Effect of Hypercholesterolemia on Myocardial Necrosis and Apoptosis in the Setting of Ischemia-Reperfusion

Robert M. Osipov, MD; Cesario Bianchi, MD, PhD; Jun Feng, MD, PhD; Richard T. Clements, PhD; Yuhong Liu, MD; Michael P. Robich, MD; Hilary P. Glazer; Neel R. Sodha, MD; Frank W. Sellke, MD, FAHA, FACS

Background—Hypercholesterolemia is prevalent in patients who experience myocardial ischemia-reperfusion injury (IR). We investigate the impact of dietary-induced hypercholesterolemia on the myocardium in the setting of acute IR.

Methods and Results—In normocholesterolemic (NC, n=7) and hypercholesterolemic (HC, n=7) Yucatan male pigs, the left anterior descending coronary artery was occluded for 60 minutes, followed by reperfusion for 120 minutes. Hemodynamic values were recorded, and TTC staining was used to assess necrosis. Oxidative stress was measured. Specific cell death and survival signaling pathways were assessed by Western blot and TUNEL staining. Infarct size was 45% greater in HC versus NC (42% versus 61%, P<0.05), whereas the area at risk (AAR) was similar in both groups (P=0.61). Whereas global LV function (+dP/dt, P<0.05) was higher during entire period of IR in HC versus NC, regional function deteriorated more following reperfusion in HC (P<0.05). Ischemia increased indices of myocardial oxidative stress such as protein oxidation (P<0.05), lipid peroxidation (P<0.05), and nitrotyrosylation in HC versus NC, as well as the expression of phospho-eNOS (P<0.05). The expression of myeloperoxidase, p38 MAPK, and phospho-p38 MAPK was higher in HC versus NC (all P<0.05). Ischemia caused higher expression of the proapoptotic protein PARP (P<0.05), and lower expression of the prosurvival proteins Bcl2 (P<0.05), phospho-Akt, (P<0.05), and phospho-PKCe (P<0.05) in the HC versus NC. TUNEL-positive cell count was 3.8-fold (P<0.05) higher in the AAR of HC versus NC.

Conclusions—This study demonstrates that experimental hypercholesterolemia is associated with increased myocardial oxidative stress and inflammation, attenuation of cell survival pathways, and induction of apoptosis in the ischemic territory, which together may account for the expansion of myocardial necrosis in the setting of acute IR. (Circulation. 2009;120[suppl 1]:S22–S30.)

Key Words: apoptosis ■ hypercholesterolemia ■ ischemia ■ myocardial infarction ■ risk factors

Coronary artery disease (CAD) and acute myocardial infarction (AMI) are the largest cause of death in the world today. Prompt reperfusion via angioplasty, thrombolytic therapy, or coronary artery bypass surgery to salvage the ischemic myocardium is considered the optimal management for AMI.1

In recent years many studies have shown that hypercholesterolemia is not only detrimental for CAD progression but is also a risk factor for higher mortality and poor left ventricular systolic function in patients after AMI.2,3 This suggests that hypercholesterolemia may adversely influence the evolution of AMI even after patency of an occluded coronary artery is successfully reestablished. However, the mechanisms responsible for enhanced cardiomyocyte injury after ischemia-reperfusion (IR) in patients with hypercholesterolemia are poorly understood.

To date, myocardial IR injury studies conducted in hypercholesterolemic animals (rabbits and rodents) have yielded varied and controversial findings. Although some researchers reported that diet-induced hypercholesterolemia enhances myocardial injury by increasing oxidative stress,4 upregulation of inflammation,5 inhibition of nitric oxide synthesis,6 vascular obstruction,7 and increased cardiomyocyte apoptosis,8 others have found no additional harm after reperfusion injury8,9 or have shown that hypercholesterolemia improves myocardial function and may even be cardioprotective.10 In past decades the effects of hypercholesterolemia in a porcine model of acute myocardial IR were addressed in a single study which examined the impact of preconditioning and postconditioning on the myocardium.11 In addition, most clinical studies are confounded by multiple cardiovascular risk factors other than hypercholesterolemia. In light of the
clinical implications, these controversies deserve further investigation. In this study, we investigated the effects of hypercholesterolemia alone on the nonischemic and ischemic myocardium in a porcine model of acute IR.

