Pathophysiology Underlying the Bimodal Edema Phenomenon After Myocardial Ischemia/Reperfusion

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ABSTRACT

BACKGROUND Post-ischemia/reperfusion (I/R) myocardial edema was recently shown to follow a consistent bimodal pattern: an initial wave of edema appears on reperfusion and dissipates at 24 h, followed by a deferred wave that initiates days after infarction, peaking at 1 week.

OBJECTIVES This study examined the pathophysiology underlying this post-I/R bimodal edematous reaction.

METHODS Forty instrumented pigs were assigned to different myocardial infarction protocols. Edematous reaction was evaluated by water content quantification, serial cardiac magnetic resonance T2-mapping, and histology/immunohistochemistry. The association of reperfusion with the initial wave of edema was evaluated in pigs undergoing 40-min/80-min I/R and compared with pigs undergoing 120-min ischemia with no reperfusion. The role of tissue healing in the deferred wave of edema was evaluated by comparing pigs undergoing standard 40-min/7-day I/R with animals subjected to infarction without reperfusion (chronic 7-day coronary occlusion) or receiving post-I/R high-dose steroid therapy.

RESULTS Characterization of post-I/R tissue changes revealed maximal interstitial edema early on reperfusion in the ischemic myocardium, with maximal content of neutrophils, macrophages, and collagen at 24 h, day 4, and day 7 post-I/R, respectively. Reperfused pigs had significantly higher myocardial water content at 120 min and T2 relaxation times on 120 min cardiac magnetic resonance than nonreperfused animals. Permanent coronary occlusion or high-dose steroid therapy significantly reduced myocardial water content on day 7 post-infarction. The dynamics of T2 relaxation times during the first post-infarction week were altered significantly in nonreperfused pigs compared with pigs undergoing regular I/R.

CONCLUSIONS The 2 waves of the post-I/R edematous reaction are related to different pathophysiological phenomena. Although the first wave is secondary to reperfusion, the second wave occurs mainly because of tissue healing processes. (J Am Coll Cardiol 2015;66:816–28) © 2015 by the American College of Cardiology Foundation. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Post-myocardial infarction (MI) tissue characterization offers the possibility of predicting long-term remodeling and evaluating the impact of interventions aimed at preserving cardiac function. Reperfusion, the basis of myocardial protection during infarction, alters post-MI tissue composition compared with the nonreperfused setting (1,2). Nonetheless, most current knowledge of tissue modifications in the post-infarcted myocardium is based on observations in nonreperfused hearts. There is therefore a need for comprehensive tissue characterization in the context of ischemia/reperfusion (I/R).

The post-I/R myocardium is characterized by an intense edematous reaction confined to the post-ischemic region (3-7) assumed to appear early after I/R and persist in stable form for at least 1 week (8,9). It has been used both experimentally and clinically as a marker of ischemic "memory." Noninvasive evaluation of this post-I/R edematous reaction has become possible with the development of T2-weighted and T2-mapping cardiac magnetic resonance (CMR) sequences able to accurately detect increases in water content (10-12). Innumerable clinical and experimental reports have been based on the unimodal hypothesis of a persistent edematous reaction during the first week after I/R. However, we recently showed that the post I/R edematous reaction is not constant, but rather follows a bimodal pattern, with an initial wave of edema appearing abruptly on reperfusion and dissipating at 24 h, followed by a deferred wave of edema appearing several days after I/R, increasing to a maximum on post-reperfusion day 7 (13). These experimental observations challenge the accepted view (14) and call for a mechanistic study to unravel the pathophysiological mechanisms underlying this newly recognized phenomenon.

In the present study, we used a large animal model (pig) of MI to characterize the tissue changes taking place in the post I/R myocardium by histological and CMR methods. Under the hypothesis that the initial wave of edema is a direct consequence of reperfusion while the deferred wave is caused by healing processes, we manipulated each wave independently through the use of reperfused and nonreperfused MI models, and exposure to high-dose steroid therapy, interventions that are known to alter healing processes.

METHODS

STUDY DESIGN, PROTOCOLS, AND IMAGING ANALYSIS.
The study population was 40 castrated male Large-White pigs. The study (Figure 1) was approved by the Institutional Animal Research Committee and conducted in accordance with recommendations of the Guide for the Care and Use of Laboratory Animals. MI was generated by 40-min angioplasty-balloon occlusion of the mid-left anterior descending (LAD) coronary artery followed by balloon deflation and re-establishment of blood flow (15). Short follow-up (120 min) nonreperfused MI was achieved by maintaining balloon inflation until sacrifice and heart excision. Long follow-up (7 days) nonreperfused MI was achieved by implanting an intravascular CMR-compatible coil in the mid-LAD to generate permanent coronary occlusion. Full methods can be found in the Online Appendix.

Myocardial water content was quantified in samples from the infarcted and remote myocardium of all pigs by using the desiccating technique as the reference standard: water content (%) = [(wet weight – dry weight)/wet weight] ×100. Samples of ischemic myocardium were analyzed by histology and immunohistochemistry. Histological sections were stained with hematoxylin and eosin and Masson trichrome, and with antibodies against F4/80 (macrophages) and PM1 (neutrophils).

CMR examinations were performed immediately before MI and at subsequent post-MI follow-up time points until sacrifice. Examinations were conducted with a Philips 3-Tesla Achieva Tx whole body scanner (Philips Healthcare, Best, the Netherlands) equipped with a 32-element phased-array cardiac coil. The imaging protocol included a standard segmented cine steady-state free-precession sequence to provide high-quality anatomic references, a T2-weighted triple short-tau inversion recovery sequence, and T2 turbo spin multiecho mapping. The imaging parameters are detailed in the Online Appendix.

CMR images were analyzed using dedicated software (MR Extended Work Space 2.6, Philips Healthcare) by 2 experienced observers blinded to group allocation.

