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PRECLINICAL RESEARCH

Brief Myocardial Ischemia Produces Cardiac Troponin I Release and Focal Myocyte Apoptosis in the Absence of Pathological Infarction in Swine

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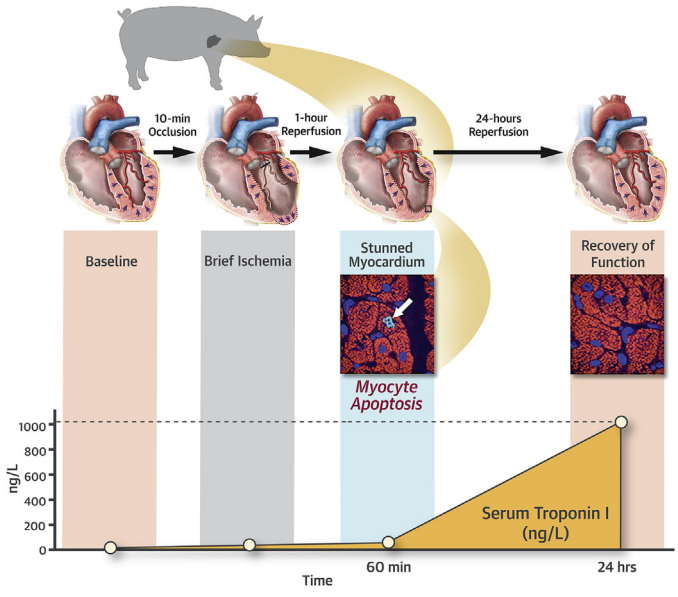
VISUAL ABSTRACT



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HIGHLIGHTS

- High-sensitivity cTnI assays have increasingly identified a rise and fall in situations not typically thought to be associated with infarction, such as exercise stress in patients with coronary disease and prolonged exercise in apparently healthy marathon runners.
- Using a porcine model of brief ischemia leading to myocardial stunning following a 10-min coronary occlusion, the authors demonstrate a delayed release of cTnI after what had previously been felt to be completely reversible ischemia.
- Although tissue necrosis, sarcolemmal disruption, and infarction are absent after brief ischemia, TUNEL staining demonstrates rare single myocytes undergoing irreversible injury from apoptosis.
- These studies demonstrate that significant cTnI release can occur after a brief duration of ischemia that could be compatible with angina.
- In the absence of an acute coronary syndrome or a prolonged myocardial supply/demand imbalance, it may be more appropriate to ascribe significant cTnI elevations after brief ischemia to myocardial injury rather than infarction.

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ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome

AIV = anterior interventricular vein

cTn = cardiac troponin

cTnI = cardiac troponin I

IV = intravenously

LAD = left anterior descending coronary artery

LADWT = left anterior descending coronary artery regional wall thickening

LV = left ventricle/ventricular

TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling

TTC = triphenyltetrazolium chloride

SUMMARY

In a porcine model of brief ischemia leading to reversible stunning in the absence of tissue necrosis, we demonstrated delayed release of cardiac troponin I (cTnI) that exceeded the 99th percentile for normal animals 60 min after reperfusion and rose to readily detectable levels 24 h later. Although tissue analysis at 60 min showed no evidence of infarction, TUNEL staining demonstrated isolated myocytes undergoing apoptosis, which was absent after 24 h. These results demonstrate that cTnI elevations occur after ischemia of a duration that is insufficient to produce myocyte necrosis and reflect myocyte injury associated with apoptosis in the absence of pathological evidence of infarction. (J Am Coll Cardiol Basic Trans Science 2017;2:105–14)

Elevated serum cardiac troponin (cTn) concentrations have traditionally been considered indicative of myocardial injury leading to myocyte necrosis (and potentially other mechanisms of myocyte death) and are currently the preferred diagnostic biomarker for the clinical detection of myocardial infarction (1). Nevertheless, recent studies using high-sensitivity cTn assays have demonstrated a rise in serum cTn in situations not typically thought to be associated with irreversible myocyte injury (2). Two examples include transient myocardial ischemia associated with physiological stress (3–5) and prolonged exercise in apparently healthy marathon runners (6). Because sarcolemmal membrane integrity remains intact for up to 15 min of no-flow ischemia (7,8) and pathological evidence of infarction is absent (9), the cardiac troponin I (cTnI) release in these circumstances suggests that cTn release may not always be indicative of myocyte necrosis.

