Cardiovascular Effects of Acute Hypercholesterolemia in Rabbits

Reversal with Lovastatin Treatment

John A. Osborne, Paul H. Lento, Martin R. Siegfried, Gregory L. Stahl, Ben Fusman, and Allan M. Lefer
Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Abstract

Hypercholesterolemia was induced in New Zealand white rabbits by feeding them a 0.5% cholesterol-enriched rabbit chow for 2 wk. Half of the cholesterol-fed rabbits were given lovastatin, a potent inhibitor of hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA reductase), the rate limiting enzyme in cholesterol biosynthesis, and the other half were given its vehicle (i.e., DMSO). At the end of 2 wk, the rabbits underwent experimental myocardial ischemia or a sham ischemia procedure. Ischemic animals fed the cholesterol-enriched diet for 2 wk experienced much greater cardiac damage than ischemic rabbits fed the control diet, despite the absence of any atherosclerosis. Lovastatin was shown to protect the ischemic rabbit myocardium by three different indices of ischemic damage: (*a*) maintenance of creatine kinase (CK) activity in the ischemic myocardium; (*b*) reduced loss of free amino-nitrogen containing compounds from the ischemic myocardium; and (*c*) blunting the rise of plasma CK activity. These effects were not due to differences in myocardial oxygen demand between the groups. Arteries isolated from animals fed the cholesterol-enriched diet developed defects in endothelium-dependent relaxation in both large vessels as well as coronary resistance vessels. Acute hypercholesterolemia increases the severity of myocardial ischemia while at the same time impairing endothelium-dependent relaxation. These deleterious changes can be significantly attenuated by treatment with lovastatin.

Introduction

Hypercholesterolemia and ensuing atherosclerosis have been implicated in the pathophysiology of coronary artery disease and myocardial ischemia (MI)[1] (1–3). Elevated plasma cholesterol concentrations may be important in the initiation of ischemia and also in the subsequent propagation of ischemic damage (1, 2, 4). There is also evidence that elevated plasma cholesterol concentrations may act to inhibit the ability of the vasculature to relax to certain humoral agents (5) and to enhance platelet aggregation (4, 6). Furthermore, it is well established that long standing hypercholesterolemia and atherosclerosis contribute to increased platelet turnover and sensitize platelets to a variety of aggregating agents (7–9).

We have recently developed a model of acute myocardial ischemia in cholesterol-fed rabbits (1). The rabbits are chronically fed a cholesterol-enriched diet that produces several of the pathophysiologic sequelae which occur during atherosclerosis. This may be important in coronary artery pathophysiology and the response to acute MI since atherosclerotic coronary vessels are more susceptible to spasm induced by ergonovine (10, 11) and may be predisposed to spontaneous coronary vasospasm (10, 11). Enhanced vasoconstrictor responses to serotonin following hypercholesterolemia have also been demonstrated (12). In addition, the vasodilator actions of many agents including acetylcholine, serotonin, and thrombin are dependent on a functional endothelium (13, 14). These endothelium-dependent dilator responses are abolished in long-term hypercholesterolemia and atherosclerosis (5, 15, 16). In these experiments, the hypercholesterolemia was of a 12–14-wk duration and achieved circulating cholesterol values > 1,000 mg/dl.

Lovastatin is a potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase; EC 1.1.1.34), an enzyme that catalyzes the rate limiting step in cholesterol biosynthesis (17). The purpose of the present study was to test whether this inhibitor of cholesterol biosynthesis, which has been shown to lower plasma cholesterol concentrations both in laboratory animals as well as in humans (17, 18) could prevent myocardial damage during acute myocardial ischemia after 2 wk of hypercholesterolemia in rabbits. We chose this short term model of hypercholesterolemia to avoid the extremely high cholesterol values usually obtained in longer term models (e.g., 1,000–1,200 mg/dl) and to avoid the further complicating effects of atherosclerotic plaque formation. We also investigated some possible mechanisms by which lovastatin may act to reduce the ischemic damage to the myocardium.

Methods

Housing and feeding. Adult male New Zealand white rabbits (2.5–3.0 kg) were fed a diet enriched with 0.5% cholesterol added to the standard Purina rabbit pellets diet for a total of 2 wk (Dyets, Inc., Allentown, PA). All animals were housed in an identical manner. Half of the rabbits fed the 0.5% cholesterol-enriched diet received 10 mg lovastatin (1,2,6,7,8,8a-hexahydro-beta, delta-dihydroxy-2,6-dimethyl-8-(2-methyl-*l*-oxobutoxy)-*l*-naphthalene-heptanoic acid delta-lactone), (Merck & Co., Rahway, NJ) once daily at 5:00 p.m. by subcutaneous injection. This dose of lovastatin was chosen based on previous studies which have shown that the maximal cholesterol lowering activity in rabbits to be in the range of 10 mg/d (18). The remainder of the animals were given an equal volume of the vehicle for lovastatin (i.e., 100 μl dimethylsulfoxide, DMSO). Additional rabbits were fed a stan-

Address reprint requests to Dr. Lefer, Department of Physiology, Jefferson Medical College, 1020 Locust Street, Philadelphia, PA 19107.

Received for publication 16 May 1988 and in revised form 12 September 1988.

1. *Abbreviations used in this paper:* ACh, acetylcholine; CK, creatine kinase; CPP, coronary perfusion pressure; EDRF, endothelium-derived relaxing factor; HMGCoA, 3-hydroxy-3-methylglutaryl-coenzyme A; MABP, mean arterial blood pressure; PAF, platelet-activating factor; PRI, pressure-rate index; KH, Krebs-Henseleit solution; MI, myocardial ischemia.

J. Clin. Invest.

0021-9738/89/02/0465/09 $2.00
Volume 83, February 1989, 465–473

dard Purina rabbit pellet diet without added cholesterol for 2 wk. Half of these animals were then given 10 mg lovastatin and half were given the vehicle. Arterial blood samples anticoagulated with heparin (50 U/ml) were obtained just before the 2-wk feeding period and again at the end of 2 wk for the determination of plasma cholesterol concentration (19), expressed in milligrams per deciliter.

Acute myocardial ischemia. At 2 wk, the rabbits were anesthetized with sodium pentobarbital injected into the marginal ear vein (30 mg/kg), tracheotomized, and ventilated by use of a small animal respirator (Harvard Apparatus Co. Dover, MA) connected to a tracheal cannula. The right femoral artery was catheterized and polyethylene tubing (PE-190) was inserted into the aorta and used for the continuous measurement of mean arterial blood pressure (MABP) in millimeters of mercury using a pressure transducer (P23AC; Statham Instruments, Oxnard, CA) and heart rate (HR) in beats per minute. Hemodynamic variables were continuously recorded on a oscillographic recorder (model R411; Beckman Instruments, Inc., Fullerton, CA). The left external jugular vein was catheterized and used for administration of additional pentobarbital as needed to maintain a constant degree of anesthesia.

