Transvalvular Ventricular Unloading Before Reperfusion in Acute Myocardial Infarction



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ABSTRACT

BACKGROUND Myocardial damage due to acute ST-segment elevation myocardial infarction (STEMI) remains a significant global health problem. New approaches to limit myocardial infarct size and reduce progression to heart failure after STEMI are needed. Mechanically reducing left ventricular (LV) workload (LV unloading) before coronary reperfusion is emerging as a potential approach to reduce infarct size.

OBJECTIVES Given the central importance of mitochondria in reperfusion injury, we hypothesized that compared with immediate reperfusion (IR), LV unloading before reperfusion improves myocardial energy substrate use and preserves mitochondrial structure and function.

METHODS To explore the effect of LV unloading duration on infarct size, we analyzed data from the STEMI-Door to Unload (STEMI-DTU) trial and then tested the effect of LV unloading on ischemia and reperfusion injury, cardiac metabolism, and mitochondrial function in swine models of acute myocardial infarction.

RESULTS The duration of LV unloading before reperfusion was inversely associated with infarct size in patients with large anterior STEMI. In preclinical models, LV unloading reduced the expression of hypoxia-sensitive proteins and myocardial damage due to ischemia alone. LV unloading with a transvalvular pump (TV-P) but not with venoarterial extracorporeal membrane oxygenation (ECMO) reduced infarct size. Using unbiased and blinded metabolic profiling, TV-P improved myocardial energy substrate use and preserved mitochondrial structure including cardiolipin content after reperfusion compared with IR or ECMO. Functional testing in mitochondria isolated from the infarct zone showed an intact mitochondrial structure including cardiolipin content, preserved activity of the electron transport chain including mitochondrial complex I, and reduced oxidative stress with TV-P-supported reperfusion but not with IR or ECMO.

CONCLUSIONS These novel findings identify that transvalvular unloading limits ischemic injury before reperfusion, improves myocardial energy substrate use, and preserves mitochondrial structure and function after reperfusion. (J Am Coll Cardiol 2020;76:684–99) © 2020 by the American College of Cardiology Foundation.



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yocardial damage due to acute STsegment elevation myocardial infarction (STEMI) remains a significant global health problem. For every 30-min delay from symptom onset to reperfusion, 1-year mortality increases by 7.5%, and infarct size increases by nearly 30% (1,2). For every 5% increase in infarct size, 1-year mortality and 1-year heart failure hospitalization increase by 20% in STEMI (3). These data are particularly sobering given that the prevalence of heart failure is projected to exceed 8 million individuals by 2030 in the United States alone (4). New approaches to limit infarct size and reduce progression to heart failure after STEMI are needed.

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Within minutes of coronary occlusion, oxygen delivery is reduced, and cardiac metabolism switches from high energy, producing fatty acid oxidation, to low energy, yielding anaerobic glycolysis, thereby limiting energy production through the citric acid cycle and electron transport chain (ETC). Reperfusion begins to recover glucose and fatty acid oxidation; however, a burst of mitochondria-generated reactive oxygen species (ROS) combined with calcium overload increases permeability of the inner mitochondrial membrane (IMM), leading to mitochondrial swelling and rupture. Reperfusion also reduce levels of cardiolipin, an integral lipid that maintains IMM structure and ETC function (5-7).

Mitochondrial complex I (CI) (NADH [nicotinamide adenine dinucleotide (NAD) + hydrogen]: ubiquinone oxidoreductase) is a multiunit protein that transfers electrons from NADH to ubiquinone, which triggers a conformational change in CI, thereby facilitating proton transfer across the IMM. During myocardial ischemia, CI becomes deactivated, which limits proton transfer and dissipates the transmembrane proton gradient that drives mitochondrial adenosine triphosphate (ATP) generation. Upon reperfusion, reactivation of CI increases ROS production and cellular damage (5,8).

The use of percutaneous circulatory support pumps that decrease left ventricular (LV) workload and increase systemic perfusion is increasing. Transvalvular pumps (TV-Ps) displace blood from the left ventricle into the ascending aorta. Venoarterial extracorporeal membrane oxygenation (ECMO) pumps displace and oxygenate venous blood into the aorta. Recent reports identified that compared with immediate reperfusion, first unloading the LV with a TV-P and delaying reperfusion reduce infarct size in preclinical models of STEMI (9-11). Proposed mechanisms for reduced infarct size with transvalvular unloading include reduced LV work and oxygen consumption, activation of cardioprotective signaling (10), and increased collateral coronary blood flow and myocardial perfusion (12,13). Given the central importance of mitochondria in the pathogenesis of reperfusion injury, studies exploring the impact of LV unloading on mitochondrial integrity in acute myocardial infarction are required.

