Conclusions—In human ventricular fibrillation, we found an increase in complexity of electric activation patterns during global myocardial ischemia, and this was not reversed during reflow despite an increase in activation rate.  

Key Words: ventricular fibrillation ■ arrhythmia ■ reentry ■ heart ■ myocardial ischemia

Global myocardial ischemia is an inevitable consequence of naturally occurring ventricular fibrillation (VF) and has been shown to profoundly alter the dynamics of activation wavefronts.1–8 An understanding of the mechanisms that operate during the first few minutes of VF is important in the clinical management of cardiac arrest victims.9–12 Extensive experimental work, focusing mainly on models of nonischemic VF, has identified several different mechanisms of VF, including mother rotors, multiple wavelets, compound rotors, and focal activity,13,14 as well as focal activity after several minutes of ischemia.15 Although most of these studies provide evidence for either one or another mechanism at any one time, our previous observations in humans suggest that multiple mechanisms are present during the early stages of VF in the human heart.16

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Studies of ischemic VF in both canine and porcine hearts have shown that during the first few minutes of global ischemia, VF dynamics evolve through a series of stages with changing activation frequency, increasing organization, and a reduction in the number of waves.5,8 On the other hand, studies in isolated human hearts from patients with cardiomyopathy,17 isolated rabbit hearts,4 and computational models of cardiac tissue with simulated regional ischemia18 have reported an increase in the number of activation waves during ischemic VF.

Ionic and molecular mechanisms may be of critical importance in determining the nature and time course of electrophysiological changes during a specific episode of ischemic VF.19 In view of the many well-known electrophysiological differences between humans and experimental models,20 we hypothesized that important differences between the nature and characteristics of VF in experimental models and the intact human heart were likely to exist as a result of the effects of ischemia. In this report, we present the results of multielectrode ventricular epicardial mapping in patients...
undergoing routine cardiac surgery with 3.5 minutes of VF with coronary perfusion, occlusion (global myocardial ischemia), and reflow. Our results provide clear evidence for the coexistence of rotors and multiple wavelets during early ischemic VF in humans and show striking differences in wave dynamics compared with experimental models described in the literature. We propose a hypothesis whereby molecular mechanisms with opposite effects compete during VF in the globally ischemic human heart.

**Methods**

**Patients**

Ten patients 30 to 79 years of age (mean ± SD 64 ± 16 years; 8 men) were studied during routine cardiac surgery. Written informed consent was obtained from all patients, and the study was approved by the local hospital ethics committee (REC 01/0130). Five patients (all men) had graft procedures for coronary artery disease, 4 (3 men, 1 woman) had aortic valve replacement, and 1 patient (a woman) had a mitral valve replacement. None of the patients undergoing surgery for valve disease had any hemodynamically significant coronary artery disease (>50% stenosis in any 1 major vessel). Individual patient details are given in the Table.

**Epicardial Mapping Protocol**

As in our previous studies, an epicardial sock with 256 electrodes (inter-electrode spacing approximately 10 mm) was fitted over the left and right ventricles immediately after cannulation and before cardiopulmonary bypass. Unipolar electrograms were sampled continuously at 1 kHz from each electrode with a UnEmap system (Uniservices Ltd, Auckland, New Zealand) with the reference channel connected to the chest retractor. After the institution of cardiopulmonary bypass to replace the function of heart and lungs, VF was induced with 50-Hz burst pacing. After 30 seconds of control VF with myocardial perfusion, the aorta was cross-clamped at a position between the coronary sinus and cardiopulmonary bypass cannula, which prevented arterial blood from the bypass machine from perfusing the coronary arteries. This produced global myocardial ischemia, and after a further 150 seconds, the cross-clamp was removed to enable coronary reflow. Electrogram recording was continued for a further 30 seconds after the removal of the cross-clamp and before removal of the electrode sock and ventricular defibrillation.

**Signal Processing**

The recorded electrograms were preprocessed as described previously to remove transients that resulted from the burst stimulus, as well as other low-frequency fluctuations such as respiratory artifact. Signals from electrodes with poor contact or low-amplitude signals that were dominated by noise were identified and rejected by use of a dominant frequency (DF; see below) of <2 Hz or >20 Hz as a rejection criterion. On average, 234 of the 256 electrodes were available for analysis (range 213–249).

