

# Hypothermia Modulates Arrhythmia Substrates During Different Phases of Resuscitation From Ischemic Cardiac Arrest

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**Background**—We designed an innovative porcine model of ischemia-induced arrest to determine dynamic arrhythmia substrates during focal infarct, global ischemia from ventricular tachycardia or fibrillation (VT/VF) and then reperfusion to determine the effect of therapeutic hypothermia (TH) on dynamic arrhythmia substrates and resuscitation outcomes.

**Methods and Results**—Anesthetized adult pigs underwent thoracotomy and regional plunge electrode placement in the left ventricle. Subjects were then maintained at either control (CT; 37°C, n=9) or TH (33°C, n=8). The left anterior descending artery (LAD) was occluded and ventricular fibrillation occurred spontaneously or was induced after 30 minutes. Advanced cardiac life support was started after 8 minutes, and LAD reperfusion occurred 60 minutes after occlusion. Incidences of VF/VT and survival were compared with ventricular ectopy, cardiac alternans, global dispersion of repolarization during LAD occlusion, and LAD reperfusion. There was no difference in incidence of VT/VF between groups during LAD occlusion (44% in CT versus 50% in TH;  $P=1$ s). During LAD occlusion, ectopy was increased in CT and suppressed in TH ( $33\pm 11$  ventricular ectopic beats/min versus  $4\pm 6$  ventricular ectopic beats/min;  $P=0.009$ ). Global dispersion of repolarization and cardiac alternans were similar between groups. During LAD reperfusion, TH doubled the incidence of cardiac alternans compared with CT, with a marked increase in VF/VT (100% in TH versus 17% in CT;  $P=0.004$ ). Ectopy and global dispersion of repolarization were similar between groups during LAD reperfusion.

**Conclusions**—TH alters arrhythmia substrates in a porcine translational model of resuscitation from ischemic cardiac arrest during the complex phases of resuscitation. TH worsens cardiac alternans, which was associated with an increase in spontaneous VT/VF during reperfusion. (*J Am Heart Assoc.* 2017;6:e006472. DOI: 10.1161/JAHA.117.006472.)

**Key Words:** arrhythmia • hypothermia • myocardial infarction • resuscitation • ventricular fibrillation

Sudden cardiac death is a major cause of mortality in the United States.<sup>1,2</sup> In 2014, >600 000 deaths were caused by heart disease, with half of those being sudden. Risk-stratifying these patients can be difficult, because nearly half of the patients experiencing sudden cardiac death did not have previously known heart disease.<sup>3</sup> Although, relative to other arrest rhythms, the incidence of sudden cardiac death

secondary to ventricular fibrillation (VF) is declining, it remains a major public health concern.<sup>4</sup> VF secondary to acute myocardial infarction (AMI) is devastating, with many patients not reaching the hospital; most of those who do reach the hospital experience poor outcomes.<sup>2</sup> Recent improvements in resuscitation care have saved lives, but there has been little advancement in the antiarrhythmic therapy of advanced cardiac life support (ACLS) during the past 2 decades.<sup>5–9</sup> Thus, improved understanding of the mechanisms of ischemia-induced arrhythmias during resuscitation is imperative to develop novel ACLS therapies and further improve resuscitation outcomes.

Therapeutic hypothermia (TH), or targeted temperature management, has become the standard of care for comatose patients who experience sudden cardiac death and have subsequent return of spontaneous circulation (ROSC).<sup>10,11</sup> The 2015 ACLS Guidelines recommend induction of targeted temperature management (32°C–36°C) after ROSC for all patients who remain comatose, broadening the recommendation from 2010. Generally, there have been minimal reports of increased risk of arrhythmias during targeted temperature

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## Clinical Perspective

### What Is New?

- This study suggests that different phases of resuscitation, from focal ischemia, as seen in acute myocardial infarction, through reperfusion, as seen after percutaneous intervention, have different arrhythmia substrates.
- Therapeutic hypothermia can affect each phase of resuscitation differently.
- Furthermore, cardiac alternans was the arrhythmia substrate associated with increased ventricular fibrillation after reperfusion.

### What Are the Clinical Implications?

- Resuscitation from cardiac arrest is a dynamic process, with different arrhythmia substrates dominating different phases of resuscitation.
- Therefore, certain antiarrhythmic therapies may work differently at different times.
- Therapies targeted towards different phases of resuscitation may provide a benefit during resuscitation.

management, although there are case reports suggesting that in some selected patients TH can promote VF.<sup>12,13</sup> Most hypothermia-related arrhythmias, including VF, are encountered in patients with severe hypothermia (<30°C).<sup>14,15</sup> Hypothermia is well known to prolong action potential duration and QT intervals and affects numerous cardiac ion channels, many of which are central to arrhythmogenesis, and intracellular Ca<sup>2+</sup> regulation.<sup>16–20</sup> We previously developed a model of severe hypothermia (cooling to 26°C) that demonstrated hypothermia profoundly increases transmural dispersion of repolarization (DOR), which could be casually linked to a reentrant excitation, and of VF in severe hypothermia.<sup>21</sup>

Paradoxically, we also identified TH (cooling to 32°C) as beneficial during ischemia by limiting ischemia-induced ventricular conduction velocity (CV) slowing and increases in transmural DOR.<sup>22</sup> This improvement can be attributed to maintenance of cell-to-cell coupling via gap junctions and improvement of sodium channel function during TH.<sup>23</sup> This in vitro observation provides molecular insight into one potential benefit of hypothermia. But further insight into more complex mechanisms in the intact heart, where effects on regional electrical heterogeneities, nodal activity, and His-Purkinje system conduction, is needed to understand the effects of temperature during resuscitation.