Materials and Methods

Animals
Intact Yucatan male mini-swine (20 weeks old; Sinclair Research Center, Inc, Columbia, Mo) were divided into 2 groups: normocholesterolemic (NC, n=7), fed with normal chow (Sin. Purina) and hypercholesterolemic (HC, n=7), fed a high-fat/high-cholesterol chow (Sinclair Research Center, Inc) from age 16 to 20 weeks. Animal weight was evenly matched between the 2 groups (NC 22.4±1.3 kg versus HC 21.4±0.9 kg, P=0.5).

Animals were housed individually and provided with laboratory chow and water. All experiments were approved by the BIDMC IACUC and conformed to the U.S. National Institutes of Health guidelines regulating the care and use of laboratory animals (NIH publication 5377-3, 1996).

Surgical Protocol
Pigs were sedated with Telazol and weighed before induction of anesthesia (4 mg/kg, IM) followed by endotracheal intubation and ventilation with a volume-cycled ventilator (North American Dragger). Anesthesia was established and maintained with 2.0% isoflurane (Ulltane; Abbott Laboratories). The right common femoral artery was used for arterial blood sampling and continuous monitoring of intraarterial blood pressure (Millar Instruments). Arterial blood gas analysis, hematocrit, and core temperature were assessed every 30 minutes. Before left anterior descending (LAD) coronary artery occlusion, each animal received a 1-liter bolus of Lactated Ringer solution followed by continuous infusion (15 mL/kg/h). A phenylephrine drip (0.25 µg/kg/min) to prevent hypotension induced by isoflurane, heparin (80 U/kg bolus), and lidocaine (1.5 mg/kg) to control ventricular dysrhythmia, were administered. A median sternotomy was performed. A catheter-tipped manometer (Millar Instruments) was introduced through the apex of the left ventricle (LV) to record LV pressure. The LAD was occluded 3 mm distal to the origin of the second diagonal branch by a Rommel tourniquet. After 60 minutes of ischemia the tourniquet was released and the myocardium was reperfused for 120 minutes. LAD flow was monitored by a transonic Doppler probe. At the end of reperfusion the LAD was reestablished, and monastryl blue pigment (Engelhard Corp) was injected into the aortic root to demarcate the area at risk (AAR). The heart was rapidly excised and sliced into 1-mm-thick slices perpendicular to the LAD up to the area of ligation. Tissue from the second slice was used in molecular and coronary microvascular reactivity studies. The remaining tissue was incubated in 1% triphenyl tetrazolium chloride (TTC; Sigma Chemical Co) solution for 30 minutes, and infarct size was assessed as previously described. Ventricular dysrhythmia (ventricular fibrillation or pulseless ventricular tachycardia) events were recorded and treated with immediate electric cardioversion with 20 to 50 J.

Measurement of Global and Regional Myocardial Function
Indices of global and regional myocardial function were monitored and recorded during the entire experiment: mean arterial pressure (MAP); developed LV pressure (DLPV); positive (+dP/dt) and negative (−dP/dt) first derivatives of LV pressure; longitudinal and horizontal segmental shortening (SS) in the AAR, for 10 sequential beats at baseline and then every 30 minutes (Occlusion-O1 30 minutes and O2 60 minutes; Reperfusion- R1 30, R2 60, R3 90, and R4 120 minutes) using a Sonometrics system (Sonometrics Corp) as previously described. Tissue from the second slice was used in molecular and coronary microvascular reactivity studies. The remaining tissue was incubated in 1% triphenyl tetrazolium chloride (TTC; Sigma Chemical Co) solution for 30 minutes, and infarct size was assessed as previously described. Ventricular dysrhythmia (ventricular fibrillation or pulseless ventricular tachycardia) events were recorded and treated with immediate electric cardioversion with 20 to 50 J.