**DF Analysis**

DFs are indices of local activation rate and were computed for each electrode signal by use of a fast Fourier transform operating on a rectangular window with a width of 4096 ms and advancing through the recording in 10-ms time increments. DF was calculated as the frequency of the largest-amplitude peak in the power spectrum and had a frequency resolution of approximately 0.24 Hz. Figure 1 shows example electrograms, together with their frequency spectra and DFs. Additional example electrograms and their spectra are provided in the online-only Data Supplement, as a figure showing that the electrogram amplitude was not markedly affected by global myocardial ischemia.

**Phase Singularities and Wavefronts**

The electrogram voltage at each electrode was detrended, interpolated over the epicardial surface, and transformed into phase with the Hilbert transform as described previously. Trajectories in phase space were not markedly affected by global myocardial ischemia.
ischemia (see online-only Data Supplement for details). Phase singularities (PS) were identified by a method based on topological charge, and wavefronts were identified as lines of zero phase. Wavefront lengths were estimated by adding together the distances between pixels located on the wavefronts. The analysis sometimes produced very short-lived pairs of closely spaced PS that were considered to be artifacts of the interpolation. PS of opposite chirality separated by a distance of ≤5 pixels were therefore removed from the analysis. In Figure 2, we show 2 example snapshots of interpolated electrogram voltage, together with the corresponding phase information, PS, and wavefront.

Statistical Analysis
Each recording was split into 3 epochs to capture VF characteristics during coronary perfusion (first 30 seconds), ischemia (30–180 seconds), and reflow (180–210 seconds). For each recording, an average value of DF was calculated from the values obtained for all electrodes during each 1-second interval. In addition, an average value of the number of PS (nPS) was calculated over each of the same 1-second intervals. A mixed-effects linear model was then fitted to the time series data for each epoch. The model variable of interest (ie, DF or nPS) was set as the response variable, with time as a covariate. The fixed effects for the model included an overall intercept, a time effect (ie, slope), an effect for pathology (coronary artery disease versus valve disease), and an interaction effect for pathology×time. The random effects for the model were a random intercept and intercept for each patient. After a model was fitted, the results were examined, the fixed effects that were not significant (P>0.05) were removed, and then the fitting procedure was repeated. When a model in which all the remaining fixed effects were significant (P<0.05) was obtained, the significance of the random effects was investigated. For each random effect, a test model was constructed in which the random effect was removed. If an ANOVA indicated that the inclusion of a random effect significantly improved the model fit (P<0.05), then the random effect was retained. All statistical analysis was performed with R (version 2.8.0). For all the models fitted in the present report, the fixed effects of pathology and pathology×time were not significant and are not included in the results. Parameters are reported as mean±SE.

Results
The findings in the present study showed that VF in the perfused human heart before global myocardial ischemia appeared to be sustained by multiple mechanisms, and this is consistent with the idea that both relatively long-lasting rotors and increasing numbers of wavelets coexist during early global ischemia in humans. During early ischemia, the overall effect was persistent coexistence of rotors and wavelets with a progressive increase in the number of wavelets. Figure 3A shows a sequence of snapshots showing a single cycle of reentry. The other panels of Figure 3 are all taken from the same recording and illustrate the subsequent 4 cycles of this rotor, which had a period of approximately 140 ms (Figure 3B), and more mobile activity subsequently (Figure 3C).

Change in DF
The average activation rate over the ventricular epicardium measured by mean DF showed a consistent pattern of change in all patients (Figure 4). Consistent with observations from isolated myopathic human hearts,7 there was an increase of DF during perfused VF, a fall during global myocardial ischemia, and then a dramatic increase during reflow, with an overshoot to greater than control values. A detailed analysis of the distribution of DF domains across the epicardial surface is given below.