STATISTICAL ANALYSIS.
Normal distribution of each data subset was checked using graphical methods and a Shapiro-Wilk test. For quantitative variables showing a normal distribution, data are expressed as mean ± SD. For quantitative variables showing a non-normal distribution, data are expressed as median and interquartile range. A Kruskal-Wallis test was conducted for comparison of hemorrhage, number of inflammatory cells, and collagen content among groups over the histopathological time course. Given the hypothesis-driven nature of the study, comparisons between groups of pigs sacrificed at
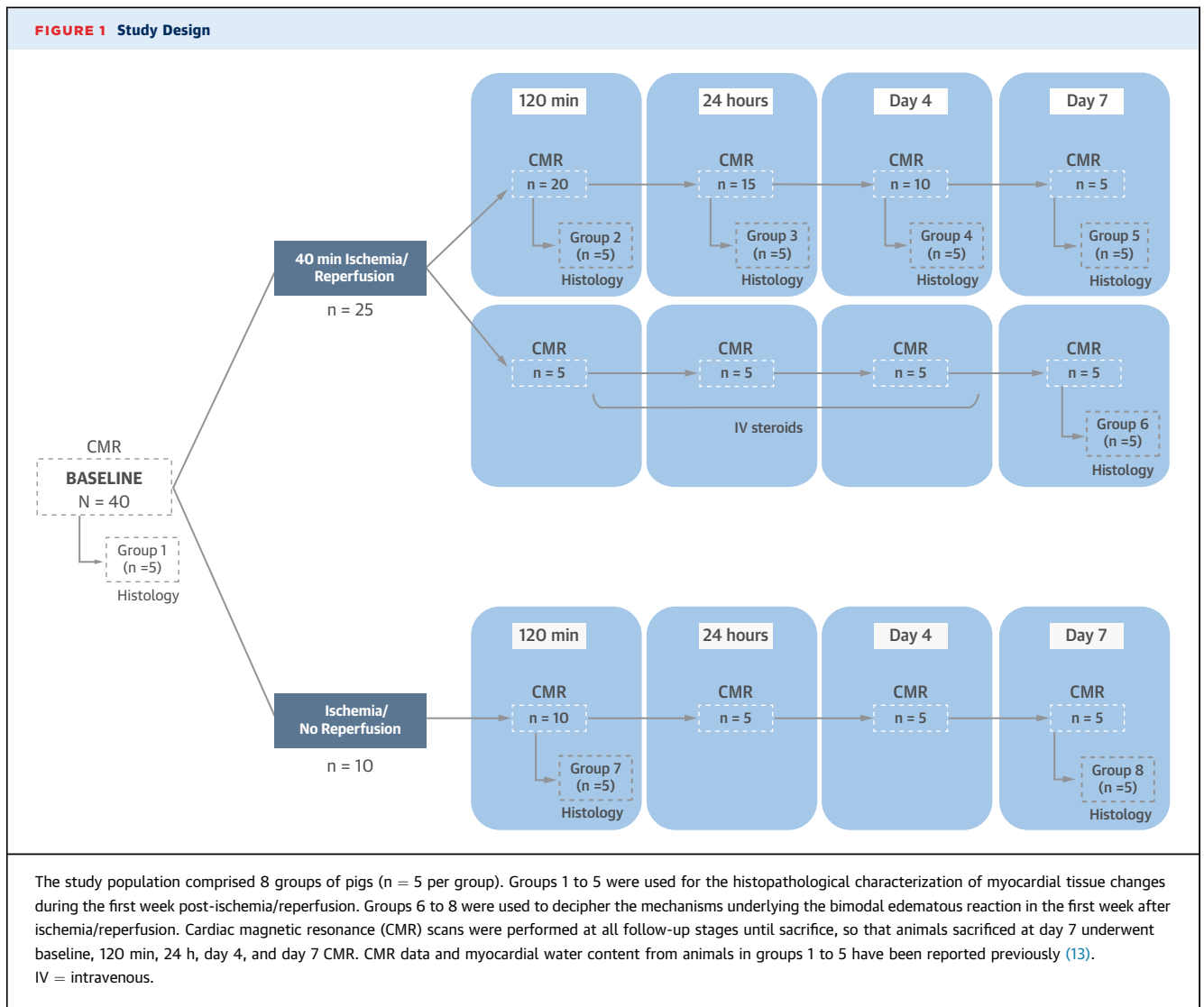
ABBREVIATIONS AND ACRONYMS

CMR = cardiac magnetic resonance

I/R = ischemia/reperfusion

LAD = left anterior descending

MI = myocardial infarction

FIGURE 1   Study Design

The study population comprised 8 groups of pigs (n = 5 per group). Groups 1 to 5 were used for the histopathological characterization of myocardial tissue changes during the first week post-ischemia/reperfusion. Groups 6 to 8 were used to decipher the mechanisms underlying the bimodal edematous reaction in the first week after ischemia/reperfusion. Cardiac magnetic resonance (CMR) scans were performed at all follow-up stages until sacrifice, so that animals sacrificed at day 7 underwent baseline, 120 min, 24 h, day 4, and day 7 CMR. CMR data and myocardial water content from animals in groups 1 to 5 have been reported previously (13). IV = intravenous.

post-infarction day 7 were planned in advance. Quantitative variables showing a normal distribution were therefore compared using a two-tailed Student *t* test, whereas quantitative variables showing a non-normal distribution were compared using a

Wilcoxon rank sum test. All statistical analyses were performed with Stata 12.0 (StataCorp, College Station, Texas).

RESULTS

DYNAMICS OF TISSUE CHANGES DURING THE FIRST WEEK AFTER REPERFUSED MI. To characterize changes associated with the 2 waves of edema, we first studied histological changes during the first week after standard I/R. Samples for this analysis came from a group of animals whose bimodal CMR and water content results were previously reported (13). The time course of tissue changes in the post-I/R myocardium is summarized in **Table 1** and illustrated in **Figure 2A** with representative staining images in **Figure 2B**. The density of infiltrating neutrophils was maximal at 24 h post-I/R and remained high at day 4

TABLE 1   Tissue Changes in the Ischemic Myocardium During Week 1 Post-I/R

	R-120 Min	R-24 H	R-Day 4	R-Day 7	p Value
Hemorrhage, score 0-5	0 (0-1)	2 (2-3)	4 (3-5)	1 (0-2)	0.02
Neutrophils,* n/mm^2	69.0 ± 94.7	677.6 ± 318.3	454.5 ± 312.5	94.9 ± 86.8	<0.01
Macrophages,* n/mm^2	5.5 ± 11.0	20.6 ± 30.1	154.4 ± 295.8	12.6 ± 20.5	<0.01
Collagen,† %	0 ± 0	0 ± 0	4.5 ± 1.1	13.3 ± 3.2	<0.01

