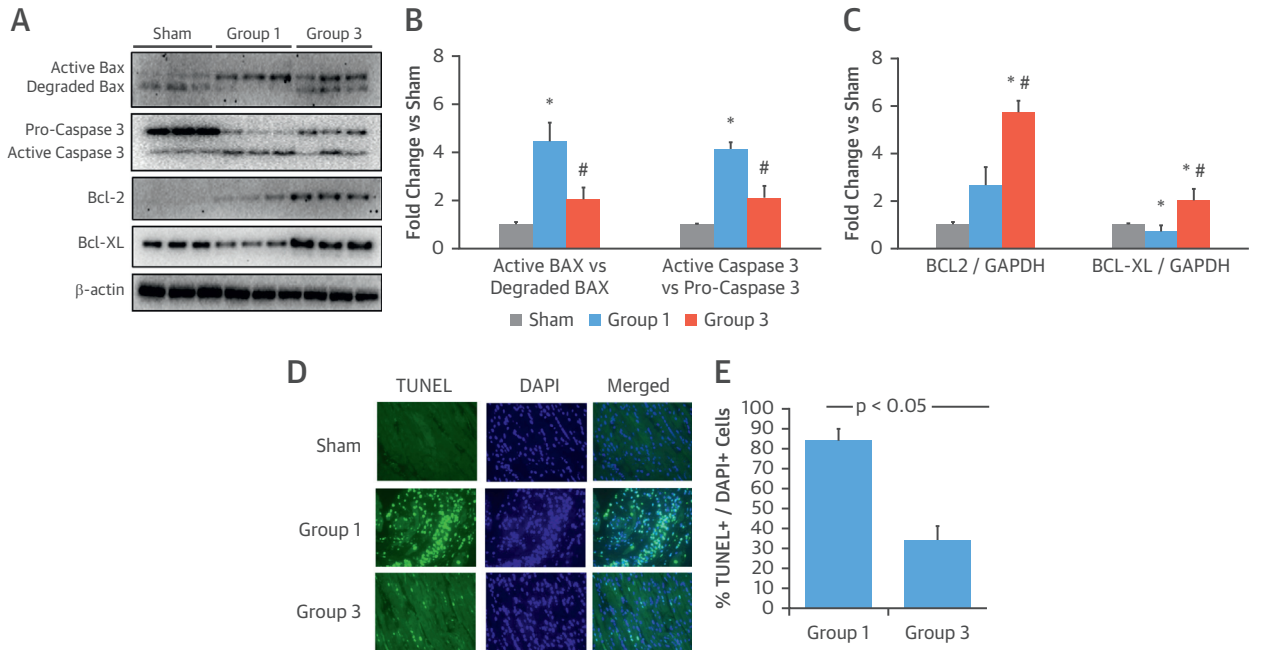
LV UNLOADING INDUCES A GLOBAL SHIFT IN GENE EXPRESSION ASSOCIATED WITH REDUCED INJURY WITHIN THE INFARCT ZONE AFTER AMI. To begin exploring cardioprotective mechanisms associated with LV unloading before reperfusion, we analyzed whole transcriptomes from within the infarct zone among sham controls, group 1, and group 3 to identify genes that were differentially expressed between treatment groups. A heat map of all differentially regulated genes showed that compared with sham controls, LV unloading for 30 min before reperfusion attenuates changes in the gene expression associated with reperfusion alone (Figure 2A).

Relative to reperfusion alone, LV unloading for 30 min before reperfusion limited down-regulation of genes associated with mitochondrial function and cellular respiration (Online Table 1). Consistent with these observations, real-time PCR of LV tissue samples from the infarct zone confirmed that compared with group 1, group 3 exhibited increased messenger ribonucleic acid (mRNA) levels of key genes associated with cellular respiration (Figure 2B). Electron microscopy further showed loss of mitochondrial integrity within the infarct zone from group 1 (but not group 3) (Figure 2C). These findings identify that

