High-Density Lipoproteins Protect Isolated Rat Hearts From Ischemia-Reperfusion Injury by Reducing Cardiac Tumor Necrosis Factor-α Content and Enhancing Prostaglandin Release

Laura Calabresi, Giuseppe Rossoni, Monica Gomaraschi, Francesca Sisto, Ferruccio Berti, Guido Franceschini

Abstract—The incidence and severity of primary cardiac events are inversely related to the plasma concentration of high-density lipoproteins (HDLs). We investigated whether HDLs may exert a direct cardioprotection in buffer-perfused isolated rat hearts, which underwent a 20-minute low-flow ischemia followed by a 30-minute reperfusion. The administration of HDLs at physiological concentrations (0.5 and 1.0 mg/mL) during the 10 minutes immediately before ischemia rapidly and remarkably improved postischemic functional recovery and decreased creatine kinase release in the coronary effluent. Reconstituted HDLs containing apolipoprotein A-I (apoA-I) and phosphatidylcholine, but not lipid-free apoA-I or phosphatidylcholine liposomes, were also effective in protecting the heart from ischemia-reperfusion injury. HDLs at reperfusion were less effective than when given before ischemia. HDLs caused a dose-dependent reduction of ischemia-induced cardiac tumor necrosis factor-α (TNF-α) expression and content, which correlated with the improved functional recovery. A parallel increase of TNF-α release in the coronary effluent was observed, due to a direct binding of cardiac TNF-α to HDLs. Taken together, these findings argue for a cause-effect relationship between the HDL-mediated removal of TNF-α from the ischemic myocardium and the HDL-induced cardioprotection. Indeed, etanercept, a recombinant TNF-α–blocking protein, caused a dose-dependent improvement of postischemic functional recovery. HDLs also enhanced ischemia-induced proteaglandin release, which may contribute to the cardioprotective effect. A low plasma HDL level may expose the heart to excessive ischemia-reperfusion damage, and HDL-targeted therapies may be helpful to induce immediate or delayed myocardial protection from ischemia-reperfusion injury. (Circ Res. 2003;92:330-337.)

Key Words: high-density lipoproteins • myocardial ischemia • reperfusion • tumor necrosis factor-α • prostaglandins

Several prospective studies have clearly established that plasma high-density lipoprotein (HDL) cholesterol levels are inversely related to the incidence of primary cardiac events.1 In addition to the strong epidemiological data, there is compelling clinical trial evidence that coronary event rates may be favorably influenced by raising plasma HDL levels, especially in subjects with low HDL cholesterol and elevated triglycerides.2–4 The protective effect of HDLs is believed to be due to their capacity to promote reverse cholesterol transport, the process by which cholesterol in peripheral tissues, including the arterial wall, is routed to the liver for excretion from the body. Through this pathway, HDLs retard formation of lipid-rich arterial lesions, thus preventing plaque rupture and coronary events.5

Besides being a strong independent predictor of the occurrence of primary coronary events, a low plasma HDL cholesterol level is also associated with unfavorable prognosis in patients who have recovered from a myocardial infarction.6–8 Whether this association reflects accelerated atherogenesis or a direct detrimental effect of a low HDL level on postischemic myocardial function is unknown. A low HDL level adversely influences postinfarct left ventricular function in patients with a first myocardial infarction, independent of the severity of coronary atherosclerosis,9,10 and is an independent predictor of left ventricular dysfunction in angina patients with normal coronary angiograms,11 suggesting that HDLs might influence cardiac function through extra-atherosclerotic mechanisms. With the use of the isolated heart model, we demonstrate in the present study a direct cardioprotective effect of HDLs against ischemia-reperfusion injury, which is independent of their major function in lipid/lipoprotein metabolism.