SEE PAGES 115 AND 118

Some have hypothesized that elevations in serum cTn in circumstances not associated with a myocardial infarction could reflect the rapid release of an exchangeable pool of unbound cytosolic cTn due to sublethal changes in cell membrane permeability or vesicular release from myocytes (6,10–12). In these scenarios, serum cTn concentrations would be expected to rise and fall towards baseline fairly quickly (i.e., within 24 h) (12,13), in contrast to the prolonged elevation seen in reperfused myocardial infarction (14). Alternatively, ischemia-induced cTn release in the absence of necrosis could reflect mechanisms of irreversible myocyte injury such as cardiomyocyte apoptosis that are not detectable with routine light microscopy. Although apoptosis has not been evaluated after brief ischemia, we have previously demonstrated that it is increased in hibernating myocardium subjected to chronic repetitive ischemia, which can lead to substantial regional myocyte loss in the absence of infarction (15). Apoptosis has also been implicated as a significant source of myocyte loss during myocardial infarction (16) and leads to myocyte death in normally perfused remote regions of the heart during post-infarction left ventricular remodeling (17).

With this background, we determined whether a single brief episode of myocardial ischemia having a time course consistent with angina (e.g., from coronary vasospasm) could produce measurable elevations in serum cardiac troponin I (cTnI). We employed a 10-min period of supply-induced ischemia followed by reperfusion for up to 24 h in swine. Serial coronary venous blood sampling from the anterior interventricular vein (AIV) was employed to assess early transcoronary cTnI release to determine whether myocardial release kinetics were consistent with the proposed rapid release from an exchangeable

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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cytosolic pool of unbound cTnI versus delayed release from myocyte injury. Because previous studies employing light and electron microscopy have clearly established the absence of myocyte necrosis when the duration of ischemia is <15 min (8), we determined whether brief ischemia induces myocyte apoptosis, which would not have been detected using standard pathological approaches.

METHODS

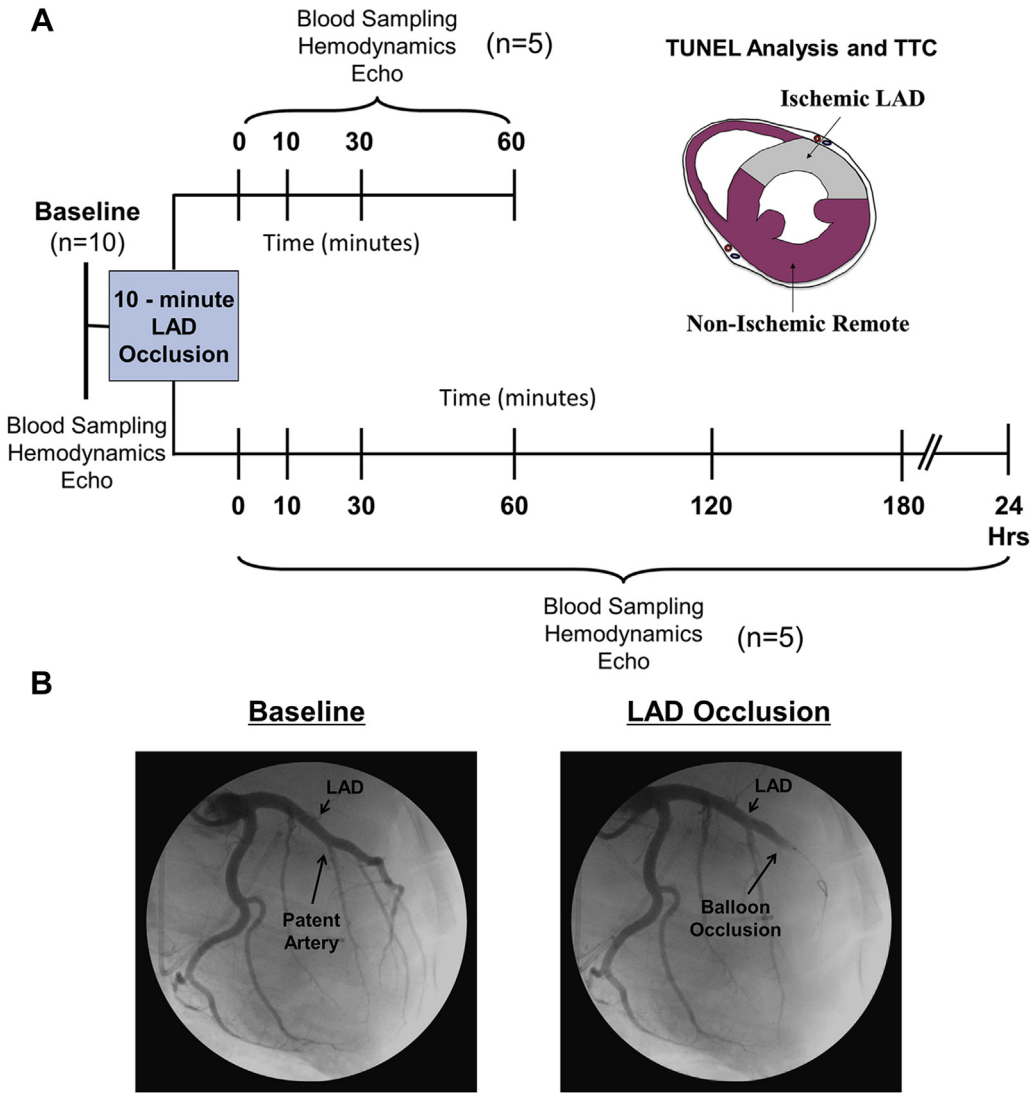
All procedures and protocols conformed to institutional guidelines for the care and use of animals in research and were approved by the University at Buffalo Institutional Animal Care and Use Committee.