FIGURE 3 Continued

(A and B) Western blots and quantification graphs for left ventricular (LV) protein levels of stromal-derived factor-1α (SDF1a) and CXCR4 normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for sham controls and each of the 4 acute myocardial infarct groups with quantification (group 1: reperfusion alone; group 2: LV unloading for 15 min before reperfusion; group 3: LV unloading for 30 min before reperfusion; and group 4: LV unloading 30 min after reperfusion; n = 4 per group). **(C and D)** Quantification of mRNA levels of SDF1 and CXCR4 taken from sham controls and from tissue within the infarct zones of Group 1 and Group 3 (n = 4 per group). **(E and F)** Gel zymography with quantification for LV matrix metalloprotease (MMP)-9 and MMP-2 activity from samples taken from sham controls and the infarct zones of group 1 and group 3. **(G and H)** Quantification of dipeptidyl peptidase-4 (DPP4) protein levels and activity from samples taken from sham controls and the infarct zones of groups 1 and 3 (n = 4 per group). *$p < 0.05$ versus sham; #$p < 0.05$ versus group 1. **(I)** Quantification of infarct size as a percentage of the area at risk among groups subjected to LV unloading for 30 min with intracoronary delivery of either vehicle or the CXCR4 inhibitor AMD3100 followed by reperfusion (n = 4 per group). **(J)** Quantification of phosphorylated and total Akt, phosphorylated and total extracellular-regulated kinase (ERK), and phosphorylated and total glycogen synthase kinase 3β (GSK3b) (n = 4 per group). *$p < 0.05$ versus LV unloading + vehicle.

FIGURE 4 Compared With Reperfusion Alone, LV Unloading and Delayed Reperfusion Is Associated With an Antiapoptotic Phenotype



(A–C) Western blots and corresponding quantification of left ventricular (LV) protein levels of pro-apoptotic (Bax, Caspase-3) and antiapoptotic (B-cell lymphoma-2 [BCL-2] and B-cell lymphoma-extra-large [BCL-XL]) normalized to beta-actin levels from sham controls and the infarct zones of groups 1 and 3 (n = 3 per group). *p < 0.05 versus sham; #p < 0.05 versus group 1. **(D and E)** TUNEL-positive staining for deoxyribonucleic acid fragmentation from LV tissue from sham controls and from within the infarct zone in group 1 and group 3 (n = 3 per group).

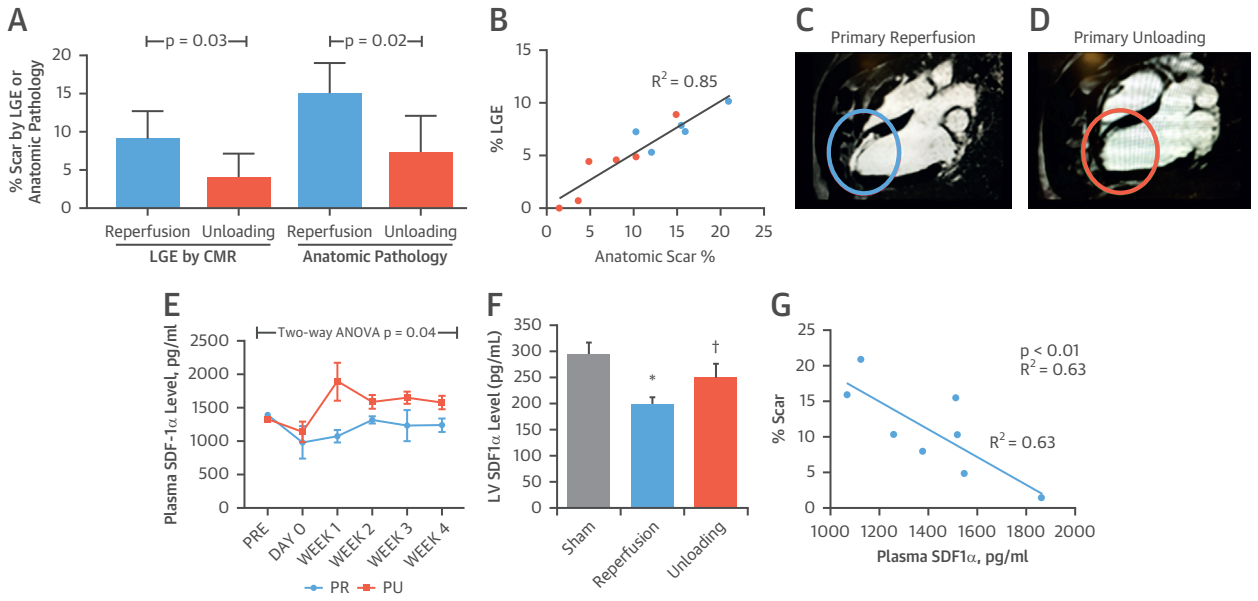
compared with reperfusion alone, LV unloading for 30 min before reperfusion triggers a broad shift in gene expression within the infarct zone, with significant protection of genes associated with mitochondrial function.

