Mechanically Unloading the Left Ventricle Before Coronary Reperfusion Reduces Left Ventricular Wall Stress and Myocardial Infarct Size

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- **Background**—Ischemia/reperfusion injury worsens infarct size, a major determinant of morbidity and mortality after acute myocardial infarction (MI). We tested the hypothesis that reducing left ventricular wall stress with a percutaneous left atrial-to-femoral artery centrifugal bypass system while delaying coronary reperfusion limits myocardial injury in a model of acute MI.
- *Methods and Results*—MI was induced by balloon occlusion of the left anterior descending artery in adult male swine. In the MI group (n=4), 120 minutes of left anterior descending artery occlusion was followed by 120 minutes of reperfusion without mechanical support. In the mechanically supported group (MI+unload; n=4), percutaneous left atrial-to-femoral artery bypass was initiated after 120 minutes of ischemia, and left anterior descending artery occlusion was prolonged for an additional 30 minutes, followed by 120 minutes of reperfusion with device support. All animals were euthanized after reperfusion, and infarct size was quantified by triphenyltetrazolium chloride staining. Compared with baseline, mean left ventricular wall stress and stroke work were not changed at any point in the MI group but were decreased after reperfusion in the MI+unload group (mean left ventricular wall stress, 44658 versus 22963 dynes/cm²; stroke work, 2823 versus 655 mmHg·mL, MI versus MI+unload). Phosphorylation of reperfusion injury salvage kinase pathway proteins from noninfarcted left ventricular tissue was unchanged in the MI group but was increased in the MI+unload group. Compared with the MI group, total infarct size was reduced in the MI+unload group (49% versus 28%, MI versus MI+unload).

Conclusions—These data support that first unloading the left ventricle despite delaying coronary reperfusion during an acute MI reduces myocardial injury. (*Circulation*. 2013;128:328-336.)

Key Words: heart-assist devices ■ myocardial infarction ■ reperfusion injury

n acute myocardial infarction (AMI) affects nearly 8 mil-A nacute myocalular inflation (Land) Alion individuals annually worldwide and is commonly caused by thrombotic occlusion of a coronary artery, leading to myocardial ischemia, cardiomyocyte necrosis, and infarction within minutes of onset.^{1,2} Beginning with the open artery theory in the 1970s, the field of AMI management has been dominated by the principle that time is muscle, recognizing that prolonged coronary occlusion leads to ongoing myocardial injury.^{3,4} For this reason, the well-established paradigm of contemporary management of AMI focuses on rapid coronary reperfusion via balloon angioplasty and stenting to limit myocardial injury. The metric for success in AMI therapy is a door-to-balloon time, defined as the interval from patient arrival in the emergency department to mechanical reperfusion of the occluded coronary artery, of <90 minutes. The door-to-balloon time is a standard part of American College of Cardiology/American Heart Association guidelines and a core quality measure of hospital performance.⁵ However, despite timely reperfusion, nearly 10% of AMI subjects die during their index hospitalization, and 25% of survivors progress to develop chronic heart failure.⁶ One explanation for these poor outcomes is that reperfusion of ischemic myocardium can also cause cardiomyocyte death and microvascular damage through a process referred to as myocardial ischemia/reperfusion injury.^{6,7} These data suggest that despite progress in the treatment of AMI, a major scientific problem is the need for novel approaches to limit myocardial damage resulting from reperfusion injury.

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Pharmacological approaches to limit reperfusion injury have focused on attenuating mitochondrial permeability transition pore generation and promoting activity of reperfusion injury salvage kinases (RISK), including extracellular

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regulated kinase (ERK) and the serine/threonine kinase Akt.8-12 Other approaches to limit ischemia/reperfusion injury include systemic hypothermia, which has also shown promise as a method to reduce total body metabolic demand¹³; however, this approach does not specifically target myocardial injury in AMI. Alternatively, the generation of repeated periods of myocardial ischemia and reperfusion with balloon angioplasty, known as ischemic conditioning, has also been shown to promote activity of the RISK pathway.^{14–16} Although promising, critical barriers to these cardioprotective strategies include the multifactorial nature of reperfusion injury, thereby limiting the impact of a single-target pharmacological strategy; the potential for coronary vascular injury (dissection or perforation) with ischemic conditioning; and the mandate for rapid coronary reperfusion and thus insufficient time for a drug to penetrate into myocardial injury zones. A need exists for improved cardioprotective strategies that broadly affect the multiple levels of reperfusion injury without causing further myocardial damage. In this study, we explore the central hypothesis that initially reducing left ventricular (LV) wall stress by mechanically reducing LV preload while delaying coronary reperfusion activates endogenous anti-ischemia/reperfusion injury signaling pathways and limits myocardial injury in the setting of AMI.