Quantification of Myocardial Infarct Size
Heart slices (1 cm thick) were incubated in 1% TTC solution as previously described. Briefly, necrotic (pale), the AAR (bright red), and nonischemic portion of the heart specimens (purple) were photographed and measured. The size of the AAR and percent necrosis in the AAR was calculated in each individual slice by planimetry (Image J 1.4, NIH) using the following equations: [(LV necrosis surface+noninfarct AAR surface area)/LV total surface×100] and [(LV necrosis surface area/LV AAR surface area)×100].

Coronary Microvascular Reactivity Studies
Coronary microvascular reactivity was examined in the ischemic territory as previously described. Briefly, coronary arterioles (80 to 130 µm) were dissected with a 40× microscope. Microvessels were mounted on dual glass micropipettes and examined in a pressurized isolated microvessel chamber. Adenosine diphosphate (ADP, 1 µmol/L to 100 µmol/L), substance P (0.1 pmol/L to 10 nmol/L), A23187 (1 µmol/L to 10 µmol/L), and sodium nitroprusside (SNP, 1 nmol/L to 100 µmol/L) were applied extraluminal after precontraction by 20% to 50% of the baseline diameter with the thromboxane A2 analog U46619 (0.1 to 1 µmol/L).

Western Blotting
Myocardial samples were homogenized in RIPA buffer (Boston Bioproducts) and protein concentration was determined by BCA assay (Pierce). Equal amounts of lysate were subjected to SDS-Page and immunoblotting as previously described. Primary antibodies were used according to the manufacturer’s recommendation. Levels of Bcl-2, caspase-3, cleaved caspase-3 (Asp175), PARP, cleaved PARP (Asp214), Akt and phospho-Akt (Ser473), BNip3, eNOS and phospho-eNOS (Ser1177), PKCe and phospho-pan-PKC (epi, Ser729), p38 MAPK and phospho-p38 (Thr180/Tyr182; Cell Signaling Technology), Erk 1/2 and phospho-Erk 1/2 (Thr185/Tyr187; Invitrogen), myeloperoxidase (Dako) were assessed. Immunoblotting for vinculin was used to confirm equal protein loading (Santa Cruz Biotechnology, Inc). Band intensities were normalized to Ponceau staining intensity. Data are presented as mean±SEM in arbitrary density units (AU).

Protein Oxidation and Lipid Peroxidation
Protein oxidation (OxyBlot, Chemicon International, Inc) and lipid peroxidation (Peroxidation colorimetric microplate assay, Oxford Biomedical Research) in myocardial samples were measured according to the manufacturer’s recommendation. Data analyzed using Student t test.

Nitrotyrosine Staining
Formalin-fixed paraffin embedded heart tissue was processed as previously described (4 slides from each group, nonischemic and ischemic territories) and was incubated overnight at 4°C with monoclonal nitrotyrosine antibody (USBiological). Data analyzed using Student t test.

TUNEL Staining
Apoptotic cells were identified using the ApopTag detection kit according to manufacturer’s specifications (Chemicon, Inc). At least 1 cm² of tissue from the AAR was analyzed from each animal (4 per group). The number of TUNEL-positive cardiomyocytes is expressed as positive cells per cm² Data analyzed using Student t test.

Serum Lipid Profile
Serum lipid profile was measured by Siemens Advia 1200 chemistry system (Accell, Inc). Data are presented as mean±SEM and were analyzed using Student t test.

Serum Lipid Profile
Serum lipid profile was measured by Siemens Advia 1200 chemistry system (Accell, Inc). Data are presented as mean±SEM and were analyzed using Student t test.

Statistical Analysis
Functional and microvascular reactivity data were analyzed using repeated measures ANOVA. Infarct size and Western blot densitometry were analyzed using unpaired Student t test. The incidence of VT/VF was analyzed using χ² test. Western blot data between the
HC versus NC groups were compared within the same myocardial territory (either nonischemic or ischemic territories). Data are reported as mean±SEM, and $P<0.05$ was considered significant (Systat).