LV UNLOADING LIMITS SDF-1α DEGRADATION IN AMI. Given the importance of SDF-1α/CXCR4 signaling in cardioprotection during ischemia-reperfusion injury, SDF-1α and CXCR4 protein levels were quantified within the infarct zone. We observed that compared with sham controls, reperfusion alone (group 1), LV unloading for 15 min (group 2), or LV unloading after reperfusion (group 4) were associated with reduced protein levels of SDF-1α within the infarct zone (Figures 3A and 3B). In contrast, compared with sham controls, only LV unloading for 30 min before reperfusion (group 3) maintained SDF-1α protein levels within the infarct zone. CXCR4 levels remain unchanged across all 4 study groups compared with sham controls.

To determine whether increased SDF-1α levels are transcriptionally regulated, we quantified mRNA expression by using real-time PCR between groups and observed no difference in SDF-1α or CXCR4 gene expression (Figures 3C and 3D). Because SDF-1α is highly regulated by proteolytic degradation, we next explored expression of key proteases known to degrade SDF-1α. Compared with sham controls, reperfusion alone increased MMP-2 and MMP-9 activity levels, but LV unloading for 30 min before reperfusion did not (Figures 3E and 3F). Reperfusion alone increased DPP-4 expression and activity levels within the infarct zone compared with sham controls (Figures 3G and 3H). LV unloading for 30 min before reperfusion limited up-regulation of DPP-4 expression and activity. These data suggest that LV unloading for 30 min before reperfusion may preserve SDF-1α protein levels by limiting the activity of proteases known to degrade SDF-1α.

LOSS OF SDF-1α/CXCR4 ACTIVITY ATTENUATES THE CARDIOPROTECTIVE EFFECT OF LV UNLOADING. To explore whether SDF-1α/CXCR4 signaling is necessary for the cardioprotective effect of LV unloading, in a separate group of animals, we blocked CXCR4 activity using intracoronary delivery of AMD3100. Compared with vehicle-treated controls subjected to LV unloading for 30 min before reperfusion, loss of CXCR4 activity increased infarct size and reduced

FIGURE 5 Primary Unloading Reduces LV Scar by CMR and Anatomic Pathology



(A) Quantification of LV scar size 28 days after either primary reperfusion or primary unloading using late gadolinium enhancement (LGE) by cardiac magnetic resonance imaging (CMR) or according to anatomic pathology (n = 6 per group). **(B)** Regression plot showing correlation between LGE-CMR and anatomic pathologic quantification of LV scar size. **(C and D)** Representative CMR images showing LV scar within the **blue** or **red circles** (Online Videos 1 and 2). **(E)** Circulating levels of SDF-1α over 28 days after either P-reperfusion (PR) or P-unloading (PU) (n = 4 per group). **(F)** Protein levels of SDF-1a within the infarct zone 28 days after sham operation, P-reperfusion, or P-unloading (n = 6 per group). **(G)** Regression plot showing the correlation between LV scar size as a percentage of the total left ventricle versus plasma SDF-1α levels 28 days after myocardial infarction. *p < 0.05 versus sham; †p < 0.05 versus P-reperfusion. Abbreviations as in Figure 3.

cardioprotective signaling via the RISK pathway, including Akt, extracellular-regulated kinase, and glycogen synthase kinase 3b (Figures 3I and 3J). These findings suggest that SDF-1α/CXCR4 signaling is required for the cardioprotective effect of LV unloading before reperfusion.

LV UNLOADING LIMITS PROAPOPTOTIC SIGNALING. To further explore whether LV loading for 30 min reduces levels of proteins associated with apoptosis within the infarct zone, we observed that compared with sham controls, reperfusion alone (group 1) increased levels of proapoptotic proteins, including BAX and active caspase-3, and further reduced levels of antiapoptotic proteins, including BCL-2 and BCL-XL (Figures 4A to 4C). Compared with group 1, group 3 exhibited reduced levels of BAX and active caspase-3 and increased levels of the antiapoptotic BCL-2 and BCL-XL proteins. Compared to P-reperfusion, P-unloading reduced the number of TUNEL-positive cells within the infarct zone (Figures 4D and 4E).