Values are median (interquartile range) or mean ± SD. Interstitial hemorrhage is graded from 0 (absence) to 5 (very severe). p value for among-group comparison. *In the lesion area. †In the granulation tissue area.
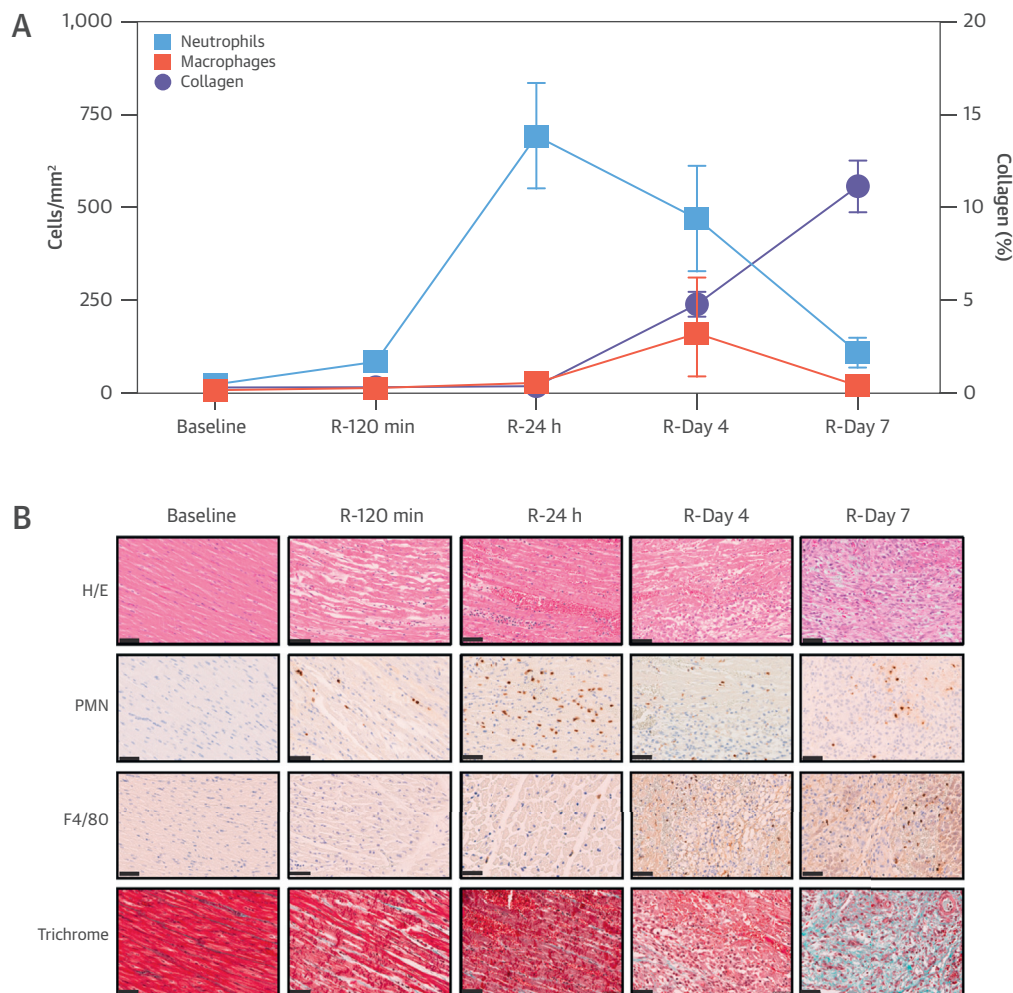I = ischemia; R = reperfusion.

before dropping significantly by day 7 post-I/R. The density of infiltrating macrophages also increased significantly from day 1 to a peak on day 4 before dropping significantly at day 7. Interstitial hemorrhage was apparent at 24 h, peaking on day 4 post-I/R. There was no evidence of increased collagen deposition until day 4, with a further increase in content observed at post-I/R day 7.

INITIAL WAVE OF EDEMA AND REPERFUSION. To study the potential association between the initial wave of edema and reperfusion, a new group of 5 pigs (Group 7 in **Figure 1**) underwent ischemia (angioplasty-balloon mid-LAD coronary occlusion) and were sacrificed after 120-min CMR without being reperfused. When compared with animals undergoing 40-min coronary occlusion followed by 80-min reperfusion (Group 2 in **Figure 1**), myocardial water content in the ischemic region of the nonreperfused pigs was significantly lower than in reperfused animals sacrificed at the same time ($80.8 \pm 0.4\%$ vs. $84.5 \pm 0.5\%$; $p < 0.0001$) (**Figure 3A**).

CMR results agreed with histological water content data: although baseline (pre-ischemia) T2 relaxation times in nonreperfused and reperfused pigs were similar (45.3 ± 1.4 ms vs. 48.7 ± 0.6 ms), the 120-min CMR T2 relaxation times in the ischemic region of nonreperfused pigs were significantly lower than those of reperfused pigs (57.0 ± 3.0 ms vs. 73.3 ± 10.0 ms;

FIGURE 2 Histopathological Changes in the Ischemic Myocardium During the First Week After Ischemia/Reperfusion

Neutrophil and macrophage density in the lesion area and collagen content in the granulation tissue **(A)** and representative images of histological changes in the ischemic myocardium **(B)** during the first week post-ischemia/reperfusion. For each time point, images are shown of staining with hematoxylin and eosin (H/E), anti-PM1, anti-F4/80, and Masson trichrome. Data are mean ± SEM. PMN = polymorphonuclear leukocytes; R = reperfusion.

FIGURE 3   Effect of Preventing Reperfusion on the Initial Wave of Post-Myocardial Infarction Edema

Mean differences in absolute water content at 120 min from ischemia onset between the ischemic and remote myocardial regions were 5.2% for reperfused pigs and 1.5% for nonreperfused pigs **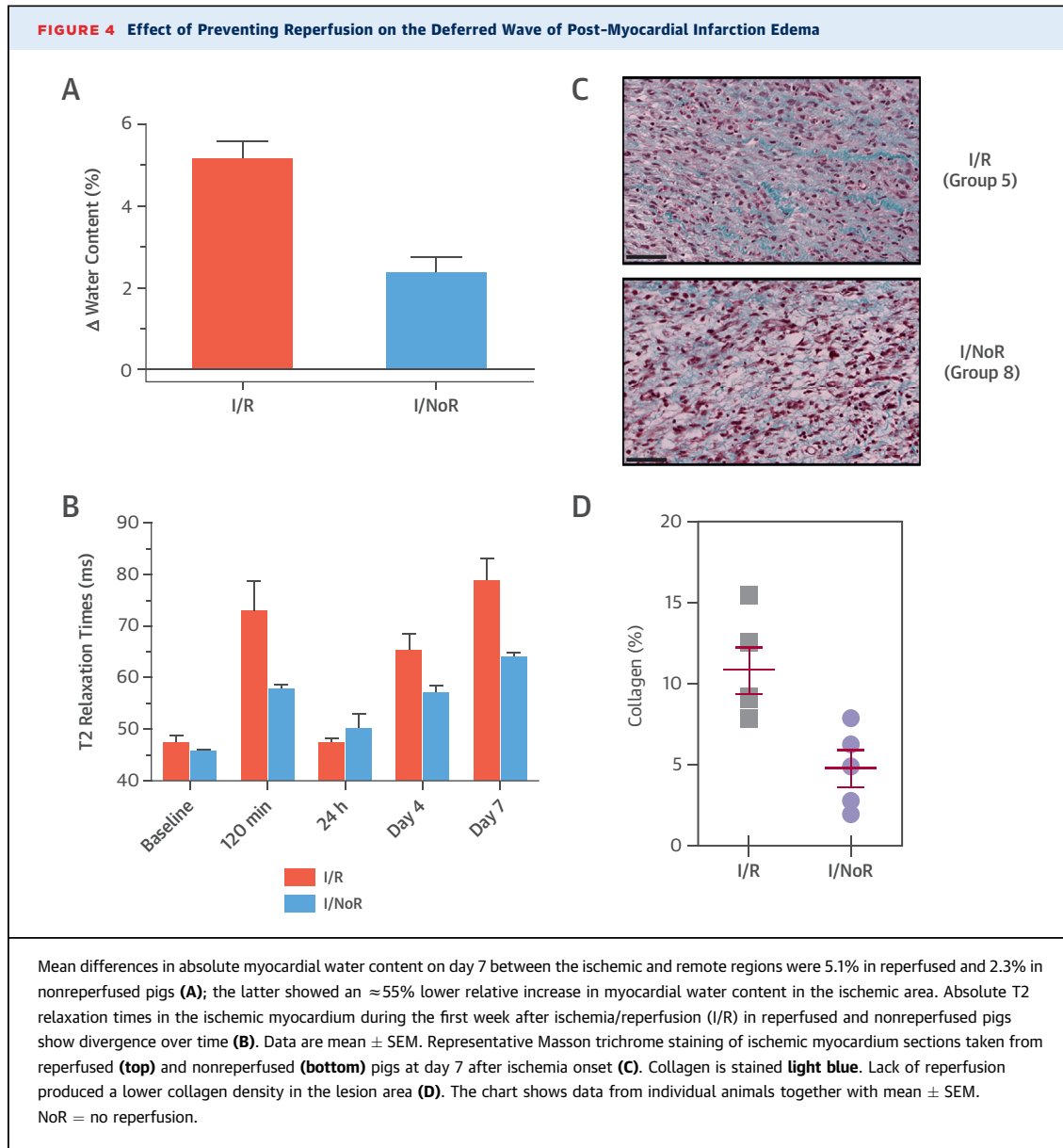(A)**; the latter showed an ≈70% lower relative increase in myocardial water content in the ischemic area. The nonreperfused pigs also demonstrated significantly lower 120-min cardiac magnetic resonance T2 relaxation times in the ischemic region **(B)**. Representative T2-weighted triple short-tau inversion recovery (STIR) and T2-mapping images **(C)** from representative pigs subjected to ischemia with reperfusion (40-min ischemia plus 80-min reperfusion) and with no reperfusion (120-min ischemia). Data are mean ± SEM; all T2 maps were scaled between 30 and 120 ms. Representative images of ischemic myocardial tissue stained with hematoxylin and eosin from 2 reperfused **(D, top)** and 2 nonreperfused **(D, bottom)** pigs sacrificed at 120 min after ischemia onset. Note the evident increase in extracellular space, most probably caused by extracellular edema, in reperfused pigs compared with nonreperfused animals.