Change in nPS
Epicardial reentry was present in all recordings, with a varying number of PS. In contrast to the reduction in DF associated with global myocardial ischemia and the rapid increase of DF with reflow, in Figure 5 we show that the overall trend in nPS was a gradual increase throughout VF.

Comparison of DF and nPS
Figure 6 shows the linear statistical models that were fitted to the observed DF and nPS data. Each model was a significant fit (P<0.05), and the models show 3 different associations between the time courses of DF and nPS shown in Figures 4 and 5.

During perfused VF, both DF and nPS increased. DF increased from a fitted mean intercept of 5.3 Hz at a fitted linear rate of 0.030±0.005 Hz s⁻¹ (P<0.001). At the same time, fitted mean nPS increased from 5.5 to 7.7 at a fitted rate of 0.11±0.03 PS s⁻¹ (P<0.01).

In global myocardial ischemia, DF decreased, whereas nPS continued to increase. The fitted mean DF decreased from 6.4 Hz to 4.7 Hz at a rate of −0.011±0.002 Hz s⁻¹ (P<0.001), whereas nPS increased from a fitted mean of 7.7 to 9.7 at a rate of 0.013±0.005 PS s⁻¹ (P<0.01).
With reflow, there was a rapid increase in DF that overshot the control value during perfusion, whereas nPS remained constant. Mean DF increased at a fitted linear rate of $0.078 \pm 0.008$ Hz s$^{-1}$ ($P<0.001$) to a fitted mean value of 7.5 Hz. In contrast, there was no significant linear change in nPS, with a fitted mean value of 10.3 throughout reflow, which overshot control values. There was no significant effect of pathology on either the initial value or the rate of change of DF and nPS. These 3 different types of associations indicate that the electrophysiological changes during global myocardial ischemia and reflow influenced the mechanism of human VF, as discussed below.

Number and Size of Epicardial Wavefronts

The time course of the number of epicardial wavefronts was similar to the time course of nPS (Figure 7A). In Figure 7, we show the distribution of wavefront lengths during perfused VF, the last 30 seconds of ischemic VF, and reflow. The shape of the distribution shows that epicardial activity during VF in the human heart was mainly composed of short wavefronts. The number of wavefronts increased from perfused VF to the end of ischemic VF, and again from ischemic VF to reflow VF, which is consistent with the gradual increase in nPS shown in Figures 5 and 6. There was a small change in the proportion of wavefronts less than 50 mm in

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**Figure 3.** Snapshots from patient H062 showing wavefronts (red lines) and phase singularities (PS; blue, clockwise; yellow, anticlockwise). **A,** Top row of snapshots highlight a wavefront (circled in white) at 4 stages during a single anticlockwise rotation of a rotor located on the anterior wall with a period of $\approx 140$ ms, at approximately 2 o’clock (after 72.15 seconds of ischemia, ie, 102.15 seconds after VF initiation), 10 o’clock (72.2 seconds), 5 o’clock (72.25 seconds), and 12 o’clock (72.3 seconds). **B,** Snapshots after 72.30 seconds of ischemia and at 140-ms intervals show 4 cycles of this rotor, indicated by white circles. **C,** Snapshots after 73.49 seconds of ischemia and at 140-ms intervals subsequently show multiple mobile PS. LV indicates left ventricle; RV, right ventricle.

**Figure 4.** Time course of mean dominant frequency (DF) during perfused ventricular fibrillation, global ischemia, and reflow. Central line indicates mean DF; dashed lines, standard deviations.

**Figure 5.** Data from all patients showing time course of number of phase singularities during perfused ventricular fibrillation, global ischemia, and reflow. Central line indicates mean number of phase singularities; dashed lines, standard deviations.
length, from 59% during perfused VF to 62% in the last 30 seconds of ischemic VF and to 63% with reflow.

**DF Domains**

Previous work in rabbit hearts has suggested that during global myocardial ischemia, there is a transition from type I VF, sustained by multiple wavelets, to type II VF, sustained by a mother rotor.24 One of the features associated with mother rotor VF is a domain structure in which regions of myocardium (domains) are activated at distinct frequencies and the size and boundaries of individual domains remain stable.25 To assess whether there was a trend toward a domain structure, we calculated the number and size of contiguous regions with the same DF. We found an overall increase in the number of domains coupled with the decrease in the size of the largest domains, consistent with a trend toward more fragmented activity, with more PS and more, shorter wavefronts. (Details of this calculation and these results are provided in the online-only Data Supplement.)