COMPARED WITH PRIMARY REPERFUSION, PRIMARY UNLOADING REDUCES MYOCARDIAL INFARCT SIZE AND PRESERVES CARDIAC FUNCTION 28 DAYS AFTER AMI. To confer clinically relevant cardioprotection, the observed effect of P-unloading on infarct size reduction must be maintained beyond the acute treatment phase. To test this theory, adult male swine were treated with either P-reperfusion or P-unloading, and LV scar size, LV function, and molecular changes associated with heart failure were quantified 28 days after MI. Fourteen animals completed the ischemia-reperfusion phase of the protocol. Two animals in the P-reperfusion group died within 6 h after reperfusion and 12 animals survived to 28 days (6 per group).

Compared with P-reperfusion, P-unloading reduced LV scar size quantified by using LGE (3.9 ± 3.2% vs. 9 ± 3.7%; p = 0.03) and anatomic pathology (7.2 ± 4.9% vs. 14.9 ± 4.1%; p = 0.02) (Figure 5A). Histological planimetry of infarct size correlated directly with percentage LGE from CMR (R^2 = 0.85) (Figures 5B to 5D, Online Videos 1 and 2). Using CMR-derived volumes, end-diastolic volume and end-systolic volume were similar between groups (end-diastolic volume: 152 ± 29 ml vs. 142 ± 14 ml; P-reperfusion vs. P-unloading [p = NS]; end-systolic volume: 86 ± 26 ml vs. 74 ± 6 ml; P-reperfusion vs. P-unloading [p = NS]). CMR-derived LV mass did not

TABLE 1 Hemodynamic Variables 28 Days After Acute Myocardial Infarction

	Primary Reperfusion	Primary Unloading	p Value
Heart rate, beats/min	63 ± 9	73 ± 12	NS
LV EDV, ml	190 ± 13	248 ± 54	NS
LV ESV, ml	150 ± 15	195 ± 47	NS
LV stroke volume, ml	40 ± 6	54 ± 7	0.02
LV cardiac output, l/min	2.5 ± 0.2	3.9 ± 0.6	0.006
LV stroke work, ml × mm Hg	2,195 ± 307	3,075 ± 339	0.008
LV systolic pressure, mm Hg	79 ± 3	78 ± 10	NS
LV end-diastolic pressure, mm Hg	11.3 ± 2.5	7.4 ± 1.5	0.02

Values are mean ± SD.

EDV = end-diastolic volume; ESV = end-systolic volume; LV = left ventricular; NS = not significant.

differ between groups (90.4 ± 10.6 g vs. 84.4 ± 8.6 g; P-reperfusion vs. P-unloading [p = NS]). Compared with P-reperfusion, hemodynamic analysis using LV conductance catheters showed that P-unloading was associated with higher stroke volume (54 ± 7 ml vs. 40 ± 6 ml; p = 0.02), cardiac output (3.9 ± 0.6 l/min vs. 2.5 ± 0.2 l/min; p = 0.006), and stroke work (3,075 ± 339 ml × mm Hg vs. 2,195 ± 307 ml × mm Hg; p = 0.008) (Table 1).

PRIMARY UNLOADING INCREASES CIRCULATING AND TISSUE LEVELS OF SDF-1α LEVELS ACUTELY AND 28 DAYS AFTER AMI. Compared with P-reperfusion, P-unloading increased circulating SDF-1α levels during the 28 days after AMI with a peak SDF-1α level 1 week after AMI (Figure 5E). In contrast, P-reperfusion failed to increase circulating SDF-1α levels at any time point after AMI. Compared with sham controls, P-reperfusion decreased SDF-1α protein levels within the infarct zone of the left ventricle, but P-unloading did not. Circulating SDF-1α levels on day 28 after AMI correlated inversely with LV scar size (Figures 5F and 5G).