p < 0.01) (**Figure 3B**). In agreement with quantitative T2 maps, qualitative T2-weighted signal intensity in nonreperfused pigs was significantly lower than that in reperfused pigs, with the intensity at 120-min CMR in the former similar to that observed pre-ischemia (baseline) (**Figure 3C**). Histological analysis revealed significantly less interstitial edema in the ischemic area of nonreperfused pig hearts (**Figure 3D**).

DEFERRED WAVE OF EDEMA AND MI HEALING. To study the potential association between the deferred wave of edema and MI healing, we modified tissue healing in the post-infarcted myocardium with 2 independent strategies: permanent coronary occlusion and high-dose steroid therapy.

Five pigs were subjected to chronic occlusion without reperfusion by delivery of a CMR-compatible coil to the mid-LAD (Group 8 in **Figure 1**). All animals underwent serial CMR examinations (baseline, 120 min, days 1, 4, and 7) and were sacrificed after day 7 CMR. Permanent occlusion of the coronary artery significantly attenuated the deferred wave of edema, with myocardial water content in the ischemic region at day 7 significantly lower in the nonreperfused pigs than in reperfused pigs—Group 5 (82.5 ± 1.0% vs. 85.2 ± 0.9%; p < 0.01) (**Figure 4A**).

Permanent coronary occlusion altered the dynamics of serial CMR T2 relaxation times (**Figures 4B and 5**). In agreement with the outcome of acute occlusion experiments, T2 relaxation times in the ischemic region on 120-min CMR were significantly lower in nonreperfused pigs, whereas T2 relaxation times at 24-h CMR did not differ between nonreperfused and reperfused pigs. Thereafter, T2 relaxation times diverged; at day 4, a trend toward lower T2 relaxation

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FIGURE 4 Effect of Preventing Reperfusion on the Deferred Wave of Post–Myocardial Infarction Edema

Mean differences in absolute myocardial water content on day 7 between the ischemic and remote regions were 5.1% in reperfused and 2.3% in nonreperfused pigs **(A)**; the latter showed an ≈55% lower relative increase in myocardial water content in the ischemic area. Absolute T2 relaxation times in the ischemic myocardium during the first week after ischemia/reperfusion (I/R) in reperfused and nonreperfused pigs show divergence over time **(B)**. Data are mean ± SEM. Representative Masson trichrome staining of ischemic myocardium sections taken from reperfused **(top)** and nonreperfused **(bottom)** pigs at day 7 after ischemia onset **(C)**. Collagen is stained **light blue**. Lack of reperfusion produced a lower collagen density in the lesion area **(D)**. The chart shows data from individual animals together with mean ± SEM. NoR = no reperfusion.

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times was detected in the ischemic myocardium of chronically nonreperfused pigs. Consistent with the water content analysis, at day 7 this difference was statistically significant (63.8 ± 2.5 ms in nonreperfused pigs vs. 78.4 ± 10.6 ms in reperfused pigs; p = 0.02) (**Figures 4B and 5**).

Histological analysis at day 7 post-infarction revealed different healing patterns per reperfusion status. Permanent occlusion resulted in lower collagen density in the lesion area (4.7 ± 2.4% vs. 10.8 ± 3.1%; p = 0.01) (**Figures 4C and 4D**). The extent of necrosis within the lesion area was significantly higher in the nonreperfused pigs (68.9 ± 21.7% vs. 8.9 ± 12.7%; p < 0.001) (**Figure 6**), and the proportion

of granulation tissue was correspondingly lower (31.1 ± 21.7% vs. 91.1 ± 12.7%; p < 0.001) (**Figure 6**).

Five pigs underwent 40-min/7-day I/R and received high intravenous doses of steroids, commencing after the 120-min CMR (Group 6 in **Figure 1**). All animals underwent serial CMR examinations (baseline, 120 min, days 1, 4, and 7) and were sacrificed after day 7 CMR. The pigs received 3 doses of intravenous methylprednisolone (30 mg/kg/dose) within the first 24 h post-I/R, followed by 1 daily dose of intravenous methylprednisolone (30 mg/kg) on the following 3 days.

Administration of steroids significantly altered the deferred wave of edema, with myocardial water

FIGURE 5   Effect of Preventing Reperfusion on Myocardial T2 Relaxation Time
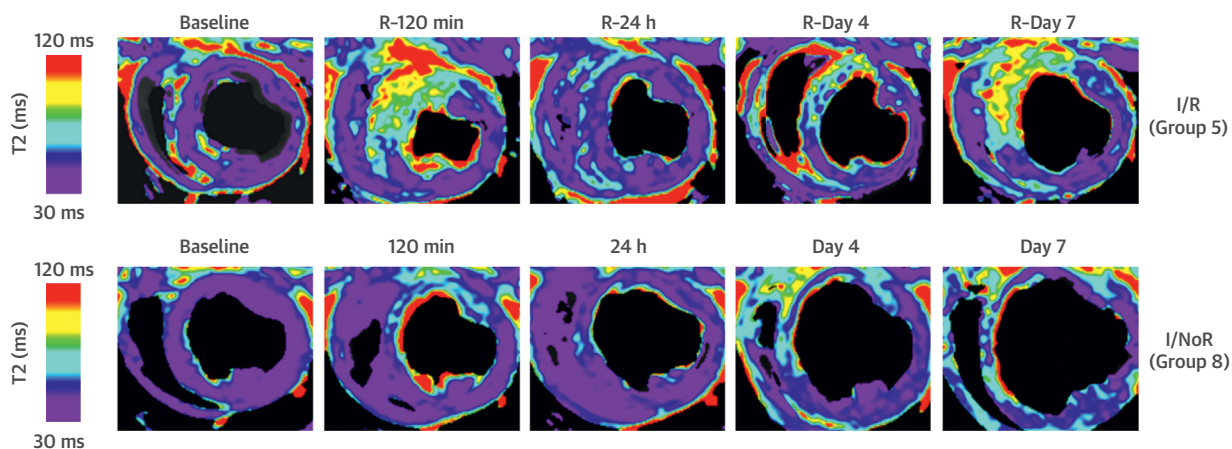
Permanent coronary occlusion altered the dynamics of serial cardiac magnetic resonance T2 relaxation times in the ischemic region as it is shown by these representative T2-mapping images from reperfused and nonreperfused pigs for all time points. All T2 maps were scaled between 30 and 120 ms. Abbreviations as in **Figures 2 and 4**.