The recently completed STEMI-DTU (Primary Unloading and Delayed Reperfusion in ST-Elevation Myocardial Infarction) pilot study randomized 50 patients with anterior STEMI to LV unloading and immediate reperfusion (IR) (the U-IR arm) or unloading followed by a 30-min delay before reperfusion (unloading and delayed reperfusion [the U-DR arm]) (14). Despite delayed reperfusion, the U-DR arm showed no increase in infarct size compared with the U-IR arm. Among large anterior STEMIs, defined by a sum of precordial ST-segment elevation (STE) ≥ 6 mm. U-DR decreased infarct size normalized to the area at risk compared with U-IR. These results support the need for mechanistic studies to understand how LV unloading attenuates myocardial damage despite a delay to reperfusion.

We hypothesized that compared with immediate reperfusion, mechanically unloading the left ventricle before reperfusion improves myocardial energy substrate use and preserves mitochondrial structural and functional integrity.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS. The following chemicals were purchased: carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone FCCP (C2920, Sigma-Aldrich, St. Louis, Missouri), antimycin A (A8674, Sigma-Aldrich), rotenone (R8875, Sigma-Aldrich), TMPD (N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride) (87890, Sigma-Aldrich), sodium ascorbate (PHR 1279, Sigma-Aldrich), NADH (606-60-8, Sigma-Aldrich), Ru360 (557440, Sigma-Aldrich), and cyclosporine A (59865-13-3, Sigma-Aldrich).

SUBANALYSIS OF THE STEMI-DTU PILOT TRIAL

To study the relationship between the time from symptom onset to balloon angioplasty, the duration of LV unloading, and the magnitude of infarct size normalized to the area at risk as determined by 3- to 5-day cardiac magnetic resonance (CMR) imaging, we analyzed data from patients enrolled in the STEMI-DTU pilot study. STE served as a surrogate for the

ABBREVIATIONS AND ACRONYMS

ATP = adenosine triphosphate
CI = mitochondrial complex I
CMR = cardiac magnetic resonance
ECMO = extracorporeal membrane oxygenation
ETC = electron transport chain
HIF-1α = hypoxia-inducible factor 1-alpha
IMM = inner mitochondrial membrane
IR = immediate reperfusion
LAD = left anterior descending
LV = left ventricular
PHD = prolyl hydroxylase domain
ROS = reactive oxygen species
STE = ST-segment elevation
STEMI = ST-segment elevation myocardial infarction
TV-P = transvalvular pump
U-DR = unloading and delayed reperfusion
U-IR = unloading and immediate reperfusion



total group with S1-segment elevation (S1E) > 7 mm and unloading followed by immediate reperfusion (U-IR) or delayed reperfusion (U-DR). (U) infarct size normalized to the area at risk. (D) A plot comparing infarct size/area at risk and the duration of unloading before reperfusion. AAR = area at risk; CMR = cardiac magnetic resonance; IS = infarct size; LAD = left anterior descending; LV = left ventricular.

area at risk. We included patients with STE \geq 7 mm, available CMR data, and documented left anterior descending (LAD) artery occlusion in the analysis (n = 23 of 50) (Figure 1A).

The Institutional Review Boards at each site approved the trial, and each enrolled patient provided written informed consent. The study was designed by the principal investigators and Abiomed (Danvers, Massachusetts). The study steering committee members and an independent data and safety monitoring board oversaw the safety and feasibility of the trial. A clinical events committee, blinded to randomization group, independently adjudicated clinical safety endpoints in the study. The sponsor was responsible for the study conduct, reporting, and monitoring and managed the database. Angiographic, CMR, echocardiographic, and electrocardiographic studies were analyzed by core laboratories blinded to the treatment allocation. The study design was approved by the Food and Drug Administration and registered accordingly (NCT03000270).

SWINE MODELS OF MYOCARDIAL ISCHEMIA AND REPERFUSION INJURY. Preclinical study protocols were approved by the Institutional Animal Care and Use Committee at Tufts Medical Center, Boston, Massachusetts. All experiments were performed according to the committee's guidelines. Adult male Yorkshire swine were anesthetized, mechanically ventilated, and prepped for myocardial infarction studies as described in the Supplemental Methods. Using a coronary catheter and wire, an angioplasty balloon was deployed in the mid-LAD after the first diagonal branch with angiographic confirmation of occlusion. Angiography performed immediately after reperfusion and at the end of the study protocol confirmed LAD patency.

To determine the impact of circulatory support on infarct size during prolonged LAD occlusion with and without reperfusion, 4 groups of adult male swine (n = 5 per group) were studied (Figure 2A). In Group A, animals were subjected to 210 min of LAD occlusion without reperfusion. In one-half of these animals, a



TV-P (Impella CP, Abiomed) was initiated 120 min before termination of the protocol. In Group B, 210 min of LAD occlusion was followed by 120 min of reperfusion with and without TV-P activation 120 min before reperfusion.