Resuscitation from cardiac arrest is complex, with hemodynamic, autonomic, and electrophysiologic factors all playing a role during resuscitation. More important, there are multiple causes of cardiac arrest, which can sometimes be difficult to initially determine, and impeding initiation of specific

therapeutic interventions. Even when acute ischemia is the most likely cause, a mix of focal ischemia and global ischemia may promote different and varying arrhythmia substrates not accounted for by ACLS. For example, the patient undergoing resuscitation from ischemia-induced VF initially undergoes regional ischemia (MI), then global ischemia during VF, global reperfusion during resuscitation, and hopefully subsequent reperfusion of the culprit lesion. These electrophysiologically distinct phases are not accounted for by current ACLS protocols, and the interplay of arrhythmia substrates and susceptibilities during each phase is unknown. For example, rearrest after ROSC is common (20%–70%), but its mechanisms are poorly understood.<sup>24–26</sup> Each phase of resuscitation may require different, tailored, antiarrhythmic therapy. Improved understanding of these phases and their complex arrhythmia mechanisms is paramount to the identification of novel antiarrhythmic targets for resuscitation.

Although in vivo porcine models have provided important results on resuscitation outcomes, no models have demonstrated the effects of TH on global and regional whole-heart arrhythmia mechanisms.<sup>27–30</sup> Furthermore, no in vivo model has determined these effects during the different phases of resuscitation. We aimed to better understand these potentially dynamic arrhythmia substrates in the common and clinically important scenario of sudden cardiac arrest related to AMI. We designed an innovative porcine translational model of ischemia-induced sudden cardiac arrest to determine dynamic arrhythmia substrates during focal infarct, global ischemia from VF, global reperfusion after ROSC with maintained focal ischemia, and then focal reperfusion. Herein, this model was used to determine the effect of TH on arrhythmia substrates and electrophysiologic resuscitation outcomes after sudden cardiac arrest from AMI.

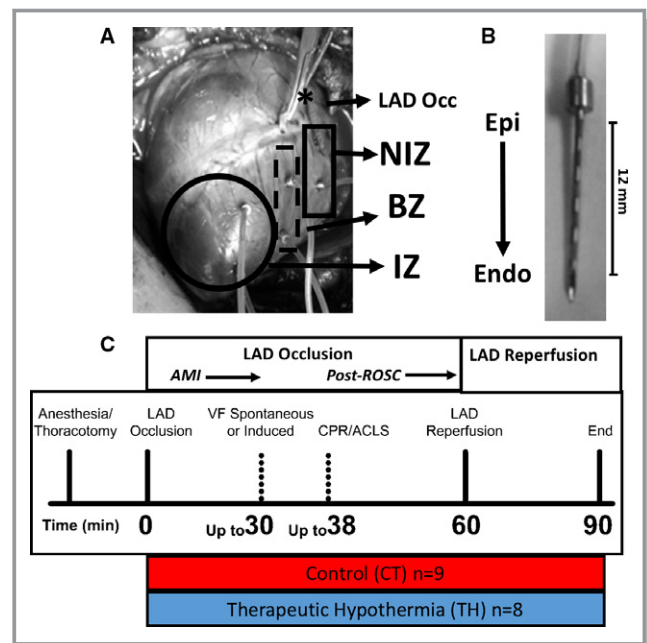
## Methods

All procedures in this protocol were approved by the Case Western Reserve University School of Medicine (Cleveland, OH) Institutional Animal Care and Use Committee. Healthy adult pigs (≈40 kg; n=9 for control [CT], n=8 for TH) were sedated with the following (in mg/kg): a combination of tiletamine hydrochloride and zolazepam hydrochloride (Telazol 1.5 IM), xylazine 1.5, and/or ketamine 1.5. They were intubated and ventilated, and anesthesia was maintained with isoflurane (0.5%–2%). The subjects were then fully instrumented for electrophysiologic assessments. After median sternotomy, multipolar needle plunge electrodes (Figure 1A) were placed on the basis of coronary anatomical features at the infarct zone (IZ), border zone, and non-IZ (NIZ) (Figure 1B). Position of the electrodes was verified by fluoroscopy, and depth was assessed after the procedure by direct visualization and measurement. Arterial cannulation for

blood pressure (BP) monitoring and central venous access was obtained. Pacing electrodes were placed in the right atrium. Baseline electrophysiologic measurements and hemodynamic measurements were performed. Subjects in the TH group were cooled using a cooling blanket, ice packs, and cold saline. Subjects underwent reversible occlusion of the proximal left anterior descending artery (LAD) between the first and second diagonal branch using a vascular tie to produce AMI (Figure 1B). To ensure homogeneous infarcts between subjects, the tie was always placed between 6 and 6.5 cm from the apex, accounting for anatomical variability. Experimental timeline is shown in Figure 1C. Ischemia continued for a total of 60 minutes (AMI phase of LAD occlusion). After 30 minutes of ischemia, if spontaneous VF did not occur, then VF was induced (see below). VF continued without compressions or pharmacologic intervention for 8 minutes to simulate out-of-hospital cardiac arrest (VF phase of LAD occlusion).<sup>27,30</sup> After 8 minutes, subjects were then resuscitated under ACLS with 3 modifications: (1) Because this was an open chest model, cardiopulmonary resuscitation (CPR) was delivered via cardiac massage. (2) Defibrillation was performed with internal open chest shocks in a step-wise manner (10, 30, and 50 J with a monophasic defibrillator) to assess for ease of defibrillation. (3) Epinephrine (EPI; 0.01 mg/kg of the 1:1000 IV) was used during CPR as standard therapy, but no antiarrhythmic medication was used because our model was designed to determine the effects of TH on arrhythmia substrates during resuscitation. Amiodarone and lidocaine were not used because the efficacy of these antiarrhythmic medications during targeted temperature management and how they might affect arrhythmia substrates and triggers are unknown. The goal of this study was to determine the effect of hypothermia itself on arrhythmia substrates during resuscitation. ACLS was continued until ROSC (post-ROSC phase of LAD occlusion). If VF recurred, ACLS was resumed. Pulseless electrical activity (PEA)<sup>31</sup> was defined as systolic BP <50% of baseline BP. If PEA occurred, then ACLS was started. Arrhythmias were recorded without intervention only if BP remained 50% of baseline. LAD occlusion was continued for a total of 60 minutes. The LAD tie was then opened, and the heart was reperfused for a total of 30 minutes (LAD reperfusion phase). If VF/pulseless ventricular tachycardia (VT) or PEA recurred, ACLS was resumed. Protocols ended after 30 minutes of reperfusion or 20 minutes of arrest without reperfusion. All events (eg, VF initiation, PEA, and defibrillation) were recorded during the entire protocol.