content significantly lower in the ischemic region at day 7 than in the untreated pigs in Group 5 (83.3 ± 0.8% vs. 85.2 ± 0.4% for steroid-treated and untreated pigs, respectively; p = 0.01) (**Figure 7A**). This effect was not accompanied by significant differences in T2 relaxation times on day 7 CMR (77.3 ± 13.0 ms steroid-treated vs. 78.4 ± 10.6 ms untreated; p > 0.10) or at any of the earlier time points evaluated (**Figures 7B and 8**).
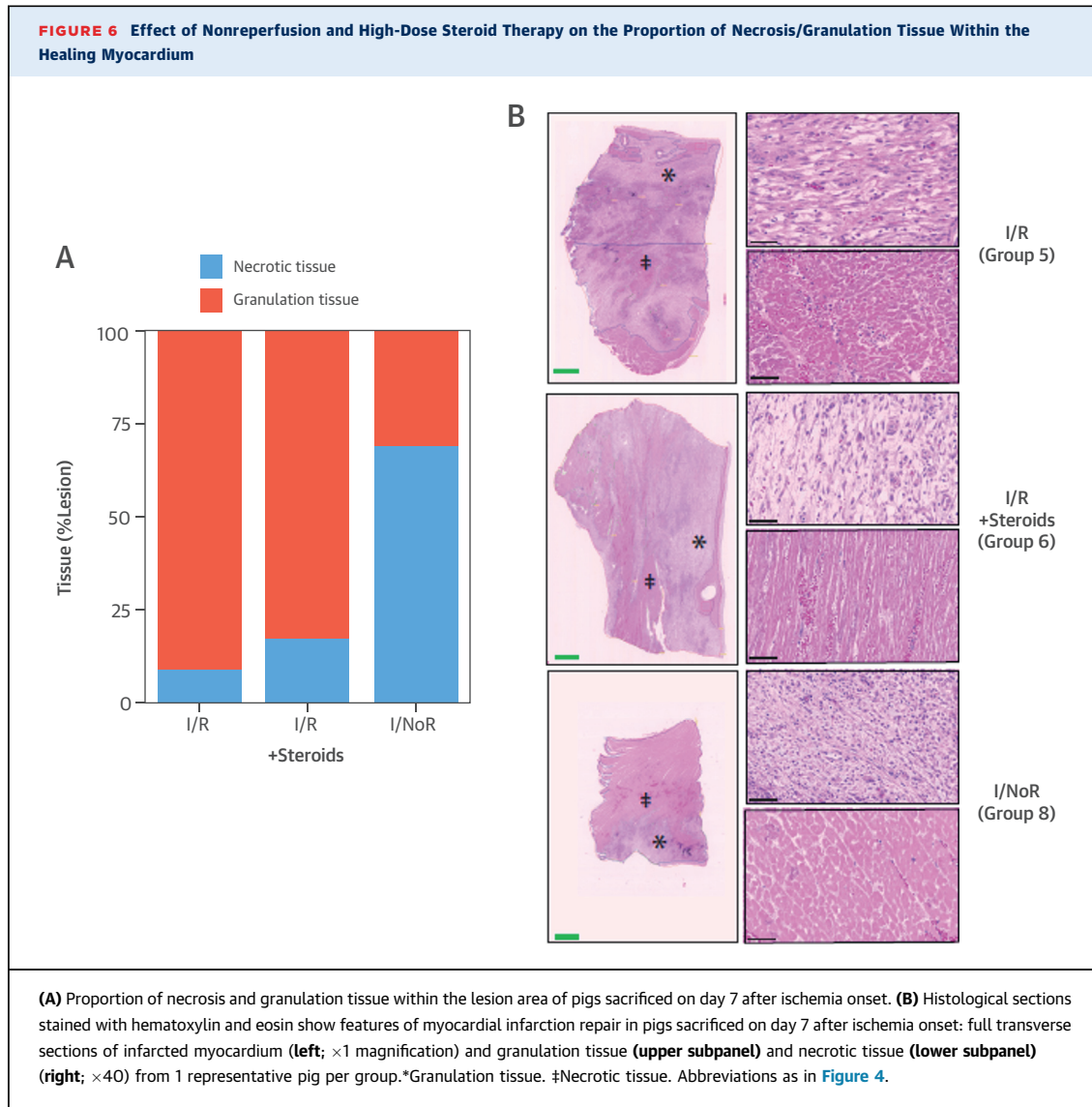
According to the histological analysis, high-dose steroid therapy resulted in lower collagen density in the lesion area (6.1 ± 3.4% vs. 10.8 ± 3.1%; p = 0.05) (**Figures 7C and 7D**). The proportions of necrosis and granulation tissue within the lesion area were similar in the 2 groups: 17.3 ± 18.7% and 82.7 ± 18.7%, respectively, in steroid-treated pigs vs. 8.9 ± 12.7% and 91.1 ± 12.7% in nontreated pigs (p > 0.10) (**Figure 6**). Water content and hemorrhage scores in the myocardium of pigs sacrificed on day 7 after ischemia onset is summarized in **Table 2**.

DISCUSSION

Recent work has shown that the edematous reaction in the reperfused post-infarcted myocardium is not stable, but follows a bimodal pattern. An initial wave of edema appears on reperfusion, abating almost completely by 24 h, followed by a deferred wave that initiates days after I/R and peaks around 1 week after infarction (13). In the present study, we explored the possible mechanisms underlying these 2 waves of