Methods

Studies were conducted in 15 adult male Yorkshire swine weighing 45±4 kg. The study protocol was approved by the Institutional Animal Care and Use Committee at Tufts Medical Center. All experiments were performed according to the committee's guidelines.

Experimental Protocol of MI and Mechanical Circulatory Support

Animals were premedicated with Telazol (0.8 mL/kg IM). General anesthesia was induced and maintained with isoflurane (1%-2%). All animals were intubated and mechanically ventilated (Harvard Apparatus Inc) with room air and supplemented oxygen to maintain physiological pH and oxygen saturation. Surface ECG leads, an orogastric tube, peripheral 18-gauge 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 (10F), left carotid artery (7F), and both femoral arteries (7F) and veins (10F). A pulmonary artery catheter was deployed via the right internal jugular vein. Unfractionated heparin boluses with a goal activated clotting time of 300 to 400 seconds, continuous lidocaine infusion (1 mg/kg), and noradrenaline (0.16 µg/min) were initiated in all animals. A 6F Judkins right coronary catheter (Boston Scientific) engaged the left coronary artery via the right femoral artery, and baseline angiograms were recorded. A 90-cm guidewire was delivered into the distal left anterior descending artery (LAD), and a 3.0×8-mm bare metal stent (Boston Scientific) 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. Length of the LAD distal to the stented segment was measured in the anteroposterior projection offline with Encompass quantitative angiography software (Heartlab, Inc, Agfa HealthCare, Westerly, RI). Animals were then euthanized with pentobarbital and phenytoin after 120 minutes of reperfusion (Figure 1A).

To explore the impact of ischemia/reperfusion injury in a clinically relevant model of AMI, 12 adult male swine were subjected to 120 minutes of mid-LAD occlusion, and 3 animals served as sham controls. Of the 12 animals in which MI was induced, 4 animals died within 60 minutes (at 40, 20, 30, and 60 minutes) of LAD occlusion as a result of refractory ventricular fibrillation. The surviving 8



Figure 1. A, Swine model of acute myocardial infarction (MI) and MI with activation of a percutaneous left atrial-to-femoral artery (pLA-FA) centrifugal bypass pump after 120 minutes of left anterior descending artery (LAD) occlusion with persistent occlusion of the LAD for an additional 30 minutes, followed by 120 minutes of reperfusion. Left ventricular (LV) unloading continued throughout reperfusion (MI+LV unloading; n=4 per group at each time point: baseline, occlusion, and reperfusion). B, Illustration of pLA-FA bypass. C, Fluoroscopic image of a swine heart with full instrumentation for hemodynamic analysis with pLA-FA bypass during mid-LAD occlusion. PA indicates pulmonary artery; PTCA: percutaneous transluminal coronary angioplasty; and RFA, right femoral artery.

animals were randomly assigned to either the MI-alone group (n=4) or the MI+unload group (n=4) (defined below). In the MI group, 120 minutes of LAD occlusion was directly followed by 120 minutes of reperfusion without mechanical support, at which time the animals were euthanized and LV samples were obtained. In the mechanically supported group (MI+unload), we used a centrifugal-flow pump (TandemHeart, Cardiac Assist Inc), as described below, which is used currently in clinical practice as a percutaneous left atrial-to-femoral artery (pLA-FA) support system.¹⁷⁻¹⁹ In the MI+unload group, the centrifugal-flow pump was deployed and clamped until the completion of 120 minutes of LAD occlusion, at which time the pump was activated. A flow probe attached to the outflow cannula directly measured device flow. Coronary reperfusion was then delayed for an additional 30 minutes in this group. After reperfusion, the mechanical pump remained active throughout the 120 minutes of reperfusion, followed by euthanasia and tissue harvesting as in the MI group (Figure 1A). To deploy the pLA-FA support system, transseptal puncture of the interatrial septum was performed with a Brockenbrough needle and a transseptal dilator (Boston Scientific) via the right femoral vein. An Inoue wire was deployed into the left atrium, and a staged 14F-to-21F dilator was used to place a 21F inflow cannula into the left atrium (Figure 1B and 1C). Next, a 15F arterial cannula was placed into the left femoral artery. Both cannulas were then connected to a desired centrifugal-flow pump and clamped (Figure 1B). Finally, 3 shamoperated animals were intubated, anesthetized, and mechanically ventilated without MI or mechanical unloading. LV tissue samples obtained from sham controls were used for Western blot analysis.