### Temperature Modulation

Exquisite care was taken to continuously monitor and CT temperature. Temperature was monitored by a rectal probe. To maintain baseline temperatures, a warming blanket and



**Figure 1.** Experimental design and timeline. A, Multipolar plunge electrode placement near the noninfarct zone (NIZ; solid rectangle), border zone (BZ; dashed rectangle), and infarct zone (IZ; circle). Left anterior descending artery (LAD) occlusion site (asterisk) is placed between the first and second diagonal branch of the LAD. B, Stainless steel transmural needle electrodes with 5 recording sites. C, Experimental timeline. LAD is occluded during the first 60 minutes and reperfused during the final 30 minutes. ACLS indicates advanced cardiac life support; AMI, acute myocardial infarction; CPR, cardiopulmonary resuscitation; CT, control; ENDO, endocardium; EPI, epinephrine; ROSC, return of spontaneous circulation; TH, therapeutic hypothermia; and VF, ventricular fibrillation.

heating lamps were used, as required. Care was taken to not promote hyperthermia. TH at 33°C was initiated before ischemia to determine the effect of temperature on ischemia itself. At 4°C, 0.9% saline (30 mg/kg), ice packs, and a cooling blanket were simultaneously used in each subject in the TH group to induce and maintain TH. TH was then maintained at 33°C with a cooling blanket and ice packs, as needed.

### VF Induction Using Programmed Electrical Stimulation

Programmed electrical stimulation was performed if spontaneous VF did not occur by 30 minutes of ischemia. The right atrium was paced at a cycle length of 600 ms from the intracardiac mean arterial pressure catheter, and premature stimuli (up to 7) were applied at successively decreasing coupling intervals until failure to capture or VF was induced. If VF did not occur, then epicardial premature stimuli were applied in a similar manner. If no VF was induced, rapid atrial

pacing was performed; finally, if VF did not occur, it was induced by a 9-V battery, which was placed on the epicardium.

## Resuscitation Outcome Analysis

All subjects (n=9 CT, n=8 TH) underwent hemodynamic and resuscitation outcome analysis. Temperature, heart rate (HR), arterial BP, end tidal CO<sub>2</sub>, and oxygen saturation were continuously assessed throughout the experimental protocol. Hemodynamic measurements were recorded at baseline, after induction of TH in that group, every 2 minutes during ischemia, every 2 minutes after ROSC, and during any event (eg, pharmacologic intervention and arrhythmia).

Resuscitation outcomes measured were as follows: VF reinitiation, defibrillation success, time in arrest, and survival. These outcomes were examined during (1) LAD occlusion and (2) LAD reperfusion (Figure 1C).

## Electrophysiologic Outcome Analysis

### Ventricular ectopy

Spontaneous ventricular ectopy was analyzed over 8 to 30 seconds during each recording to determine its frequency at baseline, during ischemia, post-ROSC, and reperfusion.

### Activation recovery interval, CV, and DOR

Multipolar plunge electrodes placed in the IZ, NIZ, and border zone were used to determine activation recovery intervals (ARIs) from the electrograms (EGs) to measure ventricular conduction and repolarization. Endocardial and epicardial monophasic action potential catheters were used to verify EG measurements. Unipolar EGs (filtered at 0.05–300 Hz) and bipolar EGs (filtered at 10–300 Hz) were recorded at baseline and every 2 minutes until protocol end.<sup>32</sup> To eliminate confounding effects of variations in heart on APD (action potential duration) and DOR, atrial pacing was performed for 10 s at 600-ms cycle length during recordings. ARIs were determined by previously validated techniques.<sup>33</sup> Briefly, the greatest negative first derivative of the EG QRS complex was determined as the activation time (AT). Repolarization time was determined by maximal positive upstroke the EG T wave. ARIs were assessed for each electrode for a complete transmural analysis. Global DOR was determined by the difference between the longest and shortest recorded ARI across the left ventricle. Left ventricular ATs were assessed by the difference between the earliest and latest AT of the multipolar plunge electrodes and related to their baseline measurements. To avoid confounding effects of cardiac alternans on APD and DOR, these measurements were made during recordings that did not exhibit cardiac alternans (>90%

of recordings used this analysis) whenever possible. If alternans was present, then the average ARI was used for 2 subsequent beats. All subjects underwent multipolar plunge electrode placement. However, in a subset of 7 subjects in the CT group and 5 subjects in the TH group, recordings were digitally recorded and a more detailed analysis was obtained. ARI, DOR, and CV were reported for this subset.