post-I/R edema through a comprehensive histopathological analysis and state-of-the-art CMR serial evaluations in the pig model. Our results show that the 2 waves of post-I/R edema are produced by different processes that can be independently modulated (**Central Illustration**). The absence of reperfusion almost completely abolishes the initial wave, indicating that it is a direct consequence of the reperfusion process. The deferred wave is substantially altered by interventions that impair healing in the post-infarcted myocardium (absence of reperfusion and high-dose steroid therapy), thus implicating tissue healing processes in this second phase of edema. Elucidation of the pathophysiology underlying bimodal post-I/R edema has potential translational implications in diagnosis, prognosis, and therapy. Pharmacological or other interventions targeting the pathways implicated in the initial or deferred waves of post-I/R edema could contribute to better cardiac recovery and remodeling, and thus improve prognosis in patients experiencing an acute MI. Additionally, this study provides a comprehensive histological characterization of the dynamic changes occurring in a large animal model of reperfused MI.
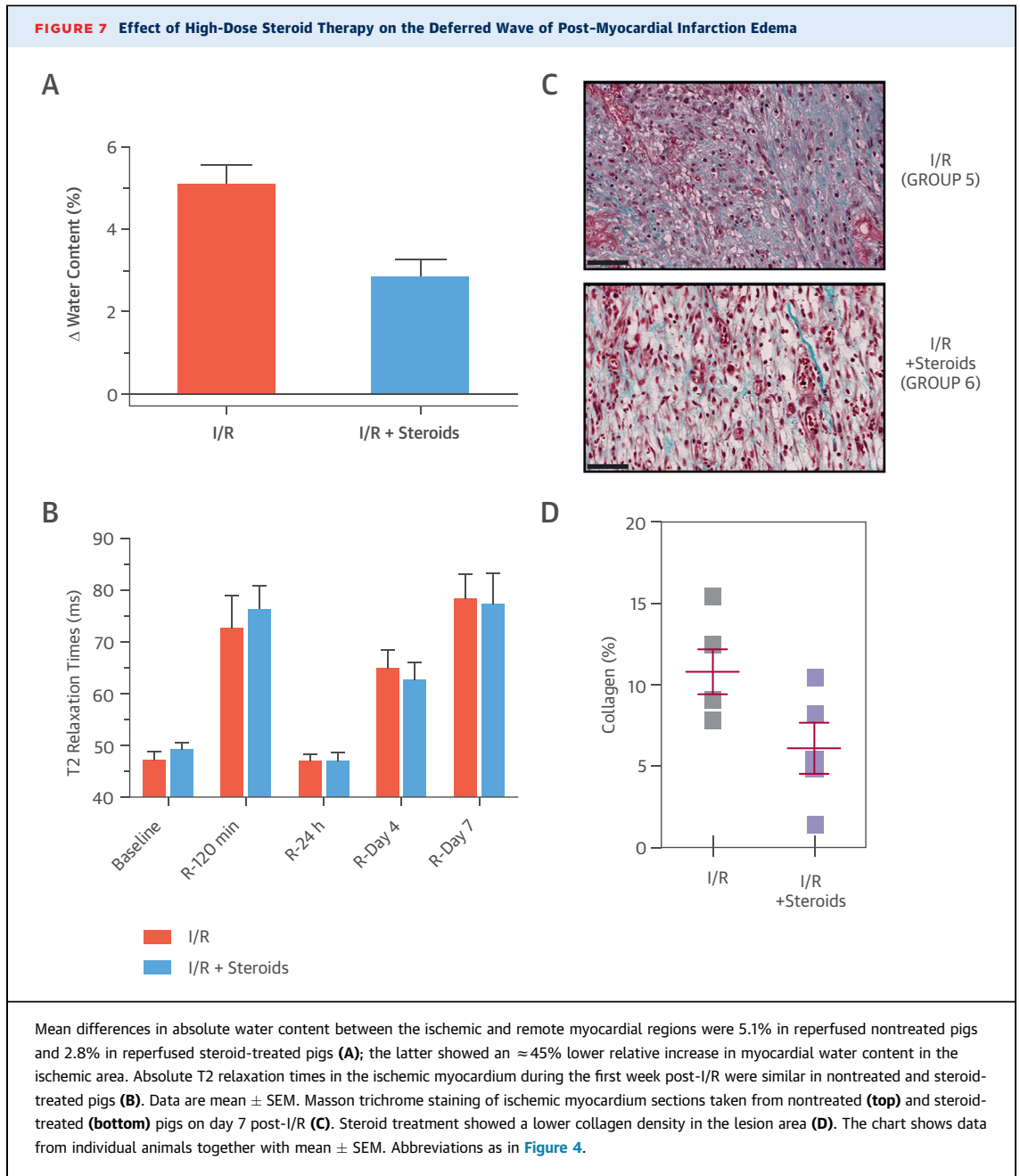
Water is the major component of healthy cardiac tissue. In steady-state conditions, myocardial water content is stable and mostly intracellular, with only a very small interstitial component in the extracellular matrix. During MI, edema occurs initially as cardiomyocyte swelling during the early stages of

FIGURE 6 Effect of Nonreperfusion and High-Dose Steroid Therapy on the Proportion of Necrosis/Granulation Tissue Within the Healing Myocardium

(A) Proportion of necrosis and granulation tissue within the lesion area of pigs sacrificed on day 7 after ischemia onset. (B) Histological sections stained with hematoxylin and eosin show features of myocardial infarction repair in pigs sacrificed on day 7 after ischemia onset: full transverse sections of infarcted myocardium (**left**; ×1 magnification) and granulation tissue (**upper subpanel**) and necrotic tissue (**lower subpanel**) (**right**; ×40) from 1 representative pig per group.*Granulation tissue. ‡Necrotic tissue. Abbreviations as in **Figure 4**.

ischemia (7). Myocardial edema is then exacerbated significantly on restoration of blood flow to the ischemic region. This reperfusion-associated increase seems to be caused by increased cell swelling (5) and, more importantly, by interstitial edema secondary to reactive hyperemia and leakage from capillaries damaged when hydrostatic pressure is restored on reperfusion (3,6). For many years, this myocardial edema was believed to remain stable for at least 1 week (8-10); this view led many experimental studies and clinical trials to rely on the ability of CMR and other imaging tools to retrospectively evaluate post-MI edema (10-12). The concept of stable post-I/R edema was recently challenged in an experimental analysis by our group in a pig model of I/R, revealing the bimodal pattern of the edematous response (13).

The present study confirms our original description of a bimodal response and provides insight into the underlying pathophysiological mechanisms.

The association between the initial, hyperacute edematous response and the reperfusion process has been reported previously (2,16). In agreement with those studies, here we show that the absence of reperfusion almost completely abolishes the early increase in water content and the CMR-measured regional T2 relaxation times in the ischemic myocardium. Indirect histological detection of interstitial edema (increased extracellular volume) was consistent with the water content and CMR data. Our study demonstrates that the reperfusion-driven hyperacute edematous reaction (4) is exhausted after 24 h; moreover, these processes can be visualized and

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FIGURE 7 Effect of High-Dose Steroid Therapy on the Deferred Wave of Post-Myocardial Infarction Edema

Mean differences in absolute water content between the ischemic and remote myocardial regions were 5.1% in reperfused nontreated pigs and 2.8% in reperfused steroid-treated pigs **(A)**; the latter showed an ≈45% lower relative increase in myocardial water content in the ischemic area. Absolute T2 relaxation times in the ischemic myocardium during the first week post-I/R were similar in nontreated and steroid-treated pigs **(B)**. Data are mean ± SEM. Masson trichrome staining of ischemic myocardium sections taken from nontreated **(top)** and steroid-treated **(bottom)** pigs on day 7 post-I/R **(C)**. Steroid treatment showed a lower collagen density in the lesion area **(D)**. The chart shows data from individual animals together with mean ± SEM. Abbreviations as in **Figure 4**.

quantified by state-of-the-art contemporaneous T2 CMR sequences, a finding of special translational relevance. Although water content in the ischemic myocardium decreases significantly during the first 24 h post-reperfusion, it does not return to normal values, whereas T2 relaxation time does decrease to values seen in the pre-ischemia baseline scan (13). These data indicate that myocardial water content may not be the only factor influencing post-infarction T2 relaxation time. The fact that the interstitial hemorrhage was more pronounced at 24 h than

early after reperfusion (**Table 2**) suggests a possible contribution from the well-described paramagnetic effect of hemoglobin denaturation products (17).

