Journal of the American College of Cardiology
© 2002 by the American College of Cardiology Foundation
Published by Elsevier Science Inc.
Vol. 40, No. 3, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(02)01985-X

EXPERIMENTAL STUDIES

Simvastatin Preserves Myocardial Perfusion and Coronary Microvascular Permeability in Experimental Hypercholesterolemia Independent of Lipid Lowering

Piero O. Bonetti, MD,* Stephanie H. Wilson, MBBS,* Martin Rodriguez-Porcel, MD,* David R. Holmes, JR, MD, FACC,* Lilach O. Lerman, MD, PHD,† Amir Lerman, MD, FACC*
Rochester, Minnesota

OBJECTIVES This study was designed to assess the lipid-independent effects of simvastatin on myocardial perfusion (MP) and coronary microvascular permeability index (PI) at baseline and during episodes of increased cardiac demand in experimental hypercholesterolemia.

BACKGROUND Simvastatin preserves coronary endothelial function in experimental hypercholesterolemia independent of its lipid-lowering effect. However, the functional significance of this observation is unknown.

METHODS Pigs were randomized to three groups: normal diet (N), high-cholesterol diet (HC) and HC diet plus simvastatin (HC+S) for 12 weeks. Subsequently, cardiac electron beam computed tomography was performed before and during intravenous infusion of adenosine and dobutamine, and MP and PI were calculated.

RESULTS Total and low density lipoprotein cholesterol levels were similarly and significantly increased in HC and HC+S animals compared with N. Basal MP was similar in all groups. Myocardial perfusion significantly increased in response to either adenosine or dobutamine in N and HC+S animals. Dobutamine also significantly increased MP in HC animals. However, the changes of MP in response to either drug were significantly lower in the HC group compared with the other two groups ($p < 0.01$ for adenosine and $p < 0.05$ for dobutamine vs. N and HC+S). Basal PI was similar in all groups and was not altered by either drug in N and HC+S animals. In contrast, PI significantly increased in HC pigs during infusion of either adenosine ($p < 0.001$) or dobutamine ($p < 0.05$).

CONCLUSIONS These findings demonstrate that chronic administration of simvastatin preserves myocardial perfusion response and coronary microvascular integrity during cardiac stress in experimental hypercholesterolemia independent of lipid lowering. (J Am Coll Cardiol 2002;40:546–54)

Several large-scale trials have demonstrated that 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, commonly referred to as statins, significantly reduce cardiovascular morbidity and mortality in patients with a wide range of cholesterol levels (1–5). Plaque regression induced by cholesterol reduction alone is not considered sufficient to explain the striking benefit in these patients (6). Indeed, recent evidence suggests that statins exert a multiplicity of favorable effects on the vascular system that are not directly related to their impact on lipid metabolism. These pleiotropic properties may contribute to the observed benefit in coronary artery disease beyond the reduction of cholesterol levels (7).

Hypercholesterolemia is associated with the development of coronary endothelial dysfunction (8), which is characterized by impaired endothelium-dependent vasorelaxation (9) and an increase in microvascular permeability (10). We recently demonstrated that changes in coronary vascular function associated with hypercholesterolemia in a porcine model may also lead to an attenuated increase in myocardial perfusion (MP) and an exaggerated augmentation of coronary microvascular permeability in response to an intravenous infusion of adenosine (11). In humans with coronary artery disease, altered endothelial function contributes to ischemic manifestations and also unfavorably modulates the course of acute coronary syndromes (12). Moreover, endothelial dysfunction of the coronary resistance vessels is associated with myocardial ischemia (13) and an adverse prognosis (14), even in the absence of obstructive coronary artery disease. Cholesterol lowering with statins improves endothelial function in hypercholesterolemia (15), and is also associated with an improvement of MP (16) in patients with coronary artery disease. Recently, we demonstrated that preservation of endothelial function of epicardial coronary arteries and coronary arterioles in experimental hypercholesterolemia is one of the statin effects mediated at least partly by lipid-independent mechanisms (17). However, the functional significance of this lipid-independent

From the *Division of Cardiovascular Diseases and the †Division of Hypertension, Department of Internal Medicine, Mayo Clinic and Foundation, Rochester, Minnesota. This study was supported by the National Institutes of Health (grant numbers NIH R01 HL-63911 and HL-63282); the Miami Heart Research Institute; Mayo Foundation; Bruce and Ruth Rappaport Program in Vascular Biology; Margarete und Walther Lichtenstein, Switzerland; Freiwillige Akademische Gesellschaft Basel, Switzerland and an unrestricted medical school grant from Merck.