### Cardiac alternans

In the same subset of experiments as above, cardiac alternans was determined from continuously recorded unipolar EGs (filtered at 0.05–300 Hz) and ECG leads. Cardiac alternans magnitude was defined as beat-to-beat changes of >10% of maximal signal voltage during the T wave of the EG. To eliminate confounding effects of variations in HR on alternans, atrial pacing was performed for 10 beats at 600-ms cycle length at periodic intervals during the resuscitation protocol. Cardiac alternans was recorded as the maximal change in voltage between beats 1 and 2 compared to beat 1 and reported as a percent change of arbitrary units (A.U.%) Cardiac alternans was determined for the following time points: (1) baseline, (2) 16 to 18 minutes after AMI, (3) 6 minutes after VF termination, (4) 2 minutes after LAD reperfusion, and (5) immediately before any occurrence of VF/VT.

## Statistical Analysis

Continuous variables are reported as means±SEM. Continuous resuscitation outcomes were compared between temperature groups using the Student *t* test. Categorical variables were compared using the Fisher exact test. Ventricular ectopy was analyzed with ANOVA. Student *t* tests were used to compare 2 continuous variables at baseline and when required. Repeated-measures ANOVA was performed for regional and global DOR, CV, and ARI because these were continuous variables determined by the multipolar plunge electrodes. When comparing 2 means, effect size was calculated using pooled SD and then classified according to Cohen *d* classifications (small=0.2, medium=0.4, large=0.6).<sup>34</sup> All statistics were performed using SPSS 24.

## Results

### Hemodynamic Outcomes

Temperature was appropriately maintained for both the CT and TH groups (37.1±0.1°C versus 33.4±0.3°C; *P*<0.001; effect size=15.5, large). Baseline HR was slower in the TH group (64±2 versus 79±4 beats/min; *P*=0.01; effect size=1.4, large) and remained slower during the AMI phase only. No differences were seen in mean arterial pressure and

**Table 1.** Hemodynamic Variables During Resuscitation

Variable	Group	Baseline	LAD Occlusion (6 Min)	VF (8 Min)	CPR (2 Min)	LAD Reperfusion (8 Min)
Temperature, °C	CT	37.1±0.1	36.9±0.2	36.9±0.2	36.9±0.3	37±0.2
	TH	33.4±0.3	32.8±0.1	32.4±0.2	32.2±0.4	31.8±0.2
	<i>P</i> value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
HR, bpm	CT	79±4	80±4	NA	98±4	100±16
	TH	64±2	65±2	NA	113±13	90±7
	<i>P</i> value	0.01*	0.01*	...	0.16	0.4
MAP, mm Hg	CT	55±4	47±4	NA	46±5	37±13
	TH	49±2	45±2	NA	41±3	46±8
	<i>P</i> value	0.19	0.51	...	0.08	0.9
etCO <sub>2</sub> , mm Hg	CT	34±2	34±2	3.4±0.2	32±2	35±3
	TH	36±1.6	32±2	4.7±0.7	31±3	28±4
	<i>P</i> value	0.84	0.34	0.18	0.86	0.14
SO <sub>2</sub> , %	CT	97±1	97±1	NA	97±1	98±1
	TH	100±0.2	99±0.2	NA	99±0.7	99±0.8
	<i>P</i> value	0.035*	0.052	...	0.34	0.36

Data are given as mean±SEM. bpm indicates beats/min; CPR, cardiopulmonary resuscitation; CT, control; etCO<sub>2</sub>, end tidal CO<sub>2</sub>; HR, heart rate; LAD, left anterior descending artery; MAP, mean arterial pressure; NA, not applicable; SO<sub>2</sub>, oxygen saturation; TH, therapeutic hypothermia; and VF, ventricular fibrillation.

\*denotes statistical significance.

end tidal CO<sub>2</sub> (etCO<sub>2</sub>) between groups at baseline, and there were no differences in etCO<sub>2</sub>, mean arterial pressure, or oxygen saturation during AMI. During VF, etCO<sub>2</sub> decreased similarly in each group (from 34±2 to 3.4±0.2 mm Hg in CT versus 36±1.6 to 4.7±0.7 mm Hg in TH). Adequate CPR was similarly maintained between groups, with no significant differences in HR or etCO<sub>2</sub> (98±4 beats/min and 32±2 mm Hg for CT and 113±13 beats/min and 31±3 mm Hg for TH; *P*=0.16 and *P*=0.86, respectively). During reperfusion, no differences were seen in HR, mean arterial pressure, or etCO<sub>2</sub>. Hemodynamic variables are shown in Table 1 for baseline, ischemia, VF, CPR, and reperfusion phases. All significant differences were categorized as a large effect size.

