Ultrastructural Evidence of Increased Tolerance of Hibernating Myocardium to Cardioplegic Ischemia-Reperfusion Injury

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OBJECTIVES
The goal of this study was to investigate the effects of ischemia-reperfusion on myocardial ultrastructure in patients with and without hibernating myocardium.

BACKGROUND
It is generally accepted that chronically dysfunctional, hibernating myocardium may remain nonetheless viable for a long time. It has been postulated that hibernating myocytes may survive, despite being subtended by a severe coronary artery stenosis, as they might be less susceptible to ischemic insults. However, whether hibernating myocardium is indeed more resistant to ischemia has never been investigated.

METHODS
Myocardial biopsies were taken before cardiac arrest and after reperfusion from the anterior wall of the left ventricle in patients undergoing coronary artery bypass surgery, divided according to presence (n = 7) or absence (n = 7) of hibernating myocardium. Ultrastructural changes were studied by electron microscopy. Because ischemia-reperfusion injury is related to oxidative stress, we also evaluated coronary sinus concentration of the antioxidants alpha-tocopherol, beta-carotene, and ubiquinol, and of lipid peroxidation products pre-ischemia and after reperfusion.

RESULTS
Both groups were similar with respect to length of ischemia and changes in the various indexes of oxidative stress. In normally contracting myocardium, ischemia/reperfusion induced moderate overall ultrastructural changes, and marked alterations at the mitochondrial level. In contrast, post-reperfusion biopsies of hibernating myocardium displayed only minor overall ultrastructural changes, and scored significantly better on mitochondrial damage.

CONCLUSIONS
Despite similar severity of ischemia/reperfusion, hibernating myocardium showed significantly less ultrastructural evidence of cell injury compared with normally contracting myocardium. These data indicate that human hibernating myocardium is intrinsically more resistant to ischemia/reperfusion injury. (J Am Coll Cardiol 2004;43:2329–36) © 2004 by the American College of Cardiology Foundation

Rahimtoola (1) described hibernation as a state of persistently impaired contractile function of myocardium subtended by severely stenotic coronary arteries that can be restored to normal function upon revascularization. Since then, a number of studies have sought to address the apparent paradox of dysfunctional myocardium showing nevertheless preserved viability and ultimate potential for recovery (2–7). Carefully conducted ultrastructural investigations in patients subjected to cardiac surgery have provided evidence that hibernating myocardium shows very distinctive morphology, with loss of sarcomeres and myofibrils, which have been interpreted as indication of “dedifferentiation” of myocytes toward a non-contractile phenotype (8–16). These features would explain both the loss of contractile function and its slow recovery after revascularization.

Hibernating myocardium also shows another seemingly paradoxical characteristic. It is generally assumed that hibernating myocytes may remain viable for months or longer. To explain preserved viability in myocytes distal to a critical coronary artery stenosis, and, hence, possibly subjected to frequent episodes of ischemia, it has been hypothesized that the non-contractile phenotype may render hibernating myocytes more tolerant to ischemia (5,13,17). However, this crucial issue has never been directly investigated.

In the present study, we evaluated the ultrastructural alterations brought about by ischemia and reperfusion on human hibernating myocardium, and compared them to the effects on normally contracting myocardium. Electron microscopy was performed on cardiac biopsies taken before cardiac arrest and after reperfusion in patients undergoing...
coronary artery bypass graft surgery. Because oxidative stress may play an important role in myocardial ischemia/reperfusion injury (4,18,19), we also assessed changes in coronary sinus concentrations of soluble antioxidants and products of lipid peroxidation.

**METHODS**

**Patient selection.** Patients scheduled for elective coronary artery bypass surgery were included. Patients had to have: 1) a history of chronic (>3 months) exercise-induced angina; 2) angiographic evidence of the left anterior descending (LAD) coronary artery requiring surgical revascularization; 3) persistent R waves in the anterior leads of the electrocardiogram (ECG); 4) no evidence of myocardial scar in the anterior wall of the left ventricle (LV) upon inspection at surgery. Patients with unstable angina or valvular disease were excluded. Patients treated with carvedilol, angiotensin-converting enzyme inhibitors, or antioxidant drugs were also excluded. Written, informed consent was obtained from each patient. The study protocol was approved and controlled by the ethics committee of the Argentine Society of Cardiology.