LARGE ANIMAL INSTRUMENTATION. Pigs (44 ± 2 kg) were sedated with a Telazol (100 mg/ml; Tiletamine HCl and Zolazepam HCl, Zoetis, Inc., Kalamazoo, Michigan)/xylazine (100 mg/ml; MWI, Boise, Idaho) mixture (0.04 ml/kg intramuscularly) maintained on a continuous intravenous infusion of propofol (5 to 10 mg/kg/h), and mechanically ventilated with supplemental oxygen. A 7-F sheath was placed into the right carotid artery through which a 5-F catheter (Millar, Houston, Texas) was positioned in the left ventricle (LV) for continuous pressure measurement. The side port of the introducer was used to measure arterial pressure. Next, a 7-F sheath was placed into the right jugular vein through which a 5-F multipurpose catheter (Cordis Corporation, Miami Lakes, Florida) was advanced into the AIV for coronary venous blood sampling. The side-port was used for systemic blood samples. A third 7-F sheath was then placed into the left carotid artery to advance a balloon angioplasty catheter into the left anterior descending coronary artery (LAD) as described later in the text. Animals were heparinized (100 U/kg intravenously [IV]), and hemodynamics were allowed to equilibrate (~20 min) before beginning the protocol. To minimize the occurrence of lethal arrhythmias, all animals were pretreated with amiodarone (5 mg/kg IV) and lidocaine (1.5 mg/kg IV) boluses followed by continuous infusions (amiodarone: 0.04 mg/kg/min, lidocaine: 0.05 mg/kg/min) during coronary occlusion and 10 min into the reperfusion period. Hemodynamic parameters and systolic wall thickening using echocardiography (GE Vivid 7, GE Healthcare, Little Chalfont, United Kingdom) were assessed as previously described (18).

EXPERIMENTAL PROTOCOL. The timeline of the experimental protocol is summarized in Figure 1A and described in detail later in the text. After baseline measurements, an appropriately sized balloon angioplasty catheter (Maverick, 3.0 to 4.0 mm, Boston Scientific, Natick, Massachusetts) was advanced distal to the second diagonal branch of the LAD through a 6-F guiding catheter (Cordis Corporation). Balloon occlusion was documented with contrast angiography (Figure 1B), and hemodynamic and functional measurements were repeated. After 10 min, the balloon was deflated and reperfusion confirmed angiographically. In one series of experiments (n = 5), blood sampling, hemodynamics, and echocardiography were repeated at the end of ischemia, and 10 min, 30 min, and 1 h after reperfusion. Hearts were then arrested with intracardiac KCl under deep isoflurane anesthesia. Myocardial tissue was excised for histopathology and 2,3,5-triphenyltetrazolium chloride (TTC) staining (19). In the other series of experiments (n = 5), blood sampling, hemodynamics, and echocardiography were repeated 30 min, 1 h, 2 h, and 3 h after reperfusion. We removed the catheters, and the pigs were brought back to the animal facility. Animals returned 24 h later when they were reanesthetized for blood sampling, assessment of hemodynamic and echocardiographic parameters, and excision of the heart for pathology and TTC analysis.

MYOCARDIAL HISTOPATHOLOGY. Samples from the ischemic LAD region and a nonischemic remote region (inferior wall) of the left ventricle (illustrated in Figure 1A) were fixed with formalin and embedded in paraffin for histopathology. Hematoxylin and eosin-stained tissue sections from animals sacrificed 1 h after reperfusion were evaluated for evidence of necrotic cell death, including loss of myocyte nuclei, inflammatory cell infiltration, and contraction bands. Cardiomyocyte apoptosis was assessed using the In-Situ Cell Death Detection Kit (Roche Diagnostics, Indianapolis, Indiana) according to the manufacturer's guidelines (15). Briefly, apoptotic cells were detected by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) and epifluorescence with a FITC filter. Samples were costained with an F-Actin antibody conjugated to fluorescent Alexa Fluor 555 dye (Alexa Fluor 555 Phalloidin, Thermo Fisher Scientific, Waltham, Massachusetts) and colocalization with TUNEL to quantify apoptotic cardiomyocytes. Approximately 100 microscopic fields (200×) were examined per sample and the number of apoptotic myocytes was expressed as $TUNEL^+$ myocytes per cm^2. Only $TUNEL^+$ nuclei that could be definitively confirmed to be of myocyte origin were included. In addition, sections from animals sacrificed 1 h after reperfusion were immunostained for active caspase-3 (Cell Signaling Technology, Danvers, Massachusetts) according to the manufacturer's guidelines to corroborate the results of TUNEL staining in these samples.