Materials and Methods

Lipoprotein Preparation

Human HDLs (density \(d=1.063\) to \(1.21\) g/mL) and low-density lipoproteins (LDLs, \(d=1.019\) to \(1.063\) g/mL) were isolated from...
plasma of fasting healthy male volunteers by salt density ultracentrifugation. Volunteers were fully informed of the modalities and end points of the study, which was approved by the Institutional Review Board, and signed an informed consent form. Apolipoprotein A-I (apoA-I) was purified from human blood plasma; reconstituted HDLs (rHDLs) containing apoA-I and egg-yolk phosphatidylcholine (EPC) and EPC liposomes were prepared by the cholate dialysis technique, as previously described. Lipoprotein preparations were dialyzed against sterilized saline immediately before use. Protein and EPC contents were measured as previously described.

**Experimental Protocol**

Male Sprague-Dawley rats (Charles River Italia, Calco, Italy) weighing 200 to 250 g were anesthetized with sodium pentobarbital (50 mg/kg IP). The hearts were rapidly excised, placed in ice-cold Krebs-Henseleit (K-H) buffer, and mounted for retrograde perfusion using a modified Langendorff method. The hearts were equilibrated with K-H buffer at a flow rate of 15 mL/min for 30 minutes. Left ventricular pressure (LVP), coronary perfusion pressure (CPP), and left ventricular developed pressure (LVDP) were monitored as previously described. A moderate ischemia was induced by reducing the perfusion flow rate to 1 mL/min for 20 minutes; the normal flow rate (15 mL/min) was then restored, and reperfusion continued for 30 minutes. Control hearts (n=5) were mounted and perfused with K-H buffer at a flow rate of 15 mL/min for 80 minutes. Immediately at the end of the experiment, hearts were snap-frozen in liquid nitrogen and stored at −80°C. Cardiac homogenates were prepared by grinding tissue under liquid nitrogen with a Micro-Dismembrator II (B. Braun Biotech).

Creatine kinase (CK) activity was measured on coronary effluent aliquots collected every 150 seconds of reperfusion.

HDLs diluted into K-H buffer at a final protein concentration of 0.5 mg/mL and 1.0 mg/mL were perfused through the hearts (n=5 for each treatment group) during the 10 minutes immediately before ischemia. Groups of five hearts were similarly perfused with LDLs (1.0 mg protein/mL), rHDLs (1.0 mg apoA-I/mL, 2.5 mg EPC/mL), apoA-I (1.0 mg/mL), EPC liposomes (2.5 mg/mL), or with an equal volume of saline diluted into K-H buffer. In another group of five hearts, HDLs (1.0 mg protein/mL) were administered during the first 10 minutes of reperfusion.

In separate experiments, groups of three hearts were perfused during the 10 minutes immediately before ischemia with increasing concentrations (10 to 100 μg/mL) of etanercept, a recombinant tumor necrosis factor-α (TNF-α)–blocking protein containing the extracellular ligand-binding portion of the TNF-α receptor, or with an equal volume of saline diluted into K-H buffer.

All animals received humane care as described in the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 80-23, revised 1985).

**Prostaglandins and TNF-α in the Coronary Effluent**

Generation of prostaglandins I2 and E2 (PGI2 and PGE2) by the cardiac tissue was assessed by measuring 6-keto-PGF1α and PGE2 on coronary effluent aliquots collected in the 5 minutes immediately before ischemia and during the first 10 minutes of reperfusion by ELISA (Endogen Inc). TNF-α was measured on pooled aliquots collected every 10 minutes during reperfusion by a sandwich ELISA specific for rat TNF-α (Endogen Inc).

**TNF-α in Cardiac Tissue**

The myocardial homogenate was suspended in PBS containing aprotinin 31 nmol/mL, PMSF 1 mmol/L, and 1% Triton X-100. After 1-hour incubation at 4°C, the homogenate was centrifuged for 20 minutes at 20000g at 4°C. The supernatant was dialyzed overnight against PBS to eliminate Triton X-100, which interferes with the following analyses. Protein and TNF-α contents were measured by the method of Lowry et al and by ELISA, respectively.