To investigate the effects of TV-P or venoarterial ECMO before reperfusion in acute myocardial infarction, 18 swine underwent 120 min of LAD occlusion followed by 180 min of reperfusion (Figures 3A and 3B). After 90 min of LAD occlusion, subjects were randomly assigned to continued occlusion for 30 min (IR) or continued occlusion for 30 min with activation of a TV-P or ECMO (n = 6 per group). In the 2 device arms, pumps remained active throughout the 180 min after reperfusion. The TV-P was inserted via the right carotid artery and activated at maximal support (44,000 rpm). ECMO was initiated using a 19-F arterial cannula and 25-F multistage venous cannula in the left femoral artery and right femoral vein, respectively. ECMO was activated at 7,500 rpm using a centrifugal pump (CardiacAssist, Pittsburgh, Pennsylvania)

and membrane oxygenator (Maquet, Rastatt, Germany). LV pressure and volume relationships were measured using a 5-F conductance catheter system (CD Leycom, Hengelo, the Netherlands) as previously described (9).

MYOCARDIAL INFARCT SIZE QUANTIFICATION. Upon study protocol completion, balloon occlusion was performed within the mid-LAD and 0.5% Evans blue injected into both coronary vessels to depict the area at risk. The left ventricle was removed and sectioned into 4 1-cm slices from the anteroapical left ventricle distal to the site of stent deployment (infarct zone). Tissue samples were consistently collected from the center of the infarct zone and the remote noninfarct zone. LV slices were stained and the total myocardial area, area at risk, and infarct zone quantified (9).

RNA ISOLATION. For RNA isolation, tissue samples were homogenized using a PowerGen 125 (Fischer Scientific, Waltham, Massachusetts) homogenizer in ice-cold 600 μ l RTL buffer provided by the RNeasy



Mini Kit (Qiagen, Hilden, Germany). Isolated RNA was transcribed using the First Strand cDNA Synthesis Kit (Applied Biosystems, Foster City, California) and transcript levels analyzed by quantitative real-time polymerase chain reaction (Supplemental Table 1) as previously described (9).

IMMUNOBLOT ANALYSES. Protein from homogenized tissue (Protein Extraction Reagent, Thermo Scientific, Waltham, Massachusetts) was supplemented with protease and phosphatase inhibitors. Protein concentrations were determined and analyzed as previously described (9). Succinate levels were quantified using a commercial kit. The Supplemental Methods section and Supplemental Table 2 list the assays and antibodies used.

MITOCHONDRIAL ISOLATION, OXYGEN CONSUMPTION RATE, AND CI ACTIVITY ANALYSIS. Mitochondrial isolation and oxygen consumption rate analysis using the Seahorse XF96 Extracellular Flux Analyzer (Seahorse Bioscience, Billerica, Massachusetts) and direct mitochondrial CI activity analyses are described in the Supplemental Methods.

QUANTIFICATION OF REDUCED GLUTATHIONE/ OXIDIZED GLUTATHIONE RATIO DETECTION ASSAY. Oxidative stress reduces the ratio of reduced versus oxidized glutathione. To quantify levels of oxidative stress, we used a fluorometric glutathione assay. Homogenates from the infarct and noninfarct zones were treated with a dye that fluoresces when reacting with glutathione (ab138881, Abcam, Cambridge, United Kingdom).

CATALASE ACTIVITY ASSAY. Catalase activity in tissue isolated from the infarct zone core were determined as per the manufacturer's instructions (ab83464, Abcam). Catalase reacts with hydrogen peroxide to produce H_2O and oxygen. Unconverted hydrogen peroxide is measured calorimetrically at an optical density of 570 nm.

METABOLOMIC SCREEN. Tissue samples were inventoried and stored at -80° C, processed, and submitted to Metabolon Inc. (Morrisville, North Carolina) in blinded fashion for analysis as described in the Supplemental Methods.

ACTIVATED/DEACTIVATED STATE OF MITOCHONDRIAL CI. To analyze the activated and deactivated state of mitochondrial CI, oxidation of NADH was determined spectrophotometrically as a decrease in absorption at 340 nm as described in the Supplemental Methods.

STATISTICS. Data are presented as mean \pm SD. Statistical analyses were performed using the Student's 2-tailed *t*-test. One-way analysis of variance analysis (Bonferroni post hoc test) was performed in cases of comparisons with more than 2 groups. Values of p < 0.05 were considered statistically significant. Every calculation is based on n = 4 to 6 animals from each group as indicated in the figure legends; p values presented in this report have not been adjusted for multiplicity, and, therefore, inferences drawn from these statistics may not be reproducible. For analysis of the DTU-STEMI pilot trial, all continuous variables were compared between treatment groups using appropriate parametric or nonparametric tests. Categoric variables were compared between treatment groups using the Pearson chi-square test for contingency tables or the Fisher exact test. All statistical tests and confidence intervals were performed at $\alpha = 0.05$ (2 sided).