**Results**

**Serum Lipid Profile**
Levels of total serum cholesterol (13.8±1.8 versus 1.5±0.3 mmol/L, $P<0.05$), D-LDL (5.5±0.7 versus 0.5±0.04 mmol/L, $P<0.05$), and D-HDL (1.8±0.1 versus 0.5±0.05 mmol/L, $P<0.05$) were significantly higher in pigs fed a high-fat/high-cholesterol chow compared to those fed a normal chow. The level of triglycerides (0.3±0.03 versus 0.3±0.1 mmol/L, $P=0.67$) was not different between groups.

**Myocardial Infarct Size**
Although the AAR was similar between groups (Figure 1A), the area of necrosis was 45% greater in the HC versus NC (Figure 1B). Transmural necrosis involving the LV wall and septum was present in 7/7 HC pigs but only in 4/7 NC pigs (Figure 1C and 1D, arrows).

**Arterial Blood Gas, Hematocrit, Core Temperature, and Hemodynamic Parameters**
There were no differences among groups at any time point with respect to arterial blood gas, hematocrit, and core temperature. Heart rate ($P=0.5$) and LAD flow ($P=0.4$) were similar between groups at all recorded time points. LAD blood flow

---

**Figure 1.** Percent of LV wall area at the risk (AAR; $P=0.6$; A) and necrosis of AAR in the NC and HC animals (B). Shown are representative images of the myocardium after TTC staining in NC (C) and HC (D). Black arrow pointed to the nonischemic (purple), white arrow to AAR (bright red), and gray arrow to the necrotic area (pale). Data presented as mean±SEM and compare NC (n=7) and HC (n=7) groups. $^*P<0.05$.

**Figure 2.** Global and regional left ventricular function: Mean arterial pressure (MAP, $P=0.08$; A), developed left ventricular pressure (DLVP, $P=0.08$; B), positive first-order derivative of ventricular pressure (+dP/dt; C), negative first-order derivative of ventricular pressure (−dP/dt, $P=0.08$; D), percent of segmental shortening (SS) in horizontal axis (E), and percent of SS in longitudinal axis (P=0.4; F). Baseline- Pre, 30 and 60 minutes into ischemia/LAD occlusion- O1/O2, R1–30, R2–60, R3–90, and R4–120 minutes into reperfusion. Data presented as mean±SEM and compare NC (n=7) and HC (n=7) groups. $^*P<0.05$. 
increased 2.8-fold during the first minute of reperfusion (hyperemic response) and then steadily declined to reach the baseline levels by the end of the experiment in both groups. There was a trend toward higher mean arterial pressure (MAP) and higher developed left ventricular pressure (DLVP) in the HC group (Figure 2A and 2B).

Global and Regional Myocardial Function
Global systolic LV function, as determined by +dP/dt, was greater in the HC group versus NC group. There was no significant difference in −dP/dt between groups (Figure 2C and 2D). Segmental shortening (SS) in the horizontal axis was significantly decreased in HC animals beginning 1 hour.

Figure 3. Microvascular responses to endothelium-dependent agents: adenosine diphosphate (ADP, \( P = 0.7; A \)), substance P (\( P = 0.8; B \)), and \( \text{Ca}^{2+} \) ionophore A23187 (\( P = 0.2; C \)), microvascular response to endothelium-independent agent sodium nitroprusside (SNP, \( P = 0.2 \)). Data presented as percent of relaxation and compare NC (n=7) and HC (n=7) groups.

Figure 4. Myocardial oxidative stress indices: protein oxidation (1.7-fold increased in the HC versus NC; A), lipid peroxidation (1.4-fold increased in the HC versus NC; B) in the ischemic territory, and nitrotyrosine staining (C). The HC samples have more intense nitrotyrosine staining (brown) in the ischemic territory. Yellow arrow denotes monastryl blue pigment in the capillary in the nonischemic territory (original magnification 20×). D, Expression of phospho-eNOS(Ser1177) in the ischemic territory. Representative Western blots (insets, 3 samples from each group) are shown. Data compare NC (n=7) and HC (n=7) groups. *\( P < 0.05 \).
into reperfusion (Figure 2E), whereas no difference was observed in the longitudinal axis (Figure 2F).