In addition to fulfilling the above inclusion criteria, patients were selected based on evidence of hibernating myocardium, according to: 1) presence of akinetic areas in the anterior wall of the LV (by Tc99m-gated equilibrium radionuclide angiography and LV angiography); and 2) evidence (by Tl201 scintigraphy) of reduced perfusion at rest. Radionuclide studies were performed with a gamma-camera interfaced to a dedicated computer using ECG gating. Sixteen frames per R-R interval were taken for assessment of ventricular wall motion and ejection fraction at rest.

**Radioisotopic studies. GATED EQUILIBRIUM RADIONUCLIDE ANGIOGRAPHY.** Autologous red blood cells labeled with Tc99m were administered to the patients. Acquisitions were performed with a gamma-camera interfaced to a dedicated computer using ECG gating. Radioisotopic studies were performed four to six days before surgery.

**MYOCARDIAL PERFUSION IMAGING.** Thallium201 was used as perfusion tracer. Images were recorded in anterior, left-anterior oblique, and left-lateral views. Images were acquired with a gamma-camera equipped with a high-resolution parallel-hole collimator. Images were evaluated for areas of decreased Tl201 uptake and for changes in regional count density.

**Surgical technique.** Anesthesia protocols were similar in all patients. After sternotomy, cardiopulmonary bypass was instituted under mild hypothermia (32°C to 33°C). Biopsies and blood samples were taken before inducing cardiac arrest, and 10 min after reperfusion. Full-thickness biopsies were obtained from the anterior wall of the LV near the apex (Travenol Tru-Cut needle, Baxter Corp., Valencia, California) (20–23). Direct inspection confirmed that no scar tissue was present in the territory subtended by the LAD coronary artery. Biopsies were immediately immersed in cold glutaraldehyde (3% w/v in 0.1 mol/l phosphate buffer; pH 7.4). Blood (10 ml) was obtained from a coronary sinus perfusion cannula introduced through a purse-string suture in the right atrium and manually guided into the coronary sinus. Blood samples were immediately centrifuged, the plasma frozen and stored for biochemical assays.

**CARDIAC ARREST.** Immediately after aortic cross-clamping, patients received warm (33°C to 35°C) blood containing 40 mEq/l potassium, hematocrit ranging between 20% to 25%. Approximately 300 ml were initially administered into the aortic root at a pressure of 70 to 90 mm Hg; an additional 200 ml were administered into the coronary sinus at a pressure <40 mm Hg. Every 20 min an additional 300 ml of the same infusion was given in the same fashion. Five minutes before removal of the clamp, 300 ml of warm blood was administered. The LAD coronary artery was always revascularized using the left internal mammary artery.

**Biochemical assays. ANTIOXIDANTS.** Standard solutions of alpha-tocopherol were prepared by dissolving the pure compound (Sigma Chemical Co., St. Louis, Missouri) in methanol:ethanol 50:50 to yield final concentrations of 10 to 50 μM. Concentrations were checked spectrophotometrically using the molar extinction coefficients 292 to 294 nm = 71 to 76. A 200-μl-aliquot of plasma was mixed with 500 μl of methanol. The mixture was vortexed for 30 s, added with 4 ml of hexane, vortexed for 1 min, and centrifuged for 5 min at 1,000 × g to separate phases. A 3-ml aliquot of the hexane layer was transferred to another tube and dried under N2. The residue was re-dissolved in 0.5 ml methanol/ethanol 1/1 (v/v) and filtered through a 0.22-μm-pore nylon membrane. High performance liquid chromatography conditions were: isocratic reverse-phase high performance liquid chromatography; column: supelcosil LC-8, 3.3 cm × 4.6 mm, 3 μm; pre-column: Supelguard LC-8; mobile phase: 20 mM lithium perchlorate in methanol/water 99/1 (v/v); flow rate: 1 ml/min; retention time (min): alpha-tocopherol = 0.8; electrochemical detection: oxidation potential +0.6V; UV detection: 290 nm. A similar procedure was carried out for beta-carotene and ubiquinol determinations (24).
Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Hibernation</th>
<th>Control</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>59.6 ± 5.3</td>
<td>65 ± 2.3</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>39.2 ± 1.3</td>
<td>57 ± 2.5*</td>
</tr>
<tr>
<td>Time of ischemia (min)</td>
<td>50.2 ± 2.7</td>
<td>47.6 ± 3</td>
</tr>
<tr>
<td>Number of grafts</td>
<td>2.8 ± 0.2</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>Previous MI</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, *p = 0.0003 by two-sample test.

