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

Running title: Kapur et al.; Reducing wall stress in AMI

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Abstract:

**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 (LV) wall stress with a percutaneous left atrial-to-femoral artery centrifugal (pLA-FA) 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 (LAD) in adult, male swine. In the MI group (n=4), 120 minutes of LAD occlusion was followed by 120 minutes of reperfusion without mechanical support. In the mechanically supported group (MI+Unload)(n=4), pLA-FA bypass was initiated after 120 minutes of ischemia and LAD occlusion prolonged for an additional 30 minutes, followed by 120 minutes of reperfusion with device support. All animals were sacrificed after reperfusion and infarct size quantified by triphenyltetrazolium chloride staining. Compared to baseline, mean LV 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 LV wall stress: 44658 vs 22963 dynes/cm²; stroke work: 2823 vs 655 mmHg x mL, MI vs MI+Unload). Phosphorylation of reperfusion injury salvage kinase (RISK) pathway proteins from non-infarcted LV tissue was unchanged in the MI group, but increased in the MI+Unload group. Compared to MI alone, total infarct size was reduced in the MI+Unload group (49% vs 28%, MI vs MI+Unload).

**Conclusions**—These data support that first unloading the LV despite delaying coronary reperfusion during an acute MI reduces myocardial injury.

**Key words**: mechanical circulatory support, acute myocardial infarction, reperfusion injury
An acute myocardial infarction (AMI) affects nearly 8 million 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. Beginning with the ‘open artery theory’ in the 1970’s, 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. 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 (DTB) less than 90 minutes, which is defined as the interval from patient arrival in the emergency department to mechanical reperfusion of the occluded coronary artery. The DTB time is a standard part of ACC/AHA guidelines and a core quality measure of hospital performance. However, despite timely reperfusion, nearly 10% of AMI subjects die during their index hospitalization and 25% of survivors progress to develop chronic heart failure. 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 (IRI). 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 due to reperfusion injury.

Pharmacologic approaches to limit reperfusion injury have focused on attenuating mitochondrial permeability transition pore (mPTP) generation and promoting activity of rapidly inducible salvage kinases (RISK), including extracellular regulated kinase (ERK) and the serine/threonine kinase, Akt. Other approaches to limit IRI include systemic hypothermia which has also shown promise as a method to reduce total body metabolic demand, 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 (IC), has also been shown to promote activity of the RISK pathway\textsuperscript{14-16}. While promising, critical barriers to these cardioprotective strategies include: 1) the multifactorial nature of reperfusion injury, thereby limiting the impact of a single-target pharmacologic strategy, 2) the potential for coronary vascular injury (dissection or perforation) with IC, and 3) the mandate for rapid coronary reperfusion and therefore insufficient time for a drug to penetrate into myocardial injury zones. There exists a need for improved cardioprotective strategies that broadly impact the multiple levels of reperfusion injury without causing further myocardial damage. In this study, we explore the central hypothesis that initially reducing LV wall stress by mechanically reducing LV preload, while delaying coronary reperfusion activates endogenous anti-IRI 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 (IACUC) at Tufts Medical Center. All experiments were performed according to the committee’s guidelines.

**Experimental Protocol of Myocardial Infarction and Mechanical Circulatory Support**

Animals were premedicated with Telazol (0.8ml/kg, intramuscular). 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 physiologic 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 (10Fr), left carotid artery (7Fr), and both femoral arteries (7 Fr) and veins (10Fr). A pulmonary artery catheter was deployed via the right internal jugular vein. Unfractionated heparin boluses with a goal activated clotting time of 300-400 seconds, continuous lidocaine infusion (1mg/kg), and noradrenaline (0.16 mcg/min) were initiated in all animals. A 6Fr Judkins right coronary catheter (Boston Scientific) engaged the left coronary artery via the right femoral artery and baseline angiograms were recorded. A 90cm guidewire was delivered into the distal left anterior descending artery (LAD) and a 3.0 x 8mm bare-metal stent (Boston Scientific) 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 antero-posterior projection offline using 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 (40; 20; 30; 60 minutes) of LAD occlusion due to refractory ventricular fibrillation. The surviving 8 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 obtained. In the mechanically supported group
(MI+Unload), we employed a centrifugal flow pump (TandemHeart; Cardiac Assist Inc), as described below, which is utilized 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, trans-septal puncture of the inter-atrial septum was performed using a Brockenbrough needle and a trans-septal dilator (Boston Scientific) via the right femoral vein. An Inoue wire was deployed into the left atrium and a staged 14-to-21Fr dilator used to place a 21Fr inflow cannula into the left atrium (Figures 1B and 1C). Next, a 15Fr arterial cannula was placed into the left femoral artery. Both cannulas were then connected to a de-aired centrifugal flow pump and clamped (Figure 1B). Finally, 3 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 western blot analysis.