A midline sternotomy was performed, the pericardium opened, and a 4-O silk tie was loosely placed around the left anterior descending (LAD) coronary artery 12–14 mm from its origin. The LAD supplies almost all of the blood flow to the anterior free wall of the heart in the rabbit (2). After a 30-min stabilization period, rabbits were either subjected to MI by totally occluding the LAD coronary artery, or to a sham MI procedure in which the identical surgical procedures were performed except for the coronary artery ligation. In those animals undergoing MI, the ligature remained tied tightly for the entire 5-h observation period. This procedure allowed us to achieve a consistent ischemic zone in the MI rabbits (1, 20). The pressure-rate index (PRI), calculated as the product of MABP and HR divided by 1,000, was used as an index of myocardial oxygen demand. 2-ml arterial blood samples for the measurement of plasma creatine kinase (CK) and total protein were drawn at 0, 1, 2, 3, 4, and 5 h. The volume of blood removed by sampling was replaced by an equal volume of 0.9% NaCl. All blood samples were anticoagulated with heparin (50 U/ml), kept on ice until centrifuged at 2,500 *g* for 20 min at 4°C to separate the plasma from the formed blood elements.

5 h postligation the rabbits were killed with an overdose of pentobarbital and the hearts and aortae were removed. Tissue biopsies were taken from the ischemic region of the heart (MI region) perfused by the LAD coronary artery, and from a nonischemic region of the heart perfused by the circumflex artery (NMI region). The 200-mg tissue biopsies were homogenized in ice-cold 0.25 mol/liter sucrose (1:10, wt/vol) containing 1 mmol/liter EDTA and 0.1 mmol/liter mercaptoethanol using a (PCU-2 Polytron; Brinkmann Instruments, Westbury, NY) homogenizer. Homogenates were centrifuged at 36,000 *g* at 4°C for 30 min. The supernatant was decanted and assayed for myocardial creatine kinase (CK) activity, total protein, and free amino-nitrogen concentration. The loss of myocardial CK activity and free amino-nitrogen content are reproducible markers of myocardial ischemic damage (21–23).

Myocardial and plasma CK activity was analyzed by the Rosalki method (24), whereas free amino-nitrogen concentration was analyzed by the ninhydrin method after deproteinization with 5% TCA (25). Protein concentrations were measured on all samples by the biuret method (26). CK activity is expressed in international units per milligram of protein and free amino-nitrogen concentration in units per milligram of protein where 1 U is equal to 1 μeq of serine.

Histology. To determine whether any histological changes occurred during the 2 wk of cholesterol feeding, sequential 10-μm thick sections of the aorta were surveyed. Segments of the thoracic aorta from three animals in each group were fixed in 10% formalin and dehydrated. Fixed blocks of tissue were subsequently sectioned and stained with hematoxylin and eosin. Photomicrographs were taken using an Axioplan microscope (Carl Zeiss, Inc., Thornwood, NY).

Isolated aortic rings. In separate experiments the thoracic aortae from rabbits were removed and placed in ice-cold Krebs-Henseleit (KH) solution consisting of 118 mM NaCl, 4.75 mM KCl, 2.54 mM $CaCl_2 \cdot H_2O$, 1.19 mM KH_2PO_4, 1.19 mM $MgSO_4 \cdot 7H_2O$, 12.5 mM $NaHCO_2$, and 10 mM glucose, at pH 7.3. The surrounding tissue was carefully dissected away and great care was taken not to injure the endothelium. The aorta was then cut into transverse rings 2–3 mm in width. The rings were then mounted isometrically in 20-ml organ baths containing oxygenated KH warmed to 37°C and gassed with 95% O_2 and 5% CO_2. The rings were suspended under a resting force of 1.0 g and allowed to equilibrate for 2 h before the administration of any agents. Isometric contractions were measured on a model 7 oscillographic recorder using FT-03 force transducers (both from Grass Instruments, Quincy, MA). Fresh KH solution was added to the bath periodically during the equilibration period and after each test response. In some of the experiments, the endothelium was gently removed with a moistened cotton-tipped applicator. This technique caused no loss of vasoconstrictor tone in the vessel, but abolished endothelium-dependent relaxation to acetylcholine chloride (0.01–10.0 μM). All rings were precontracted with 100 nM norepinephrine prior to addition of other vasoactive agents (e.g., acetylcholine, $NaNO_2$ at pH 2.0).

Heart perfusion. Rabbits were given 1,000 IU/kg of heparin injected into the marginal ear vein 15 min before induction of anesthesia with pentobarbital sodium (30 mg/kg, i.v.). After a midline sternotomy, the hearts were rapidly excised and placed in ice-cold oxygenated (95% O_2 + 5% CO_2) KH solution. Within 30 s, the hearts were transferred to a perfusion apparatus where they were perfused retrogradely via the aorta with oxygenated (95% O_2 + 5% CO_2) KH solution at 37°C. All hearts were perfused by the Langendorff technique for 10 min at a constant pressure perfusion of 55 mmHg. After this equilibration period, the hearts were perfused at a constant flow rate of 50–60 ml/min without recirculation of the buffer. The perfusate employed was KH buffer solution consisting of: 118 mM NaCl, 4.7 mM KCl, 0.5 mM EDTA, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot H_2O$, 2.9 mM $CaCl_2 \cdot H_2O$, 25 mM $NaHCO_3$, 11 mM glucose, at pH 7.4. Coronary perfusion pressure (CPP) was continuously monitored from a side-arm in the aortic inflow line which was connected to a pressure transducer and a oscillographic recorder. The stable thromboxane A_2-mimetic, U-46619 (300 nM), was used to preconstrict the coronary vasculature before the addition of acetylcholine chloride (Sigma Chemical Co., St. Louis, MO) (0.01–10.0 μM), the calcium ionophore A-23187 (0.1 nM–1.0 μM) (Calbiochem-Behring Corp., La Jolla, CA), or $NaNO_2$ (0.1–100 μM, pH 2.0).

Vascular prostacyclin production. Segments of thoracic aortae were trimmed of excess fat and connective tissue and preincubated in KH buffer (5 ml) for 2 h to remove substances formed during the removal of the vessel. The vessels were then weighed, placed in KH buffer (0.1 ml/l mg tissue) and incubated in a metabolic shaker bath at 37°C for 15 min. Aliquots (250 μl) of buffer were then removed at 0, 2.5, 5, 7.5, 10, and 15 min. The concentration of 6-keto-$PGF_{1\alpha}$, the stable breakdown product of prostacyclin, was determined using a specific radioimmunoassay according to previously established methods (27). The limit of detection using this method is 1 pmol 6-keto-$PGF_{1\alpha}$/ml.