Conductance Catheter Assessment of LV Pressure and Volume

Changes in LV pressure and volume were assessed with a 5F conductance catheter system (Sigma-M, CD Leycom, the Netherlands) deployed via the left carotid artery into the LV (Figure 1C). Ventricular pressure and volume were measured at baseline, after 120 minutes of LAD occlusion, and after 120 minutes of reperfusion with a solidstate pressure transducer and dual-field excitation mode, respectively, as previously described.^{20,21} Briefly, the method measures time-varying electric 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 LV was confirmed by 3-dimensional (3D) echocardiography and fluoroscopy. Timevarying segmental conductance has been shown to reflect segmental LV volumes in prior preclinical and clinical studies.^{20,21} Parallel conductance was assessed by injecting 20 mL hypertonic (6%) saline into the right internal jugular vein.22 Absolute LV volumes were measured by subtracting parallel conductance from total conductance volumes and further confirmed by direct volumetric measurements using 3D echocardiography as described below. Stroke volume is calculated as the difference in conductance volumes at dP/dtmax and -dP/dtmin. An estimated end-systolic pressure-volume relationship was calculated as peak LV end-systolic pressure divided by stroke volume for a single cardiac cycle. LV stroke work was calculated as the product of peak LV peak systolic pressure and stroke volume.

3-Dimensional Echocardiography

To evaluate LV myocardial mechanics, 3D speckle tracking echocardiography was performed with the Artida 4D System (Toshiba Medical Systems, Tustin, CA) and matrix-array PST-25SX transducer. For optimal imaging, all animals underwent a 4-cm midline abdominal incision for subdiaphragmatic placement of the transducer in proximity to the LV apex. The 3D data sets consisted of apical full volumes created by the combination of 6 ECG-gated, wedge-shaped subvolumes during a single breath hold. Along with the combination of 6 subvolumes, both optimization of 3D images and adjustments in depth and full volume sector angles yielded a temporal resolution of 20 to 30 volumes per second. Offline processing for speckle tracking analysis was performed with the Wall Motion Tracking software (Toshiba Medical Systems) as previously described.23-25 Longitudinal and circumferential LV strain with 3D speckle tracking echocardiography was quantified as myocardial deformation, defined as the percent change in length between pairs of points throughout the cardiac cycle relative to the initial length in the longitudinal and circumferential directions, respectively, relative to the endocardial contour. The 3D speckle tracking echocardiography-derived global values for longitudinal and circumferential strain were obtained from the rendered 16-segment model with exclusion of the apical-most segment resulting from imaging dropout. LV peak wall stress, mean wall stress, and systolic wall tension were also calculated as previously described.26-28

Determination of MI Size

On completion of the study protocol, animals were euthanized, and hearts were immediately excised and manually flushed with normal saline. Both atria and the right ventricle were removed, and the LV was cross-sectioned into 1-cm segments perpendicular to the long axis. Coronary stents were visualized to confirm the location of the mid-LAD occlusion segment. Biopsy specimens were obtained from the anteroapical LV distal to the site of stent deployment (infarct zone) and from the posterobasal wall (noninfarct zone) for molecular analysis, and LV slices were then incubated in 1% triphenyltetrazolium chloride at 37°C for 5 minutes for noninfarct staining. LV slices were then photographed, and digitized planimetry was used to quantify infarct zones expressed as a percentage of total myocardium. Blood samples obtained during the study protocol were centrifuged at 5000 rpm for 15 minutes for isolation of serum and plasma, which were then stored at -80°C. Cardiac biomarkers (creatine kinase-MB and troponin-I) were measured by Anilytics (Gaithersburg, MD).