Manuscript received February 27, 2002; revised manuscript received April 18, 2002, accepted May 7, 2002.

Abbreviations and Acronyms

EBCT = electron beam computed tomography
ECG = electrocardiogram
HC = hypercholesterolemia
HC+S = hypercholesterolemia and simvastatin therapy
HDL = high density lipoprotein
LDL = low density lipoprotein
LVEF = left ventricular ejection fraction
LVEDV = left ventricular end-diastolic volume
LVESV = left ventricular end-systolic volume
LVMM = left ventricular muscle mass
MAP = mean arterial pressure
MP = myocardial perfusion
PI = permeability index

effect of statins on MP and coronary microvascular permeability remains unclear.

The current study was designed to test the hypothesis that chronic administration of simvastatin would preserve MP response and coronary microvascular integrity during situations of increased myocardial demand in an animal model of porcine experimental hypercholesterolemia in vivo independent of lipid lowering. For this purpose, we used electron beam computed tomography (EBCT), an ultrafast computed tomography technique that has been shown to enable accurate quantification of global and regional MP and coronary microvascular permeability in animals as well as in humans (11,18–22).

METHODS

Animals. All the study procedures were designed in accordance with the National Institutes of Health Guidelines and approved by the Mayo Foundation Institutional Animal Care and Use Committee. Eighteen female domestic crossbred pigs, three months of age, were used for the experiments. These were randomized into three groups and subsequently treated for 12 weeks: group 1 (N group; n = 6) was fed a normal diet. Group 2 (HC group; n = 6) was placed on an atherogenic diet of 2% cholesterol and 15% lard by weight (TD 93296, Harlan Teklad, Madison, Wisconsin). In addition to a high-cholesterol diet, animals of group 3 (HC+S group; n = 6) orally received simvastatin, 40 to 80 mg/d. This dosage was based on our previous study (17) and on clinical practice in humans. Plasma lipid profiles (Roche, Nutley, New Jersey) and in vivo studies were performed after 12 weeks.

EBCT studies. Electron beam computed tomography studies were performed as previously described (11,18–24). In brief, animals were anesthetized with ketamine (30 mg/kg) and xylazine (5 mg/kg). Anesthesia was maintained with a constant infusion of ketamine (0.3 mg/kg/min) and xylazine (0.04 mg/kg/min) in saline. Animals were intubated and ventilated with room air. The carotid artery and the jugular vein were exposed by cutdown and cannulated with 8F and 7F sheaths, respectively. After injection of 10,000 U of heparin intravenously and initiation of a continuous intravenous infusion of 1,000 U heparin per hour, a guiding catheter was placed in the descending aorta for online blood pressure measurement and a pigtail catheter was placed in the right atrium for injection of contrast media.

Subsequently, animals were positioned supine in the EBCT (Imatron C-150, Imatron Inc., South San Francisco, California) gantry and an intravenous infusion of saline (5 ml/min) was initiated. With localization scans, cross-sectional images at two adjacent mid-left ventricle levels were identified. After a right atrial bolus injection (0.3 ml/kg over 2 s) of nonionic, low-osmolar contrast media iopamidol (Isovue-370, Squibb Diagnostics, Princeton, New Jersey), 40 consecutive electrocardiogram (ECG)-triggered end-diastolic scans were obtained over the preselected levels at one to three heartbeat intervals. Ten minutes after this baseline study an intravenous infusion of adenosine (400 μg/kg/min) was started and after hemodynamic stabilization EBCT flow studies were repeated. Subsequently, adenosine infusion was discontinued and ECG-triggered cine CT images of the heart were acquired during central venous injection of contrast agent at rest for the determination of left ventricular volumes and left ventricular muscle mass (LVMM). Finally, an intravenous infusion of dobutamine (5 to 25 μg/kg/min, to achieve a heart rate of $\geq$150 beats/min) was started and EBCT flow studies were repeated after hemodynamic stabilization.