Washed rabbit platelets. Whole blood was collected from the rabbit's central ear artery in acid citrate dextrose buffer (9:1, vol/vol) consisting of the following (in mmol/liter): trisodium citrate, 85; citric acid, 78; and glucose, 110 at pH 5.2. Citrated blood was centrifuged at 500 *g* for 15 min at room temperature. The platelet-rich plasma was removed and centrifuged at 2,000 *g* for 15 min at room temperature. The platelet button was resuspended in 10 ml of Ca^{2+}-free KH buffer and 0.05 ml of 2% EDTA consisting of the following composition (in mmol/liter): NaCl, 120; glucose, 10; $NaHCO_3$, 12.5; KCl, 4.7; KH_2PO_4, 1.2; and $MgSO_4 \cdot 7H_2O$, 1.2 at pH 7.4. The platelet suspension was centrifuged at 2,000 *g* for 10 min at room temperature. The button was then resuspended in 10 ml of a 0.025% albumin-Tyrode's buffer solution consisting of the following composition (in mmol/liter): KCl, 2.6; $MgCl_2 \cdot H_2O$, 1.04; NaCl, 136.9; $CaCl_2 \cdot 2H_2O$, 1.3; Tris (hydroxymethyl)-aminomethane, 7.68; and glucose, 5.55 at pH 7.4.

The platelet suspension was centrifuged at 2,000 *g* for 10 min at room temperature and the resulting button resuspended in 10 ml of Tyrode's-albumin buffer. This platelet suspension was counted with an autoanalyzer particle counter (Technicon Instruments, Tarrytown, NY) and the platelet count adjusted to 300,000/ml. The mixture was then warmed to 37°C for at least 10 min before aggregation studies.

Platelet aggregation was studied in a dual channel (Payton Associates, Inc., Buffalo, NY) aggregometer at 37°C with continuous stirring at 1,000 rpm. Known amounts of freshly prepared platelet activating factor (PAF) or the thromboxane A_2-mimetic, U-46619, were used to generate a dose-response curve. The changes in light transmission occurring during platelet aggregation were continuously recorded and the results normalized to the amount of light transmission through the Tyrode's-albumin buffer alone and expressed as percent aggregation.

Statistics. All values in the text are means±standard error of the mean (SEM). Differences among multigroup means were compared by analysis of variance (ANOVA) (28). Tukey's pairwise comparison was used to determine significance between specific pairs of data (28). All P values < 0.05 were considered statistically significant.

Results

Table I illustrates the plasma cholesterol concentrations developed in the six groups of rabbits after 2 wk of feeding with either the control diet or the 0.5% cholesterol-enriched diet. The initial plasma cholesterol concentration before the feeding period was 51±6 mg/dl for the animals on the control diet and 47±6 mg/dl for the animals on the cholesterol-enriched diet. The two groups fed the control diet for 2 wk demonstrated no significant change in plasma cholesterol concentration. In the 0.5% cholesterol-fed groups given only the lovastatin vehicle, the plasma cholesterol concentrations were 319±25 and 301±21 mg/dl for the sham MI and the MI group, respectively, at the time of the experimental procedure. These plasma cholesterol concentrations are significantly elevated above the values obtained from the same animals at week 0 ($P < 0.001$). The cholesterol-fed, lovastatin-treated animals experienced much smaller increases in plasma cholesterol levels with the final values reaching 139±19 and 124±13 mg/dl for the sham MI and MI group, respectively, $P < 0.01$ from vehicle-treated animals. Thus, all groups began the dietary regimen with equivalent plasma cholesterol concentrations, and the 0.5% cholesterol diet given over a 2-wk period significantly increased the plasma cholesterol concentration on the order of sixfold in the animals given only the vehicle for lovastatin. In those animals receiving lovastatin, there was a significantly smaller ($P < 0.01$) rise in the circulating plasma cholesterol concentration reaching ~ 60% of the values obtained in cholesterol-fed animals given the vehicle. Nevertheless, these values were double those observed in the control diet group.

Table I. Plasma Cholesterol Concentrations after 2 Wk of Feeding 0.5% Cholesterol Supplement to Control Diet

Diet	Plasma cholesterol concentration (mg/dl)		
	Vehicle (DMSO)	Lovastatin	Significance (Vehicle vs. Lovastatin)
		10 mg/d	
Control diet	47±5 (11)	44±4 (7)	NS
0.5% Cholesterol (Sham MI)	319±25 (7)	139±19 (7)	$P < 0.01$
0.5% Cholesterol (MI)	302±21 (7)	124±13 (7)	$P < 0.01$

All values are means±SEM.
Numbers in parentheses represent number of rabbits studied.

The hypercholesterolemia that develops in the cholesterol-fed animals is neither of sufficient intensity nor duration to produce any histological changes in the arterial wall of the cholesterol-fed animals as demonstrated in Fig. 1. A representative photomicrograph of a section of thoracic aorta from an animal fed the 0.5% cholesterol diet for 2 wk and given the vehicle for lovastatin is shown in Fig. 1 *A*. Note the normal architecture of the smooth muscle and elastic fibers of the media. There is no evidence of fatty deposits or lipid filled vacuoles, which usually occur in atherosclerotic vessels. The intima also appears normal with no sign of subintimal thickening. Aortae obtained from animals fed the cholesterol-supplemented diet and given lovastatin daily (Fig. 1 *B*) are histologically similar to the artery obtained from a rabbit fed the cholesterol diet and demonstrate no atherosclerotic changes.

In all rabbits subjected to MI, regardless of dietary or drug treatment, the pressure-rate index (PRI, MABP × HR/1000), an index of myocardial oxygen demand, decreased comparably over the 5-h observation period for each group. These data are summarized in Table II. Therefore, the presence of lovastatin did not alter the basic hemodynamics and did not decrease the myocardial oxygen demand of the treated animals, as MABP, HR, and their product was not different from controls either initially or during 5 h of MI. Similarly, sham MI rabbits given either lovastatin or its vehicle, did not exhibit significant changes in the PRI over the course of the 5-h observation period. In addition, the diet had no demonstrable effect on the PRI.

To determine whether lovastatin itself had any effect on the degree of ischemic damage in the rabbit heart, we gave lovastatin or its vehicle to animals that had received only the control diet (i.e., no additional cholesterol) for 2 wk. These data are summarized in Table III. Treatment with 10 mg/ml lovastatin for 2 wk had no effect on the accumulation of plasma CK activity, myocardial CK activity, or free amino-nitrogen content with respect to the vehicle-treated animals. Therefore, it is unlikely that lovastatin itself induces a direct protective effect on the ischemic myocardium in the absence of hypercholesterolemia.