Immunoblot Analysis (Western)

Total protein was extracted from tissue homogenates isolated as previously described.¹⁴ Immunoblot analysis was then performed

as previously described^{29,30} with antibodies against porcine total ERK, phosphorylated ERK-1/2, total AKT, phosphorylated Akt, and GAPDH. Expression of phosphorylated ERK-1/2 and phosphorylated Akt was normalized to both total protein levels and GAPDH.

Statistical Analysis

Results are presented as mean±SD. All data within groups were analyzed by nonparametric 2-way repeated-measures ANOVA on ranks followed by a Holm-Sidak comparison if warranted. Simple linear regression analysis was used to evaluate for a correlation between 2 parameters. Given the small sample size (n=4 per group), any *P* values throughout the article from comparisons of the randomized groups are unreliable and should be interpreted with caution. All statistical analyses were performed with SigmaStat version 3.1 (Systat Software, Inc). An α level of *P*<0.05 was considered to indicate a significant effect or between-group difference.

Results

Mechanical Unloading in AMI Promotes Hemodynamic Stability and Reduces LV Wall Stress

The hemodynamic effects of ischemia and reperfusion injury with and without mechanical unloading are shown in the Table. In both the MI and MI+unload groups, 120 minutes of LAD occlusion did not significantly change mean arterial pressure, dP/dtmax, LV systolic pressure, or estimated endsystolic pressure-volume relationship (Table). Compared with baseline values, LV end-diastolic pressure was increased in the MI group after 120 minutes of reperfusion but not in the MI+unload group. In the MI+unload group, pLA-FA bypass provided 3.2±0.2 L/min of flow. Furthermore, after 120 minutes of reperfusion, native cardiac output was decreased whereas mean arterial pressure and estimated endsystolic pressure-volume relationship were increased in the MI+unload group compared with the MI group (Table and Figure I in the online-only Data Supplement). Compared with baseline values, LV stroke work was not significantly altered at either time point in the MI group. In contrast, in the MI+unload group, after 120 minutes of reperfusion, LV stroke work was significantly reduced compared with baseline values and compared with the MI group (Figure 2). After 120 minutes of reperfusion, LV mean wall stress (44658±13925 versus 22963±8610 dynes/cm², MI versus MI+unload; P=0.009) and LV systolic wall tension (1595±789 versus 736±119 dynes/cm, MI versus MI+unload; P=0.02) were significantly reduced, and a trend toward reduced LV peak wall stress (94±37 versus 43±18×10³ dynes/cm², MI versus MI+unload; P=0.06) was observed in the MI+unload group compared with the MI group (Figure 2). Given the small sample size (n=4 per group), any P values throughout the article from comparisons of the randomized groups are unreliable and should be interpreted with caution. These findings support that despite identical degrees of hemodynamic compromise resulting from LAD occlusion, pLA-FA bypass in the MI+unload group reduced LV wall stress and stroke work while supporting mean arterial pressure.

Reperfusion Impairs Myocardial Strain

We next examined the functional impact of ischemia/reperfusion injury in AMI on LV volumes and strain with 3D speckle tracking echocardiography. In both the MI and MI+unload

	Baseline (n=4 per group)		120 Minutes of Occlusion (n=4 per group)		<i>P</i> Value			120 Minutes of Reperfusion (n=4 per group)		P Value		
	MI	MI+Unload	MI	MI+Unload	*	†	‡	MI	MI+Unload	*	†	‡
Heart rate, bpm	82±15	88±20	74±5	85±13				91±45	78±11			
Mean arterial pressure, mm Hg	70±11	71±12	57±8	58±10				43±5	52±3*	0.03	0.01	0.02
LV stroke volume, mL	61±20	62±41	73±16	60±13				53±18	22±16*	0.01		
Cardiac output, L/min	6±2	6±3	6±1	5±2				5±1	3±1*	0.03		
dP/dtmax	1206±289	1063±253	1015±434	1029±283				946±391	614±453			
dP/dtmin	1095±244	1037±62	741±395	807±117				736±465	381±266			
PA diastolic pressure, mm Hg	12±2	12±3	20±5	21±3		0.03	0.02	31±4	19±3*	0.02	0.04	0.03
LV end-systolic pressure, mm Hg	92±8	89±8	73±19	85±10				71±22	56±41			
LV end-diastolic pressure, mm Hg	14±4	12±4	19±3	18±3				27±6	10±9*	0.04	0.03	
LV end-systolic volume, mL	70±8	66±7	79±13	76±5				91±26	44±16*	0.04		
LV end-diastolic volume, mL	130±17	129±41	153±13	136±9				145±21	83±35*	0.03		
ESPVR, mm Hg/mL	1.5±0.3	1.4±0.3	1.0±0.3	1.1±0.2				0.8±0.1	1.7±0.5*	0.03	0.02	