EBCT data analysis. The methods have been previously described in detail (11,18–25). In brief, EBCT images were transferred to a Sun workstation and regions of interest were traced in the anterior wall and chamber of the left ventricle using the image analysis software package Analyze (Biomedical Imaging Resource, Mayo Foundation, Rochester, Minnesota), and time-density curves were generated for both regions. The left ventricular curve was fitted with a standard gamma-variate curve-fit, whereas the curve obtained from the anterior wall was fitted using a custom-designed extended gamma-variate curve-fitting algorithm implemented in commercially available computer software (KaleidaGraph, Synergy Software, Reading, Pennsylvania) to obtain curves representing intramyocardial distribution of contrast media in both the intra- and extravascular compartments (11,25). The areas enclosed under the intravascular myocardial and left ventricular cavity curves were subsequently used to calculate an index of intramyocardial mean transit time(s) and vascular blood volume (ml/cc tissue). Myocardial perfusion (ml/g/min) was then computed as: 60 $\times$ (blood volume/mean transit time)/[1.05 $\times$ (1 $-$ blood volume)]. The factor (1 $-$ blood volume) served to correct for dynamic changes in blood volume occurring in the heart in vivo (19–21,25). Coronary microvascular permeability index (PI) (arbitrary units) was calculated as contrast extraction rate/blood volume, where contrast extraction rate (analogous to permeability-surface area product) was derived from the curve depicting the extravascular compartment of the contrast agent as 60 $\times$ 1.05 $\times$

(maximal slope of ascending part of extravascular curve × mean transit time)/area under left ventricular curve (11).

By using commercially available software (Imatron Inc., South San Francisco, California), left ventricular end-diastolic and end-systolic frames were visually identified from each cine-mode movie that spanned the cardiac cycle at the level. Left ventricular volumes were measured by planimetry from the left ventricular apex to the left ventricular base and the tomographic measurements of end-diastolic and end-systolic volume at each level were added by use of a modified Simpson's formula to determine left ventricular end-diastolic (LVEDV) and end-systolic (LVESV) volumes. Stroke volume was then calculated as LVEDV − LVESV, cardiac output as stroke volume × heart rate, and left ventricular ejection fraction (LVEF) as stroke volume/LVEDV (19,24). In addition, left ventricular myocardial area on each end-diastolic frame was measured by planimetry after tracing the outer border of the left ventricular myocardium, and the mass of each short-axis slice was calculated as the product of the myocardial area, scan slice thickness (0.8 cm) and specific gravity of the myocardium (1.05 g/cm^3). Left ventricular muscle mass was then computed as the sum of the masses of the individual scanned sections according to a modified Simpson's formula (19,23).

Statistical analysis. Results are expressed as mean ± SEM. Comparisons within groups were performed using paired Student *t* test. One-way analysis of variance followed by the Bonferroni *t* procedure, if indicated, was used for comparisons among the three different groups. All analyses were done by using SigmaStat statistical software, version 2.03 (SPSS Inc., Chicago, Illinois). Statistical significance was accepted for a value of $p < 0.05$.

RESULTS

Plasma lipid profiles. After 12 weeks of high cholesterol diet, total cholesterol, low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol levels were significantly increased in the HC group and the HC+S group compared with pigs fed a normal diet. The differences between the two groups fed a high-cholesterol diet were not significant ($p = 0.91$, $p = 0.78$, and $p = 1$ for total, LDL and HDL cholesterol), indicating that simvastatin had no lipid-modulating effect. In contrast, triglyceride levels were similar in all experimental groups after the 12-week study period (Table 1).

Body weight and systemic hemodynamics. After the 12-week study period body weight was similar in all three experimental groups. Basal mean arterial pressure (MAP) and basal heart rate did not differ significantly between the groups ($p = 0.11$ and $p = 0.12$, respectively). Adenosine infusion led to a significant and similar decrease in MAP, which was associated with a significant increase in heart rate in all experimental groups. As intended, dobutamine infusion was associated with a significant increase in heart rate, whereas it did not affect MAP. There was no significant difference of LVEF, cardiac output or LVMM between the different groups (Table 2).

Table 1. Plasma Lipid Profiles in the Three Experimental Groups at 12 Weeks

	N	HC	HC+S
Total cholesterol, mg/dl	79 ± 4	379 ± 71*	464 ± 67*
LDL cholesterol, mg/dl	34 ± 4	276 ± 67*	362 ± 60*
HDL cholesterol, mg/dl	39 ± 3	95 ± 7*	98 ± 13*
Triglycerides, mg/dl	26 ± 4	45 ± 9	23 ± 4

Values are mean ± SEM. *$p < 0.05$ vs. N.
HC = hypercholesterolemic pigs; HC+S = hypercholesterolemic pigs receiving simvastatin; HDL = high density lipoprotein; LDL = low density lipoprotein; N = normocholesterolemic pigs.