Fig. 2 demonstrates the plasma CK activities in the 4 groups of rabbits fed the cholesterol-enriched diet. Both groups of sham-operated (sham MI) rabbits did not develop any significant alteration in circulating CK activities over the 5-h observation period. However, the plasma CK activity of rabbits undergoing myocardial ischemia and receiving the vehicle (MI + V) exhibited a fivefold increase. In contrast, the plasma CK activity of rabbits undergoing MI and receiving lovastatin (MI + L) rose only threefold over the 5-h ischemic period. These values were significantly lower at 2, 3, 4, and 5 h compared to those MI animals not receiving the drug. Thus, a significant increase was observed in plasma CK activity in the rabbits receiving only the vehicle than in lovastatin-treated MI rabbits, suggesting a significant degree of myocardial protection with lovastatin treatment.

Fig. 3 summarizes the effect of MI on myocardial tissue CK activity in rabbits receiving either lovastatin or its vehicle.

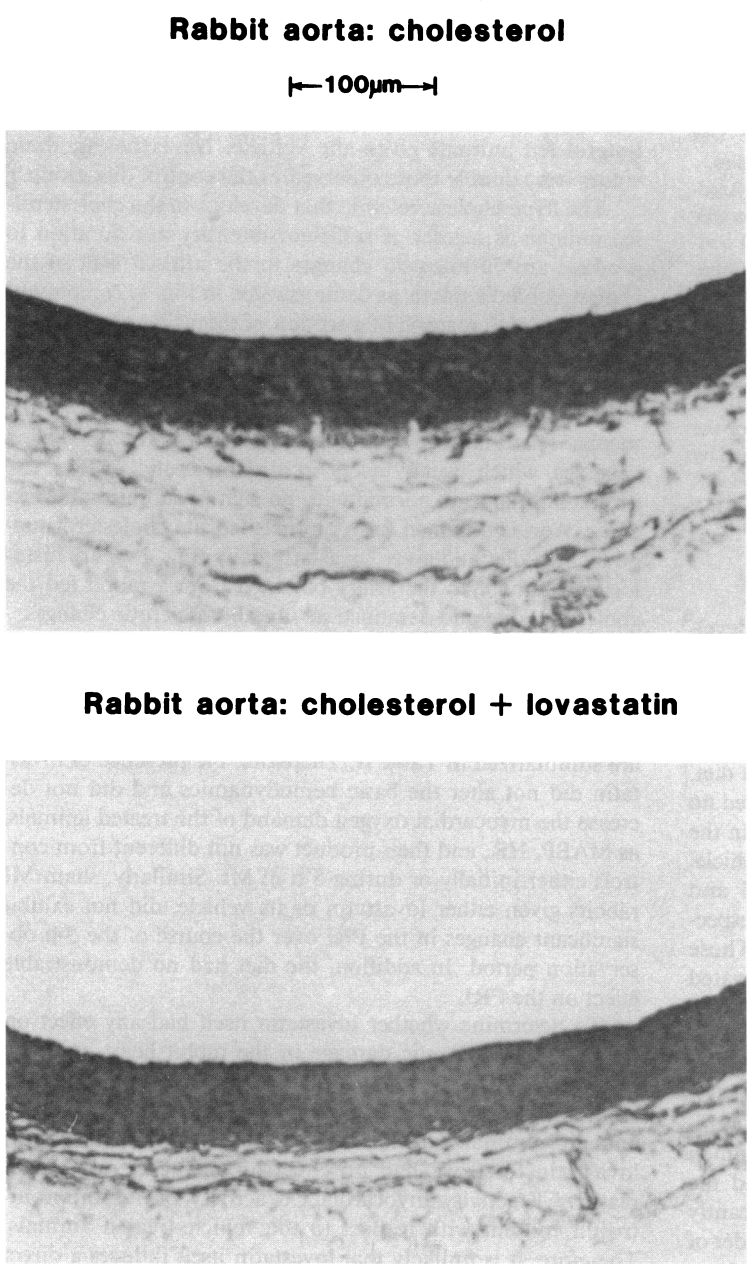

Figure 1. Representative light micrographs of thoracic aortae obtained from rabbits undergoing myocardial ischemia for five hours. (*Top*) Aorta from rabbit fed the cholesterol-enriched diet and given the vehicle for lovastatin. (*Bottom*) Aorta from rabbit fed the cholesterol-enriched diet and given lovastatin 10 mg/d. ×300.

The values expressed are the difference in myocardial CK activity between the nonischemic region of the left ventricle (NMI; i.e., that area perfused by the circumflex artery) and the ischemic region of the left ventricle (MI; i.e., that area perfused by the LAD coronary artery). Rabbit hearts undergoing ischemia and given only the vehicle exhibited a much greater loss of myocardial CK than the comparable lovastatin-treated animals 5 h after coronary artery occlusion ($P < 0.01$). Thus, the evidence points to a greater degree of myocardial cell injury in the untreated group of cholesterol-fed rabbits subjected to 5 h of coronary artery occlusion compared with the MI rabbits given lovastatin ($P < 0.01$). Neither group of sham MI rabbits developed any significant degree of myocardial cell injury as evidenced by small values for CK loss which are not significantly different from zero.

In order to verify the changes in myocardial CK activity, we analyzed the myocardial tissue samples for total free amino-nitrogen concentration as a second index of myocardial cell damage. The differences in myocardial amino-nitrogen between the NMI and the MI regions are shown in Fig. 4. Neither of the sham MI groups developed any significant loss of myocardial amino-nitrogen. The loss of myocardial amino-nitrogen from the ischemic zone of the MI group receiving only the vehicle was significantly greater than the MI group given the drug ($P < 0.01$). Thus, a similar degree of myocardial tissue injury was observed with amino-nitrogen, and the protective effect of lovastatin was evident with this index of ischemic injury.

In order to investigate the effects of short-term hypercholesterolemia on endothelium-dependent relaxation, we studied a well characterized endothelium-dependent vasodilator agent, acetylcholine (ACh, 0.01–10.0 μM), in isolated rabbit aortic rings precontracted with 100 nM norepinephrine. The response of aortic rings obtained from rabbits fed the choles-

Table II. Summary of Pressure-Rate Index Data in Anesthetized Rabbits Undergoing Myocardial Ischemia (MI) or Sham Myocardial Ischemia (SMI)

Group	N	Time (min) 0	60	120	180	240	300
Control diet							
MI + vehicle	11	24±2	23±2	22±2	23±1	21±2	20±1
MI + lovastatin	7	25±2	24±2	23±1	22±2	23±3	20±1
Cholesterol diet							
Sham MI + vehicle	7	23±2	22±1	21±2	23±1	20±1	21±2
MI + vehicle	7	25±1	22±2	23±1	22±1	21±1	20±1
Sham MI + lovastatin	7	24±1	23±1	21±1	23±1	22±1	20±1
MI + lovastatin	7	24±2	24±2	22±2	23±1	22±2	21±1

All values are means±SEM. N = number of rabbits studied.

terol-enriched diet and treated with lovastatin or its vehicle is shown in Fig. 5. The responses in which the endothelium was present were compared to rings denuded of endothelium. The data clearly demonstrate that ACh dilates only rings having a functionally intact endothelium. Moreover, the vasodilator effect of acetylcholine in rings from cholesterol-fed rabbits is significantly reduced at the higher concentrations of ACh (i.e., $P < 0.05$ at 1 μM; $P < 0.01$ at 10 μM), even in the presence of an intact endothelium. Thus, there is a significant difference in the responsiveness of the arteries obtained from hypercholesterolemic animals to ACh. However, the impaired vasodilator response to ACh can be normalized in the hypercholesterolemic animals by treatment with lovastatin.