Table. Hemodynamic Variables at Baseline, After 120 Minutes of LAD Occlusion, and After Completion of 120 Minutes of Reperfusion for Both the MI-Only and MI-Unload Groups

No significant differences were observed between groups at baseline. Data are expressed as mean±SD. ESPVR indicates end-systolic pressure-volume relationship; LAD, left anterior descending artery; MI, myocardial infarction; MI+unload, myocardial infarction with mechanical unloading of the left ventricle; and PA, pulmonary artery.

*MI vs MI+unload; †MI vs baseline; ‡MI+unload vs baseline.

groups, 120 minutes of LAD occlusion did not significantly change LV end-systolic and end-diastolic volumes from baseline. In the MI group, after 120 minutes of reperfusion, LV end-systolic and end-diastolic volumes remained unchanged. In contrast, compared with baseline values, LV end-diastolic volume was significantly reduced after 120 minutes of reperfusion in the MI+unload group (Table and Figure I in the online-only Data Supplement). Both LV end-systolic and enddiastolic volumes were lower in the MI+unload group compared with the MI-alone group. We next examined changes in circumferential and longitudinal LV strain. In both groups, 120 minutes of LAD occlusion did not significantly change either circumferential (Figure 3A and 3B) or longitudinal (Figure IIA in the online-only Data Supplement) strain. Longitudinal



Figure 2. Changes in pressure-volume (PV) loops during myocardial infarction (MI) with and without mechanical unloading. **A**, **Top**, Representative PV loops from a swine subjected to ischemia/reperfusion alone (MI). **Bottom**, Representative PV loops with ischemia and reperfusion during left ventricular (LV) unloading with percutaneous left atrial-to-femoral artery bypass (MI+unload). **B**, Left ventricular stroke work (LV SW), (**C**) LV mean wall stress (LVMWS), (**D**) LV peak wall stress (LVPWS), and (**E**) LV wall tension in the MI and MI+unload groups. *P<0.05 vs MI reperfusion; †P<0.05 vs baseline.

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Figure 3. Three-dimensional echocardiography strain analysis. **A**, Representative changes in percent circumferential global left ventricular (LV) strain during myocardial infarction (MI) and MI with LV unloading using a 12-segment analysis with exclusion of the apical 4 segments. **B**, Changes in circumferential global LV strain with MI alone vs MI+unload. **P*<0.05 vs MI reperfusion; †*P*<0.05 vs baseline. **C**, Regression plot of circumferential global LV strain and LV mean wall stress (*R*=0.72, *P*=0.004). Ant indicates anterior; inf, inferior; lat, lateral; post, posterior; and sept, septal.

strain remained unchanged after 120 minutes of reperfusion in the MI group. Circumferential LV strain in the MI and MI+unload groups was reduced compared with baseline values after reperfusion (P=0.003) with a greater reduction observed in the MI+unload group compared with the MI-alone group ($-12\pm-2\%$ versus $-7\pm-3\%$, MI versus MI+unload; P=0.03; Figure 3B). Changes in global circumferential LV strain correlated directly with LV mean wall stress (R=0.72, P=0.004; Figure 3C) and stroke work (R=0.61, P=0.03; Figure IIB in the online-only Data Supplement). Consistent with our findings with pressure-volume loop analysis, these findings confirm that mechanical support during reperfusion reduces LV volumes and circumferential LV strain and further identify a potentially important correlation between our invasive and noninvasive measures of LV function.