RESULTS

DURATION OF LV UNLOADING BEFORE REPERFUSION IS INVERSELY ASSOCIATED WITH INFARCT SIZE IN LARGE **ANTERIOR STEMI.** To explore the relationship between ischemic time, the duration of LV unloading before reperfusion, and reduced infarct size, we analyzed data from patients enrolled in the STEMI-DTU pilot study with documented LAD occlusion, STE \geq 7 mm, and available 3- to 5-day infarct size quantification data by CMR (n = 23). The total symptom onset to angioplasty time was 210 \pm 78 min (Figures 1A and 1B). The time from symptom onset to angioplasty was longer in the U-DR group than in the U-IR group (p < 0.01) (Figure 1B). Despite the increased ischemic time in the U-DR arm, the infarct size normalized to the area at risk was smaller in the U-DR group (p < 0.01) (Figure 1C). The duration of LV unloading before reperfusion was inversely associated with infarct size (R = -0.52, p < 0.01) (Figure 1D). These findings suggest that LV unloading may attenuate myocardial injury by reducing ischemia before reperfusion.

TRANSVALVULAR UNLOADING LIMITS MYOCARDIAL INJURY BEFORE REPERFUSION. To explore mechanisms by which prolonged unloading reduces infarct size before reperfusion, we performed LAD occlusion for a total of 210 min without reperfusion in adult male swine (Group A) (Figure 2A). Compared with LAD occlusion alone, activation of a TV-P beginning after 90 min of LAD occlusion reduced infarct size $(9.75 \pm 5.9\% \text{ vs. } 2 \pm 1.9\%, \text{ } p < 0.01)$ (Figure 2B). We further quantified levels of proteins known to increase within minutes of tissue hypoxia including hypoxia-inducible factor 1-alpha (HIF-1α) and prolyl hydroxylase domain (PHD) enzymes 2 and 3 without reperfusion (Group A) (15). LAD occlusion without TV-P activation increased the levels of all 3 hypoxia-sensitive proteins in the infarct zone. No increase in these proteins was observed after 210 min of LAD occlusion with TV-P activation. Compared with LAD occlusion without TV-P activation, succinate levels were lower with TV-P activation (Supplemental Figure 1).

To study the impact of prolonged unloading on myocardial injury after reperfusion, 2 additional groups were subjected to 210 min of LAD occlusion followed by 120 min of reperfusion with and without unloading beginning after 90 min of LAD occlusion (Group B) (Figure 2A). Compared with reperfusion alone, activation of a TV-P before reperfusion reduced infarct size ($60.1 \pm 7.04\%$ vs. $27.3 \pm 9.7\%$, p < 0.001) (Figure 2B). Compared with ischemic injury alone, reperfusion increased infarct size by 7.4-fold without TV-P versus 3-fold with TV-P activation (p < 0.001). These findings suggest that TV-P activation may reduce myocardial injury by first reducing ischemia before reperfusion.

LV UNLOADING VIA ECMO DOES NOT REDUCE INFARCT SIZE. To study whether ECMO can reduce infarct size, we used a large-bore multiporous venous cannula that unloads the left ventricle by reducing cardiac preload. Adult male swine were subjected to LAD occlusion for 120 min followed by 180 min of reperfusion (immediate reperfusion [IR]). In 2 separate groups, either a TV-P or ECMO was activated 30 min before reperfusion followed by 180 min of reperfusion (Figures 3A and 3B). Compared with IR alone, TV-P support reduced LV stroke work and decreased both the infarct size normalized to the area at risk and the infarct size normalized to the total LV area (Figure 3C). ECMO activation reduced right atrial pressure and LV stroke work but increased the infarct size normalized to the area at risk and the infarct size normalized to the total LV area compared with IR (Table 1).