**RT-PCR**

Total RNA was extracted from myocardial homogenate with TRIzol reagent (Life Technologies). CDNA was prepared by reverse transcription of 1 μg of total RNA with Moloney murine leukemia virus reverse transcriptase (M-MVL RT, Promega Corporation) at 42°C for 60 minutes. M-MVL RT was then inactivated by incubation at 95°C for 5 minutes; cDNA was chilled on ice and stored at −20°C. cDNA was amplified for 30 cycles with Taq DNA polymerase (Promega Corporation) in a Mastercycler Gradient (Eppendorf). The following primers were used: TNF-α sense 5′-ATGAGACCGAAAGCATGATCCGA-3′, antisense 5′-CCAAAGTAGACCTGCGGACTC-3′, producing a 692-bp fragment; GAPDH sense 5′-ACGACCCCTTACGACC-3′, antisense 5′-TGCTTCACCCCTTCTTG-3′, producing a 691-bp fragment.

PCR products were separated by electrophoresis on 1% agarose gel, stained with ethidium bromide, visualized by ultraviolet irradiation and photographed with Polaroid film. To evaluate band densities, a GS-690 Imaging Densitometer and Multi-Analyst software were used (Bio-Rad Laboratories). All TNF-α band intensities were normalized by their respective GAPDH values. Each PCR reaction was performed twice.

**Binding of TNF-α to HDLs**

Microtiter wells were coated with 25 ng of recombinant rat TNF-α (Endogen Inc) in buffer A (15 mmol/L Na2CO3, 35 mmol/L NaHCO3, and 3 mmol/L Na2PO4, pH 9.6) at room temperature for 18 hours. Nonspecific binding sites were blocked by incubation with 0.5% gelatin (wt/vol) in buffer B (10 mmol/L Tris, 2 mmol/L CaCl2, and 150 mmol/L NaCl, pH 7.2) for 1 hour at room temperature. HDLs (0 to 200 ng) were then added together with 0.5% gelatin, washed three times with 300 μL of 0.5% gelatin. HDL binding to coated TNF-α was evaluated by reaction of HDLs with sheep anti-human apoA-I (Roche Diagnostics), followed by incubation with a peroxidase-conjugated rabbit anti-sheep secondary antibody (Dako) and TMB substrate (Endogen Inc). Wells saturated with a rabbit anti-rat TNF-α (Endogen Inc) before adding HDLs serve as negative controls.

Alishots of the coronary effluent from HDL- and saline-perfused hearts were run on a nondenaturing 4% to 20% polyacrylamide gradient gel and then electrophoretically transferred to a nitrocellulose membrane. After electrotobbling, the membrane was incubated with 5% fat-free dry milk, incubated with rabbit anti-TNF-α at 4°C overnight, and then with peroxidase-conjugated goat anti-rabbit secondary antibody (Dako) for 1 hour at 37°C. The immunocomplexes were detected using an enhanced chemiluminescence kit (Amersham). After TNF-α detection, the membrane was stripped, reacted with a sheep anti-human apoA-I followed by a peroxidase-conjugated rabbit anti-sheep secondary antibody, and developed with an enhanced chemiluminescence kit.

**Statistical Analyses**

Results are reported as mean±SEM. Group differences in continuous variables were determined by analysis of variance (ANOVA). Pearson correlation coefficients were computed to assess the association between parameters. Group differences or correlations with a value of P<0.05 were considered as statistically significant.

**Results**

**Effects of HDLs on Cardiac Function During Ischemia and Reperfusion**

The basal values of CPP, LVEDP, and LVDP were similar in all groups of hearts and did not change throughout the 80-minute experiment in control hearts (data not shown).