Myocardial perfusion. Basal MP of the anterior wall was similar in all three groups ($p = 0.74$). Intravenous adenosine infusion led to a significant increase in MP in normal pigs that was not observed in HC pigs. In contrast, in HC+S pigs the MP response to adenosine was normalized. Relative change of MP in response to adenosine was similar in the N and the HC+S groups and significantly differed from that in the HC group (Fig. 1). Intravenous dobutamine led to a significant increase in MP in all experimental groups. However, the increase was more pronounced in the N and the HC+S groups compared with the HC group. Consequently, similar to adenosine, the relative change of MP during dobutamine infusion observed in the N and the HC+S groups was significantly higher than that in the HC group (Fig. 2).

Coronary microvascular permeability. Under basal conditions coronary microvascular PI did not differ substantially among the experimental groups ($p = 0.42$). Intravenous adenosine infusion did not alter coronary microvascular PI in the N group, but led to a significant increase in this parameter in the HC group. However, similar to normals, no change in coronary microvascular PI in response to adenosine was observed in the HC+S group. Compared with N and HC+S animals, the relative change of coronary microvascular PI in response to adenosine was significantly

Table 2. Body Weight and Systemic Hemodynamics in the Three Experimental Groups at 12 Weeks

	Normal	HC	HC+S
Body weight, kg	61 ± 2	61 ± 2	59 ± 2
MAP, mm Hg			
Baseline	111 ± 4	112 ± 7	94 ± 7
Adenosine	101 ± 4*	91 ± 8*	83 ± 6*
Dobutamine	105 ± 7	114 ± 15	103 ± 10
Heart rate, beats/min			
Baseline	75 ± 4	68 ± 6	85 ± 7
Adenosine	88 ± 5*	79 ± 5*	96 ± 6*
Dobutamine	156 ± 3†	153 ± 1†	157 ± 5†
LVEF, %	55 ± 1	58 ± 2	59 ± 1
Cardiac output, l/min	4.4 ± 0.2	4.2 ± 0.5	4.7 ± 0.7
LVMM, g	103.1 ± 3.3	108.9 ± 3.8	104.4 ± 4.6

Values are mean ± SEM. *$p < 0.05$ vs. baseline; † $p < 0.001$ vs. baseline.
HC = hypercholesterolemic pigs; HC+S = hypercholesterolemic pigs receiving simvastatin; LVEF = left ventricular ejection fraction; LVMM = left ventricular muscle mass; MAP = mean arterial pressure; N = normocholesterolemic pigs.