M = myocardial infarction.

LIPID PEROXIDATION PRODUCTS. Di-keto compounds produced by oxygen radical peroxidation of cell membrane lipids (25,26) were measured spectrophotometrically as thiobarbituric acid-reactive substances (TBARS) (25,26).

Ultrastructure analysis. Biopsies were taken as full-thickness, transmural specimens. Biopsy dimension ranged between 10 and 30 mm². No attempts were made to analyze endocardium and epicardium separately. The methodology for electron microscopy study has been previously described (20–23). Briefly, biopsies were fixed in cold 3% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4), post-fixed in 1% osmium tetroxide, dehydrated, and embedded in Epon resin. From each block, 1-μm-thick sections were cut, stained with 1% toluidine-borax, and examined by light microscopy to select appropriate areas for thin sectioning. Ultrathin sections (15 for each biopsy) were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined under a Jeol Jem-100C, Japan, electron microscope.

Electron micrographs were taken systematically at 5,000× magnification, to permit comparative evaluation of pre-ischemic and post-reperfusion biopsy samples from both groups. Images were analyzed by two experienced investigators unaware of sequence of sampling (i.e., pre-ischemic or post-reperfusion) and of clinical data of patients. Cell injury representative of specific ischemic alterations was ranked on 0 to 4 scale, as follows (27): grade 0 = normal; 1 = minimal changes (glycogen loss, nuclear chromatin clumping/margination); 2 = moderate ischemic changes (as in grade 1, plus intermyofibrillar and sarcoplasmic reticulum edema); 3 = severe ischemic changes (as in grade 2, plus subsarcolemmal blebs, sarcosomal gaps, and marked edema; and 4 = complete architectural disruption and nuclear lysis. Mitochondrial damage was scored assigning a numerical value of 0 through 4 to each mitochondrion, according to the degree of morphologic alterations (27). Grading scale was: 0 = normal; 1 = initial swelling (separation of cristae, decreased matrix density); 2 = more marked swelling than in grade 1; 3 = massive swelling with architectural disruption; and 4 = findings as in grade 3 plus rupture of inner and outer mitochondrial membranes. The average obtained from two observers was expressed for each grade as a percentage of the total number of mitochondria counted per sample. Approximately 150 mitochondria per sample were graded.

Statistical analysis. Statistical analyses were performed using GraphPad Instat version 3.01 software. Baseline characteristics of patients were evaluated using unpaired t test. Biochemical data were evaluated by analysis of variance (ANOVA) followed by Tukey’s multiple-comparison test (Kramer’s correction for unequal sample size was employed when appropriate). Ultrastructural data were compared using the Kruskal-Wallis test (nonparametric ANOVA) with Dunn’s post-test. A value of p < 0.05 was considered statistically significant.

RESULTS

Clinical characteristics. Baseline characteristics of patients are described in Table 1. There were no statistical differences between groups concerning age, ischemic time during surgery, and number of grafts. The incidence of previous myocardial infarction was higher among patients with hibernating myocardium; however, areas of fibrosis when present were always confined to the diaphragmatic wall of the LV.

By entry criteria, global and regional LV function was preserved in control patients. In contrast, all patients in the hibernation group showed presence of large dysfunctional areas in the anterior wall. Ejection fraction was normal in patients from the control group, but it was significantly lower in the hibernation group (Table 1). Follow-up data were available in five patients in the hibernation group, in whom LV ejection fraction rose from 40.2 ± 1.1% before surgery to 49.0 ± 1.3% after revascularization (p < 0.05). Improvement in global LV function was documented in each patient. In these patients, regional wall motion in the anteroseptal area (i.e., the area subtended by the stenotic LAD coronary artery where biopsies were taken) was severely depressed at baseline, and it recovered to a large extent three to four months after revascularization. On a four-point semiquantitative score, regional function in the anteroseptal segments averaged 2.7 ± 0.2 at baseline, and it significantly decreased to 1.2 ± 0.3 at follow-up. Again, improvement was observed in each patient.