FIGURE 1 Experimental Protocol



(A) Following baseline data collection, the left anterior descending coronary artery was occluded distal to the second diagonal branch with an inflatable angioplasty balloon for 10 min, followed by reperfusion for 1 h (n = 5) or 24 h (n = 5). Post-mortem TUNEL analysis was performed on myocardial tissue sections collected from ischemic and non-ischemic regions of the left ventricle. **(B)** Example fluoroscopic images illustrating the percutaneous coronary occlusion procedure. LAD = left anterior descending coronary artery; TTC = triphenyltetrazolium chloride; TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling.

ASSESSMENT OF SERUM cTnI. Blood samples were allowed to clot at room temperature for 40 min, centrifuged at 1,500 *g* for 15 min, aliquoted, and frozen for storage at −80°C. Serum was thawed once and cTnI quantified in duplicate with a porcine-specific cTnI ELISA kit for serum (Life Diagnostics, West Chester, Pennsylvania) according to the manufacturer's instructions.

STATISTICAL ANALYSIS. Data are expressed as mean ± SEM. Differences between time points were assessed by repeated measures analysis of variance and the post hoc Holm-Sidak test. Because cTnI concentrations were not normally distributed, nonparametric testing (Friedman test with post hoc paired Wilcoxon sign rank test) was employed to assess differences in serum cTnI

TABLE 1 Serial Hemodynamic Changes During and After Reversible Myocardial Ischemia

			Post-Reperfusion		
	Baseline (n = 10)	Ischemia (n = 10)	30-min (n = 10)	60-min (n = 10)	24-h (n = 5)
Heart rate, beats/min	87 ± 6	67 ± 4*	61 ± 3*	60 ± 4*	73 ± 7
Mean arterial pressure, mm Hg	101 ± 4	71 ± 4*	80 ± 4*	80 ± 4*	106 ± 7
LV peak systolic pressure, mm Hg	118 ± 5	93 ± 4*	100 ± 4*	101 ± 4*	130 ± 10
LV end-diastolic pressure, mm Hg	15 ± 1	25 ± 1*	18 ± 1	17 ± 1	15 ± 2
+dP/dt, mm Hg/s	2,351 ± 136	1,152 ± 102*	1,196 ± 50*	1,265 ± 62*	2,119 ± 180
−dP/dt, mm Hg/s	−2,182 ± 159	−1,353 ± 108*	−1,688 ± 107*	−1,678 ± 97*	−2,636 ± 260
Ejection fraction, %	67 ± 3	38 ± 3*	54 ± 2*	59 ± 2*	68 ± 3

Values are mean ± SEM. *p < 0.05 vs. baseline.
LV = left ventricular.

between sampling locations (i.e., AIV vs. jugular vein) and time points. Post hoc tests were not adjusted for multiple comparisons. Statistical analysis was performed with IBM SPSS Statistics 23 (IBM, Armonk, New York), and the acceptable type 1 error rate was prospectively set at 5%.