Table III. Effect of Lovastatin or its Vehicle on Indices of Ischemic Damage in Non-cholesterol-fed Animals Subjected to Myocardial Ischemia

Diet	Vehicle (DMSO)	Lovastatin	Significance
		10 mg/d	
Plasma CK activity	Initial	Initial	
(IU/mg protein)	2.6±0.5	2.3±0.4	NS
	Final	Final	
	10.5±1.9	11.0±3.0	NS
	(11)	(7)	
	5.1±0.9	4.7±1.0	NS
Myocardial CK activity (IU/mg protein) (NMI − MI)	(11)	(7)	
	0.23±0.04	0.22±0.05	NS
Free amino-nitrogen content (U/mg protein) (NMI − MI)	(11)	(7)	

All values are means±SEM. Numbers in parentheses represent number of rabbits studied. NMI − MI = non-ischemic region − ischemic region. Initial plasma CK values were obtained just before coronary artery occlusion; final values were obtained after 5 h of ischemia.

The ability of the coronary vasculature of the rabbit heart to react to vasodilators was investigated using the isolated Langendorff-perfused heart. The thromboxane A_2-mimetic, U-46619, at 300 nM produced coronary vasoconstriction as evidenced by an increase in CPP. This increase in CPP was then reversed by ACh in the hearts from the rabbits fed the control diet (Fig. 6 *A*). However, in the hearts obtained from rabbits fed the 0.5% cholesterol diet, the vascular response to ACh was further constriction. Therefore, the hypercholesterolemia produced during the cholesterol feeding results in a defect in the ability of the coronary vasculature to relax to ACh. This defect was reversed by treating the animals with lovastatin as demonstrated by the significant vasodilation produced after ACh infusion in the animals fed cholesterol and administered the reductase inhibitor. To insure that the ability of the coronaries to relax was undiminished by the dietary regimen, the vasodilator responses of a non-endothelium-dependent vasodilator (i.e., acidified $NaNO_2$) was evaluated. Acidified $NaNO_2$ produced nearly complete relaxation in all groups of

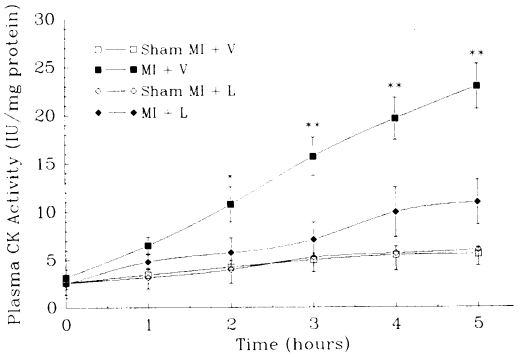

Figure 2. Plasma CK activity over 5 h of sham myocardial ischemia (Sham MI) or myocardial ischemia (MI) in rabbits fed a 0.5% cholesterol diet with either lovastatin (L) or its vehicle (V). Sham-operated controls (Sham MI) given lovastatin or its vehicle showed no difference in plasma CK activity over the course of the experiment. Rabbits undergoing MI without lovastatin treatment showed large increases in plasma CK activity that were significantly greater than those in the MI group given lovastatin. All values are means±SEM with $n = 7$ animals per group. *$P < 0.05$ from MI rabbits given lovastatin. **$P < 0.01$ from MI rabbits given lovastatin.

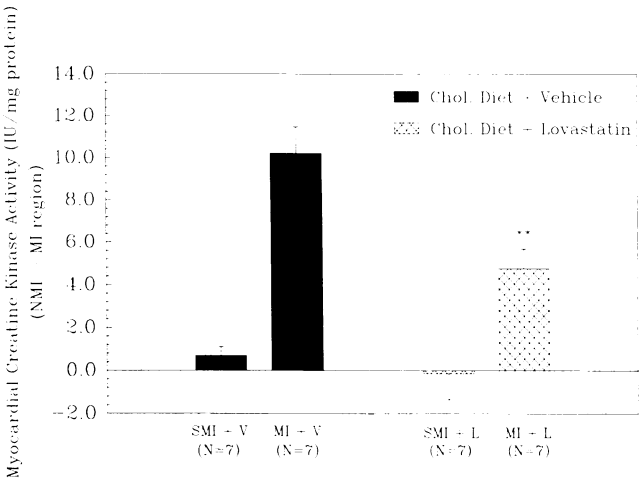

Figure 3. Difference in myocardial CK activities between the non-ischemic region (NMI) and the ischemic region (MI) in rabbit hearts 5 h postligation or sham ligation. All animals were fed 0.5% cholesterol as a dietary supplement. Both MI groups differed significantly from the sham operated control (SMI) animals ($P < 0.01$). Rabbits fed 0.5% cholesterol also developed a significantly greater CK loss ($P < 0.01$) than control diet rabbits (Table II). Bar heights represent means; brackets indicate ±SEM; and N = number of rabbits used in each group. **$P < 0.05$ from MI rabbits given vehicle alone (MI + V).

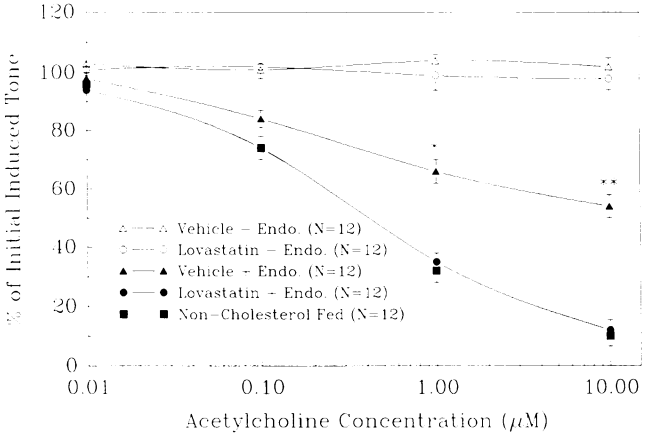

Figure 5. Concentration-response curves to acetylcholine (0.01–10.0 μM) on isolated rabbit aortic rings. All values are means±SEM; N = number of rings. *$P < 0.05$ from rings obtained from vehicle-treated animals. **$P < 0.01$ from vehicle.

animals independent of dietary or pharmacologic treatment (Fig. 6 *B*), indicating that the vasorelaxant properties of the smooth muscle are unimpaired by hypercholesterolemia.