After the beginning of low-flow ischemia, LVDP declined to zero within <5 minutes and did not change until the end of the ischemic period (Figure 1). No differences in cardiac function could be detected up to this time point among saline-
and lipoprotein-perfused hearts (Figure 1). On reperfusion, LVDP recovered only partially in saline-perfused hearts, reaching a maximum (45±4% of the preischemia value) at the end of the observation period; CPP rapidly increased, reaching a maximum (189±5% of the preischemia value) after 4 to 5 minutes of reperfusion, slowly declining thereafter (Figure 1). These functional changes were accompanied by the release of CK into the coronary effluent during reperfusion (Table 1), indicative of a disruption of the cardiac myocyte membrane.

The administration of HDLs during the 10 minutes immediately before ischemia caused a rapid and dose-dependent improvement of postischemic left ventricular functional recovery at reperfusion (Figure 1). At the maximum dose (1.0 mg protein/mL), HDLs preserved LVDP and blunted the increase of CPP compared with saline-perfused hearts (Figure 1, Table 1); the recovery of LVDP at the end of reperfusion was 84±6% of the preischemia value, and the CPP maximum was 135±2% of the preischemia value. A dose-dependent reduction of CK release into the coronary effluent was also observed in HDL-perfused hearts (Table 1). These effects were specific for HDLs, as LDLs, isolated from the same human plasma, did not improve postischemic functional recovery and did not reduce cardiac CK release (Table 1), even at high concentrations (2 mg/mL; data not shown).

In the attempt of identifying the effective component of HDLs, isolated hearts were perfused with rHDLs containing apoA-I and EPC or with the isolated components, lipid-free apoA-I and EPC liposomes. rHDLs, but not apoA-I or EPC liposomes, were effective in improving postischemic functional recovery and in decreasing cardiac CK release, although at a lower extent compared with plasma-derived HDLs (Table 1).

HDLs administered during the first 10 minutes of reperfusion also improved postischemic functional recovery and reduced CK release, but less effectively than when given before ischemia (Table 1). After these observations, experiments were performed to identify the mechanism(s) involved in the direct HDL-mediated cardioprotection. It should be noted that the functional studies were performed in hearts that were buffer-perfused according to the Langendorff technique, thus excluding the influence of hemodynamic factors, blood cells, and constituents. Therefore, in this particular experimental setting, the postischemic impairment of cardiac function is the result of a balance between the effects of damaging and protective responses generated within the heart.

Table 1. Postischemic Cardiac Function and CK Release in Isolated Rat Hearts

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>LVDP (mm Hg)</th>
<th>CPP (mm Hg)</th>
<th>CK Release (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>908±74</td>
<td>1588±131</td>
<td>5573±496</td>
</tr>
<tr>
<td>HDLs (0.5 mg/mL)</td>
<td>1309±95†</td>
<td>1127±72†</td>
<td>3093±208†</td>
</tr>
<tr>
<td>HDLs (1.0 mg/mL)</td>
<td>1881±112‡</td>
<td>317±28‡</td>
<td>1364±97‡</td>
</tr>
<tr>
<td>HDLs (1.0 mg/mL) at reperfusion</td>
<td>1199±86‡</td>
<td>1045±79‡</td>
<td>3749±223‡</td>
</tr>
<tr>
<td>LDLs (1.0 mg/mL)</td>
<td>1106±103</td>
<td>1324±118</td>
<td>4606±405</td>
</tr>
<tr>
<td>rHDLs (1.0 mg/mL)</td>
<td>1467±93†</td>
<td>947±66†</td>
<td>3155±183†</td>
</tr>
<tr>
<td>apoA-I (1.0 mg/mL)</td>
<td>978±56</td>
<td>1606±103</td>
<td>4998±267</td>
</tr>
<tr>
<td>EPC (2.5 mg/mL)</td>
<td>912±69</td>
<td>1615±167</td>
<td>4947±379</td>
</tr>
<tr>
<td>Etanercept (10 µg/mL)</td>
<td>1155±118</td>
<td>1321±165</td>
<td>4718±302</td>
</tr>
<tr>
<td>Etanercept (30 µg/mL)</td>
<td>1413±75†</td>
<td>1003±92†</td>
<td>3975±235†</td>
</tr>
<tr>
<td>Etanercept (100 µg/mL)</td>
<td>1702±141‡</td>
<td>815±56‡</td>
<td>2884±261‡</td>
</tr>
</tbody>
</table>