TABLE 1 Hemodynamic Data									
		IR (n = 4)							
		LAD Oc							
	Baseline	90 min	120 min	Reperfusion					
Heart rate, beats/min	68.5 ± 2.1	67.75 ± 2.8	69.25 ± 3.2	$\textbf{79.75} \pm \textbf{6}$					
MAP, mm Hg	83.5 ± 7.2	81.75 ± 8.3	$\textbf{79.75} \pm \textbf{7.3}$	71 ± 5.5					
RA, mm Hg	8 ± 1	7.5 ±1.6	$\textbf{6.75} \pm \textbf{1.84}$	7 ± 2.3					
LVEDV	263 ± 10	256 ± 10	256 ± 12	$290 \pm 10 \ddagger\ddagger$					
LVESV	220 ± 7	215 ± 9	211 ± 9	237 ± 8					
LVESP	86 ± 8	80 ± 8	84 ± 10	72 ± 4					
LVEDP	11 ± 2	14±1	14 ± 1*	13 ± 1					
LVSW, mm Hg	$\textbf{3,214}\pm\textbf{600}$	$\textbf{2,728} \pm \textbf{548}$	$\textbf{3,727} \pm \textbf{550}$	$\textbf{3,147} \pm \textbf{297}$					
+dP/dt	761 ± 75	715 ± 84	733 ± 81	772 ± 54					
Device flow, l/min									

Values are mean \pm SD. *p < 0.05 versus baseline. †p < .05 versus 90 min. ‡p < 0.05 versus 120 min.

IR = immediate reperfusion; LAD = left anterior descending; LVEDP = left ventricular end-systolic pressure; LVEDV = left ventricular end-diastolic volume; LVESP = left ventricular end-systolic pressure; LVESV = left ventricular end-systolic volume; LVESP = left ventricular stroke work; MAP = mean arterial pressure; RA = right atrial pressure; VA-ECMO = venoarterial extracorporeal membrane oxygenation.

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TRANSVALVULAR UNLOADING PRESERVES MYOCARDIAL **ENERGY SUBSTRATE LEVELS.** To explore mechanisms governing the cardioprotective effect of unloading, we performed untargeted metabolomics using tissue samples from the infarct zone after reperfusion. Principal component analysis identified distinct metabolite profiles between samples from the 3 groups (Figure 4A). Hierarchical analysis identified distinct metabolite profiles between TV-P and sham controls, IR, and ECMO (Figure 4B). Pathway analysis identified that compared with IR, TV-P significantly altered multiple metabolic pathways involving glycolysis, the citric acid cycle, and ETC function (Figure 4C). Compared with sham controls, IR reduced levels of key metabolites associated with glycolysis, glucose oxidation, fatty acid transport and oxidation, and amino acid use (Figure 4D, Supplemental Figure 2). Compared with IR, TV-P activation preserved levels of these metabolites, but ECMO did not. These findings suggest that compared with IR or ECMO, transvalvular unloading preserves substrate levels for oxidative phosphorylation.

TRANSVALVULAR UNLOADING PRESERVES MITOCHONDRIAL STRUCTURE. Compared with the noninfarct zone, electron micrographs showed that mitochondria from the infarct zone were swollen and disrupted with reduced membrane potential in the IR and ECMO groups but preserved in the TV-P group (Figures 5A and 5B). Compared with sham controls, the total cardiolipin content and the glycerol 3-phosphate levels (a cardiolipin precursor) were reduced in the infarct zone of both the IR and ECMO groups (Figures 5C and 5D). In contrast, the total cardiolipin content and the glycerol 3-phosphate levels were preserved in the TV-P group. Compared with the sham controls, levels of monolysl-cardiolipin, an intermediate of cardiolipin remodeling, normalized to total cardiolipin were increased with IR and ECMO but unchanged in the TV-P group (Figure 5E). Compared with the sham controls, messenger RNA levels of ETC complex subunits were decreased with IR and ECMO but preserved with TV-P (Figures 5F through 51). Supplemental Figure 3 shows comparisons with samples from the noninfarct zones. These findings suggest that transvalvular unloading preserves mitochondrial structural integrity.

TRANSVALVULAR UNLOADING PRESERVES MITOCHONDRIAL FUNCTION. To explore the functional integrity of the ETC, we used specific agonists and antagonists for ETC complexes. Compared with the noninfarct zones, the rate of basal oxygen consumption in the presence of CI substrates required was reduced in the infarct zone by IR (Figure 6A). The CI inhibitor rotenone reduces the rate of oxygen consumption in both the infarct and noninfarct zones, thereby confirming intact CI function in the noninfarct zones. Functions of CII, III, and IV were intact in both the noninfarct and infarct zones after IR. In contrast to IR, CI through IV function remained intact in both the infarct and noninfarct zones in the