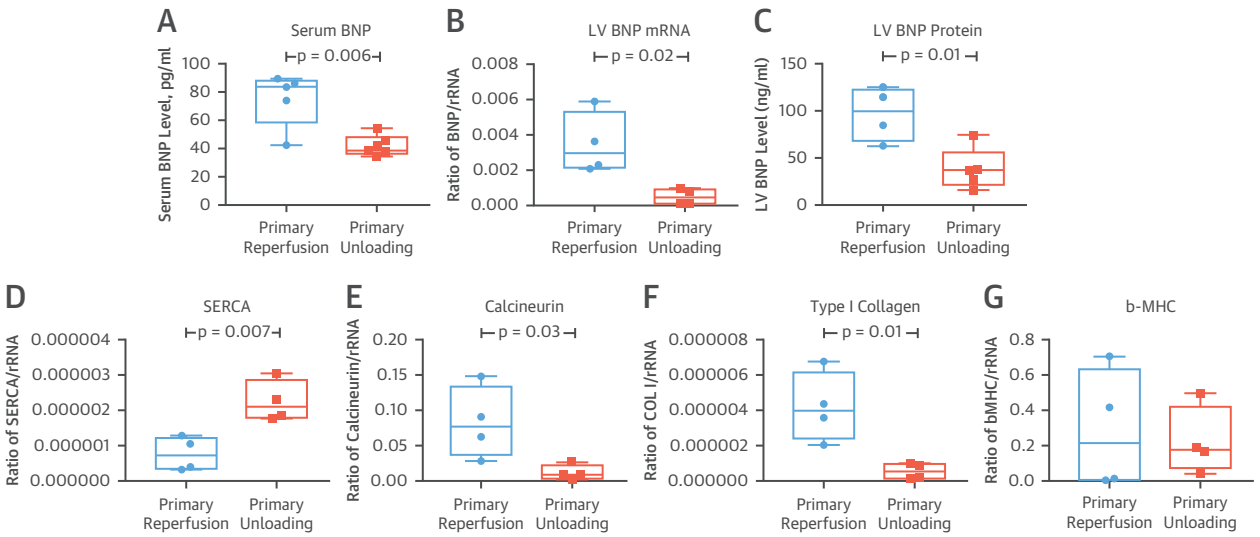
PRIMARY UNLOADING LIMITS MALADAPTIVE CARDIAC REMODELING. Compared with P-reperfusion, P-unloading reduced circulating levels of B-type natriuretic peptide (BNP) 28 days after AMI (Figure 6A). Compared with sham controls, P-reperfusion increased BNP mRNA and protein levels within the noninfarct zone (Figures 6B and 6C). In contrast, P-unloading attenuated any increase in tissue levels of BNP within the noninfarct zone of the left ventricle. Compared with P-reperfusion, P-unloading increased mRNA levels of sarcoplasmic/endoplasmic reticulum calcium ATPase and reduced levels of calcineurin and type I collagen without affecting levels from the noninfarct region of the left ventricle (Figures 6D to 6F).

DISCUSSION

The central finding of this report is that P-unloading for 30 min before reperfusion alters several key biological pathways involving cellular respiration and post-translation regulation of SDF-1α levels, thereby reducing acute infarct size (Central Illustration). Furthermore, P-unloading reduced LV scar size and improved cardiac function 28 days after AMI. Specifically, we report that: 1) 30 min of P-unloading is necessary and sufficient before reperfusion to limit infarct size; 2) P-unloading triggers a global shift in gene expression associated with protection of mitochondrial integrity within the infarct zone; 3) compared with P-reperfusion, P-unloading for 30 min preserves SDF-1α protein levels without changing SDF-1α mRNA levels within the infarct zone and further promotes a shift toward antiapoptotic signaling within the infarct zone; 4) P-unloading reduces activity levels of proteases known to degrade SDF-1α; and 5) P-unloading reduces LV scar size, preserves cardiac output, reduces BNP expression, and limits expression of genes and proteins associated with maladaptive remodeling within the noninfarct zone 28 days after AMI. These data identify P-unloading as a novel approach to enhance cardioprotective mechanisms that may preserve cardiac function after AMI.

In 2013, we reported for the first time that compared with P-reperfusion, unloading the left ventricle using a percutaneously delivered extracorporeal, centrifugal pump (TandemHeart, LivaNova, London, United Kingdom) and delaying coronary reperfusion (P-unloading) reduces myocardial infarct size in a nonsurgical swine model of AMI (21). We next tested the reproducibility and translational potential of P-unloading by activating a more efficient percutaneously delivered transvalvular pump (Impella) directly into the left ventricle and delaying coronary reperfusion for 60 min (9). In both reports, infarct size was reduced by 40% to 50% despite delayed reperfusion and correlated with reduced LV wall stress and increased SDF-1α levels and activity. Based on the population studies described here, if a fraction of the infarct reduction observed in these preclinical models is evident in humans, P-unloading could improve survival and reduce ischemic heart failure after AMI. In this report, we sought to address the following: 1) the importance of delayed reperfusion after LV unloading; 2) the impact of P-unloading on global myocardial gene expression; 3) the mechanism by which P-unloading increases SDF-1α levels; and 4) whether P-unloading can affect late-term LV remodeling weeks after AMI.