**Incidence of VF/VT**
VF/VT requiring cardioversion tended to be higher in the HC group versus NC group. (5 of 7 versus 2 of 7, $P=0.37$). Generally, VF/VT appeared earlier in the ischemic period in the HC group compared to the NC group, in which it appeared early into reperfusion. All ventricular dysrhythmias were successfully terminated with electric cardioversion.

**Coronary Microvessel Function**
There were no significant differences between groups in endothelial-dependent and -independent relaxation of microvessels taken from the ischemic territory (Figure 3A through 3D).

**Myocardial Oxidative Stress Indicators**
Protein oxidation (Figure 4A) and lipid peroxidation (Figure 4B) were higher in the HC group versus NC group in the ischemic territory only. Nitrotyrosine staining in the ischemic territory was also more intense in the HC group (Figure 4C) but not in the nonischemic territory. Although the expression of eNOS in the ischemic territory was similar between groups (NC 0.01 ± 0.002 versus 0.01 ± 0.002 AU, $P=0.1$), the expression of phospho-eNOS (Ser1177) was higher in the HC group (Figure 4D).

**Profile of Proinflammatory Proteins**
Myeloperoxidase (MPO) expression was higher in the HC group versus NC group in the nonischemic myocardium. There was a trend of lower MPO expression ($P=0.097$) in the ischemic territory (Figure 5A). The expression of p38 was higher in the HC group versus NC group in both territories (Figure 5B). The expression of phospho-p38(Thr180/Tyr182) was higher in the HC group in the nonischemic territory (Figure 5C), whereas no bands of phospho-p38 was seen in the ischemic territory in either group.

**Profile of Proapoptotic Signaling Proteins**
Total PARP expression was higher in the HC group versus NC group in both territories (Figure 6A). However, cleaved PARP(Asp214) was lower in the HC group (nonischemic and ischemic territories; Figure 6B). The expression of total caspase-3 and its active form, cleaved caspase-3(Asp175), was not different between 2 groups in both territories (Figure 6C and 6D). The expression of BNip3 was higher in the HC group in the nonischemic territory, but was similar in the ischemic territory (Figure 6E).

**Profile of Prosurvival Signaling Proteins**
The expression of antiapoptotic Bcl-2 was lower in the HC group in both territories (Figure 6F). Total Akt expression was lower in the HC group compared to the NC group in the ischemic territory. The expression of phospho-Akt(Ser473) was higher in the nonischemic territory, but lower in the ischemic territory in the HC group (Figure 7A and 7B). The expression of total Erk 1/2 was higher in the HC group in both territories (Figure 7C). The expression of phospho-Erk 1/2(Thr185/Tyr187) was higher in the nonischemic territory in the HC group, but similar in the ischemic territory (Figure 7D). The expression of PKCe was not different between groups in either the nonischemic or the ischemic territories, whereas the expression of phospho-PKCe(Ser729) was higher in the nonischemic territory and lower in the ischemic territory in the HC group (Figure 7E and 7F). All Western blot data from the nonischemic and the ischemic myocardium are summarized in supplemental Tables I and II, respectively (see Online Supplemental Data).

**TUNEL Staining for Apoptosis**
The apoptotic cell counts in the AAR was 3.8-fold higher in the HC compare to NC (59.5 ± 14.9 cells/cm² versus 15.7 ± 4.3 cells/cm², $P<0.05$). Most apoptotic cells were cardiomyocytes and mainly located near the necrotic area.