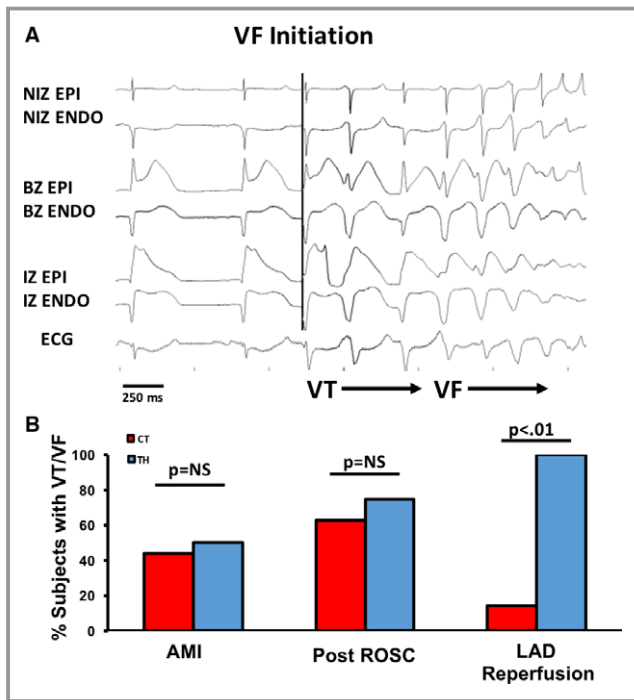
### Electrophysiologic Outcomes

Spontaneous VF/VT occurred with different frequency throughout the different phases of resuscitation, and this frequency was altered by TH. Figure 2A shows VF initiation in a CT experiment, where VF occurs after a ventricular ectopic beat (VEB) originating from the ischemic zone. Figure 2B is a summary of the number of experiments in which spontaneous VF/VT was observed during the different phases of resuscitation. During ischemia, the rate of initial spontaneous VF/VT was 44% (4/9) in CT versus 50% (4/8) in TH (*P*=1). The remainder of subjects in each group required initiation, as per

methods above. Of those subjects who were able to be resuscitated, no difference was again seen after ROSC during continued LAD occlusion (63% [5/8] versus 75% [6/8]; *P*=1). Of those subjects who survived to LAD reperfusion (7/9 in CT versus 8/8 in TH), 14% (1/7) of the CT group underwent spontaneous VF/VT while 100% (8/8) of the TH group had spontaneous VF/VT (*P*=0.001). Not surprisingly, the TH group required more defibrillation attempts during reperfusion as well (0.1±0.1 versus 2±0.5 shocks per experiment; *P*=0.004; effect size=1.7, large) and spent more time in arrest during reperfusion (1.6±0.5 versus 8±2.3 minutes; *P*=0.03; effect size=1.7, large). However, there was no difference seen in survival (77% in CT versus 88% in TH; *P*=1), number of PEA arrests, or amount of EPI used during either LAD occlusion or LAD reperfusion (Table 2).

### Ventricular ectopy

The incidence of ventricular ectopy changed during the phases of resuscitation, and TH altered the incidence of VEBs. Figure 3A shows a representative spontaneous VT after a series of VEBs. Figure 3B shows summary data between CT and TH during the different phases of resuscitation. At baseline, there were few VEBs observed in either group. During ischemia, ectopy increased in CT but not TH (33±8 VEB/min in CT versus 4±2 VEB/min in TH; *P*=0.009; effect size=1.5, large). After ROSC during LAD occlusion, ectopy remained elevated at 27±4 VEB/min in the



**Figure 2.** Spontaneous ventricular tachycardia/fibrillation (VT/VF) initiation. A, Electrogram tracings are shown in the noninfarct zone (NIZ), border zone (BZ), and infarct zone (IZ) as well as transmurally (epicardial (EPI) and endocardial (ENDO)). ECG tracing is shown. A ventricular ectopic beat (black line) originates in the IZ ENDO, before VT initiation, which quickly degenerates to VF. B, Summary data for the initiation of spontaneous VT/VF during each phase of resuscitation. AMI indicates acute myocardial infarction; LAD, left anterior descending artery; NS, p value not significant; for AMI  $p=1$ , for Post ROSC  $p=1$  and ROSC, return of spontaneous circulation.

CT group versus  $5 \pm 3$  VEB/min in the TH group ( $P=0.003$ ; effect size=2.0, large). During LAD reperfusion, ectopy for the CT group remained elevated, but ectopy for TH increased such that the difference between groups was no longer significant. These data suggest that ectopy changes during each phase of resuscitation and that TH reduces ectopy during regional and global ischemia, but not reperfusion.

### Cardiac alternans

Cardiac alternans is a beat-to-beat alternation in the ECG T-wave and cellular repolarization that is a well-established cause of arrhythmias.<sup>35,36</sup> Figure 4A is an example of cardiac alternans in EG traces during LAD reperfusion. In this example, significant alternans is followed by spontaneous VF in TH, whereas no alternans or VF is seen in CT. Summary data for alternans magnitude are shown in Figure 4B. During AMI, cardiac alternans increases from baseline to a similar magnitude with TH or CT. In post-ROSC, alternans remains increased, again similarly at both temperatures. However, with subsequent LAD reperfusion, alternans declines during

**Table 2.** Resuscitation Outcomes During the LAD Occlusion Phase and the LAD Reperfusion Phase

Outcomes	CT Group	TH Group	P Value
LAD occlusion			
Time before VF, min	32±3	27±5	0.39
PEA arrests	0.625±0.25	0.875±0.35	0.35
Total No. of shocks (per experiment)	4.4±1	4.0±0.7	0.72
Doses of EPI (per experiment)	1.8±0.5	2.6±0.7	0.34
Time in arrest, min/experiment	14.4±1	18.9±3	0.15
Survival to reperfusion, %	88	100	1
LAD reperfusion			
PEA arrests	1±0.22	1.125±0.25	0.62
Total No. of shocks (per experiment)	0.1±0.1	2.0±0.5	0.007*
Doses of EPI (per experiment)	1.4±0.5	1.5±0.2	0.34
Time in arrest, min/experiment	1.6±0.5	8±2.4	0.03*
Survival to end, %	77	88	1