Mechanical Unloading Promotes RISK Pathway Activity

To begin exploring whether mechanical unloading promotes myocardial salvage in AMI, we quantified RISK pathway activation. Compared with sham controls, LV tissue from the noninfarct zone showed no change in phosphorylated ERK and phosphorylated Akt expression in the MI group. However, compared with sham controls or the MI group, phosphorylated ERK and phosphorylated Akt expression was increased in LV samples from the noninfarct zone in the MI+unload group (Figure 4). Compared with sham controls, levels of phosphorylated ERK and phosphorylated Akt were not significantly changed in the infarct zone from both groups (data not shown). These findings suggest that mechanical unloading before reperfusion activates signaling



Figure 4. Reperfusion injury salvage kinase pathway activation. A, Western blot of phosphorylated extracellular signal-regulated kinase (pERK) and phosphorylated Akt (pAkt) normalized to total ERK (tERK), total Akt (t-Akt), and GAPDH. B and C, Quantification of Western blots for pERK and pAkt normalized to GAPDH. pERK/GAPDH ratio: 0.04 vs 0.04 vs 0.12, sham vs myocardial infarction (MI) vs MI+unload. *P=0.02 and †P=0.01 for sham and MI vs MI+unload, respectively. pAkt/ GAPDH ratio: 0.02 vs 0.03 vs 0.06, *Sham vs +MI vs MI+unload. P=0.03 and P=0.04 for sham and MI vs MI+unload, respectively.

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pathways that protect against reperfusion injury in the noninfarct zone during AMI.

Mechanically Reducing LV Wall Stress and Delaying Coronary Reperfusion Reduces Myocardial Infarct Size

We next explored whether mechanically reducing LV wall stress and stroke work while delaying coronary reperfusion affects myocardial infarct size. To account for regional perfusion of the LAD, no differences in LAD length (80±3 versus 78±3 mm, MI versus MI+unload; P=0.4) or LV mass (32±6 versus 31 ± 6 g, MI versus MI+unload; P=0.72) distal to the stent were observed between the MI and MI+unload groups. Compared with the MI-alone group, the percent of total LV myocardium infarcted was reduced by 42% in the MI+unload group (49±14% of all myocardium versus 28±7% of all myocardium, MI versus MI+unload; P=0.03; Figure 5A and 5B). Among all animals, a direct correlation was observed between LV wall stress and percent MI (Figure 5C). In both the MI and MI+unload groups, LAD occlusion led to a similar increase in both creatine kinase-MB (4+2% versus 4+1%, MI versus MI+unload; P=0.53) and troponin-I (0.3+0.4 versus 0.6+0.4, MI versus MI+unload; P=0.6) values after 120 minutes of ischemia. In contrast, after 120 minutes of reperfusion, creatine kinase-MB (8.7±1% versus 5.1±0.5%, MI versus MI+unload; P=0.03) and troponin-I (9.7±2 versus 4.5±1.4, MI versus MI+unload; P=0.001) values were significantly lower in the mechanically supported group (Figure 5D and 5E).

Discussion

Our central finding is that mechanically unloading the LV despite delaying coronary reperfusion reduces LV wall stress and activates signaling pathways that promote myocardial salvage, leading to significantly reduced myocardial injury in a preclinical model of acute MI. Specifically we report the following: (1) Reducing LV preload with a percutaneously delivered left atrial to femoral artery circuit driven by a centrifugal pump significantly reduces LV volume, pressure, and native stroke volume, which in turn reduce LV wall stress, stroke work, and circumferential strain in AMI while maintaining mean arterial pressure; (2) unloading the LV and delaying reperfusion during an AMI promote phosphorylation of proteins involved in the RISK pathway; and (3) this approach significantly limits myocardial damage as measured by triphenyltetrazolium chloride staining and cardiac biomarker release. These findings suggest that initially reducing LV wall stress despite delaying coronary reperfusion reduces myocardial injury alone. The clinical implications of these findings require further study.