We hypothesized that the deferred wave of edema might be related to early healing, a process coinciding with the deferred edema wave. To test this hypothesis, we used 2 independent strategies to manipulate this biological phenomenon: high-dose steroid therapy and absence of reperfusion. Steroids are well known to interfere with collagen deposition in healing infarcts (18,19) and absence of reperfusion is

FIGURE 8 Effect of High-Dose Steroid Therapy on Myocardial T2 Relaxation Time
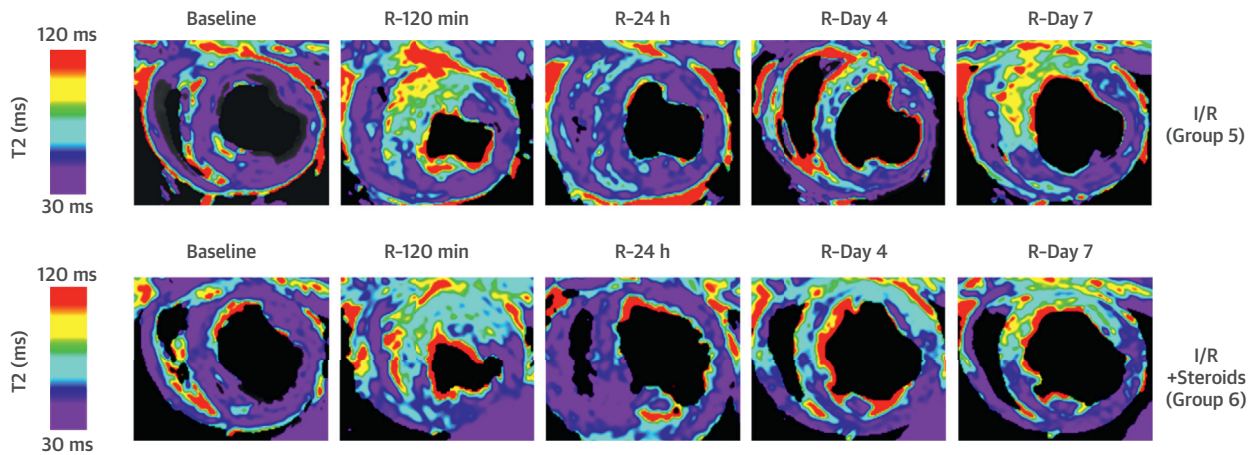
Treatment with high-dose steroid therapy did not alter the dynamics of serial cardiac magnetic resonance T2 relaxation times in the ischemic region as it is shown by these representative T2-mapping images from reperfused nontreated and treated pigs for all time points. All T2 maps were scaled between 30 and 120 ms. Abbreviations as in **Figures 2 and 4**.

associated with significant delay to healing (1,2,20), in contrast with reperfusion, which accelerates this process. Both interventions altered the deferred wave of edema, as demonstrated by the significant reductions in water content in the ischemic myocardium of steroid-treated and nonreperfused animals compared with reperfused animals not receiving steroids. CMR data in nonreperfused pigs paralleled the water content measures, with T2 relaxation times at day 7 significantly lower than in reperfused pigs.

However, T2 relaxation times in steroid-treated pigs did not differ from values in untreated pigs. This apparent discrepancy might be explained by the effect of steroid therapy on collagen content: myocardial tissue from steroid-treated pigs showed a significant reduction in collagen content compared with untreated pigs, in agreement with previous reports (18). Collagen deposition correlates inversely with CMR T2 relaxation times (21-23); this effect may thus have compensated for the reduced water content, resulting in no net alteration in CMR T2 relaxation time in untreated pigs. The counterbalancing effects of water and collagen content highlight the likelihood that CMR T2 relaxation time is a composite measure of several phenomena occurring in the post-I/R myocardium. Collagen deposition was also reduced by permanent occlusion, where CMR T2 relaxation times and water content were consistent with each other. Notably, the infarct composition was different in

steroid-treated and nonreperfused hearts at day 7. The proportions of necrosis and granulation tissue within the myocardial lesion were similar in steroid-treated and standard reperfused pigs, whereas lesions in the nonreperfused pigs had a much higher proportion of necrotic tissue, indicating a delay to healing. Several factors might contribute to the discrepancies in water content and T2 relaxation time observed between nonreperfused pigs and steroid-treated reperfused pigs: distinct healing characteristics; different myocardial perfusion status related to circulation-induced increases in T2 relaxation time (24); and indirect influences, such as inflammation (25,26) and angiogenesis (27).

The results of the present study show that the 2 waves of post-I/R edema are caused by different phenomena that can be independently modulated.

**TABLE 2 Myocardial Water Content and Hemorrhage Score in Pigs***

	40 Min/7 Days I/R (Group 5)	40 Min/7 Days I/R + Steroid Therapy (Group 6)	7-Day Coronary Occlusion (Group 8)	p Value†	p Value‡
Water content, ischemic, %	85.2 ± 0.9	83.3 ± 0.8	82.5 ± 1.0	0.01	<0.01
Water content, remote, %	80.1 ± 0.3	80.4 ± 0.3	80.2 ± 0.4	NS	NS
Hemorrhage, score 0-5	1 (0-2)	0 (0-3)	1 (1-2)	NS	NS

Values are mean ± SD or median (interquartile range). Interstitial hemorrhage is graded from 0 (absence) to 5 (very severe). *Sacrificed on day 7 after ischemia onset. †Comparison between Group 5 (I/R with no other intervention) and Group 6 (I/R plus steroid therapy). ‡Comparison between Group 5 (I/R with no other intervention) and Group 8 (I/No R).

NS = not significant; other abbreviations as in **Table 1**.