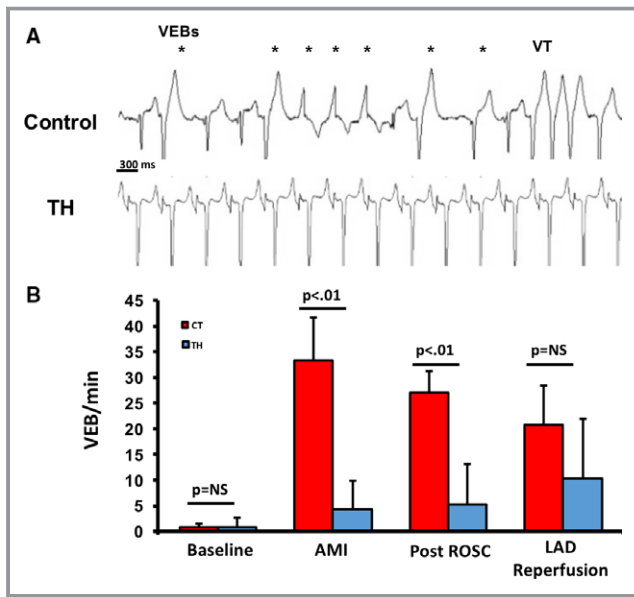
Data are given as mean±SEM unless otherwise indicated. CT indicates control; EPI, epinephrine; LAD, left anterior descending artery; PEA, pulseless electrical activity; TH, therapeutic hypothermia; and VF, ventricular fibrillation.

\*denotes statistical significance.

CT. In sharp contrast, alternans dramatically increases on LAD reperfusion with TH (TH=45+8 A.U.%, CT=16+7 A.U.%;  $P=0.022$ ; effect size=1.7, large). More important, the magnitude of alternans during resuscitation closely parallels the occurrence of VF (Figure 2B), and occurrences of spontaneous VT/VF during all phases of resuscitation were frequently preceded by alternans (>75% occurrences). Taken together, these data suggest that cardiac alternans is affected by temperature during reperfusion, but not focal or global ischemia; regardless of temperature, it remains an important mechanism of rearrest in this model.

### Regional ARIs and global DOR and AT

Transmural EGs in the IZ, border zone, and NIZ were recorded during the phases of resuscitation to determine regional ARIs, global DOR, and global left ventricular AT. Figure 5A, top panels, shows representative local EGs in the IZ at baseline and during 24 minutes of LAD occlusion. At baseline in CT, there is a small difference in the epicardial and endocardial ARIs. The global DOR equals the difference between the longest and shortest ARI of any region. TH increased the ARI at baseline. During LAD occlusion, the ARIs shorten and ARI duration is no longer different between temperatures. During LAD occlusion, ARI duration shortens significantly in the IZ,

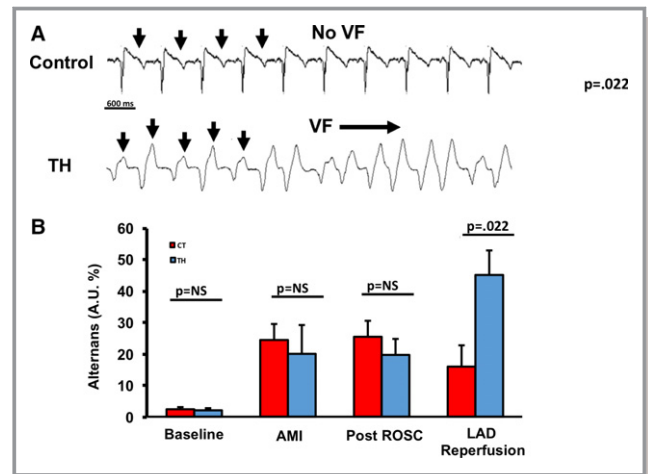


**Figure 3.** Ventricular ectopy during resuscitation. A, Representative in vivo electrogram tracings from control (CT) and therapeutic hypothermia (TH) at 6 minutes after return of spontaneous circulation (ROSC). In CT, there are frequent ventricular ectopic beats (VEBs; asterisks). B, Summary data for ventricular ectopy. VEBs/min is shown during the phases of resuscitation. VEB/min is significantly increased from baseline during each phase of resuscitation (ANOVA  $P<0.05$ ). AMI indicates acute myocardial infarction; LAD, left anterior descending artery; NS, not significant,  $p=.29$ ; and VT, ventricular tachycardia.

with the ARIs in the NIZ remaining relative constant during both CT and TH. However, because the ARIs were longer in the NIZ at baseline, the global DOR during ischemia is relatively larger in TH. Figure 5B shows summary data for global DOR. Global DOR is similar at baseline between the 2 temperatures. During ischemia, global ventricular DOR increases in both groups, with the increase in TH being greater ( $P<0.05$  by repeated measures), and then DOR returns to near baseline levels during the post-ROSC and LAD reperfusion phases of resuscitation. Changes in transmural DOR were not different between CT and TH. Global activation was longer at baseline in the TH group ( $18\pm 1$  versus  $37\pm 5$  ms;  $P=0.002$ ; effect size=2.1, large). Both groups slowed during ischemia, and there were no differences between groups in conduction during ischemia or reperfusion.