There were no differences between groups regarding incidence of postoperative myocardial infarction, mechanical support, or death. Three patients in the hibernation

Table 2. Coronary Sinus Concentrations of Antioxidants and Lipid Peroxidation Products

<table>
<thead>
<tr>
<th></th>
<th>Alpha-Tocopherol</th>
<th>Beta-Carotene</th>
<th>Ubiquinol</th>
<th>TBARS</th>
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<tbody>
<tr>
<td></td>
<td>µM</td>
<td>µM</td>
<td>µM</td>
<td>µM</td>
</tr>
<tr>
<td>Hibernation pre-ischemia</td>
<td>32.18 ± 4</td>
<td>0.28 ± 0.1</td>
<td>0.79 ± 0.1</td>
<td>2.7   ± 0.3</td>
</tr>
<tr>
<td>Hibernation reperfusion</td>
<td>28.8 ± 3.7</td>
<td>0.42 ± 0.2</td>
<td>0.72 ± 0.13</td>
<td>3.7   ± 0.2*</td>
</tr>
<tr>
<td>Control pre-ischemia</td>
<td>27.8 ± 2</td>
<td>0.33 ± 0.1</td>
<td>0.71 ± 0.04</td>
<td>2.5   ± 0.2</td>
</tr>
<tr>
<td>Control reperfusion</td>
<td>27.1 ± 2</td>
<td>0.32 ± 0.09</td>
<td>0.65 ± 0.05</td>
<td>3.5   ± 0.3*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, *p < 0.05 vs. pre-ischemia.
Biochemical determinations. Coronary sinus blood was sampled before cardiac arrest and 10 min into reperfusion. No significant differences in coronary sinus concentrations of major endogenous antioxidants (i.e., alpha-tocopherol, beta-carotene, and ubiquinol) were observed in patients from the control group, nor in patients in the hibernation group (Table 2).

Ischemia/reperfusion was associated with cardiac lipid peroxidation, as indexed by increased TBARS concentration in the coronary sinus in post-reperfusion samples (Table 2). However, the degree of lipid peroxidation was comparable in both groups, as no differences were observed concerning either relative or absolute increase in TBARS concentration (Table 2).

Electron microscopy studies. Qualitative evaluation. Pre-ischemia specimens. Biopsies from hibernating myocardium showed the typical ultrastructural characteristics reported in previous studies (8–16), consisting of loss of contractile material (sarcomeres) without decrease of cell size (i.e., myocytolysis). Sarcomere loss was particularly evident at the perinuclear region, extending toward the periphery of myocytes. Loss of sarcoplasmic reticulum was also evident. The space left by myolysis tended to become occupied by amorphous material. Another typical feature was the presence of numerous smaller-than-normal mitochondria, along with normal or larger-than-normal mitochondria. Some nuclei lost their normal contour, showing instead a tortuous appearance, with evenly distributed chromatin (Figs. 1A and 1B, and 2C).

In normally contracting myocardium, biopsies taken before ischemia largely showed normal ultrastructure (Fig. 2A). We mostly observed regular intercalated disks, with sarcomere preservation and normal mitochondrial morphology. Contraction bands and/or focal fragmentation of sarcomeres were seen at times. Mild cytosolic and intermyofibrillar edema, modest T-tubule dilation, and nuclear chromatin margination were occasionally observed.

Reperfusion specimens. Biopsies from hibernating myocardium taken after reperfusion showed only minor changes compared with ultrastructural findings seen before ischemia. Mild cytosolic and intermyofibrillar edema were observed, indicative of minimal/moderate myocardial damage. Mitochondria tended to retain their normal structure, with signs of only mild or moderate edema (Figs. 2C and 2D). In contrast, biopsies taken after reperfusion from nonhibernating myocardium showed more evident signs of injury; in particular, cytosolic and intermyofibrillar edema was moderate to marked, and mitochondria showed more extensive damage compared with post-ischemic biopsies from hibernating myocardium (Fig. 2B). To better quantitate these differences in ultrastructural appearance, specimens were further characterized and scored (27) (see the following text).

Quantitative analysis. Thirty fields at 5,000× were scored for each biopsy. As expected, before inducing ischemia, myocyte score with specific focus on ischemic changes (ranked separately from mitochondrial alterations) was low in both groups, as it averaged 1.4 ± 0.4 in specimens from hibernating myocardium, and 1.5 ± 0.4 in biopsies from normally contracting myocardium (p = NS). After reperfusion, myocardial injury score was significantly higher (i.e., worse) in biopsies taken in normally contracting areas (1.9 ± 0.3; p < 0.05 vs. pre-ischemia); specimens from hibernating regions also tended to show a higher score upon reperfusion; however, the difference did not reach significance (1.7 ± 0.5; p = NS).