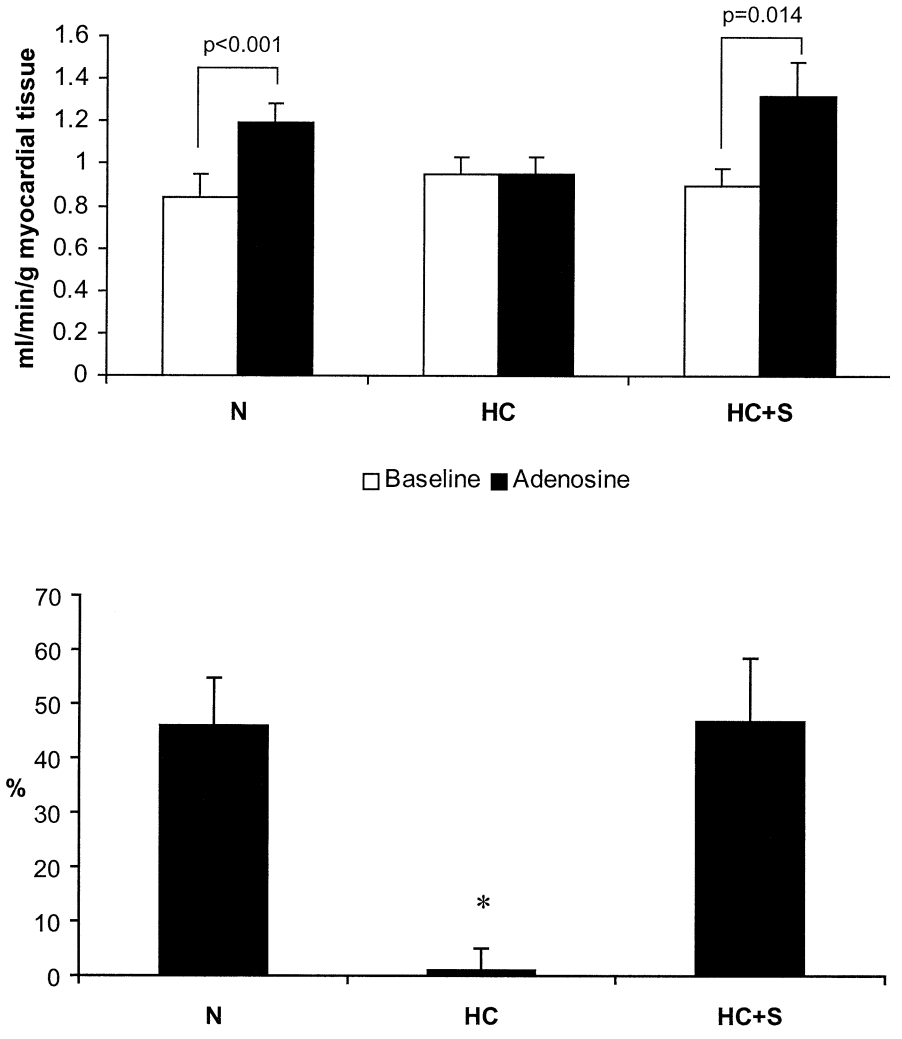

Figure 1. (Upper) Anterior wall myocardial perfusion at baseline and in response to intravenous adenosine in the three experimental groups. **(Lower)** Relative change of anterior wall myocardial perfusion in response to intravenous adenosine in the three experimental groups (compared to baseline). HC = hypercholesterolemic pigs; HC+S = hypercholesterolemic pigs receiving simvastatin; N = normocholesterolemic pigs. *$p < 0.01$ versus N and HC+S.

increased in HC animals (Fig. 3). Similar to adenosine, intravenous infusion of dobutamine was associated with a significant increase in coronary microvascular PI only in the HC group, but not in the N or the HC+S group. The relative change of this parameter in response to dobutamine in HC pigs significantly differed from that in N pigs, whereas the magnitude of the microvascular permeability response in HC+S animals was similar to that in N animals (Fig. 4).

DISCUSSION

The current study demonstrates that simvastatin prevents the attenuation of the MP response and the increase in coronary microvascular permeability during cardiac stress associated with experimental hypercholesterolemia, independent of any lipid-lowering effect.

Assessment of pleiotropic effects of statins in vivo. Statins have been shown to significantly reduce morbidity and mortality from coronary events in patients with a wide range of cholesterol levels (1–5). It is now clear that the clinical benefit of statins cannot be attributed to cholesterol lowering alone and that these drugs exert pleiotropic effects that clearly are independent of their impact on cholesterol levels (7). However, despite growing knowledge about numerous lipid-independent properties of this class of drugs in vitro, little is known about their consequences in vivo. One of the problems in identifying lipid-independent effects of statins in vivo is differentiating them from the indirect effects related to lipid lowering. Another confounding factor is that a direct effect of a statin may complement an indirect effect (26). In accordance with one of our previous studies (17), simvastatin did not alter plasma cholesterol levels in our animal model of dietary hypercholesterolemia, which may be attributable to the pure dietary nature of hypercholesterolemia in our model and the fact that lipid-lowering potency and efficacy of statins are much lower in pigs than in humans (27). Hence, our animal model provides a unique opportunity to study lipid-independent effects of statins in vivo.