Another vasoactive mediator released by the vessel wall is prostacyclin (PGI_2). PGI_2 is a potent, naturally occurring platelet antiaggregatory agent and vasodilator. In order to determine whether the cholesterol-enriched diet had any effect on the production of PGI_2 by the vasculature, we incubated sections of the thoracic aorta in warmed KH solution and assayed for the stable breakdown product of PGI_2, 6-keto-$PGF_{1\alpha}$. The results are shown in Table IV. 2 wk of cholesterol-feeding did not reduce the ability of the vessel to produce prostacyclin as compared to the control diet animals. However, in the animals fed cholesterol for two weeks, there was a significantly greater PGI_2 production in aortae from lovastatin-treated animals as compared to those obtained from rabbits receiving vehicle alone. Thus, lovastatin, by reducing circulating cholesterol levels, may foster conditions favoring the endothelial release of prostacyclin during appropriate stimuli (e.g., myocardial ischemia).

Lastly, we wished to test whether the platelets of these cholesterol-fed animals had any increased sensitivity to platelet aggregating agents, such as the thromboxane A_2-mimetic

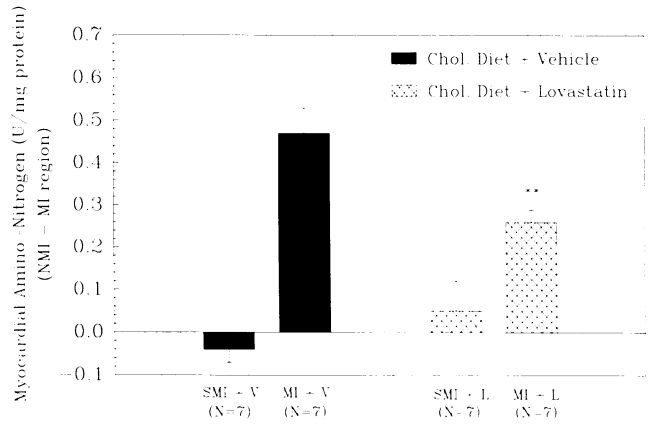

Figure 4. Difference in myocardial free amino-nitrogen concentrations between the nonischemic region (NMI) and the ischemic region (MI) in rabbit hearts 5 h postligation or sham ligation. All animals were fed the 0.5% cholesterol-enriched diet. The rabbits fed a 0.5% cholesterol diet and given only vehicle (V) developed a loss of free amino-nitrogen during MI, which was significantly greater than that of the MI group given lovastatin (L) ($P < 0.01$). No difference existed between the sham MI (SMI) rabbits given lovastatin and the sham MI rabbits given the vehicle. Bar heights represent means; brackets indicate±SEM; and N = number of rabbits used in each group. **$P < 0.05$ from MI rabbits given vehicle alone (V).

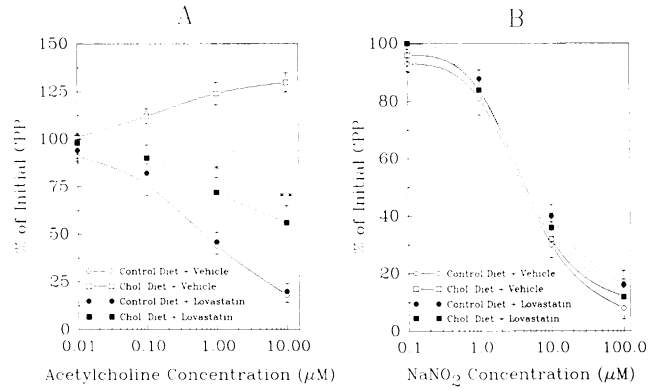

Figure 6. Change in CPP as percentage of initial response to 300 nM U-46619 (U-46) in the Langendorff perfused rabbit heart. (*A*) The concentration-response curve to acetylcholine (ACh, 0.01–10 μM); (*B*) the concentration-dependent decrease in CPP in response to $NaNO_2$ (0.1–100 μM, pH 2.0). All values are means±SEM with n = 7 animals per group. *$P < 0.05$ from cholesterol fed rabbits given vehicle. **$P < 0.01$ from cholesterol rabbits given vehicle.

Table IV. Prostacyclin Release from Isolated Thoracic Aortae of Rabbits Fed Either the Control Diet or the Cholesterol-enriched Diet and Treated with Either Lovastatin or Its Vehicle

Time	Medium 6-keto-$PGF_{1\alpha}$ concentration (*pmol/ml*)			
	Control diet + vehicle	Control diet + lovastatin	Cholesterol diet + vehicle	Cholesterol diet + lovastatin
(min)				
0	12.6±0.5	12.3±0.4	11.2±0.6	13.6±0.5
2.5	14.0±0.8	16.8±0.7	13.3±0.9	17.5±0.8
5.0	15.6±1.3	19.8±1.8	14.0±1.6	22.0±1.5*
7.5	16.5±1.5	21.3±2.6	15.9±2.0	24.8±2.6*
10.0	18.8±1.8	22.3±2.8	19.6±2.2	26.8±2.6*
15.0	20.5±1.5	26.4±3.0	23.0±2.3	28.5±2.1*

All values are means±SEM from five experiments. 0.5% cholesterol-enriched diet. * $P < 0.05$ from cholesterol diet + vehicle.

U-46619 or platelet activating factor (PAF). The data are summarized in Fig. 7. Washed platelets from cholesterol-fed and control diet animals exhibited equivalent sensitivity to U-46619 over the range of 10 nM to 10 μM. Similarly, aggregation to PAF was similar for both groups, regardless of dietary treatment, although PAF was a much more potent aggregator of platelets, being active over the range of 0.05 to 50 nM. Thus, after 2 wk of cholesterol feeding, the sensitivity of platelets obtained from cholesterol-fed animals to these two agents is not different from that seen in non-cholesterol-fed animals. Therefore, the enhanced degree of ischemic damage occurring in hypercholesterolemic rabbits does not appear to be due to enhanced platelet aggregability.

Discussion

We have presented data which show that lovastatin is effective in reducing the cardiac damage associated with acute myocardial ischemia in the hypercholesterolemic rabbit. The exact mechanisms involved in the preservation of the ischemic myocardium is complex although in vitro data seem to indicate that the ability of lovastatin to preserve endothelial function in arteries and in the coronary vascular bed may play a role in its beneficial effects in MI. This endothelial protective effect is manifested by an improved endothelium derived relaxing factor (EDRF) release in response to vasoactive agents as well as a better maintained production of the vasodilator and cytoprotective eicosanoid, prostacyclin.