LVDP indicates left ventricular developed pressure; CPP, coronary perfusion pressure; and CK, creatine kinase. *Concentrations are reported as mg of protein/mL of buffer, except for EPC liposomes, which were administered at the same EPC concentration as rHDLs. Results are expressed as areas under the curve, estimated according to the trapezoid method (LVDP and CPP; in ordinate, mm Hg; in abscissa, time; CK; in ordinate, mU·min⁻¹·g⁻¹ wet tissue; in abscissa, time) from data recorded during the 30 minutes of reperfusion (mean±SEM, n=5 for each treatment group, except for etanercept at different doses, where n=3). †P<0.05; ‡P<0.01 vs Saline.
specific immunoassay. Ischemia-reperfusion in saline-perfused hearts caused a remarkable increase of cardiac TNF-α content compared with control hearts (Figure 2). The administration of HDLs during the 10 minutes immediately before ischemia caused a dose-dependent reduction of cardiac TNF-α content compared with saline-perfused ischemic hearts (Figure 2); at the maximum dose, HDLs completely blunted the ischemia-induced rise of cardiac TNF-α content. HDLs administered during the first 10 minutes of reperfusion reduced cardiac TNF-α content, but less effectively than when given before ischemia (Figure 2). No significant changes in cardiac TNF-α content were detected after LDL administration. When data from all experiments were analyzed together, the cardiac TNF-α content correlated negatively with postischemic LVDP (r = −0.804, P < 0.0001) and positively with postischemic CPP (r = 0.757, P < 0.0001). A highly significant positive correlation was also found between cardiac TNF-α content and the postischemic release of CK in the coronary effluent (r = 0.837, P < 0.0001).

TNF-α was also measured in the coronary effluent of saline- and HDL-perfused hearts. The administration of HDLs during the 10 minutes immediately before ischemia caused a significant, dose-dependent increase of TNF-α release into the coronary effluent (saline: 3.11 ± 0.05 pg/mL; HDLs 0.5 mg/mL: 10.74 ± 0.43 pg/mL; HDLs 1.0 mg/mL: 18.06 ± 1.15 pg/mL). A significant inverse correlation was found between perfusate and cardiac TNF-α contents (r = −0.824, P < 0.0001).

HDLs Downregulate Cardiac TNF-α mRNA
To investigate whether the decreased cardiac TNF-α content of HDL-perfused hearts was associated with a downregulation of TNF-α mRNA, an RT-PCR analysis was performed on control and ischemic hearts. TNF-α mRNA was practically absent in control hearts (Figure 3). Ischemia-reperfusion induced a 4-fold increase in TNF-α mRNA, which was almost completely blunted in HDL-perfused hearts (Figure 3).

HDLs Bind TNF-α
The ability of HDLs to bind TNF-α was tested by performing binding experiments in microtiter wells. The addition of increasing amounts of HDLs to TNF-α–coated wells resulted in a progressive and saturable HDL binding to TNF-α (Figure 4). Little HDL binding was observed in control wells saturated with anti-TNF-α before adding HDLs.

To prove that HDLs indeed bind the TNF-α released by the ischemic myocardium, aliquots of the coronary effluent from HDL- and saline-perfused hearts were analyzed by nondenaturing gradient gel electrophoresis followed by immunodetection with antibodies against apoA-I and TNF-α. ApoA-I

Figure 2. TNF-α content of nonischemic rat hearts (Control) and of ischemic hearts perfused with saline, HDLs (1.0 or 0.5 mg/mL) or LDLs (1.0 mg/mL) during the 10 minutes immediately before ischemia, or HDLs (1.0 mg/mL) during the first 10 minutes of reperfusion. Results are mean ± SEM, n = 5 for each treatment group. * P < 0.0001 vs saline.