FIGURE 6 Primary Unloading Is Associated With a Reduction in Biomarkers and Genes Linked to Heart Failure and Maladaptive Cardiac Remodeling at 28 Days' Post-AMI

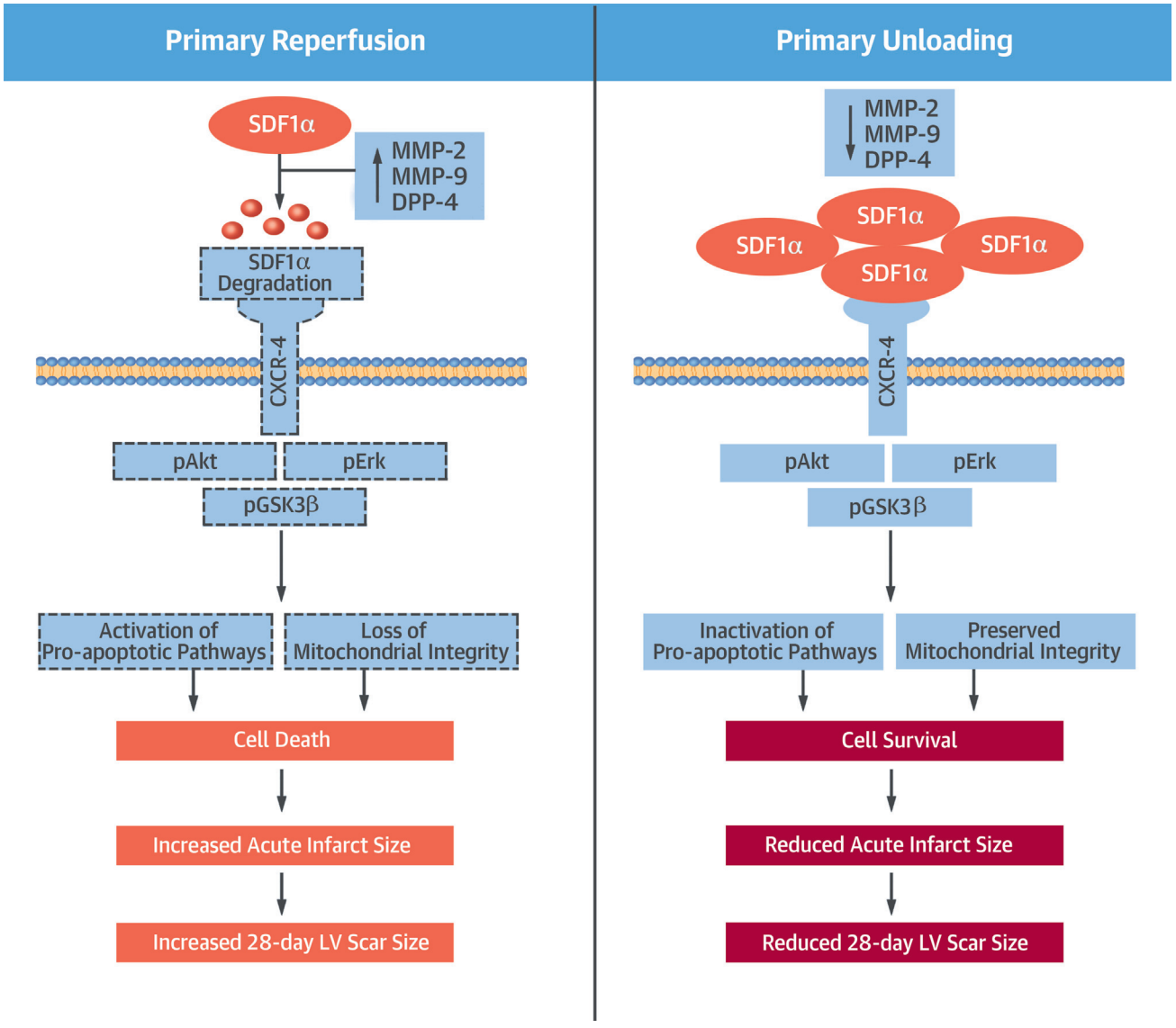


(A to C) Circulating, mRNA, and protein levels of B-type natriuretic peptide (BNP) from LV tissue (noninfarct zone) 28 days after primary reperfusion or primary unloading. **(D to G)** Messenger ribonucleic acid (mRNA) levels of sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), calcineurin, type I collagen (COL1), and beta-myosin heavy chain (b-MHC) from LV tissue (noninfarct zone) 28 days after primary reperfusion or primary unloading.