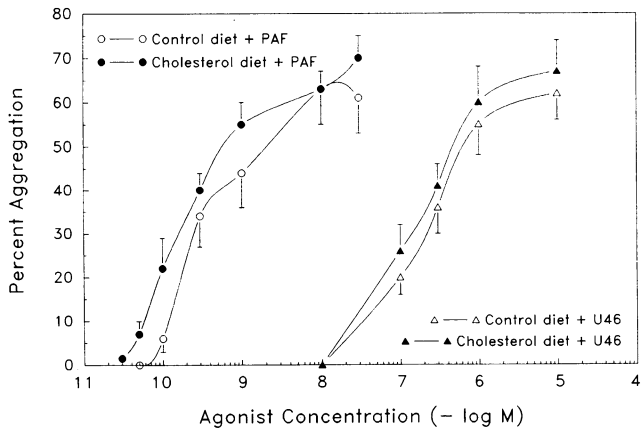

Figure 7. Dose-response curve of PAF- and U-46619-induced platelet aggregation in washed rabbit platelets obtained from rabbits fed either the control diet of the 0.5% cholesterol-enriched diet for 2 wk. Points equal means of six to eight experiments; brackets are ±SEM.

In the present study we employed three indices to measure the degree of myocardial ischemic damage: plasma CK activity, myocardial CK activity, and myocardial free amino-nitrogen concentration. All three indices demonstrate the ability of lovastatin to reduce the severity of ischemic damage associated with coronary artery occlusion. Since hemodynamic variables were neither altered by the diet nor by lovastatin, the mechanism of protection in ischemia does not appear to be due to a reduction in myocardial oxygen demand. It is therefore reasonable to hypothesize that the protective effects are due to the effects of lovastatin on the vasculature, specifically its ability to attenuate the impaired endothelium-dependent relaxation. It is unlikely that the beneficial effect of lovastatin is related to a decrease in platelet aggregation since we saw no evidence of enhanced sensitivity to two potent platelet aggregating agents (i.e., PAF, thromboxane A_2-mimetic) in in vitro platelet aggregation. Thus, lovastatin may ameliorate a pathophysiologic condition in the hypercholesterolemic rabbit. However, it does not achieve this by inhibiting platelet activation.

Our findings are somewhat different from those reported by Golino et al. (2) who found that feeding rabbits a 2% cholesterol diet for a very short time (i.e., 2–3 d) was sufficient to cause a doubling of the area of infarct. This increase in infarct size was attributed to enhanced platelet activation causing infarct extension (4). However, there are several important differences between that study and the present study. First, the model employed by Golino and colleagues was a 90-min occlusion followed by reperfusion of the ischemic segment. It is possible that a reperfusion model may be much more sensitive to the effects of intravascular platelet activation and vascular plugging than a nonreperfusion model. In addition, the duration of cholesterol-feeding in our model is longer than the model used by Golino et al. (2). It is possible that the effects of hypercholesterolemia on the vessels do not develop with very short-term hypercholesterolemia (i.e., 2–3 d). In this regard, we tested two rabbits 2–3 d after a 0.5% cholesterol diet, and failed to observe a defect in ACh-induced vasorelaxation of aortic rings. These factors could help to explain the differences in results obtained.

Several groups have shown that platelets exposed to a cholesterol-rich environment have an enhanced sensitivity to a variety of aggregating agents including TXA_2-mimetics (6–9, 29). The present study demonstrates that the platelets isolated from cholesterol-fed animals did not show an increase in the sensitivity to either a TXA_2-mimetic, U-46619, or platelet activating factor. Thus, a longer term and perhaps more severe hypercholesterolemia is required to alter platelet membrane function.

This study is the first to show the beneficial effects of an inhibitor of cholesterol biosynthesis on myocardial ischemia in the rabbit with concomitant hypercholesterolemia. While the inhibitors of HMGCoA reductase have been shown to be effi-

cacious in lowering elevated plasma cholesterol concentrations, this is the first report of the ability of lovastatin to protect the myocardium from ischemic injury. It is possible that one of the mechanisms by which lovastatin may act to lessen the severity of ischemia is by acting as a calcium channel blocker (30). Alternately, lovastatin may protect the circulatory system by facilitating the lowering of plasma low density lipoproteins. In this regard, Andrews et al. (31) have shown that elevated low density lipoproteins impair the ability of the vasculature to dilate to endothelium-dependent agents. Another possibility is that lovastatin may prevent endothelial cell damage resulting in preservation of endothelial production of prostacyclin. Since prostacyclin is known to preserve the integrity of the ischemic myocardium (23, 31, 32) probably by a cytoprotective action, this has potential significance in our experiment.

Whether these are unique properties of lovastatin or common to other HMGCoA reductase inhibitors is unknown at this time. Moreover, caution must be taken in extrapolating these results to human subjects since we do not know whether these results apply to humans, and because our dose of lovastatin is approximately three times the maximal clinical dosage.

Acknowledgments

We gratefully acknowledge the expert technical assistance of Donna Mulloy and Judith Komlosh during the course of these studies. We also wish to thank Mr. Alfred W. Alberts of the Merck Sharp & Dohme Research Laboratories, Rahway, NJ, for the generous supply of lovastatin and Dr. John Pike of The Upjohn Co., Kalamazoo, MI, for the generous supply of 9,11-methanoepoxy PGH_2 (U-46619).

Supported by National Institutes of Health grant HL-25575 from the National Heart, Lung and Blood Institute. Mr. Osborne is a Predoctoral Fellow of the Percival E. and Ethel Brown Foerderer Foundation. Mr. Siegfried is a Gibbon Fellow of Thomas Jefferson University. Dr. Stahl is a Predoctoral Fellow (HL-07599).

References

1. Osborne, J. A., R. K. Mentley, and A. M. Lefer. 1987. Increased severity of acute myocardial ischemia in experimental atherosclerosis. *Heart Vessels.* 3:73–79.

2. Golino, P., P. R. Maroko, and T. E. Carew. 1987. The effect of acute hypercholesterolemia on myocardial infarct size and the no-reflow phenomenon during coronary occlusion-reperfusion. *Circulation.* 75:292–298.

3. Lipid Research Clinics Program. 1984. The lipid research clinics primary prevention trial results II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA (J. Am. Med. Assoc.).* 251:365–374.

4. Golino, P., P. R. Maroko, and T. E. Carew. 1987. Efficacy of platelet depletion in counteracting the detrimental effect of acute hypercholesterolemia on infarct size and the no-reflow phenomenon in rabbits undergoing coronary artery occlusion-reperfusion. *Circulation.* 76:173–180.