TABLE I CON	linucu						
Transvalvular Pump (n = 4)				VA-ECMO (n = 4)			
LAD Occlusion				LAD Occlusion			
Baseline	90 min (Pre-Pump)	120 min (On-Pump)	Reperfusion (On-Pump)	Baseline	90 min (Pre-Pump)	120 min (On-Pump)	Reperfusion (On-Pump)
72 ± 2.7	69.75 ± 1.8	75.5 ± 3.6	85.25 ± 6.6	70.2 ± 3.4	$\textbf{72} \pm \textbf{4.4}$	76.6 ± 6.4	$92.8\pm6.5^{*}\text{†}$
$\textbf{83} \pm \textbf{5.4}$	$\textbf{87.25} \pm \textbf{7.4}$	93.25 ± 6.7	83.5 ± 6.5	$\textbf{89.4}\pm\textbf{3.2}$	$\textbf{85.4} \pm \textbf{3.8}$	$\textbf{67.4} \pm \textbf{5.5} \textbf{\$}$	$\textbf{58.4} \pm \textbf{5.8*}\textbf{\dagger}$
$\textbf{6.25} \pm \textbf{1.6}$	7 ± 2	$\textbf{7.25} \pm \textbf{1.8}$	6 ± 1.7	$6.4\ \pm 1.02$	$\textbf{7.6} \pm \textbf{0.8}$	$\textbf{2.2}\pm\textbf{0.6}$	$\textbf{3.2} \pm \textbf{1.2} \textbf{\dagger}$
$\textbf{168.3} \pm \textbf{14}$	167 ± 11	144 ± 13	169 ± 17	271 ± 34	266 ± 35	203 ± 31	218 ± 30
137 ± 14	137 ± 13	125 ± 12	152 ± 12	210 ± 25	203 ± 27	180 ± 24	202 ± 25
92 ± 9	98 ± 7	90 ± 6	78 ± 5	91 ± 5	78 ± 4	$63\pm5^{*\dagger}$	56 \pm 7*†
12 ± 1	13 ± 0.2	10 ± 0.21	10 ± 1	12 ± 1	15 ± 2	$4 \pm 2^{*\dagger}$	$3.5\pm1^{*+}$
$\textbf{2,980} \pm \textbf{826}$	$\textbf{2,828} \pm \textbf{458}$	1,435 \pm 440*†	$\textbf{2,125} \pm \textbf{564}$	$\textbf{4,374} \pm \textbf{724}$	$\textbf{3,607} \pm \textbf{618}$	$\textbf{298} \pm \textbf{70*}\textbf{\dagger}$	$599\pm209^{*} \dagger$
800 ± 105	806 ± 44	757 ± 73	667 ± 57	958 ± 53	$790 \pm 43^*$	$447\pm82^{*} \dagger$	$637\pm67^{*}$
		$\textbf{3.05} \pm \textbf{0.05}$	3.1 ± 0.07			$\textbf{4.6} \pm \textbf{0.3}$	5.1 ± 0.5

TV-P group (**Figure 6B**). In the ECMO group, functions of CI, II, and III were impaired in the infarct zone compared with the noninfarct zone (**Figure 6C**).

Myocardial ischemia-reperfusion injury reduces CI-(NADH)-linked state 3 respiration. Compared with mitochondria isolated from noninfarct zones, CI state 3 respiration was reduced in the infarct zone in the IR and ECMO groups but not the TV-P group (Figures 6D to 6F). Treatment with FCCP uncouples oxidative phosphorylation and maximizes oxygen consumption. FCCP increased the rate of oxygen consumption in noninfarct zones across all 3 groups. Compared with noninfarct zones, FCCP treatment failed to increase the rate of oxygen consumption in the infarct zone in the IR and ECMO groups, suggesting impaired oxidative phosphorylation. In comparison, FCCP increased oxygen consumption in the infarct zone from the TV-P group, suggesting intact oxidative phosphorylation (Figures 6D to 6F).

Compared with the sham controls, additional assays demonstrated reduced CI function and nicotinamide adenine dinucleotide (NAD) + hydrogen levels, a product of CI activity, in the IR and ECMO groups but not the TV-P group (Figures 6G and 6H). Compared with the sham controls, deactivated CI levels were increased in all 3 groups (4.8% vs. 25.1% vs. 11.8% vs. 39.9% [sham vs. IR vs. TV-P vs. ECMO], 1-way analysis of variance <0.01). Compared with IR, TV-P had lower deactivated CI levels (p < 0.004) (Figure 61). Next, we treated isolated mitochondria with the ATP synthase inhibitor oligomycin and quantified the rate of oxygen consumption as a correlate of ATP production. Compared with mitochondria isolated from the infarct zone from the IR group, the TV-P, but not the ECMO group, had higher levels of basal respiration for ATP production (Figure **6**J). Compared with the sham controls, tissue from the infarct zone of the IR and ECMO groups demonstrated reduced catalase activity and reduced glutathione:oxidized glutathione ratios, but TV-P activation did not (Figures 6K and 6L). These findings indicate that transvalvular unloading before reperfusion preserves CI and ETC activity and reduces oxidative stress.