Figure 3. Cardiac TNF-α mRNA in nonischemic rat hearts (Control) and in ischemic hearts perfused with saline or HDLs (1.0 mg/mL) during the 10 minutes immediately before ischemia. A, Representative analysis of RT-PCR products. B, Relative optical density of TNF-α PCR signal, normalized to GAPDH PCR signal (mean ± SEM, n = 5 for each treatment group).
and TNF-α immunoreactivities colocalized at a position in the gel typical of plasma HDLs (Figure 5).

**Etanercept Improves Postischemic Functional Recovery of Isolated Hearts**

One set of isolated hearts was perfused during the 10 minutes immediately before ischemia with increasing concentrations of etanercept, a recombinant TNF-α-blocking protein containing the extracellular ligand-binding portion of the TNF-α receptor. Etanercept administration caused a significant, dose-dependent improvement of postischemic left ventricular functional recovery at reperfusion and a reduction of CK release into the coronary effluent (Table 1).

**HDLs Increase Cardiac Prostaglandin Release**

Prostanoids, generated mostly through cyclooxygenase-2,20 exert salutary actions during myocardial ischemia-reperfusion injury.21,22 The induction of ischemia in saline-perfused hearts caused the expected 4-fold increase in the release of cardiac PGI2, measured as its stable metabolite 6-keto-PGF1α, and PGE2 into the coronary effluent (Table 2). The administration of HDLs during the 10 minutes immediately before ischemia caused a significant, dose-dependent increase in the rate of prostaglandin release during both the preischemic period and at reperfusion (Table 2). No significant changes were found in hearts perfused with LDLs or when HDLs were given during the first 10 minutes of reperfusion (Table 2). When data from all experiments were analyzed together, a significant correlation was found between postischemic release of PGI2 and PGE2 and left ventricular contractile capacity, measured as LVDP (r=0.678 and 0.661, respectively, both P<0.001).

**Discussion**

The present study demonstrates that HDLs protect isolated hearts from ischemia-reperfusion injury. The protection is dose-dependent and specific for HDLs, as other lipoproteins, eg, LDLs, are totally ineffective. Reconstituted HDLs made with the major HDL components, apoA-I and phosphatidylcholine, but not their separate constituents, mimic the HDL-mediated cardioprotection. To our knowledge, this is the first demonstration of a direct cardioprotective effect of HDLs against myocardial ischemia-reperfusion injury.

The experimental setting, excluding the influence of hemodynamic and blood factors, the time course of the...
experiment, and the very rapid recovery of cardiac function in HDL-perfused hearts, indicate that HDLs affect very early events occurring within the heart during ischemia and the first minutes of reperfusion. A process typically occurring in this time frame is the release of preformed molecules that either directly damage the myocardium or trigger a cascade of events leading to subacute ischemia-reperfusion injury. Among these, TNF-α appears to be a crucial one. TNF-α is a proinflammatory cytokine that has been implicated in the pathogenesis of cardiovascular diseases, including ischemia-reperfusion injury, heart failure, cardiac allograft rejection, and sepsis-associated cardiac dysfunction. Myocardial ischemia induces degranulation of resident mast cells and release of membrane-bound TNF-α by the TNF-α cleavage enzyme (TACE), both causing the immediate release of active TNF-α in the ischemic myocardium to act in an autocrine, endocrine, and paracrine fashion. Significant amounts of TNF-α are indeed found in the coronary effluent of ischemic isolated hearts. The released TNF-α affects myocardial function, either directly, by inducing oxidant stress, calcium dyshomeostasis, disruption of excitation-contraction coupling, and myocyte apoptosis, or indirectly, by triggering the inflammatory cascade that accounts for subacute ischemia-reperfusion injury.