**Conductance Catheter Assessment of LV Pressure and Volume**

Changes in LV pressure and volume were assessed using a 5Fr-conductance catheter system (Sigma-M; CD Leycom, The Netherlands) deployed via the left carotid 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 using a solid-state pressure transducer and dual-field excitation mode respectively as previously described 20, 21. 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 LV was confirmed by 3D-echocardiography and fluoroscopy. Time-varying segmental conductance has been shown to reflect segmental LV volumes in prior preclinical and clinical studies.\(^\text{20, 21}\) Parallel conductance was assessed by injecting 20mL of hypertonic (6%) saline into the right internal jugular vein.\(^\text{22}\) 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 (ESPVR) 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.

**Three-Dimensional Echocardiography**

To evaluate LV myocardial mechanics, three-dimensional speckle tracking echocardiography (3D-STE) was performed using the Artida 4D System (Toshiba Medical Systems, Tustin, California) and matrix array PST-25SX transducer. For optimal imaging, all animals underwent a 4cm mid-line abdominal incision for sub-diaphragmatic placement of the transducer in proximity to the LV apex. Three-dimensional data sets consisted of apical full volumes created by the combination of 6 ECG-gated, wedge-shaped sub-volumes during a single breath hold. Along with the combination of 6 sub-volumes, 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. Off-line processing for speckle tracking analysis was performed through the Wall Motion Tracking software (Toshiba Medical Systems) as previously described.\(^\text{23-25}\) Longitudinal
and circumferential LV strain with 3D-STE 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 longitudinal and circumferential directions, respectively, relative to the endocardial contour. 3D-STE derived global values for longitudinal and circumferential strain were obtained from the rendered 16-segment model with exclusion of the apical-most segment due to imaging dropout. Left ventricular peak wall stress (LVPWS), mean wall stress (LVMWS), and systolic wall tension (LVSWT) were also calculated as previously described

**Determination of Myocardial Infarct Size**

Upon completion of the study protocol, animals were euthanized and hearts immediately excised and manually flushed with normal saline. Both atria and the right ventricle were removed and the LV cross-sectioned into 1 cm segments perpendicular to the long axis. Coronary stents were visualized to confirm location of the mid-LAD occlusion segment. Biopsy specimens were obtained from the anterio-apical LV distal to the site of stent deployment (infarct zone) and from the postero-basal wall (non-infarct zone) for molecular analysis and LV slices then incubated in 1% triphenyltetrazolium chloride (TTC) at 37°C for 5 minutes for non-infarct 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 rotations per minute for 15 minutes for isolation of serum and plasma, which were then stored at -80°C. Cardiac biomarkers (CK-Mb and Troponin-I) were measured by Anlytics (Gaithersburg, MD).

**Immunoblot Analysis (Western)**

Total protein was extracted from tissue homogenates isolated as previously described (Ref 14). Immunoblot analysis was then performed as previously described using antibodies against
porcine total ERK, phosphorylated ERK-1/2 (pERK-1/2), Total AKT, phosphorylated Akt (pAKT), and GAPDH. Expression of pERK-1/2 and pAKT were normalized to both total protein levels and GAPDH.

**Statistical Analysis**

Results are presented as mean±SD. All data within groups were analyzed by non-parametric two-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 two parameters. Given the small sample size (n=4/group), any p-values throughout the manuscript from comparing 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 Table 1. In both the MI and MI+Unload groups, 120 minutes of LAD occlusion did not significantly change mean arterial pressure (MAP), dP/dt maximum, LV systolic pressure or estimated ESPVR (Table 1). In the MI group, compared to baseline values, LV end-diastolic pressure (LVEDP) was increased after 120 min of reperfusion, but not in the the MI+Unload group. In the MI+Unload group, percutaneous LA-FA bypass provided 3.2±0.2 liters/minute of flow. Furthermore, after 120 minutes of reperfusion, native cardiac output was decreased, while MAP and estimated ESPVR were increased in the MI+Unload group compared to the MI group (Table 1 and Supplemental Figure 1). Compared to 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 (LVSW) was significantly reduced compared to baseline values and compared to the MI group (Figure 2). After 120 minutes of reperfusion, LVMWS (44658±13925 vs 22963±8610 dynes/cm², MI vs MI+Unload, p=0.009) and LVSWT (1595±789 vs 736±119 dynes/cm, MI vs MI+Unload, p=0.02) were significantly reduced and a trend towards reduced LVPWS (94±37 vs 43±18 10³ dynes/cm², MI vs MI+Unload, p=0.06) was observed in the MI+Unload group compared to the MI group (Figure 2). Given the small sample size (n=4/group), any p-values throughout the manuscript from comparing the randomized groups are unreliable, and should be interpreted with caution.