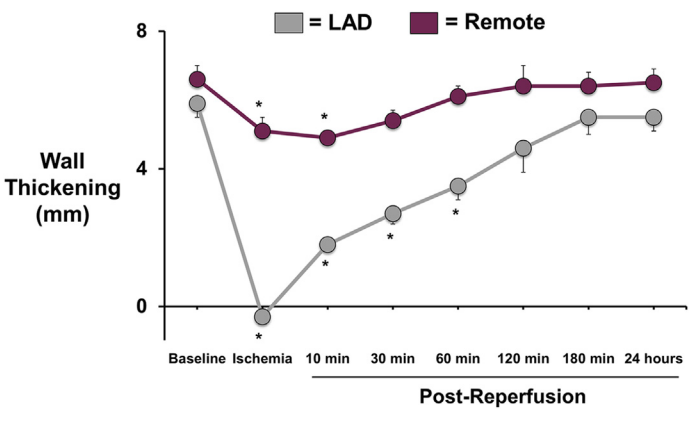
RESULTS

Serial measurements of selected hemodynamic and echocardiographic parameters before, during, and after the 10-min LAD occlusion are shown in Table 1. Baseline measurements of LV ejection fraction (67 ± 3%) and +dP/dt (2,351 ± 136 mm Hg/s) were normal. After LAD occlusion, LV ejection fraction and +dP/dt declined (to 38 ± 3% and 1,152 ± 102 mm Hg/s; both p < 0.001 vs. baseline), and LV end-diastolic pressure rose (from 15 ± 1 mm Hg to 25 ± 1 mm Hg; p < 0.001 vs. baseline). Regional wall thickening (ΔLADWT) showed a similar pattern (Figure 2). The anterior region became dyskinetic during ischemia (ΔLADWT 5.9 ± 0.4 mm to −0.3 ± 0.1 mm; p < 0.001), remained depressed 1 h after reperfusion (ΔLADWT 3.5 ± 0.4 mm; p < 0.01 vs. baseline) and normalized after 3 h (ΔLADWT 5.5 ± 0.5 mm; p = 0.63 vs. baseline) consistent with myocardial stunning. Function and hemodynamic changes completely normalized after 24 h.

Serum cTnI under baseline conditions (n = 39 normal swine) averaged 7.6 ± 1.9 ± 11.6 ng/l. Thus, the 99th percentile for the porcine assay was established at 38 ng/l. Serial measurements of serum cTnI before and after brief ischemia are summarized in Figure 3. Measurements at baseline, 30 min, and 1 h were common to both protocols and pooled (n = 10). Baseline serum cTnI concentrations were low, but detectable, in the AIV (12 ± 5 ng/l) and systemic venous samples (13 ± 6 ng/l). Transcoronary release ($\Delta cTnI = cTnI_{[AIV]} - cTnI_{[jugular\ vein]}$) was not detectable at baseline nor when assessed 10 min after reperfusion. As $cTnI_{[AIV]}$ rose, ΔcTnI slowly increased at 30 min (5 ± 3 ng/l; p = 0.07AIV vs. jugular vein), 1 h (10 ± 6 ng/l; p = 0.17), and 3 h after reperfusion (90 ± 75 ng/l; p = 0.08), indicating a delayed myocardial release of cTnI after brief ischemia (Figure 3A). Circulating cTnI concentrations became significantly elevated at 1 h (51 ± 17 ng/l; p = 0.01 vs. baseline), 2 h (148 ± 88 ng/l; p = 0.04), and 3 h (180 ± 117 ng/l; p = 0.04) post-reperfusion. The cumulative cTnI release led to levels that were easily detectable in systemic samples 24 h later (1,021 ± 574 ng/l; p < 0.01 vs. baseline) (Figure 3).

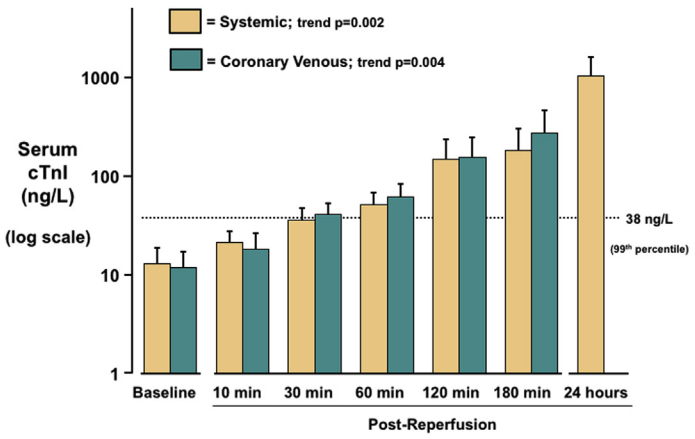
Post-mortem analyses confirmed the absence of pathological infarction by TTC staining. Similarly, light microscopic evaluation of hematoxylin and

FIGURE 2 Brief Myocardial Ischemia Produces Transient LV Dysfunction Consistent With Stunned Myocardium



A 10-min total left anterior descending coronary artery (LAD) occlusion caused a transient reduction in anterior wall thickening that persisted 1 h after reperfusion, indicative of myocardial stunning. However, regional function continued to improve and returned to normal 3 h after reperfusion. *p < 0.05 versus baseline. n = 5 at 120 min, 180 min, and 24 h; n = 10 at all other time points. LV = left ventricular.

FIGURE 3 Brief Myocardial Ischemia Elicits a Delayed Rise in Serum cTnI



Serum cTnI concentrations were low, but detectable, at baseline and did not exceed the 99th percentile of normal animals (38 ng/l) during the first 30 min of reperfusion following a 10-min LAD occlusion. However, cTnI concentrations in coronary venous and systemic venous blood rose above the 99th percentile value 60 min after reperfusion and continued to rise at subsequent time points, ultimately increasing by ~100-fold versus baseline levels 24 h post-reperfusion. Because cTnI concentrations were not normally distributed, nonparametric testing (Friedman test) was used to evaluate the trend in cTnI values after reperfusion, with post-hoc paired Wilcoxon sign rank testing to determine where significant differences were observed between specific time-points. Note the use of a logarithmic scale on the y-axis. n = 5 at 10 min, 120 min, 180 min, and 24 h; n = 10 at all other time points. cTnI = cardiac troponin I; LAD = left anterior descending coronary artery.