**Discussion**
Despite many advances in basic science and treatment options, the majority of patients with CAD have elevated cholesterol levels. Clinical studies to assess the effect of hypercholesterolemia on myocardial IR have been conducted in the setting of multiple coexisting risk factors, whereas in rabbit and rodent IR models, the effect of hypercholesterolemia has yielded varying and controversial results. The
The principal finding of this study was that hypercholesterolemia alone increased myocardial necrosis by 45% over what was observed in the NC animals. This may be explained by increased inflammation and oxidative stress in the setting of hypercholesterolemia, as has been reported in previous studies.17 This is corroborated in our study by increased indices of oxidative stress in the ischemic myocardium such as protein oxidation, lipid peroxidation, and nitrotyrosylation during IR. Our findings accentuate the importance of antioxidant therapy18 as a potential strategy to protect the myocardium before reestablishing reperfusion in hypercholesterolemic patients.13,19

A novel finding in this study was that hypercholesterolemia increased baseline indices of myocardial function. The higher peak +dP/dt, which is a good index of ventricular performance not influenced by afterload, wall motion abnormalities, or variations in ventricular anatomy and morphology, demonstrated that hypercholesterolemia had a positive inotropic effect. The dietary abundance of short-chain fatty acids, which are a primary energy source for cardiomyocytes,20 may partially explain this hyperdynamic function seen in the HC group. This also may lead to increased myocardial oxygen demand and intensify myocardial injury during hypoxia. In contrast, a previous study showed no differences with respect to myocardial function and myocardial necrosis size between normocholesterolemic control and hypercholesterolemic control pigs in the setting of 3 hours of ischemia and 2 hours of reperfusion.11 Another important finding was that myocardial necrosis size was high in both groups, above 98%.11 In our study after 1 hour of ischemia and 2 hours of reperfusion only 61% of AAR becomes necrotic in the hypercholesterolemic animals versus 42% in the normocholesterolemic. This finding from our study accentuates the importance of early reperfusion in patients with hypercholesterolemia after AMI. The majority of previously reported data from small animal models demonstrated that hypercholesterolemia has either a negative inotropic effect or no difference on basal contractile state.21,22 Moreover, the hyperdynamic pattern and extent of necrosis seen in the HC was also associated with increased and an earlier onset of VF/VT during ischemia. Though this finding warrants further investigation, it may support the need for aggressive prevention of ventricular arrhythmia during AMI in hypercholesterolemic patients.

Surprisingly, there were no differences in microvascular relaxation between control and hypercholesterolemic pigs. Numerous animal and clinical models have reported impaired endothelium-dependent and -independent relaxation in the setting of hypercholesterolemia and have implied that this may predispose to increased IR injury.23 In our study, coronary microvascular relaxation was only examined in the ischemic territory. IR is well known to result in significant impairments in coronary microvascular relaxation.24 Therefore, it is likely that the microvascular injury sustained by IR was sufficient to veil any functional differences in relaxation attributable to the addition of hypercholesterolemia.

The detrimental role of oxidative stress on the myocardium in the setting of hypercholesterolemia has long been established in the literature.14,25,4 However, the magnitude of damage conferred by oxidative stress on the ischemic myo-
cardium during IR injury is not well studied. Although some studies have shown that increased oxidative stress is associated with higher expression of MPO in ischemic myocardium,5 in this study increased oxidative stress in the ischemic myocardium was associated with lower expression of MPO after IR. In addition, previous reports conducted in rabbit models have demonstrated that hypercholesterolemia is associated with lower NO production attributable to lower phosphorylation of eNOS in the heart.26 We found that hypercholesterolemia indeed induces phospho-eNOS in the ischemic myocardium. Coupled with increased nitrotyrosine staining, this suggests that hypercholesterolemia-induced oxidant formation and eNOS activation may promote peroxynitrite formation.27,28 In addition to hypercholesterolemia-induced oxidative stress, our previous work highlighted a role for increased oxidative stress in normocholesterolemic swine treated with high-dose atorvastatin.29 If, indeed, hypercholesterolemia increases infarct size via enhanced oxidative stress pathway, it would be of value to evaluate these end points in a statin-treated hypercholesterolemia model with acute IR. In addition, Bcl-2 plays a crucial role in preventing apoptosis, by blocking the activation of executioner caspases 3, 6, and 7,31 and the release of mitochondrial cytochrome c.32 Although IR-related cardiomyocyte death is traditionally assumed to be necrotic, this and other studies have shown that a substantial proportion of cardiomyocytes deaths are apoptotic.33