These findings support that despite identical degrees of hemodynamic compromise due to LAD occlusion, pLA-FA bypass in the MI+Unload group reduced LV wall stress and stroke work, while supporting MAP.

Reperfusion Impairs Myocardial Strain
We next examined the functional impact of ischemia–reperfusion injury in AMI on LV volumes and strain using 3D-STE. In both the MI and MI+Unload groups, 120 minutes of LAD occlusion did not significantly change LV end-systolic and end-diastolic volumes (LVESV and LVEDV) from baseline. In the MI group, after 120 minutes of reperfusion, LVESV and LVEDV remained unchanged. In contrast, compared to baseline values, LVEDV was significantly reduced after 120 minutes of reperfusion in the MI+Unload group (Table 1 and Supplemental Figure 1).

Both LVESV and LVEDV were lower in the MI+Unload group compared to MI alone. 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-B) or longitudinal strain (Supplemental Figure 2A). Longitudinal strain remained unchanged after 120 of
reperfusion in the MI group. Circumferential LV strain in the MI and MI+Unload groups was reduced compared to baseline values after reperfusion (p=0.003) with a greater reduction observed in the MI+Unload group compared to MI alone (-12±2% vs -7±3%, MI vs MI+Unload, p=0.03; Figure 3B). Changes in global circumferential LV strain correlated directly with LVMWS (R=0.72, p=0.004, Figure 3C) and stroke work (R=0.61, p=0.03, Supplemental Figure 2B). Consistent with our findings using 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 non-invasive measures of LV function.

Mechanical Unloading Promotes Reperfusion Injury Salvage Kinase (RISK) Pathway Activity

To begin exploring whether mechanical unloading promotes myocardial salvage in AMI, we quantified RISK pathway activation. Compared to sham controls, LV tissue from the non-infarct zone showed no change in pERK and pAkt expression in the MI group. However, compared to sham controls or the MI group, pERK and pAkt expression was increased in LV samples from the non-infarct zone in the MI+Unload (Figure 4). Compared to sham controls, levels of pERK and pAkt were not significantly changed in the infarct zone from both groups (data not shown). These findings suggest that mechanical unloading prior to reperfusion activates signaling pathways that protect against reperfusion injury in the non-infarct 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 impacts myocardial infarct size. To account for regional perfusion of the LAD, no differences in LAD length (80±3 vs 78±3 mm, MI vs MI+Unload, p=0.4) or LV mass
(32±6 vs 31±6 grams, MI vs MI+Unload, p=0.72) distal to the stent were observed between the MI and MI+Unload groups. Compared to MI alone, the percent of total LV myocardium infarcted was reduced by 42% in the MI+Unload group (52±14% of all myocardium vs 28±7% of all myocardium, MI vs MI+Unload, p=0.03; Figure 5A-B). Among all animals, a direct correlation was observed between LV wall stress and percent myocardial infarction (Figure 5C).

In both the MI and MI+Unload groups, LAD occlusion led to a similar increase in both CK-Mb (4±2% vs 4+1%, MI vs MI+Unload, p=0.53) and Troponin-I (0.3+0.4 vs 0.6+0.4, MI vs MI+Unload, p=0.6) values following 120 minutes of ischemia. In contrast, after 120 minutes of reperfusion, CK-Mb (8.7±1% vs 5.1±0.5%, MI vs MI+Unload, p=0.03) and Troponin-I (9.7±2 vs 4.5±1.4, MI vs MI+Unload, p=0.001) values were significantly lower in the mechanically supported group (Figure 5D-E).

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 myocardial infarction. Specifically we report that: 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 reduces LV wall stress, stroke work, and circumferential strain in AMI, while maintaining mean arterial pressure, 2) LV unloading and delaying reperfusion during an AMI promotes phosphorylation of proteins involved in the RISK pathway, and 3) this approach significantly limits myocardial damage as measured by TTC 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 will require further study.

Over the past 4 decades, acute circulatory support devices have evolved from large, pulsatile systems to miniaturized, percutaneously-delivered pumps \textsuperscript{31}. These pumps, which include the intra-aortic balloon pump (IABP), veno-arterial extracorporeal membrane oxygenation (VA-ECMO), a catheter-mounted axial-flow pump (Impella; Abiomed Inc), and the pLA-FA centrifugal bypass system (TandemHeart; Cardiac Assist Inc) are used clinically to provide rapid circulatory support, while maintaining systemic perfusion. Several preclinical studies have shown that activation of either an IABP or catheter-mounted axial flow pump prior to coronary occlusion can reduce myocardial infarct size in surgical models of AMI \textsuperscript{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 IABP immediately before myocardial reperfusion does not limit infarct size in AMI \textsuperscript{35,36}. Several possible reasons for the discrepancy between preclinical and clinical observations include: (1) the differential effects of device type, timing of activation, or duration of support and (2) 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 including diabetes, dyslipidemia, or hypertension \textsuperscript{37}.”