DISCUSSION

Myocardial infarct size is directly associated with increased mortality and heart failure after acute myocardial infarction. Mechanically reducing LV workload before reperfusion is a potentially viable approach to limit infarct size. We provide new mechanistic insight into the cardioprotective effect of LV unloading before reperfusion by first identifying an inverse relationship between the duration of unloading and infarct size in patients with anterior STEMI. Using preclinical models, transvalvular unloading before reperfusion reduced the expression of proteins associated with myocardial ischemia, attenuated reperfusion injury, and protected mitochondrial structure including levels of cardiolipin. Using an unbiased metabolomics approach and functional testing in isolated mitochondria, transvalvular unloading preserved key energy substrate use pathways, maintained oxidative phosphorylation, and reduced oxidative stress compared with reperfusion alone (Central Illustration). These findings suggest that transvalvular unloading limits myocardial ischemia before reperfusion and protects myocardial metabolism and mitochondrial function, which are key requirements for myocardial recovery after STEMI.

The STEMI-DTU pilot study was the first clinical investigation of transvalvular unloading before reperfusion in anterior STEMI (14). No prohibitive



TV-P. (**D**) Illustration and bar graphs showing levels of representative metabolites for glycolysis, fatty acid oxidation, and the citric acid cycle. All analyses were performed on tissue isolated from the infarct zone after reperfusion. *p = 0.01 to 0.05, **p = 0.001 to 0.01, ***p = 0.001 to 0.0001, ***p < 0.0001.



safety signal associated with TV-P use was observed. Despite a more prolonged ischemic time compared with unloading and immediate reperfusion, patients with the largest area at risk had a significantly reduced infarct size with unloading and delayed reperfusion. We identified an inverse relationship between the duration of LV unloading before reperfusion and infarct size. These observations generated several important questions about the mechanism(s) underlying unloading as a therapeutic strategy in STEMI. First, does transvalvular unloading attenuate myocardial injury before reperfusion? Second, would more prolonged LV unloading before reperfusion further decrease infarct size? Third, can other contemporary circulatory support systems such as ECMO unload the LV and reduce infarct size?

Under normoxic conditions, HIF-1a is rapidly degraded by oxygen-dependent PHD enzymes. Tissue hypoxia impairs PHD activity and increases HIF-1a levels within minutes, which return to basal levels upon reoxygenation (15). As part of a negative feedback loop, PHD-2 and PHD-3 are HIF target genes whose levels are also increased during hypoxia. We observed that LAD occlusion for 210 min without reperfusion increased HIF-1a, PHD-2, and PHD-3 levels, whereas TV-P activation after 90 min of LAD occlusion followed by an additional 120 min of occlusion without reperfusion significantly reduced these levels and infarct size. Consistent with these observations, succinate levels were lower after LAD occlusion with transvalvular unloading (16). These findings suggest that TV-P activation reduces



myocardial ischemia in the absence of epicardial coronary reperfusion.

One explanation for this observation is that TV-Ps increase myocardial perfusion by enhancing microcirculatory and collateral blood flow without epicardial reperfusion. Mehrige and Wampler first reported that the transvalvular Hemopump (Medtronic, Minneapolis, Minnesota) increased myocardial perfusion before coronary reperfusion in canine models (17). Recent reports support this concept of functional reperfusion (12). These findings open opportunities to test whether transvalvular unloading reduces ischemic burden during a 30-min period to allow for drug delivery before reperfusion.

In contrast to cardiogenic shock, ECMO may reduce cardiac pre-load and unload the left ventricle in euvolemic subjects. We used ECMO with a large venous drainage cannula and observed reduced right atrial pressures and decreased LV stroke work. However, despite reducing LV stroke work, ECMO increased infarct size and failed to preserve myocardial energetics or mitochondrial function. One explanation may be that extracorporeal circuits with a large surface area may activate neutrophils, which mediate reperfusion injury. This possibility is supported by data showing that left atrial to femoral artery bypass without an oxygenator reduces LV stroke work and infarct size (18). Recent studies report improved survival in cardiogenic shock with the use of the Impella to decompress the left ventricle with ECMO (19). Whether initiating LV unloading with the Impella before ECMO can protect mitochondria from damage remains unknown and requires further study.



(blue) zones after (A) immediate reperfusion (IR), (B) a transvalvular pump (TV-P), and (C) extracorporeal membrane oxygenation (ECMO). Seahorse plots with quantification bar graphs showing Cl function in mitochondria isolated from the noninfarct (red) and infarct (blue) zones after (D) IR, (E) TV-P, and (F) ECMO. Bar graphs showing (G) Cl activity, (H) nicotinamide adenine dinucleotide levels, (I) percent of deactivated (D) Cl, (J) adenosine triphosphate (ATP)-linked respiration, (K) catalase activity, and (L) reduced (GSH) versus oxidized glutathione (GSSG) ratios. *p = 0.01 to 0.05, **p = 0.001 to 0.01, ***p = 0.001 to 0.0001, ***p = 0.0001.