In the present studies, we observed a substantial increase of cardiac TNF-α content in saline-perfused ischemic hearts. The administration of HDLs immediately before ischemia caused a dose-dependent reduction of cardiac TNF-α content; at the maximum dose, HDLs completely blunted the ischemia-induced rise of cardiac TNF-α. The cardiac TNF-α content of saline- and lipoprotein-perfused hearts was strongly correlated with parameters of postischemic left ventricular dysfunction and myocyte damage, indicating a cause-effect relationship between the decreased cardiac TNF-α content and the HDL-induced cardioprotection.

The reduction of cardiac TNF-α content in HDL-perfused hearts was associated with a dose-dependent recovery of TNF-α in the coronary effluent, indicating that HDLs may act as biological buffers that are capable of rapidly removing active TNF-α from the heart, thus preventing its deleterious effects on ischemic myocardium. Further support to this concept comes from (1) the specific and saturable in vitro binding of HDLs to immobilized TNF-α; (2) the observation that TNF-α is bound to HDLs in the coronary effluent of HDL-perfused hearts; and (3) the dose-dependent cardioprotective effect of etanercept, another TNF-α–binding protein.

A significant reduction of TNF-α mRNA content was observed in HDL-perfused compared with saline-perfused hearts. This effect may be due to either a direct inhibition by HDLs of TNF-α expression in the heart, as observed in blood monocytes, or to a decreased autocrine self-amplification of TNF-α expression, due to the removal of the released TNF-α by HDLs. The downregulation of TNF-α mRNA in HDL-perfused hearts is not a likely explanation for the rapid improvement of postischemic functional recovery, because TNF-α mRNA induction takes ~30 minutes and peaks at 1 to 2 hours, but may prevent late postischemic events that contribute to subacute ischemia-reperfusion injury.

HDLs may improve postischemic functional recovery of the heart not only by limiting the availability of damaging molecules, such as TNF-α, but also by amplifying natural protective responses elicited by the heart to counteract ischemia-reperfusion injury. Prostanoids, in particular PGI₂ and PGE₂, generated through the cyclooxygenase-2 enzyme, are major components of these protective mechanisms, either preventing the damaging effect of blood cells or acting directly on cardiac myocytes. Indeed, a remarkable release of PGI₂ and PGE₂ was observed immediately after ischemia in saline-perfused hearts. HDLs caused a further dose-dependent increase of these prostanoids in the coronary effluent; the effect was again specific for HDLs, as prostaglandin release did not change in LDL-perfused hearts. HDLs were shown to stimulate PGI₂ and PGE₂ production in cultured endothelial and smooth muscle cells by providing the arachidonate substrate for prostanoid synthesis and inducing the cyclooxygenase-2 enzyme. In addition, HDLs have the potential to stabilize prostaglandins, thus increasing their activity. The enhanced prostanoid availability/activity may contribute to the HDL-mediated cardioprotection, by acting directly on cardiac myocytes and/or by inhib-
iting cardiac TNF-α production\textsuperscript{38} and thus limiting its damaging effects on the myocardium. However, prostanoid induction only partially explains the cardioprotective effect of HDLs, as a significant improvement of postischemic functional recovery, despite no changes in prostaglandin release, was observed when HDLs were given after ischemia.

The direct cardioprotective effect of HDLs in isolated hearts may have clinical and therapeutic implications. It is generally believed that a high plasma HDL concentration protects against the development of primary cardiac events by removing lipids from the arterial wall, therefore preventing the formation of lipid-rich, rupture-prone atherosclerotic plaques in the coronary arteries.\textsuperscript{5} We demonstrate that HDLs, at concentrations typically found in the plasma of healthy individuals, exert a direct protective effect on the ischemic myocardium. Therefore, a low plasma HDL concentration increases cardiovascular risk not only by promoting atherogenesis but also by exposing the heart to excessive ischemic damage. The present findings suggest that drug treatments aimed at raising plasma HDL levels\textsuperscript{39} or innovative therapies based on rHDLs\textsuperscript{40} may represent useful therapeutic tools to induce immediate or delayed myocardial protection in clinical situations characterized by ischemia-reperfusion injury, such as angioplasty, cardiac surgery, or even in patients with unstable angina.

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