Based on these prior studies, the role of earlier device activation, delayed coronary reperfusion, and the magnitude of LV unloading as determinants of infarct size in AMI remain poorly understood. The model employed in the current 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 where time from symptom onset to coronary reperfusion is greater than 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 using non-surgical, percutaneous delivery of all catheters. This distinction mitigates the effect of an open-pericardium on intracardiac filling pressures and biventricular function and 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 using 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. 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 where the process of myocardial injury is slowed and delaying coronary reperfusion is now 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 such that coronary revascularization may ultimately be accomplished without triggering significant reperfusion injury. This approach also opens the possibility for implementation of other adjunctive cardioprotective strategies including systemic drug administration, intra-coronary drug delivery into an injured segment, more aggressive mechanical ischemic post-conditioning, or remote ischemic conditioning. The combination of mechanical support, adjunct cardioprotective therapy, and delayed reperfusion may ultimately lead to optimal myocardial salvage in AMI. While, 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 myocardial infarct 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 presents study include the technical limitation of measuring myocardial oxygen consumption, coronary collateral flow, and total aortic flow due to multiple intra-cardiac catheters and large-bore cannulas in the vena cava. Furthermore, the number of animals studied is small due in part to a 33% mortality rate among animals subjected to
prolonged LAD occlusion. Given the small sample size (n=4/group), any p-values throughout the manuscript from comparing 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 Jennings, Braunwald, Covell, Ross, and Reimer\(^3,4\) have established that infarct size due to a prolonged ischemic insult followed by reperfusion is determined primarily by four 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 prior to 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.

**Acknowledgements:** The authors thank Marvin Konstam and James Udelson for their thoughtful reviews of the data and manuscript.

**Funding Sources:** Funding for this study was received from Cardiac Assist Inc.

**Conflict of Interest Disclosures:** Dr. Kapur has received research funding from Cardiac Assist Inc.
Inc, HeartWare Inc, Maquet Inc, and Abiomed Inc. Dr. Pham has received research funding from HeartWare Inc.

References:


left ventricular unloading prior to reperfusion reduces infarct size in a canine infarction model. 


Table 1. Hemodynamic variables at baseline, after 120 minutes of LAD occlusion, and after completion of 120 minutes of reperfusion for both the myocardial infarction (MI) and MI with mechanical unloading of the left ventricle (MI+Unload) groups are shown (n=4/group for each condition). No significant differences were observed between groups at baseline.

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*MI vs MI+Unload; † MI vs Baseline; ‡ MI+Unload vs Baseline; Data expressed as mean ± standard deviation.
**Figure Legends:**

**Figure 1.** A) Swine model of acute myocardial infarction (MI) and MI with activation of a percutaneous left atrial-to-femoral artery centrifugal bypass pump after 120 minutes of LAD occlusion with persistent occlusion of the LAD for an additional 30 minutes, followed by 120 minutes of reperfusion. LV unloading continued throughout reperfusion (MI+LV Unloading; n=4/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: pulmonary artery; PTCA: percutaneous transcoronary angioplasty.

**Figure 2.** Changes in pressure-volume loops MI with and without mechanical unloading. A) Representative PV loops from a swine subjected to ischemia-reperfusion alone (MI). B) Representative PV loops with ischemia and reperfusion during LV unloading with pLA-FA bypass (MI+Unload). C) Left ventricular stroke work (LVSW), D) LV mean wall stress (LVMWS), E) LV peak wall stress (LVPWS), and F) LV wall tension in the MI and MI+Unload groups (*, p<0.05 vs MI-Reperfusion; †, p<0.05 vs baseline).

**Figure 3.** 3D-Echocardiography Strain Analysis. A) Representative changes in percent circumferential global LV strain during 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 versus MI+Unloading (*, 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).
Figure 4. RISK pathway activation. A) Western blot of phosphorylated ERK (pERK) and 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 MI vs MI+Unload, p=0.02 (*) and p=0.01(†) for Sham or 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 or MI vs MI+Unload respectively.

Figure 5. Myocardial Infarct Quantitation. A) Representative LV sections after staining with triphenyltetrazolium chloride (TTC). Infarct zones are outlined in black. B) Quantification of myocardial infarct size as percent of LV area and LV stroke work (LVSW) during MI with and without mechanical LV unloading (*, p<0.05 vs MI). Open squares indicate swine subjected to MI alone. Open circles indicate swine subjected to MI with LV unloading initiated before reperfusion. D) Changes in serum 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).
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Mechanically Unloading the Left Ventricle Before Coronary Reperfusion Reduces Left Ventricular Wall Stress and Myocardial Infarct Size
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Circulation. published online June 13, 2013;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/early/2013/06/13/CIRCULATIONAHA.112.000029

Data Supplement (unedited) at:
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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).