Whether the delay to reperfusion after mechanical LV unloading is required to limit infarct size remains poorly understood. To address this question, we studied the impact of mechanical LV unloading for 15 or 30 min before reperfusion and unloading 30 min after reperfusion. We identified that 30 min of mechanical LV unloading with a TV-pump before, not after, reperfusion limits acute infarct size. This observation suggests for the first time that LV unloading itself may be a therapy as opposed to simply an adjunct supportive approach for a dysfunctional left ventricle. One explanation for the beneficial effects of 30 min of mechanical LV unloading before reperfusion is that LV unloading biologically primes the myocardium for reperfusion. Future studies are required to explore the exact biological effects occurring during 30 min of LV unloading; however, we were able to determine that 30 min of P-unloading was necessary and sufficient to reduce infarct size and increase protein levels of SDF-1α within the infarct zone. Other possible explanations that require further study include an impact of LV unloading on microcirculatory coronary flow dynamics, changes in myocardial energy utilization, and reduction in oxidative stress during the period of LV unloading before reperfusion.

We next used a genomics approach to better understand the biological impact of P-unloading versus P-reperfusion. We identified that compared with P-reperfusion, P-unloading for 30 min differentially alters expression of >600 genes within the infarct zone. Pathway analysis identified that P-unloading preserved expression of genes associated with cellular respiration and mitochondrial integrity. We confirmed these observations with direct quantification of select genes from each component of the electron transport chain involved in cellular respiration. Electron microscopy of cardiomyocytes from the infarct zone further showed swollen and disrupted mitochondria after P-reperfusion but after not P-unloading. These findings are consistent with a previous report by Achour et al. (26), who identified in 2005 that activation of a transvalvular axial flow pump preserves cardiomyocyte mitochondrial integrity after ischemia and reperfusion. Multiple previous studies have established that ischemia-reperfusion injury uncouples oxidative phosphorylation and reduces adenosine triphosphate synthesis within cardiomyocytes, thereby leading to generation of reactive oxygen species and subsequent disruption of mitochondrial integrity by activation of a mitochondrial permeability transition pore (27–29). Our findings now suggest that initiation of LV unloading before reperfusion may limit the impact of ischemia-reperfusion injury on mitochondrial integrity, thereby promoting cardiomyocyte survival.

CENTRAL ILLUSTRATION **Mechanistic Insight Into the Biological Impact of Primary Unloading**



Esposito, M.L. et al. J Am Coll Cardiol. 2018;72(5):501–14.

Mechanically unloading the left ventricle for a minimum of 30 min before reperfusion limits expression of proteolytic enzymes that degrade stromal-derived factor-1α (SDF1α), thereby increasing cardioprotective signaling improving cell survival, and reducing both acute infarct size and subsequent myocardial scar size 28 days after acute myocardial infarction. DPP-4 = dipeptidyl peptidase-4; LV = left ventricular; MMP = matrix metalloproteinase.

Since 2005, multiple reports have identified that intermittent periods of myocardial or skeletal muscle ischemia, known as ischemic pre-conditioning and remote ischemic conditioning, respectively, before reperfusion may reduce myocardial infarct size (30,31). The molecular pathways governing these "ischemic conditioning" models of cardioprotection include activation of phospho-inositol-3 kinase, Akt, and extracellular-regulated kinase, which limit formation of the mitochondrial permeability transition pore. Increased expression of SDF-1α also promotes Akt-mediated phosphorylation, inactivation of glycogen synthase kinase-3β, and a subsequent shift toward antiapoptotic signaling, which prevents mitochondrial permeability transition pore formation and reduces myocardial infarct size. In the

present study, we observed that compared with P-reperfusion, P-unloading failed to increase SDF-1α mRNA levels within the infarct zone. However, we did observe that compared with sham controls, P-reperfusion reduced SDF-1α protein levels within the infarct zone. In contrast, LV unloading for 30 min before reperfusion preserved SDF-1α protein levels.