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During myocardial ischemia, reduced fatty acid oxidation and increased glycolysis lead to inefficient energy production and uncouple glycolysis from glucose oxidation in the citric acid cycle. Depending on the duration of ischemia, reperfusion may partially restore energy substrate use. In our study, principal component and ingenuity pathway analysis of tissues after reperfusion showed clear metabolic profile separation between the IR and TV-P groups among key energy-producing pathways involving mitochondrial function. Compared with IR, TV-P activation before reperfusion recovered glycolytic flux and increased pyruvate production, thereby maintaining the citric acid cycle. These findings support that transvalvular unloading improves substrate use for oxidative phosphorylation or potential myocardial recovery.

Consistent with prior reports, we observed preservation of the gross mitochondrial structure and membrane potential in tissues after reperfusion with transvalvular unloading (9). Several studies have also shown that reduced cardiolipin content impairs ETC activity and increases ROS (5-7). We now introduce that compared with IR, TV-P activation preserved cardiolipin content. ECMO increased the ratio of monolysl-cardiolipin:cardiolipin, an intermediate of cardiolipin synthesis associated with an inherited cardiac and skeletal myopathy known as Barth syndrome. ECMO further disrupted function of CI, II, and III, suggesting broader impairment of the ETC compared with IR. Recently, the EMBRACE-STEMI (Evaluation of Myocardial Effects of MTP-131 for Reducing Reperfusion Injury in Patients With Acute Coronary Events, ST-segment elevation myocardial infarction) trial tested the usefulness of MTP-131, a peptide that preserves cardiolipin, among STEMI patients and failed to show a reduction in infarct size (20). Whether compounds such as MTP-31 improve infarct size when combined with transvalvular unloading requires further study.

Mitochondrial CI accounts for 40% of proton motive force required to maintain ATP synthase in the ETC. During myocardial ischemia, CI transforms from an active to a deactivated state, thereby limiting ATP synthesis. Upon reperfusion, CI contributes to oxidative damage. We observed reduced CI function, increased levels of deactivated CI, and increased oxidative stress after reperfusion from the IR and ECMO groups but not the TV-P group. Specifically, we observed that mitochondria isolated from the IR or ECMO groups but not the TV-P group did not respire in the presence of substrate for CI but were able to respire in response to substrates for complexes II or IV, suggesting that these mitochondria were not fully uncoupled but rather exhibited impaired CI activity. These findings suggest that transvalvular unloading limits mitochondrial damage. Adjunct approaches to protect CI and mitochondrial function may further reduce infarct size. Furthermore, whether mitochondrial function continues to improve and is associated with myocardial recovery after transvalvular unloading remains unknown.

STUDY LIMITATIONS. Limitations include the relatively small number of large animals studied per group. Healthy swine without comorbidities were studied, which may limit the clinical applicability of the findings. Sex differences and myocardial salvage



inner mitochondrial membrane (IMM) and complex I (CI) function, thereby leading to myocardial damage. Transvalvular pump, but not ECMO, activation before reperfusion preserves cardiac metabolism and mitochondrial function and reduces infarct size (IS). ATP = adenosine triphosphate; ECMO = extracorporeal membrane oxygenation; LA = left atrium; RA = right atrium; RV = right ventricle.

> also require further study. Because mitochondria are subject to quality control and renewal, the long-term effects of TV-P and ECMO support on mitochondrial function also require further study. Future studies exploring the impact of combined TV-P and ECMO on mitochondrial function are required. Finally, we used balloon-mediated coronary occlusion without coronary thrombus, which may further limit the clinical applicability of the findings.

CONCLUSIONS

We provide new mechanistic insight into the cardioprotective effects of LV unloading by suggesting that transvalvular unloading and delayed reperfusion limits infarct size by preserving myocardial energetics and mitochondrial function in acute myocardial infarction. These findings support ongoing studies of LV unloading in STEMI and identify potential drug targets to synergistically reduce infarct size with LV unloading in STEMI.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Transvalvular unloading of the left ventricle before coronary reperfusion attenuates myocardial ischemia, reduces reperfusion injury, reduces energy substrate use, and preserves mitochondrial integrity in an animal model of acute myocardial infarction. **TRANSLATIONAL OUTLOOK:** Clinical studies comparing the effects of early myocardial perfusion versus transvalvular unloading and delayed reperfusion on myocardial function in patients with acute myocardial infarction.

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KEY WORDS acute myocardial infarction, cardioprotection, circulatory support, mitochondria

APPENDIX For an expanded Methods section and supplemental tables and figures, please see the online version of this paper.