Figure 3 shows the results of semiquantitative grading of
injury obtained after examination of >6,000 mitochondria. Biopsies taken before ischemia showed, as expected, that the large majority of mitochondria in both groups had minimal signs of injury. Normal or just slightly altered structure (grades 0 to 1) characterized 87 ± 3.3% of mitochondria in hibernating myocytes and 80 ± 3.1% in normally contracting myocardium supplied by a stenotic artery (p = NS) (Fig. 3). Mitochondrial injury developed during post-ischemic reperfusion, as reflected by the decrease of the proportion of mitochondria showing normal morphology, and the concomitant increase of the number of mitochondria showing higher (i.e., worse) scores (Fig. 3). In this case, the two groups showed distinct differences. Biopsies from hibernating myocardium showed a much larger proportion of preserved mitochondria (grades, 0 to 1: 74 ± 5.7% of all mitochondria) than the control group (46 ± 7.9%; p < 0.001) (Fig. 3). At the same time, reperfusion biopsies from normally contracting myocardium showed that the relative proportion of severely injured mitochondria (grade 3 to 4) was markedly greater than in biopsies taken after reperfusion from hibernating myocardium (37 ± 4.7% vs. 13 ± 2.1%; p < 0.01) (Fig. 3).

Thus, a prominent shift from normal or minimally altered morphology to marked alterations occurred in normally contracting myocardium subjected to ischemia and reperfusion, but it was not observed in specimens from hibernating areas.

DISCUSSION
Since the description by Rahimtoola (1), our understanding of myocardial hibernation has undergone substantial changes. It is now evident that hibernating myocardium either shows absolute reduction of flow at rest, or impaired coronary flow reserve (2,3,5,7). In either case, hibernating myocytes are at risk of impending ischemia. At the same time, it has been shown in patients with coronary artery disease that myocardium distal to a stenosis can become transiently stunned as a consequence of ischemia(28,29), and that repeated ischemic episodes may have cumulative effects in terms of more severe and longer-lasting dysfunction (29). Finally, in a series of elegant studies, Fallavollita and Canty (30–32) have documented in an animal model the possible transition from a physiological phenotype of stunning to hibernation. Accordingly, it has been hypothesized that hibernation might result from, or be accompanied by, repeated episodes of ischemia leaving myocytes in a condition of “chronic” dysfunction (3–5,7,33,34). Ac-
According to a prevailing theory, without revascularization, this condition would persist because hibernating myocytes undergo a process of dedifferentiation toward a non-contracting phenotype (5,10–12,17,30). Alternatively, the typical ultrastructural changes of hibernating myocytes might represent true degeneration and, therefore, would be less amenable to complete recovery (13,15,32).

To explain the pathophysiology of hibernation, however, one fundamental issue ought to be addressed: how can myocytes distal to a critical stenosis withstand the frequent increases in oxygen consumption imposed by daily activities, without ultimately incurring irreversible ischemic injury? It has been suggested that hibernation may represent a form of adaptation to ischemia, in which downregulation of metabolic and contractile activities would ensure long-term survival of myocytes (5,10,11,12,14,15,31,35). In a detailed ultrastructural characterization of biopsies from patients with hibernating myocardium, Ausma et al. (36) found no evidence of ischemic injury. This finding led the investigators to suggest that hibernating myocytes can be considered “adapted” to acute ischemia (36). Indirect support for this hypothesis comes from in vitro studies, in which isolated rat myocytes cultured under hypoxic conditions (to mimic hibernation) became tolerant to subsequent acute severe hypoxia (37). However, whether human hibernating myocardium actually displays enhanced tolerance to ischemia in vivo has never been directly investigated in patients.

In the present study, we took advantage of the procedure of open-heart surgery as a model to investigate the effects of cardiac ischemia in patients who fulfilled several criteria of myocardial hibernation. Previous investigators had already characterized the morphologic features of hibernating myocytes. However, they analyzed biopsies obtained only before ischemia, that is, either before induction of extracorporeal circulation (6,11,12,14,15), or while patients were on cardiopulmonary bypass but before reperfusion was performed (8–10). Thus, to our knowledge, this is the first ultrastructural evaluation of both pre-ischemic and post-reperfusion specimens in patients.