eosin-stained tissue sections collected 1 h after reperfusion did not show evidence of myocyte nuclear loss or inflammatory cell infiltration, consistent with the absence of myocyte necrosis. Contraction band necrosis was not observed. In agreement with previous reports (8), rare myocytes exhibited contracted myofibrils, but these were present to a similar extent in ischemic and non-ischemic remote areas of the left ventricle and consistent with tissue processing artifact. While myocyte necrosis was absent, hearts excised 1 h after reperfusion demonstrated regional myocyte apoptosis with an ~6-fold increase in TUNEL$^+$ cardiomyocytes in the LAD region (17.7 ± 3.7 myocytes/cm^2 vs. 3.1 ± 2.0 myocytes/cm^2 in the nonischemic remote region; p = 0.03) (**Figure 4**). Cardiomyocyte caspase-3 staining corroborated the TUNEL results, because active caspase-3-positive myocytes were detected in 4 of 5 samples from the LAD region (10.5 ± 4.3 cells/cm^2), but only 1 of 5 remote zone samples (3.8 ± 3.8 cells/cm^2). Active caspase-3 was not detectable in any myocytes from normal hearts (n = 4). When assessed in tissue excised 24 h after reperfusion, myocyte apoptosis returned to control levels and was not different than remote regions (5.2 ± 2.3 myocytes/cm^2 vs. 3.6 ± 1.5 myocytes/cm^2; p = 0.55). Collectively, these data indicate that brief ischemia from a 10-min LAD occlusion did not result in gross pathological evidence of myocardial infarction but produced a transient increase in cardiomyocyte apoptosis that led to a delayed cumulative rise of cTnI.

DISCUSSION

The present study clearly demonstrates that cTnI release occurs following what has traditionally been considered to be completely reversible ischemia and provides important new insights into the mechanism. First, we found minimal early transcoronary cTnI release arguing against an exchangeable cTnI pool in viable myocytes. Second, rather than the typical rise and fall pattern of cTnI that rapidly peaks between 2 and 4 h in reperfused myocardial infarcts (20), cTnI release after brief ischemia was delayed but increased to easily detectable levels between 3 and 24 h following restoration of flow. Finally, although brief ischemia did not produce any pathological evidence of necrosis or infarction, regional apoptosis of single dispersed myocytes transiently increased at 1 h and normalized 24 h after reperfusion. Thus, although brief ischemia does not lead to pathological necrosis or infarction, it can produce delayed cTnI release that is associated with irreversible myocyte injury from focal apoptosis.

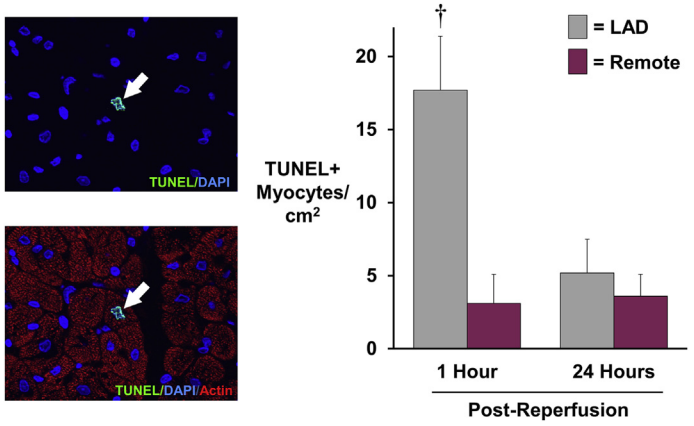
RELATION TO PREVIOUS STUDIES. Numerous preclinical studies performed in vivo as well as in vitro have demonstrated that cardiac myocytes have substantial glycogen stores that are initially able to maintain ATP production for essential membrane functions and prevent sarcolemmal disruption (8). Nevertheless, after 15 min of regional ischemia in vivo, ATP falls to critical levels leading to a wave front of necrosis progressing from the subendocardium to the subepicardium (7,21). The onset of necrosis is manifest by disruption of the sarcolemmal membrane with the entry of calcium, contraction band necrosis, and the release of intracellular proteins such as cTnI and many other enzymes (e.g., CPK and myoglobin) into the coronary circulation. Some pathological studies report the formation of sarcolemmal budding or blebs before the onset of sarcolemmal disruption in cardiomyocytes (22). Whether this reflects a reversible or irreversible stage of myocardial injury is controversial (23), but these observations have given rise to the possibility that an intracellular pool of unbound cTn could become packaged and released from viable myocytes in response to myocardial stresses insufficient to produce necrosis (22,24,25). The latter mechanism has been attractive to explain the unanticipated

transient rise and fall of cTn reported using high-sensitivity assays in otherwise normal marathon runners (26), following increased demand elicited by pacing in the cardiac catheterization laboratory (in subjects with and without coronary disease) (4), as well as by some, but not all, investigators after transient exercise-induced ischemia during routine stress testing or following coronary vasospasm (3). Troponin release in these situations could not be attributable to myocardial necrosis or infarction.