**Discussion**

Our main findings during multielectrode epicardial mapping in the in situ human heart were as follows: (1) VF during coronary artery perfusion showed a progressive increase in activation frequency, together with an increase in the number of wavelets and reentrant sources. (2) During global myocardial ischemia, the activation rate decreased progressively, whereas overall, the number of wavelets and reentrant sources increased (in contrast to most reported experimental studies in animal models). (3) During reflow, there was an immediate and dramatic reversal of activation frequency, with an overshoot to faster activation frequencies compared with control values, whereas again in contrast, the number of wavelets and reentrant sources remained constant at a level greater than control.

**Comparison With Other Studies**

**Activation Frequency**

The progressive increase in DF we observed after the initiation of VF while perfusion was maintained was similar to our previous study of VF dynamics in the nonischemic heart in a similar patient population16 and probably represents adaptation of action potential duration (APD) through a combination of restitution and memory effects. Several studies using various models and different durations of ischemia have demonstrated a reduction in DF as ischemia progresses, for example, in isolated pig hearts,2,5,10 isolated rabbit hearts,1,4,6,26 and isolated sheep hearts.3 Similar results were obtained in isolated explanted human hearts from patients with end-stage cardiomyopathy.7 Caldwell et al6 reported a differential effect with a greater reduction in DF in the left ventricle than the right ventricle, attributed to a differential distribution of the inward rectifier potassium current, $I_{K1}$. However, we were unable to detect any left ventricular/right ventricular differences, as was the case in the study by Masse and colleagues.7 We searched carefully for evidence of epicardial DF domains and were unable to detect persistent domains consistent with a mother rotor and fibrillatory conduction.

**Wavebreak and Rotors**

Although the effect of global myocardial ischemia on activation frequency in the present study was similar to that reported in experimental studies in animal models, the effect of global myocardial ischemia on wavebreak and rotor behavior was strikingly different. In contrast to our findings of an overall trend of increasing wavebreak during ischemia, several experimental studies report the opposite effect. For example, 2 studies in which relatively short periods of ischemia were investigated reported the following: (1) A
reduction in PS and rotors by approximately 50% and 75%, respectively, after 5 minutes of ischemia in isolated sheep hearts; and (2) wavefront density reduced from 1.23 to 0.93 wavefronts/cm² after 2 minutes and down to 0.55 wavefronts/cm² after 5 minutes of ischemia in isolated rabbit hearts. A study in isolated pig hearts showed an approximate 50% reduction in wavebreaks after 15 minutes of ischemia. A study of long-duration VF in canines showed a steady decrease in the number of waves after 100 seconds of ischemia. In isolated pig hearts, Huiziar et al identified 3 phases of the electrophysiological development of ischemia and observed an initial reduction in wavebreaks followed by an increase in wavebreaks. Liu et al observed an increase in wavebreaks after 10 minutes of ischemia. In isolated myopathic human hearts, Masse et al found a small increase in the nPS after 3 minutes of ischemia, although these changes were not statistically significant.

Mechanisms
Wavebreak and wavelet formation are a fundamental mechanism in the maintenance of VF. Whereas most studies in animal models have shown a decrease in wavebreak during ischemic VF, we found the opposite effect in the intact and in situ human heart. In contrast to most animal models, we did not observe increased organization as ischemia progressed but rather an increase in wavebreak with a greater number of PS and an increased proportion of shorter wavefronts. However, there was some interpatient variability, and in 2 patients, there was a transient period with a lower number of wavefronts and PS (online-only Data Supplement).

Several mechanisms have been proposed to explain increased organization during early global myocardial ischemia, including the restitution properties of APD and conduction velocity, and expansion of the spiral wave core, and stability of the scroll wave filament. We hypothesize that several of these mechanisms may operate during the same episode of VF, with the overall effect depending on the type of VF and their modulation as ischemia progresses. In this context, one factor thought to play a role, but which has received relatively little attention, is the phenomenon of postpolarization refractoriness (PRR).