Largely normal ultrastructure was observed before ischemia in biopsies taken from myocardium retaining normal contractile function. On the other hand, pre-ischemic biopsies from dysfunctional areas showed the typical morphologic characteristics of hibernation (loss of contractile material, increased intracellular glycogen content, disorganization of sarcoplasmic reticulum and cytoskeleton [8–16]). Interestingly, the effects of ischemia and reperfusion largely diverged between the two groups. While biopsies taken from normally contracting areas were characterized by development of signs of moderate-to-marked cell injury, particularly at the mitochondrial level, ultrastructural changes were decidedly less pronounced in biopsies from hibernating myocardium. Detailed analysis of >6,000 mitochondria confirmed this finding using semiquantitative scoring and provided statistical significance to the difference. Thus, ultrastructural alterations widely accepted as

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Mitochondrial score of biopsies taken from either hibernating segments (hibernation), or from normally contracting myocardium (control), before cardiac arrest, or 10 min after reperfusion. Grading scale was: 0 = normal; 1 = initial swelling (separation of cristae, decreased matrix density); 2 = more marked swelling than in grade 1; 3 = massive swelling with architectural disruption; and 4 = findings as in grade 3 plus rupture of inner and outer mitochondrial membranes. Note the shift from normal appearance to altered morphology occurring with ischemia and reperfusion. This shift is less pronounced in biopsies from hibernating segments. Data are mean ± SE of percent mitochondria scored. Values in parentheses are the number of mitochondria scored for each set. *p < 0.05 vs. control.
hallmark of ischemia–reperfusion injury were present in biopsies from normal myocardium, but they were notably less severe in biopsies from hibernating myocardium.

Our study attempted to control for confounding factors. First, all patients were operated on by the same surgeon (R.F.). More importantly, the major determinants of cardiac injury in this setting, namely severity of ischemia and magnitude of oxidant stress (18–25,38), were similar in the two groups because: 1) the number of grafts and length of cardiac arrest were comparable for the two groups; 2) coronary sinus concentrations of antioxidants were similar in the two groups, both at baseline and at the end of the procedure; and 3) cardiac production of TBARS (an index of oxidant-induced lipid peroxidation at reperfusion [25,26]) was also similar. Thus, the results of our study are unlikely to reflect differences in the severity of insult occurring during the surgical procedure; rather, they point to an intrinsic difference in the susceptibility toward ischemia–reperfusion between the two groups. In this respect, Cecconi et al. (39) have very recently documented in a similar setting that patients with hibernating myocardium subjected to coronary artery bypass surgery tend to have less creatine kinase release after cardiac arrest and reperfusion than patients with preserved LV function, also implying reduced myocyte injury in hibernation. Our data extend that observation by providing direct demonstration at the ultrastructural level that hibernating myocytes have increased tolerance toward ischemia.

Because hibernating myocardium shows reduced or absent contractile function, it has been speculated that myocytes subtended by a critical stenosis might remain viable thanks to the downregulation of contractile activity, which, in turn, would ensure survival at lower metabolic cost (11,17,30,35). While it is conceivable that reduced contractility might protect myocytes against ischemia induced by anginal episodes, it should be stressed that in our study ischemia was induced while the heart was in a non-beating, cardioplegically arrested state. Therefore, the reduced signs of injury that we observed in hibernating myocardium cannot be the reflection of reduced contractile activity; rather, it suggests that hibernating myocytes may be intrinsically more resistant to ischemia, irrespective of contractile status.

Study limitations. Our study focused on ultrastructural markers of cell injury; specific studies are necessary to determine whether better ultrastructural preservation also translates into preserved respiratory function of mitochondria. In addition, the findings of our study cannot be generalized. First, the number of patients was small; this precluded stratification or subgroup analysis of the data according to patient characteristics. Secondly, our clinical setting of surgical ischemia–reperfusion represents a condition in which myocardial protection is actively sought and degree of injury is certainly less severe than that induced by ischemia in normothermic, working myocardium. Thus, it remains to be established whether hibernating myocardium can also cope with regional ischemia secondary to angina or acute coronary artery occlusion.

In conclusion, our data show that, despite similar severity of ischemia–reperfusion insult, hibernating myocardium develops less pronounced ultrastructural injury compared with normally contracting myocytes. Although these findings cannot be extended to other clinical conditions, they provide a “proof-of-principle” and support the hypothesis that myocardial hibernation is associated with greater resistance to ischemic injury in patients with coronary artery disease.

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REFERENCES