Because SDF-1α levels are highly regulated by proteases associated with inflammation, we next explored whether protein and activity levels of key regulatory proteases such as MMP-2, MMP-9, or DPP-4 were altered by P-reperfusion and P-unloading. Consistent with previous reports, we observed that compared with sham controls, P-reperfusion increases, but P-unloading attenuates, activity of these proteases. To further establish the downstream effect of P-unloading, we also observed reduced expression of proteins associated with apoptosis within the infarct zone. These findings suggest for the first time that 30 min of P-unloading limits protease activity within the infarct zone, which limits SDF-1α degradation in the setting of an AMI.

To better understand the potential for clinical translation of P-unloading, we designed a preclinical study in which animals were assigned to P-reperfusion or P-unloading and then quantified LV scar 28 days later by using cardiac MRI. We observed for the first time that P-unloading reduced infarct scar size as blindly quantified by LGE-CMR, which tightly correlated with anatomic measurements of myocardial scar size. We further quantified well-established molecular markers of maladaptive remodeling in the noninfarct zones where the bulk of compensatory remodeling would occur in response to a large anterior MI. We observed that compared with P-reperfusion, P-unloading reduced calcineurin, beta myosin heavy chain, and BNP levels, while preserving sarcoplasmic/endoplasmic reticulum calcium ATPase levels 28 days after AMI. Furthermore, circulating and LV tissue levels of a clinically relevant biomarker of heart failure, BNP, were reduced after P-unloading but not after P-reperfusion. These findings are the first to identify that use of a transvalvular pump at the time of AMI has durable effects on both LV scar size and markers of maladaptive remodeling 28 days later. For decades, we have focused on immediate reperfusion in AMI; however, these data suggest for the first time that the "pre-reperfusion time period" is a critical moment that may allow for interventions such as LV unloading and delayed reperfusion to have a durable effect on late-term cardiac remodeling. Whether these preclinical findings translate to clinical outcomes remain to be determined.

Finally, we quantified SDF-1α levels after AMI and observed increased circulating and LV tissue levels of SDF-1α levels 28 days after P-unloading but not after P-reperfusion. Circulating SDF-1α levels correlated inversely with LV scar size. These findings identify that in addition to providing an acute reduction in infarct size after MI, P-unloading promotes a more durable reduction in LV scar size, improves cardiac function, and limits maladaptive remodeling after AMI. By using clinically relevant biomarkers of myocardial injury, including CMR and circulating BNP levels, our findings suggest that there may be a strong translational potential for P-unloading as an approach to limit ischemic heart failure after AMI.

STUDY LIMITATIONS. Limitations include the small number of animals studied in the chronic phase, which was due in part to a 37% mortality rate among animals subjected to LAD occlusion and the cost associated with large-animal studies. Another limitation of this study is the duration of ischemia. All animals were subjected to 90 min of LAD occlusion. However, duration of ischemia cannot be controlled for in the clinical setting. Future studies will further explore the functional role of SDF-1α in post-ischemic heart failure and the impact of P-unloading on other signaling pathways involving inflammation, fibrosis, and mitochondrial function. Future studies will also determine whether P-unloading can be combined with other cardioprotective approaches to incrementally reduce infarct size and to further study the late-term effects of P-unloading months after acute MI.

CONCLUSIONS

The present findings now show for the first time that activation of a transvalvular, micro-axial flow pump for 30 min before reperfusion limits both acute infarct size and subsequent scar size compared with P-reperfusion alone. We further provide new mechanistic insight into the biological impact of myocardial unloading and activation of cardioprotective pathways within the infarct zone. Based on these observations, a Phase I clinical safety and feasibility study of transvalvular pump activation in AMI is underway (The Door to Unloading Trial; NCT03000270) (32). If successful, P-unloading may shift the management paradigm of patients with AMI and provide a new approach to prevent the onset of heart failure after AMI.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Mechanically unloading the left ventricle for at least 30 min before coronary reperfusion increases signaling via the cardioprotective cytokine SDF-1α, and reduces infarct size and subsequent LV scarring.

TRANSLATIONAL OUTLOOK: Clinical studies are needed to assess the potential therapeutic value of primary unloading to prevent heart failure after AMI.

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KEY WORDS acute myocardial infarction, cardioprotection, ischemia-reperfusion injury, mechanical circulatory support

APPENDIX For an expanded Methods section, supplemental videos and their legends, and a supplemental table, please see the online version of this paper.