Finally, we found a decrease in the expression of most prosurvival proteins in the HC group in the myocardial territory subjected to IR. Total Akt, phospho-Akt, and phospho-PKCe were all significantly decreased in the ischemic myocardium in the HC group compared to the NC group. When activated Akt serves many prosurvival functions in cardiomyocytes, including the prevention of apoptosis,34 and the decreased total expression and phosphorylation at serine 473 residue may also diminish cardiomyocyte survival after IR in the setting of hypercholesterolemia. PKCe has been shown to have an important role in postconditioning cardioprotection via phosphorylation of Akt and Erk 1/2 and activation of mitochondrial K\textsubscript{ATP} channel.35,36 This may explain the previously reported finding that preconditioning and

Figure 7. The expression of prosurvival proteins: Total Akt (A), Phospho-Akt (Ser473; B), total Erk 1/2 (C), phospho-Erk 1/2 (Thr185/Tyr187; D), total PKCe (E), phospho-PKCe and vinculin (F) as a loading control in both the nonischemic and ischemic territories. Insets show representative Western blots for 3 samples from each group. Phospho-PKCe (Ser729) was identified with a phospho-pan-PKC antibody as a band at the same molecular weight as total PKCe. Data presented as mean±SEM in arbitrary density units (AU) and compare NC (n=7) and HC (n=7) groups. *P<0.05.
postconditioning fail to reduce the area of myocardial necrosis in the hypercholesterolemic pigs in the setting of IR injury. Interestingly, the nonischemic territory displayed a very different signaling profile, with hypercholesterolemia inducing higher expression of phospho-Akt, phospho-Erk 1/2, and phospho-PKCe.

In conclusion, hypercholesterolemia alone increased the susceptibility of the myocardium to infarction in the setting of acute IR. The hyperdynamic state, increased myocardial oxidative stress and inflammation, attenuation of cell-survival pathways, and induction of apoptosis may account for the further expansion of myocardial necrosis and the deterioration of regional cardiac function during IR in the setting of hypercholesterolemia.

Limitations
While providing important functional and molecular data about the role of hypercholesterolemia in acute IR, this study has several limitations. First, it should be noted that all of the control tissue was from the nonischemic territory, however it cannot be ruled out that ischemic insult may affect signaling in remote heart, including potential inflammatory insult and release of soluble mediators from the infarcted myocardium. Second, our time course for tissue harvest (3 hours after the onset of ischemia) could not account for the long-term effects of hypercholesterolemia on myocardial function and apoptosis. Finally, only a short-term diet induction of hypercholesterolemia was performed. Although this model is adept at examining changes in AMI attributable to hypercholesterolemia alone, the chronic secondary effects of hypercholesterolemia may not be developed at this time point. Whether this is similar to long-standing endogenous or exogenous hypercholesterolemia in patients needs to be further evaluated.

Acknowledgments
We thank BIDMC Animal Research Facility staff for their efforts.

Sources of Funding
Funding for this project was provided to F.W.S. by NHLBI (RO1 HL46716, HL69024, and HL85647), OrthoLogic Corp, and NIH T32-HL076130 (R.M.O, M.P.R.) and the Irving Bard Memorial Fellowship (R.M.O, R.T.C, M.P.R.).

Disclosures
F.W.S. has research support from Ikaria (Clinton, NJ) and Orthologic (Tempe, Ariz), and he is a consultant for Novo Nordisk (Princeton, NJ), Pfizer (New York, NY), and Cubist Pharmaceuticals (Lexington, Mass).