We examined the dynamics of cTnI release after brief ischemia in normal juvenile swine using a well characterized brief coronary occlusion model to produce ischemia in the absence of necrosis or infarction (9). In order to detect low levels of cTnI release at the earliest time point, we employed regional coronary venous sampling in the anterior interventricular vein that drains the region supplied by the LAD. We found no immediate cTnI release in the first 10 min after reperfusion, which stands in stark contrast to what would be anticipated for tissue necrosis. In addition, the early transcoronary cTnI gradient was small and progressively increased over the initial 3 h after reperfusion. Despite only brief ischemia, cTnI levels found in systemic samples (reflecting cumulative release competing with systemic degradation) rose from baseline (13 ng/l) to 180 ng/l at 3 h and to 1,020 ng/l at 24 h (a level easily detectable with conventional as well as high-sensitivity cTnI assays). The delayed rise of cTnI and the large increase between 3 and 24 h after reperfusion is not consistent with early release from an exchangeable pool of cTnI. It also contrasts with the early release of cTnI seen in reperfused myocardial infarcts, which, in the pig, peaks between 2 and 4 h following reperfusion (20).

Consistent with previous studies of myocardial stunning, we did not identify evidence of necrosis after brief ischemia. Although apoptosis is not visible with standard light microscopy, immunofluorescence with TUNEL staining demonstrated that apoptotic myocyte injury occurs in single dispersed myocytes throughout the ischemic area of the left ventricle. This contrasts with the confluent pattern of myocyte necrosis that occurs in myocardial infarction and provides a plausible mechanism by which cTnI is released into the circulation following brief ischemia. Although the cellular mechanism of cTnI release will require further study, the present results demonstrate rates of regional apoptosis that increased ~6-fold in hearts excised 1 h after brief ischemia. This preceded the major increases in circulating cTnI. Apoptosis returned to levels similar to the nonischemic region 24 h later. Although our studies were only able to examine apoptosis at 1 and 24 h after reperfusion, they indicate

FIGURE 4 Brief Myocardial Ischemia Is Associated With a Transient Elevation in Cardiomyocyte Apoptosis



Example images of a TUNEL+ myocyte in the LAD-supplied region of the left ventricle 1 h after reperfusion are shown in the **left panel (arrows)**. Tissue samples showed no pathological evidence of myocardial infarction. Compared with the nonischemic remote zone, there was a significant 6-fold increase in myocyte apoptosis in the ischemic LAD region 1 h after reperfusion. LAD apoptosis normalized 24 h later. †$p < 0.05$ versus remote. Abbreviations as in Figure 1.

that apoptosis is quite dynamic. This process begins fairly soon after ischemia and is completed 24 h later, making myocyte injury in the absence of necrosis challenging to identify when the heart is examined well after the recovery of stunned myocardium.

Aside from implicating apoptotic myocyte cell death as a mechanism underlying transient elevations in cTnI after brief ischemia, our results may have clinical relevance in understanding how brief repetitive ischemia can lead to myocyte remodeling in chronic coronary artery disease. Although the rates of apoptosis that occur after reversible ischemia are very low, chronic repetitive ischemia with repeated bouts of apoptosis can effect substantial regional myocyte loss. Indeed, in the setting of a chronic LAD stenosis, repetitive spontaneous ischemia leads to chronic hibernating myocardium where similar low rates of apoptosis produce significant regional myocyte cellular remodeling in swine and humans (15,27). Although infarction and necrosis are absent, apoptosis from chronic repetitive ischemia in swine leads to substantial regional myocyte loss (~30% reduction in myocyte number) with compensatory myocyte cellular hypertrophy developing to maintain myocardial mass and normal wall thickness (15). These changes occur in collateral-dependent myocardium with a critical limitation in coronary flow reserve where transient elevations in left ventricular end-diastolic pressure in the absence of ST-segment depression are the only

manifestations of demand-induced ischemia (28). In multivessel coronary disease, apoptosis-induced myocyte loss from reversible ischemia can even lead to the development of ischemic cardiomyopathy in the absence of infarction (19).