As APD shortens during myocardial ischemia, the effective refractory period becomes prolonged and often outlasts APD by many tens of milliseconds. We have previously documented the time course and extent of PRR during paced rhythm in a human model of global ischemia identical to the present studies. In that earlier report, we found that PRR in humans developed rapidly and progressively and was substantial after just 1 minute of ischemia. For example, before ischemia, APD and effective refractory period were 256 and 244 ms, respectively, whereas after 1 minute of ischemia, these values were 231 and 262 ms, respectively, with refractoriness already outlasting APD by 31 ms. After 3 minutes of global myocardial ischemia, APD and effective refractory period were 189 and 297 ms, respectively, with refractoriness now outlasting APD by 108 ms. Experimental studies using microelectrode recordings in a Langendorff pig heart model showed a widening of the diastolic interval during regional ischemia, which indicates an important role for PRR in the mechanism of ischemic VF.

A classic feature of myocardial ischemia are the marked regional differences in elevated extracellular K⁺ concentration ([K⁺]o) and refractoriness, known to facilitate wavebreak. On the basis of what we know from these studies, we propose that the early development of regional differences in PRR during VF with global ischemia could compete with other influences to determine whether there are increases or decreases in wavebreak and complexity of activation patterns. Consequently, if PRR is relatively slow to develop during ischemia and the effective refractory period remains dependent on APD, then the effects of flattening of APD restitution due to ischemia may dominate, which tends to prevent wavebreak and promote stable rotors. If, however, PRR develops early, as was shown in our previous work in humans, then regions of prolonged refractoriness may dominate and have the opposite effect, promoting wavebreak. This effect might be enhanced in patients with preexisting regional ischemia associated with coronary artery disease, but the numbers of patients in the present study were too small for us to test this hypothesis.

Although much is known about the effects of ischemia on ionic currents and molecular mechanisms, their mechanistic role in the dynamics of VF has not been fully elucidated. A combination of the effects of modulating individual ion currents on electrophysiological properties of wavefront dynamics highlights a number of potential opposing and synergistic actions on wave behavior. A recent study of the effects of hyperkalemia showed the resulting effects of I\textsubscript{K1} on organization to be opposed and overwhelmed by a concomitant prolongation of refractoriness. Our finding of increasing wavebreak during ischemia could be explained by prolonged refractoriness overwhelming the organization as described above.

Effect of Reflow on VF
Although the cellular electrophysiological changes that occur on coronary reflow are well known, information on the way that reflow influences VF is limited. We observed a rapid rebound of DF from lowered values to faster than initial control values within a few seconds of reflow. This finding is consistent with some observations in experimental VF in which a rebound was noted, but not others in which DF returned to control values during reflow.

Reflow results in rapid washout of [K⁺]o, with restoration and overshoot of resting membrane potential and hyperpolarization occurring within seconds. The rapid increase in DF and overshoot we observed after reflow is consistent with restoration of partially inactivated I\textsubscript{K1}, rapid normalization of excitability, and a slight increase above normal as a result of hyperpolarization. PRR ceases, and the effective refractory period becomes largely voltage dependent, being determined by APD. However, APD shortening as a result of ischemia is slower to recover. For example, we have previously studied humans who underwent coronary artery angioplasty that entailed a 3-minute period of ischemia during balloon occlusion of the artery. Even after 5 minutes of reflow, less than 70% of the APD shortening that occurred during the ischemic
period had been reversed, although APD restitution normalized after approximately 2 minutes. As a result, the refractory period was short during defibrill. This, together with fast conduction and rapidly steepening APD restitution, would again provide ideal conditions for wavebreak during defibrill and is consistent with our observation that the numbers of epicardial rotors (nPS) and wavefronts remained unchanged during defibrill despite the dramatic increase in DF.