Over the past 4 decades, acute circulatory support devices have evolved from large, pulsatile systems to miniaturized, percutaneously delivered pumps.³¹ These pumps, which include the intra-aortic balloon pump, venoarterial extracorporeal membrane oxygenation, a catheter-mounted axial-flow pump (Impella, Abiomed Inc), and the pLA-FA centrifugal bypass system (TandemHeart), are used clinically to provide rapid circulatory support while maintaining systemic perfusion. Several preclinical studies have shown that activation of either an intra-aortic balloon pump or a catheter-mounted



Figure 5. Myocardial infarct quantification. **A**, Representative left ventricular (LV) sections after staining with triphenyltetrazolium chloride. Infarct zones are outlined in black. **B**, Quantification of myocardial infarct size as percent of LV area and LV stroke work during myocardial infarction (MI) with and without mechanical LV unloading. *P<0.05 vs MI. \Box Indicates swine subjected to MI alone; \circ , swine subjected to MI with LV unloading initiated before reperfusion. **D**, Changes in serum creatine kinase-MB (CK-Mb) and troponin-I levels during MI with and without mechanical LV unloading. *P<0.05 vs MI reperfusion; †P<0.05 vs baseline.

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axial flow pump before coronary occlusion can reduce MI size in surgical models of AMI.³²⁻³⁴ These studies suggest that the timing of mechanical circulatory support activation is a critical determinant of myocardial salvage in AMI. However, the clinical relevance of device activation before the onset of myocardial ischemia is limited. Despite these preclinical observations, recent clinical trials have shown that activation of an intra-aortic balloon pump immediately before myocardial reperfusion does not limit infarct size in AMI.^{35,36} Several possible reasons for the discrepancy between preclinical and clinical observations include the differential effects of device type, timing of activation, or duration of support and the use of coronary ligation or balloon occlusion in healthy animal models as opposed to thrombotic coronary occlusion in aged animal models with traditional cardiovascular risk factors such as diabetes mellitus, dyslipidemia, or hypertension.37

On the basis of these prior studies, the role of earlier device activation and delayed coronary reperfusion and the magnitude of LV unloading as determinants of infarct size in AMI remain poorly understood. The model used in the present study of mechanical unloading in AMI addresses several of these issues. By creating 120 minutes of LAD ischemia, the model replicates clinical scenarios of AMI in which time from symptom onset to coronary reperfusion is >90 minutes. Further supporting the clinical relevance of the model, we observed a 33% incidence of ventricular fibrillation within 60 minutes of LAD occlusion. Next, by first initiating mechanical support and then leaving the LAD occluded for an additional 30 minutes and throughout reperfusion in the MI+unload group (150 minutes of LAD occlusion total), the model exceeds ischemic times in prior preclinical studies of AMI and places a greater burden of ischemic injury on the MI+unload group. Furthermore, in contrast to prior studies, the chest wall remains intact throughout this entire protocol through the use of nonsurgical, percutaneous delivery of all catheters. This distinction mitigates the effect of an open pericardium on intracardiac filling pressures and biventricular function, allows us to more accurately study the hemodynamic effect of percutaneously delivered mechanical support, and further makes the model clinically relevant to contemporary interventional approaches to AMI therapy.

From the moment a coronary artery becomes occluded, a proverbial clock begins to count minutes that are directly associated with ongoing myocardial cell injury and death. Using this model, we show that mechanically reducing LV preload with a pLA-FA bypass mechanism significantly reduces both LV wall stress and stroke work after reperfusion without the need to directly cannulate the LV. We further show that device activation reduces circumferential LV strain after 120 minutes of reperfusion in AMI and that circumferential global strain correlates directly with quantified LV wall stress and stroke work. These data indicate a potentially important role for circulatory support devices that target preload as a method to unload the LV. Furthermore, previous studies have shown that reduced LV wall stress and stroke work correlate with reduced myocardial oxygen consumption.38,39 Our data suggest that reducing LV wall stress, stroke work, and strain may promote myocardial salvage in AMI by reducing myocardial oxygen demand and thereby attenuating the process of ongoing myocardial ischemia.