5. Freiman, P. C., G. G. Mitchell, D. D. Heistad, M. L. Armstrong, and D. G. Harrison. 1986. Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates. *Circ. Res.* 58:783–789.

6. Moscat, J., P. Perez, P. G. Gavilanes, F. Acin, A. Schuller, and A. M. Municio. 1986. Membrane fluidity and thromboxane synthesis in platelets from patients with severe atherosclerosis. *Thromb. Res.* 44:197–205.

7. Carvalho, A. C., R. W. Colman, and R. S. Lees. 1974. Platelet function in hyperlipoproteinemia. *N. Engl. J. Med.* 290:434–438.

8. Shattil, S. J., R. Anay-Galindo, J. Bennett, R. W. Coleman, and R. A. Cooper. 1975. Platelet hypersensitivity induced by cholesterol incorporation. *J. Clin. Invest.* 55:636–647.

9. Zmuda, A., A. Dembinska-Kiec, A. Chytokowski, and R. J. Gryglewski. 1977. Experimental atherosclerosis in rabbits: platelet aggregation, thromboxane A_2 generation and anti-aggregatory potency of prostacyclin. *Prostaglandins.* 14:1035–1045.

10. Shimowawa, H., H. Tomoike, S. Nabeyama, H. Yamamoto, H. Araki, and M. Nakamura. 1983. Coronary artery spasm induced in atherosclerotic miniature swine. *Science (Wash. DC).* 221:560–562.

11. Yokoyama, M., H. Akita, T. Mizutani, H. Fukuzaki, and Y. Watanabe. 1983. Hyperreactivity of coronary arterial smooth muscles in response to ergonovine from rabbits with hereditary hyperlipidemia. *Circ. Res.* 53:63–71.

12. Heistad, D. D., M. L. Armstrong, M. L. Marcus, D. J. Piegors, and A. L. Mark. 1984. Augmented responses to vasoconstrictor stimuli in hypercholesterolemic and atherosclerotic monkeys. *Circ. Res.* 54:711–718.

13. Furchgott, R. F. 1983. Role of endothelium in responses of vascular smooth muscle. *Circ. Res.* 53:557–573.

14. Furchgott, R. F., and J. V. Zawadski. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature (Lond.).* 288:373–376.

15. Lefer, A. M., J. A. Osborne, A. Yanagisawa, and J.-Z. Sun. 1988. Influence of atherosclerosis on vascular responsiveness in isolated rabbit vascular smooth muscle. *Cardiovasc. Drugs Ther.* 1:385–391.

16. Verbeuren, T. J., F. H. Jordaens, L. L. Zoonekeyn, C. E. Van Hove, M.-C. Coene, and A. G. Herman. 1986. Effect of hypercholesterolemia on vascular reactivity in the rabbit: I. Endothelium-dependent and endothelium-independent contractions and relaxations in isolated arteries of control and hypercholesterolemic rabbits. *Circ. Res.* 58:552–564.

17. Tobert, J. A., G. D. Bell, J. Birtwell, I. James, W. R. Kukovetz, J. S. Pryor, A. Buntinx, I. B. Holmes, Y. S. Chao, and J. A. Bolognese. 1982. Cholesterol-lowering effect of mevinolin, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, in healthy volunteers. *J. Clin. Invest.* 69:913–919.

18. Kritchevsky, D., S. A. Tepper, and D. M. Klurfeld. 1981. Influence of mevinolin on experimental atherosclerosis in rabbits. *Pharmacol. Res. Commun.* 13:921–926.

19. Allain, L. A., L. S. Poon, L. S. G. Chan, W. Richmond, and P. L. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* 20:470–475.

20. Toyo-oka, T., T. Kamishiro, H. Fumino, T. Masaki, and S. Hosoda. 1984. Rabbit hearts for the critical evaluation of drugs to reduce the size of experimentally produced acute myocardial infarction. *Jpn. Heart J.* 25:623–632.

21. Kjekshus, J. K., and B. E. Sobel. 1970. Depressed myocardial creatine phosphokinase activity following experimental myocardial infarction in rabbits. *Circ. Res.* 27:403–414.

22. Mickelson, J. K., C. J. Carlson, W. Margaretten, and E. Rapaport. 1986. Streptokinase alters myocardial creatine kinase depletion after ischaemia and reperfusion in rabbits. *Clin. Exp. Pharmacol. Physiol.* 13:637–646.

23. Ogletree, M. L., and A. M. Lefer. 1978. Prostaglandin-induced preservation of the ischemic myocardium. *Circ. Res.* 42:218–224.

24. Rosalki, S. B. 1967. Improved procedure for creatine phosphokinase determination. *J. Lab. Clin. Med.* 69:696–705.

25. Kabat, E. A. 1961. Estimation of protein with the biuret and ninhydrin reactions. *In* Experimental Immunochemistry. Charles C. Thomas, Springfield, IL. 559–563.

26. Gornall, A. G., C. T. Bardowill, and M. M. David. 1949. Deter-

mination of serum protein by means of the biuret method. *J. Biol. Chem.* 177:741–766.

27. Ingerman-Wojenski, C., M. J. Silver, J. B. Smith, and E. Macarak. 1981. Bovine endothelial cells in culture produce thromboxane as well as prostacyclin. *J. Clin. Invest.* 67:1292–1296.

28. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. 6th edition. Iowa State University Press, Ames, IA.

29. Gryglewski, R. J., A. Dembinska-Kiec, A. Zmuda, and T. Gryglewski. 1978. Prostacyclin and thromboxane A_2 biosynthesis capacities of the heart, arteries and platelets at various stages of experimental atherosclerosis in rabbits. *Atherosclerosis.* 31:485–392.

30. Renaud, J.-F., A. Schmid, G. Romey, J.-L. Nano, and M. Lazdunski. 1986. Mevinolin, an inhibitor of cholesterol biosynthesis, drastically depresses Ca^{2+} channel activity in cardiac cells in culture. *Proc. Natl. Acad. Sci. USA.* 83:8007–8011.

31. Andrews, H. E., K. R. Bruckdorfer, R. C. Dunn, and M. Jacobs. 1987. Low-density lipoproteins inhibit endothelium-dependent relaxation in rabbit aorta. *Nature (Lond.).* 327:237–239.

32. Ogletree, M. L., A. M. Lefer, J. B. Smith, and K. C. Nicolaou. 1979. Studies on the protective effect of prostacyclin in acute myocardial ischemia. *Eur. J. Pharmacol.* 56:95–103.

33. Araki, H., and A. M. Lefer. 1980. Role of prostacyclin in the preservation of ischemic myocardial tissue in the perfused cat heart. *Circ. Res.* 47:757–763.