Study Limitations
In the present study, we used an electrode sock, as in our previous work,16 with a spacing of approximately 1 cm between electrodes. As a result of this spacing, we could have underestimated the nPS. However, by interpolating between electrode signals, we found coherent wavefronts (for example, see Figures 2 and 3), which is consistent with other studies of VF in the human heart in which a much smaller electrode spacing was used,39 and so we believe that the present findings are truly representative. Our observations are limited to the ventricular epicardium, and differences in the sensitivity to ischemia and VF dynamics between epicardium and endocardium have been reported.13 Other parts of the heart, such as the Purkinje system, may play an important role in VF maintenance.13 The patients were receiving medication that may have influenced the results of the study. Small epicardial and transmural temperature changes occur in this model of ischemia. We have previously performed a study using an epicardial thermistor probe during the institution of cardiopulmonary bypass and interrupted coronary flow to the myocardium. We observed a reduction in temperature of less than 0.50°C during the initial minute and no consistent further changes during a second minute.40 In the present studies, we found no evidence of a systematic difference in DF between anterior and posterior epicardium that would have potentially indicated cooling of the anterior epicardium. We also found no significant differences between patients with coronary artery disease and patients with valve disease, but the power of this statistical comparison was limited because of the small number of patients in each group.

Clinical Implications
Our studies provide an indication of the electrophysiological behavior of the hearts of patients with coronary artery disease and valve disease during the first 3 minutes of VF with global myocardial ischemia, as well as the first 30 seconds of defibrill while VF continues. This information adds to the growing knowledge of mechanisms from experimental studies and can play a part in the overall development of therapeutic strategies to prevent or terminate VF. The results also provide information relevant to cardiopulmonary resuscitation. However, the ischemic VF time in the present studies was relatively short compared with the 4- to 10-minute average time between cardiac arrest and defibrillation in the community, and in our studies, coronary defibrill was much greater than would be achieved by chest compressions alone.

Conclusions
Our results are consistent with human VF being driven by multiple mechanisms that give rise to coexisting relatively short-lived rotors and multiple wavelets. During early ischemia, rather than an increase in organization as seen in several experimental models, there is an increase in the number of wavebreaks that persists during defibrill. We propose that these results could be best explained by an important role of PRR. This notion is in keeping with growing awareness of competing mechanisms of VF maintenance from the whole heart to the molecular level.2,15,36

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Disclosures
None.

References
The present findings should provide valuable insight for this approach.
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Supplemental Material
Additional data and results

Figures S1, S2, and S3 provide an expanded view of our results for dominant frequency (DF), the number of phase singularities (nPS), and number of wavefronts (nWF). Each point plotted is the value of each quantity in a single patient, averaged over a period of 1 s. The solid lines show the fitted linear model as described in the manuscript.

Figure S4 shows additional information on the size of regions with an identical DF, which have been referred to as domains. The size of domains were measured as the number of adjacent electrodes with the same DF.

Methodological information

Figures S5 and S6 provide information about the reliability of DF estimation. In Figure S5 we show example electrograms from a single electrode in a single patient during perfused VF, after global myocardial ischemia, and during reflow. In each case the electrograms have a good amplitude, and frequency analysis shows a distinct peak at the DF. In figure S6 we show the average root mean square (RMS) for the electrogram signals over 30 s epochs during the VF recordings in each patient. RMS is a standard way to measure average signal amplitude, and these plots show that the overall signal amplitude is largely unaffected by ischemia, although in patient H065 there was a trend towards lower amplitude signals at the end of ischemia.

Analysis of VF activation patterns in the human heart is difficult because it is not practical to use voltage sensitive fluorescent dyes to map epicardial activity with high spatial resolution. As in our previous studies (Nash M. P. et al. Circulation 2006;114:536-42, and Ten Tusscher KHWJ et. al., Experimental Physiology 2009;94:553-562) we have interpolated electrogram voltage across the epicardial surface, and have used this information to obtain trajectories in phase space using the Hilbert transform that can be used to determine local phase, and hence to identify wavefronts and phase singularities.