CENTRAL ILLUSTRATION Mechanisms Underlying the Bimodal Edema Phenomenon After Myocardial Ischemia/Reperfusion
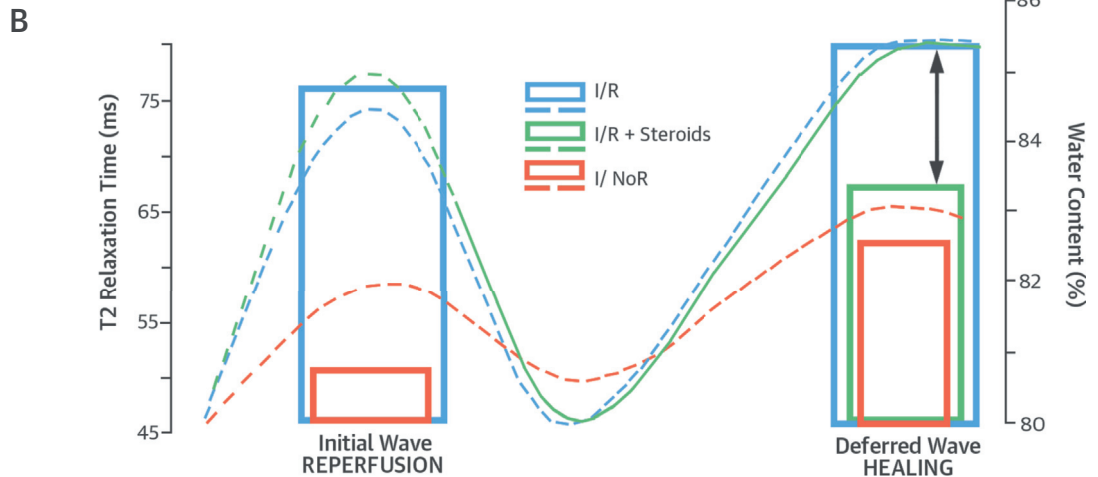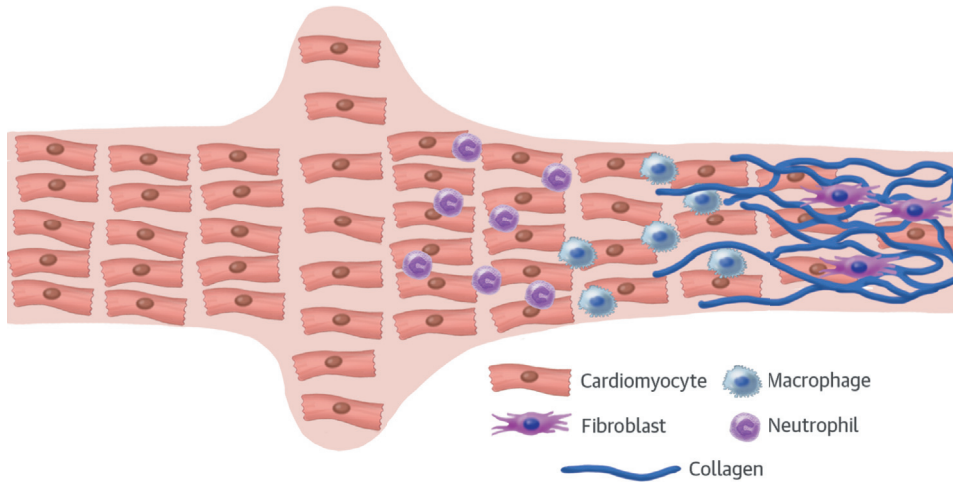
Fernández-Jiménez, R. et al. J Am Coll Cardiol. 2015; 66(7):816-28.

Two distinct waves of edema emerge within the first week after ischemia/reperfusion (I/R) because of different pathophysiological processes. The initial wave, appearing abruptly on reperfusion, is a direct consequence of the reperfusion process itself, whereas the deferred wave, appearing progressively days after I/R, is mainly caused by tissue healing processes. **(A)** Reperfusion is associated with a very abrupt edematous reaction that separates myocardial fibers from each other, resolving by day 1. Post-infarction, an initial neutrophil infiltration (peaking by day 1) is followed by macrophage infiltration (peaking by day 4). From day 4 on, necrotic cardiomyocytes are progressively replaced by granulation tissue and collagen. **(B)** Myocardial water content was evaluated by histology. **Boxes** represent water content measurements in pigs; **blue** = regular I/R protocol; **salmon** = permanent coronary occlusion (nonreperfused myocardial infarction; initial edema wave abrogated, deferred wave significantly attenuated); **green** = I/R plus steroid therapy (deferred wave significantly attenuated). The **lines** show cardiac magnetic resonance T2 relaxation time course in the ischemic myocardium; **blue** = first week after infarction (2 peaks closely track the water content changes); **salmon** = nonreperfused myocardial infarction (both waves of cardiac magnetic resonance–evaluated edema significantly attenuated); and **green** = I/R plus steroid therapy (**continuous line** after initiation of therapy). In this last case, T2 relaxation time does not track attenuation of the deferred wave as evaluated by the histological reference standard, highlighting the need for caution in interpreting cardiac magnetic resonance analysis of the post-myocardial infarction heart. **Double arrowed line** = discrepancy between water content and T2 relaxation time at day 7 in steroid group. NoR = no reperfusion.

Although the initial wave is mainly caused by the reperfusion itself, the deferred wave of edema is mainly caused by healing processes. Additionally, the present study shows that myocardial T2 relaxation time seems to be affected by dynamic factors occurring during the repair of the infarcted tissue, highlighting the need for caution in interpreting CMR analysis of the post-MI heart.

STUDY LIMITATIONS. Extrapolation of the results of this experimental study to the clinic should be done cautiously. Nonetheless, the pig is one of the most clinically translatable large animal models for the study of I/R issues given its similar coronary artery anatomy and distribution to humans and minimal pre-existing coronary collateral flow. The use of a large animal model is of great translational value (28), especially considering the difficulty of performing such a comprehensive serial CMR study (including 1 examination immediately on reperfusion) and histological validation in patients. The time course of tissue changes in the post-I/R myocardium in pigs, albeit slightly shorter, is similar to that in humans (21). Moreover, the process of infarct healing is qualitatively similar in both species, in that infarct healing involves a sequential infiltration of neutrophils and macrophages, removal of necrotic myocytes, formation of granulation tissue, and collagen deposition. The present study does not provide information on the localization of the elevated water content within the myocardial tissue; indeed, there are no reliable methods to distinguish between intracellular and interstitial increases in myocardial water content (25).

In this study, the regions of interest for T2 relaxation time quantification included the entire wall thickness and were individually adjusted by hand to carefully avoid the right and left ventricular cavities. Regions of interest might therefore include different myocardial states (e.g., hemorrhage or microvascular obstruction). We took this approach to mimic the histological evaluation of water content, which assesses the entire wall thickness. We believe that the possible inclusion of different myocardial states had little effect on the results relating to the pathophysiology of bimodal edema given that this biological process has also been measured by histological means in the present study. However, it might have had some influence on the differences in absolute T2 relaxation times between our study and others taking a different approach to region of interest selection.

CONCLUSIONS

The present study elucidates the pathophysiology underlying the bimodal edematous reaction after I/R. The initial wave of edema, appearing abruptly on reperfusion and dissipating at 24 h, is directly secondary to the reperfusion process itself. The deferred wave of edema, appearing progressively days after I/R and peaking around day 7, is mainly caused by tissue healing processes.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: After ischemic injury, the course of myocardial edema is bimodal. Acute reperfusion is responsible for the initial acute edematous reaction, whereas healing is implicated in the later wave of edema.

TRANSLATIONAL OUTLOOK: Additional research is needed to develop pharmacological or other interventions that target pathways implicated in the initial or deferred waves of edema after post-ischemic reperfusion, and evaluate the efficacy of these strategies to accelerate cardiac recovery and improve clinical outcomes of patients with acute myocardial infarction.

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APPENDIX For a supplemental Methods section, please see the online version of this article.