References


Effect of Hypercholesterolemia on Myocardial Necrosis and Apoptosis in the Setting of Ischemia-Reperfusion


_Circulation_. 2009;120:S22-S30
doi: 10.1161/CIRCULATIONAHA.108.842724
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/120/11_suppl_1/S22

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2009/09/16/120.11_suppl_1.S22.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL
Table 1. Expression of Proteins in the Non-ischemic Territory

<table>
<thead>
<tr>
<th>Proteins</th>
<th>NC</th>
<th>HC</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP</td>
<td>0.02±0.003</td>
<td>0.05±0.009</td>
<td>0.006</td>
</tr>
<tr>
<td>Cleaved PARP</td>
<td>0.06±0.01</td>
<td>0.02±0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Caspase 3</td>
<td>0.03±0.003</td>
<td>0.03±0.008</td>
<td>0.9</td>
</tr>
<tr>
<td>Cleaved caspase 3</td>
<td>0.02±0.004</td>
<td>0.04±0.01</td>
<td>0.3</td>
</tr>
<tr>
<td>Bnip-3</td>
<td>0.04±0.01</td>
<td>0.1±0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bcl 2</td>
<td>0.03±0.003</td>
<td>0.02±0.003</td>
<td>0.03</td>
</tr>
<tr>
<td>Akt</td>
<td>0.09±0.004</td>
<td>0.09±0.003</td>
<td>0.2</td>
</tr>
<tr>
<td>Phospho-Akt</td>
<td>0.03±0.004</td>
<td>0.07±0.005</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Erk 1/2</td>
<td>0.1±0.01</td>
<td>0.2±0.01</td>
<td>0.002</td>
</tr>
<tr>
<td>Phospho-Erk 1/2</td>
<td>0.14±0.01</td>
<td>0.23±0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PKCε</td>
<td>0.12±0.02</td>
<td>0.13±0.01</td>
<td>0.6</td>
</tr>
<tr>
<td>Phospho-PKCε</td>
<td>0.05±0.01</td>
<td>0.13±0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>0.03±0.01</td>
<td>0.09±0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>p38</td>
<td>0.004±0.001</td>
<td>0.067±0.013</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phospho-p38</td>
<td>0.06±0.01</td>
<td>0.96±0.01</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Data presented as mean±SEM in arbitrary units.
Unpaired Student’s t-test.
Table 2. Expression of Proteins in the Ischemic Territory

<table>
<thead>
<tr>
<th>Proteins</th>
<th>NC</th>
<th>HC</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS</td>
<td>0.01±0.002</td>
<td>0.01±0.002</td>
<td>0.9</td>
</tr>
<tr>
<td>Phospho-eNOS</td>
<td>0.03±0.01</td>
<td>0.09±0.02</td>
<td>0.005</td>
</tr>
<tr>
<td>PARP</td>
<td>0.02±0.006</td>
<td>0.06±0.01</td>
<td>0.004</td>
</tr>
<tr>
<td>Cleaved PARP</td>
<td>0.07±0.01</td>
<td>0.02±0.01</td>
<td>0.007</td>
</tr>
<tr>
<td>Caspase 3</td>
<td>0.03±0.01</td>
<td>0.04±0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>Cleaved caspase 3</td>
<td>0.01±0.004</td>
<td>0.02±0.01</td>
<td>0.4</td>
</tr>
<tr>
<td>Bnip-3</td>
<td>0.06±0.01</td>
<td>0.05±0.01</td>
<td>0.8</td>
</tr>
<tr>
<td>Bcl 2</td>
<td>0.05±0.01</td>
<td>0.02±0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Akt</td>
<td>0.08±0.01</td>
<td>0.06±0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Phospho-Akt</td>
<td>0.07±0.008</td>
<td>0.05±0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>Erk 1/2</td>
<td>0.06±0.01</td>
<td>0.08±0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Phospho-Erk 1/2</td>
<td>0.12±0.03</td>
<td>0.08±0.02</td>
<td>0.3</td>
</tr>
<tr>
<td>PKCe</td>
<td>0.1±0.02</td>
<td>0.1±0.02</td>
<td>0.9</td>
</tr>
<tr>
<td>Phospho-PKCε</td>
<td>0.12±0.03</td>
<td>0.05±0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>0.12±0.03</td>
<td>0.06±0.01</td>
<td>0.097</td>
</tr>
<tr>
<td>p38</td>
<td>0.003±0.001</td>
<td>0.06±0.02</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Data presented as mean±SEM in arbitrary units.
Unpaired Student's t-test.