## Discussion

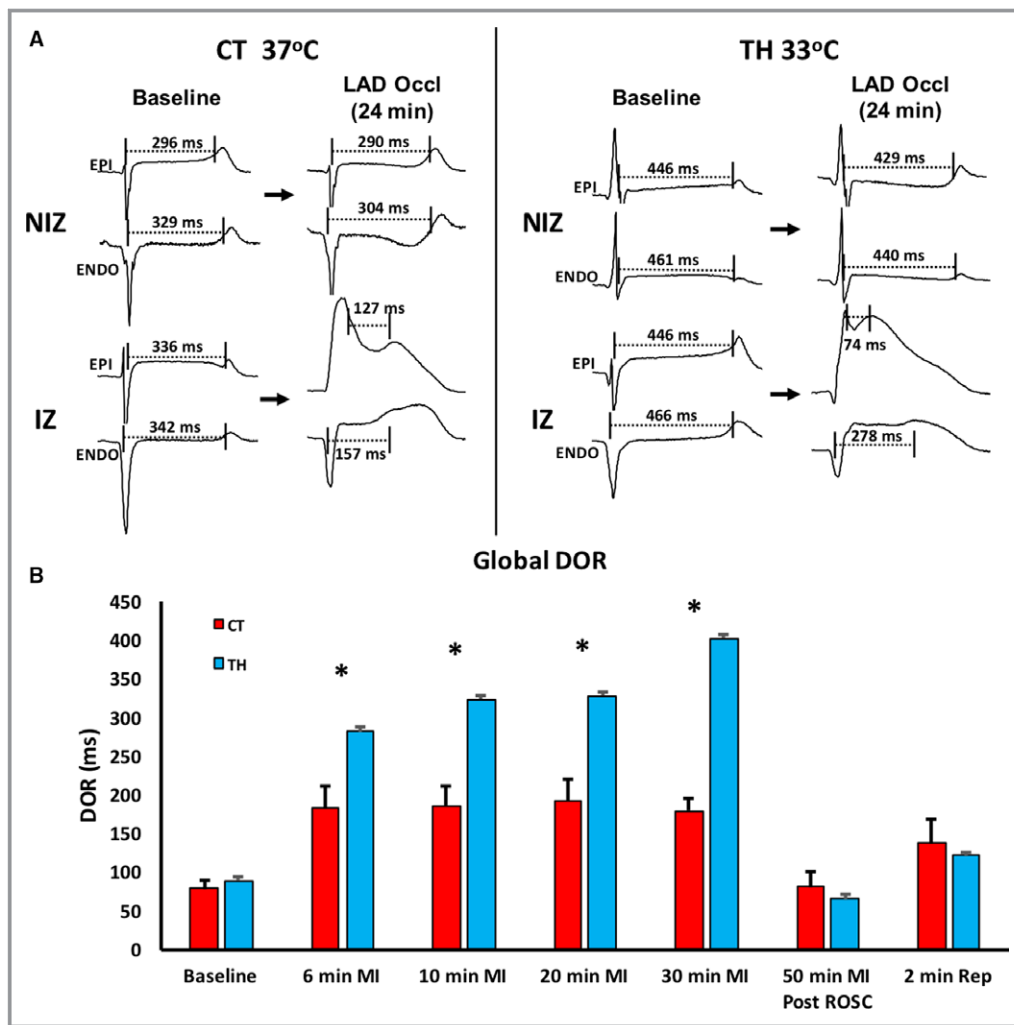
Our data suggest that global and regional arrhythmia substrates change throughout different phases of resuscitation, and that temperature alters substrates differently throughout the phases of resuscitation. At normal temperatures, LAD occlusion increases VEBs, global DOR, and cardiac alternans. TH decreases VEBs, yet increases global DOR, and



**Figure 4.** Cardiac alternans during resuscitation. A, Representative traces of alternans (arrows) and ventricular fibrillation (VF) with therapeutic hypothermia (TH) (bottom panel), and the absence of alternans and VF without TH (top panel) during left anterior descending artery (LAD) reperfusion. B, Summary data of cardiac alternans magnitude from electrogram recordings (as a percentage of full scale) in pigs achieving return of spontaneous circulation (ROSC) during each phase of resuscitation. Alternans is markedly enhanced by TH (blue bars) after LAD reperfusion. AMI indicates acute myocardial infarction; AU arbitrary units; CT, control; and NS, not significant: baseline  $p=.77$ , AMI  $p=.66$ , Post ROSC  $p=.54$ .

similarly promotes cardiac alternans. During this phase, temperature does not alter the end result of increased incidence of VT/VF. After ROSC from VF arrest, but still during LAD occlusion, there remains an increase in VEBs and cardiac alternans in CT, whereas global DOR returns to baseline. TH reduces VEBs, the increase in global DOR is no longer seen, and cardiac alternans is similarly increased. Again, the incidence of VT/VF is similarly increased in CT and TH. During LAD reperfusion, the incidence of VEBs and alternans in the CT group is greater than baseline, but global DOR returns to near baseline levels. TH markedly increases the magnitude of cardiac alternans. This is associated with a marked increase in VF/VT during reperfusion in TH compared with CT. In all phases of the protocol, the arrhythmia substrate most closely correlated with spontaneous VF/VT was cardiac alternans.

There has been little improvement in ACLS pharmacotherapy during the past decades and established treatment paradigms are being questioned. In part, this may be because it is difficult to identify effective therapies in a heterogeneous population with rapidly changing hemodynamic and electrophysiologic responses to resuscitation. Recent studies suggest EPI, a mainstay in ACLS since 1974, may actually worsen outcomes, and suggest the  $\beta$ -adrenergic effects worsen post-resuscitation myocardial dysfunction.<sup>7,8,37</sup> However, these same studies show that EPI improves ROSC, likely by its positive



**Figure 5.** Regional activation recovery interval and global dispersion of repolarization (DOR) during resuscitation. A, Electrogram (EG) recordings at baseline and 24 minutes of left anterior descending artery (LAD) occlusion in the infarct zone (IZ). At baseline, therapeutic hypothermia (TH) lengthens the activation recovery interval (ARI). During ischemia, there is similar shortening of ARIs in both groups, and significant conduction slowing during ischemia (delayed transmural conduction and widened EG) is seen in both groups. B, Global DOR is shown. TH increases DOR during ischemia but returns to baseline during reperfusion. CT indicates control; ENDO endocardium; EPI, epicardium; MI, myocardial infarction; NIZ, non-IZ; and ROSC, return of spontaneous circulation. \* $P < 0.05$ .

hemodynamic effects. EPI may not be beneficial in subsets of VF/VT arrests secondary to AMI with electrical instability, when antiarrhythmic therapy or defibrillation would be preferred, but is most likely beneficial in hemodynamic instability unrelated to arrhythmia. Clinical trials investigating pharmacologic therapy, including EPI, are currently underway. The interplay between hemodynamic and electrophysiologic improvement, and between long-term myocardial and neurological outcome, is extremely complex; a more tailored approach to the different phases of resuscitation may be required.