In Figures S7 and S8 we provide example snapshots of electrogram data from two patients. We have made an arbitrary selection of two electrodes, and show snapshots of 2 s duration recorded at the start of perfused VF, after 2 minutes of ischemia, and at the start of reflow. In Figure S7 we show data from patient H065, where the VF electrograms are close to a sinusoid, and produce clear phase trajectories. Ischemia has no major effect on the signal amplitude or the phase trajectories, although there is a trend towards a smaller amplitudes during reflow as indicated in the overall RMS data shown in Figure S6). In Figure S8 we show data from patient H064, where the electrograms from electrode 1 are noisier, but of a comparable amplitude to those shown in the previous figure. The noise has some effect of on the phase trajectories, but the origin remains at the centre. The electrograms from electrode 100 include notches, which are most likely to result from far field effects associated with the complex activation pattern of VF. These notches produce small loops in the phase trajectories, but these do not overlap the origin, shown as a red cross, and so do not have a major impact on transformation from voltage to phase.

The bottom panels of Figures S7 and S8 indicate that the numbers of PS observed fluctuate. However, the amplitude of fluctuations is not markedly affected by ischemia or reflow, and the 1 s moving average (shown as points linked by lines) acts to smooth the fluctuations while preserving the overall trend.
Figure S1. Changes in dominant frequency during perfused VF (red), ischemia (blue), and reflow (green). Each point is the mean DF in a single patient, calculated using DF measurements from each electrode over a 1 s period. Solid lines are fitted from the mixed linear model as in Figure 6.
Figure S2. Changes in number of PS during perfused VF (red), ischemia (blue), and reflow (green). Each point is the mean number of PS in a single patient, calculated using measurements over a 1 s period. Solid lines are fitted from the mixed linear model as in Figure 6.
**Figure S3.** Changes in number of wavefronts during perfused VF (red), ischemia (blue), and reflow (green). Each point is the mean number of wavefronts in a single patient, calculated using measurements over a 1 s period. Solid lines are fitted from the mixed linear model.
Figure S4. Time course of A, number of DF domains, and B, size of largest DF domain, during perfused VF, global ischaemia, and reflow. Central line indicates mean, dashed lines indicate standard deviations. To assess whether there was a trend towards a domain structure, we calculated the number of adjacent electrodes with the same DF, and the size of the largest of these regions. The overall increase in the number of domains, coupled with a decrease in the size of the largest domain is consistent with our overall theme of a trend during ischemia towards more fragmented activity, with more PS and more, shorter, wavefronts.
**Figure S5** Measurement of dominant frequency (DF). A-C, recordings from a single electrode in a single recording (H058 channel 20) during control VF (A), ischemic VF (B), and reflow VF (C). In each case a 4 s extract from the time series is shown, together with an estimate of the signal spectrum obtained using a fast Fourier transform. In each spectrum, DF is the frequency of the dominant peak as shown. Panel D shows how dominant frequency changes in this recording; solid lines show mean DF calculated across all of the electrodes, and thin lines show standard deviations.
Figure S6 Mean and standard deviation of root mean square (RMS) signal amplitude over 30 s intervals throughout recordings from each patient.
Figure S7 Example plots in phase space from patient H065 during the first 2 s of perfused VF (left panel), after 120 s global myocardial ischemia (middle panel), and during reflow (right panel. Graphs show from top; electrogram (blue) and Hilbert transform (red) for electrode 1; phase portrait for electrode 1; electrogram (blue) and Hilbert transform (red) for electrode 100; phase portrait for electrode 100; number of PS recorded every 10 ms (solid line), 1 s moving average (points, black lines), first point shows average from 0-500 ms, second average from 500-1500 ms, and third from 1500-2500 ms.
Figure S8 Example plots in phase space from patient H064 during the first 2 s of perfused VF (left panel), after 120 s global myocardial ischemia (middle panel), and during reflow (right panel). Graphs show from top; electrogram (blue) and Hilbert transform (red) for electrode 1; phase portrait for electrode 1; electrogram (blue) and Hilbert transform (red) for electrode 100; phase portrait for electrode 100; number of PS recorded every 10 ms (solid line), 1 s moving average (points, black lines), first point shows average from 0-500 ms, second average from 500-1500 ms, and third from 1500-2500 ms.