By initiating mechanical support first and sustaining systemic perfusion while reducing native LV work, we have created a window in time when the process of myocardial injury is slowed and delaying coronary reperfusion is possible while incurring only minimal additional ischemic damage. By delaying reperfusion in the setting of mechanically reduced oxygen demand, time is now available for activation of signaling pathways known to promote myocardial salvage, including phosphorylation of ERK and Akt. These findings suggest that the milieu for reperfusion injury may be modified so that coronary revascularization may ultimately be accomplished without triggering significant reperfusion injury. This approach also opens the possibility for the implementation of other adjunctive cardioprotective strategies, including systemic drug administration, intracoronary drug delivery into an injured segment, more aggressive mechanical ischemic postconditioning, or remote ischemic conditioning. The combination of mechanical support, adjunct cardioprotective therapy, and delayed reperfusion may ultimately lead to optimal myocardial salvage in AMI. Although the contemporary strategy of treating AMI is dominated by a quest to achieve rapid coronary recanalization in AMI (door-to-balloon time), we now propose that first mechanically reducing LV preload (door to unload) and then delaying coronary reperfusion will promote RISK pathway activation and significantly reduce MI size. Further study is required to establish whether this theoretical benefit of early and maximal LV unloading is a clinically viable strategy in AMI.

Limitations of the present study include the technical limitation of measuring myocardial oxygen consumption, coronary collateral flow, and total aortic flow because of the use of multiple intracardiac catheters and large-bore cannulas in the vena cava. Furthermore, the number of animals studied is small, in part a result of the 33% mortality rate among animals subjected to prolonged LAD occlusion. Given the small sample size (n=4 per group), any *P* values throughout the article from comparisons of the randomized groups are unreliable and should be interpreted with caution. Area at risk was not directly quantified in this model. Studies examining diseased models of AMI with further measurement of the area at risk using vital dyes or magnetic resonance imaging should be performed.

The pioneering work of Reimer et al⁴ and Maroko et al⁴ has established that infarct size resulting from a prolonged ischemic insult followed by reperfusion is determined primarily by 4 factors: (1) the duration of ischemia, (2) the size of the area at risk, (3) the amount of collateral circulation to the ischemic territory during coronary artery occlusion, and (4) the timing of treatment administration with respect to reperfusion. In this study, we account for each of these components of infarct size by placing a greater duration of LAD occlusion in the MI+unload group, confirming that the perfused myocardial mass was similar between groups, using a swine model with limited collateral circulation, and administering mechanical unloading 30 minutes before coronary reperfusion. Our findings extend previous observations of reduced infarct size with mechanical support by demonstrating the application of pLA-FA bypass in MI, delaying reperfusion for an extended amount of time attempting to recapitulate the clinical scenario of AMI, and showing the impact on wall stress and RISK pathway activation.

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CLINICAL PERSPECTIVE

Each year, nearly 8 million people worldwide suffer a heart attack, which is commonly caused by a blocked coronary artery leading to reduced oxygen supply to the heart, known as ischemia. Every minute of occlusion directly correlates with ongoing heart muscle damage. Treatment focuses on quickly re-establishing blood flow to the heart, known as reperfusion. However, despite timely reperfusion, $\approx 5\%$ to 10% of patients die in the hospital, and 25% develop chronic heart failure. Using a porcine model of an acute heart attack, we studied whether first reducing the workload of the heart with a mechanical pump despite delaying coronary reperfusion would reduce myocardial damage. We identified that a percutaneously delivered left atrial–to–femoral artery circuit driven by a centrifugal pump reduces left ventricular wall stress, work, and strain while maintaining systemic arterial pressure. Next, we observed that mechanically unloading the heart despite delaying reperfusion of proteins involved in myocardial salvage. Finally, we showed that compared with controls, myocardial damage was reduced with initiation of mechanical support in the setting of a heart attack. These findings suggest that initially reducing the workload of the heart despite delaying coronary reperfusion reduces myocardial injury. The clinical implications of these findings require further study.

SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Hemodynamic variables for individual animal subjects in the MI group and MI+Unload group after 120 minutes of reperfusion are shown and include: A) mean arterial pressure, B) end-systolic pressure volume relationship (ESPVR), C) left ventricular (LV) stroke volume, D) cardiac output, E) LV end-diastolic pressure, and F) LV end-diastolic volume.



Supplemental Figure 2. A) Changes in longitudinal LV strain during MI with and without mechanical LV unloading. B) Regression plot of circumferential global LV strain and LV stroke work (LVSW) (R=0.61, p=0.03).







Mechanically Unloading the Left Ventricle Before Coronary Reperfusion Reduces Left Ventricular Wall Stress and Myocardial Infarct Size

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