This study, for the first time, describes the dynamic electrophysiologic substrates during different phases of resuscitation, and the effect of temperature on these substrates. Improved understanding of the underlying substrates that

determine arrhythmia susceptibility during different, sometimes complex, phases of resuscitation is of extreme importance. Relating the arrhythmia substrates to observed arrhythmia susceptibility (and ultimately to resuscitation outcomes) during the complex timeline of AMI, VF, post-ROSC, and reperfusion offers an opportunity to develop and improve further pharmacologic interventions during ACLS. For example, suppression of ectopy by TH in this model did not decrease arrhythmia vulnerability, suggesting strategies to attenuate ectopy (an action of some Van Williams type 1 and 2 antiarrhythmics), certainly during TH and potentially during resuscitation in general, may not be efficacious. In addition, strategies to attenuate DOR (a property of some type 3 antiarrhythmics) would appear to be most efficacious only



during acute ischemia, where it was associated with VT/VF, but not after ROSC, where DOR did not predict arrhythmia susceptibility. The therapeutic strategy that may be most efficacious would be suppression of cardiac alternans, which could potentially be suppressed pharmacologically.

We have previously shown TH attenuates arrhythmia substrates in isolated canine ventricular myocardium during ischemia by attenuating ischemia-induced conduction, slowing and improving DOR. This effect, likely through preservation of gap junction coupling and improved sodium channel kinetics, demonstrates the beneficial effect of TH on a cellular level in the myocardium in a model where cell-to-cell impulse propagation primarily determines CV. Because we did not observe maintained conduction in the whole porcine heart, with an intact and transmural (unlike dogs and humans) cardiac conduction system, this suggests that these effects may not be sufficient to preserve conduction in the more complex whole heart during resuscitation. Whether hypothermia may be antiarrhythmic in the intact human heart during ischemia remains to be determined. Most patients who undergo TH are cooled in the post-ROSC phase and reach target temperatures after reperfusion. Furthermore, the complex interplay between cellular and focal arrhythmia substrates (cellular ion currents and gap junctions) with regional (NIZ, border zone, and IZ conduction and DOR) and global (His-Purkinje conduction, SA and AV nodal stimulation, and hemodynamic response) differences suggests that improved understanding of this complexity is required to develop a multifaceted approach to antiarrhythmic therapy during resuscitation.

## Limitations

Because this model is primarily designed to determine mechanisms underlying VF formation during resuscitation, there are some considerations that, by design, deviate from normal resuscitation. First, TH was performed before ischemia. This was done to identify changes in TH on arrhythmia substrates at baseline conditions to compare these conditions during resuscitation, and avoid dynamic substrates when introducing TH after ROSC. This also may contribute to the high incidence of reperfusion arrhythmias observed during TH, because they occurred during established moderate hypothermia, which is not typical during initial ROSC or post-ROSC coronary reperfusion. Second, an open chest model was required to perform our electrophysiologic assessments. CPR and defibrillation are more effective than standard closed chest CPR and defibrillation used clinically, and our survival in this large infarct model is higher than would be expected in standard resuscitation or closed chest resuscitation models.<sup>38</sup> Third, no antiarrhythmic medication was used to directly study the electrophysiologic effects of TH on resuscitation. This was by design, to identify the effect of

temperature alone on arrhythmia substrates and not the effect of temperature on pharmacologic interventions. EPI was necessary because preliminary studies in our model required pressor support to maintain adequate perfusion during the protocol, but EPI dosing did not differ between groups. Fourth, our experimental design had both spontaneous and induced VF after 30 minutes. Although the time in arrest was similar between groups, subjects who underwent spontaneous VF might have different arrhythmia susceptibility during resuscitation than subjects who required VF to be induced. However, the incidence of spontaneous VT/VF was similar during ischemia in both groups, as was total duration of ischemia, allowing for comparisons.

Fifth, pigs have a transmural Purkinje system, not present in canines or humans, and the effect of temperature on transmural conduction may differ between species. Differences between the porcine and canine models make an ideal translational model of arrhythmias in human resuscitation difficult.<sup>39</sup> In canines, transmural and regional ion currents are similar to humans, as is the conduction system; pigs have more anatomically similar coronary vascular features and do not have the extensive collaterals, as occur in canines, making a transmural infarct more similar to humans. Therefore, the ideal model is some theoretical combination of the 2 models. Finally, the number of subjects in our investigation is small, which limits the power of the study.

## Conclusions

TH alters global and regional arrhythmia substrates in a porcine translational model of resuscitation from ischemia cardiac arrest during the complex phases of resuscitation. Cardiac alternans typically preceded VT/VF during all phases of resuscitation, and TH worsens cardiac alternans, which was associated with an increase in spontaneous VT/VF during reperfusion. Further studies are required to identify which arrhythmia substrates are predominantly responsible for ventricular arrhythmias during each phase of resuscitation, and which pharmacologic interventions can improve resuscitation from cardiac arrest.

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