STUDY LIMITATIONS. Our study did not evaluate cTnI release at time points between 3 h and 24 h after reperfusion, and thus, we could not determine when cTnI peaked. Likewise, although regional LAD apoptosis returned to baseline after 24 h, we cannot exclude the possibility that it could have increased further between 1 h and 24 h after reperfusion. Although TUNEL positivity is consistent with the onset of apoptosis, others have demonstrated that it may also reflect myocyte oncosis following reperfusion after ischemia that induces pathological evidence of infarction (29). Alternatively, it is possible that focal necrosis, rather than apoptosis, is the primary form of cell death following brief ischemia, with TUNEL-positivity occurring as a secondary event following rupture of the outer mitochondrial membrane and subsequent cytochrome c release, caspase activation, and, ultimately, DNA fragmentation (30). Although the lack of light microscopic evidence of necrosis such as loss of myocyte nuclei, inflammatory cell infiltration, or contraction bands argues against a predominant role of myocyte necrosis as a primary form of cell death in the present study, it is important to acknowledge the existence of cross-talk between mitochondrial apoptosis and necrosis pathways that can complicate interpretation of TUNEL assays (31). Regardless, the isolated nature of the myocyte injury distinguishes it from the confluent nature of irreversibly injured myocytes typical of infarction. It is conceivable that a transient coronary occlusion could cause alterations in vasoreactivity or platelet aggregation that reduce microcirculatory flow in stunned myocardium upon reperfusion and delay the appearance of cTnI in the coronary sinus. Nevertheless, it is unlikely that this was a major factor underlying the delayed cTnI release observed in the present study in light of previous studies that have demonstrated that resting coronary flow returns to normal after a 10-min coronary occlusion (32). Finally, we used a porcine specific cTnI assay that does not have the sensitivity of current high-sensitivity human assays. Thus, our ability to detect very low levels of baseline transcoronary cTnI release as well as mildly elevated levels in the first few h of reperfusion may have been limited.

TRANSLATIONAL RELEVANCE. Although cTnI and cTnT are the recommended biomarkers to define the presence of a myocardial infarction, as many as 2 in three cTn elevations in patients in the United States are not related to an acute coronary syndrome (ACS) (33). It seems plausible that myocardial injury in these settings could reflect focal myocyte apoptosis that arises as a continuum in relation to the duration and severity of ischemia. Myocyte apoptosis may also arise through mechanisms not related to myocardial ischemia such as myocyte stretch (34) and increased neurohormonal stimulation (35). Thus, it is plausible that myocyte apoptosis arising in the absence of ischemia can explain elevations in cTn in many other pathological conditions (e.g., heart failure, pulmonary embolism, sepsis, and renal failure), as well as in physiological states associated with increased myocyte turnover such as aging (36) or prolonged endurance exercise (6). Although speculative, it is possible that identifying whether cTn is released in the intact form or as a specific degradation product could provide insight into the mechanism responsible for release (12,37,38). Although the prognostic significance of an elevated cTnI level appears to be independent of its underlying cause, categorizing non-ACS cTn elevations arising from focal apoptosis as “myocardial injury” rather than infarction (related to ACS and a vulnerable plaque) may help distinguish the pathophysiological basis for abnormal values and better direct the clinical approach required for patient evaluation and treatment.

CONCLUSIONS

Brief ischemia elicits delayed cTnI release that is compatible with irreversible myocyte injury as opposed to release from an exchangeable cTnI pool. Nevertheless, rather than necrosis from sarcolemmal disruption, the delayed cTnI release appears to reflect focal myocyte cell death from apoptosis and may explain cTnI elevations in the absence of an acute coronary syndrome.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Serum troponin I (cTnI) can become elevated after brief episodes of ischemia that have previously been considered to be completely reversible because pathological evidence of myocyte necrosis is absent. The findings of this study demonstrate that cTnI elevations in this circumstance do not reflect the early release of an exchangeable pool of cTnI from viable myocytes but instead arise from delayed programmed myocyte death from apoptosis.

TRANSLATIONAL OUTLOOK 1: There is a delayed release of cTnI above the 99th percentile for normal animals after brief regional ischemia. The time course differs from the early release expected for an exchangeable cTnI pool in viable myocytes. Rather, delayed cardiac cTnI release follows transiently increased regional myocyte apoptosis without pathological evidence of necrosis or infarction.

TRANSLATIONAL OUTLOOK 2: It is likely that focal myocyte apoptosis develops after many, if not all, episodes of brief ischemia, with the extent and severity of apoptosis determining the magnitude of troponin I elevation in the serum. These episodes would be considered to be angina clinically, yet some would meet our current definition of myocardial infarction despite the absence of myocyte necrosis. Many transient cTnI elevations probably occur without demonstrable electrocardiographic changes or chest pain because these are insensitive indices of brief ischemia. Still others may reflect nonischemic causes of myocyte apoptosis. Thus, in the absence of clinical suspicion of an acute coronary syndrome or prolonged demand-induced ischemia, it may be more appropriate to ascribe these cTnI elevations as due to "myocardial injury" rather than infarction.

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