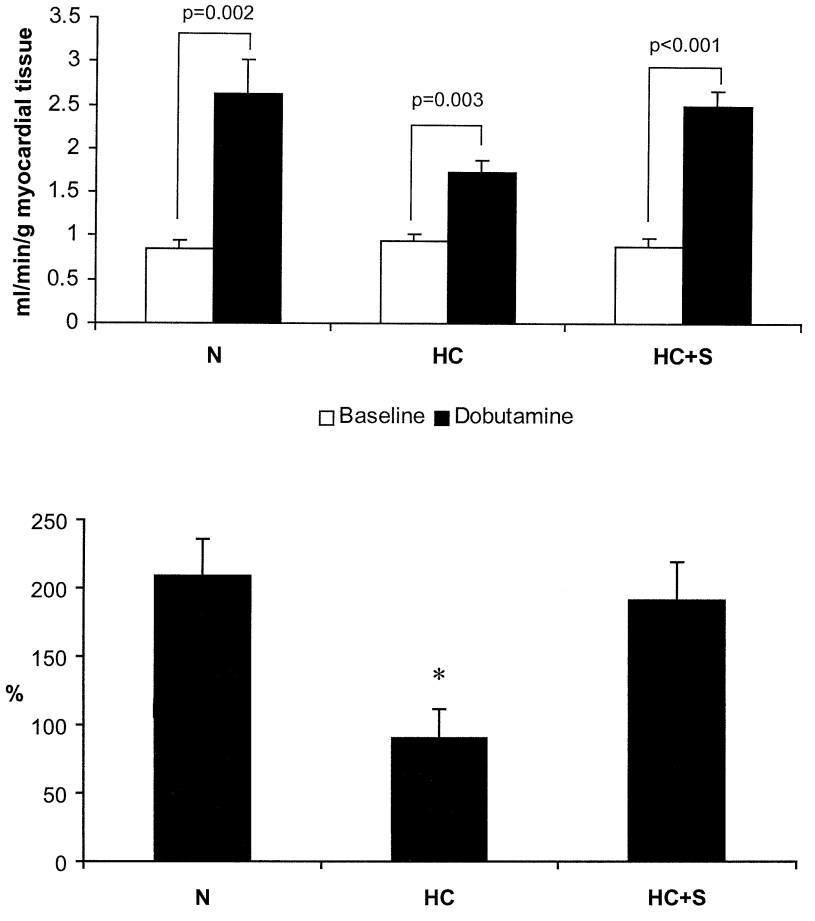

Figure 2. (Upper) Anterior wall myocardial perfusion at baseline and in response to intravenous dobutamine in the three experimental groups. **(Lower)** Relative change of anterior wall myocardial perfusion in response to intravenous dobutamine in the three experimental groups (compared to baseline). HC = hypercholesterolemic pigs; HC+S = hypercholesterolemic pigs receiving simvastatin; N = normocholesterolemic pigs. *$p < 0.05$ vs. N and HC+S.

Effect of simvastatin on MP. Our animal model of hypercholesterolemia has been well characterized and shows endothelial dysfunction of epicardial coronary arteries and coronary resistance vessels (17), without development of atherosclerotic plaques (28). Hence, the observed difference of increase in MP during simulated cardiac stress between untreated hypercholesterolemic pigs and those treated with simvastatin cannot be attributed to the presence of coronary stenoses or an inhibitory effect of simvastatin on the development of such lesions.

Several mechanisms may be responsible for the observed differences of the MP response to simulated cardiac stress between the experimental groups. The hemodynamic effects of dobutamine result from a unique and complex interaction of its alpha- and betamimetic properties (29). However, although dobutamine may have a minor direct vasodilative effect on coronary vessels, the increase in myocardial blood flow in response to this compound is due mainly to enhanced myocardial metabolic demand, which leads to dilation and/or recruitment of coronary resistance vessels, including arterioles that are ≤ 100 μm in diameter. Importantly, endothelium-derived NO has been shown to play a pivotal role for the dilative response of the coronary microvasculature associated with the infusion of dobutamine (30). Thus, the increase in MP in response to dobutamine depends to a certain degree on endothelial function. On the other hand, adenosine is primarily considered an endothelium-independent vasodilator. However, its vasodilatory effect is mediated via specific A_2-receptors that are located not only on vascular smooth muscle cells but also on endothelial cells (31,32). Moreover, several studies demonstrated that the vasorelaxant effect exerted by adenosine involves the release of NO (33,34), and is, therefore, also somewhat dependent on endothelial function. Thus, changes in endothelial function may play a role in the vasodilatory response to both dobutamine and adenosine. Furthermore, we recently demonstrated that treatment with simvastatin preserves endothelial function in epicardial arteries and resistance vessels in vitro in a lipid-independent manner (17). Hence, although direct effects of dobutamine and adenosine might have contributed to our results, it is likely that the lack of increase in MP during infusion of either drug observed in hypercholesterolemic pigs might result from coronary endothelial dysfunction, and that the

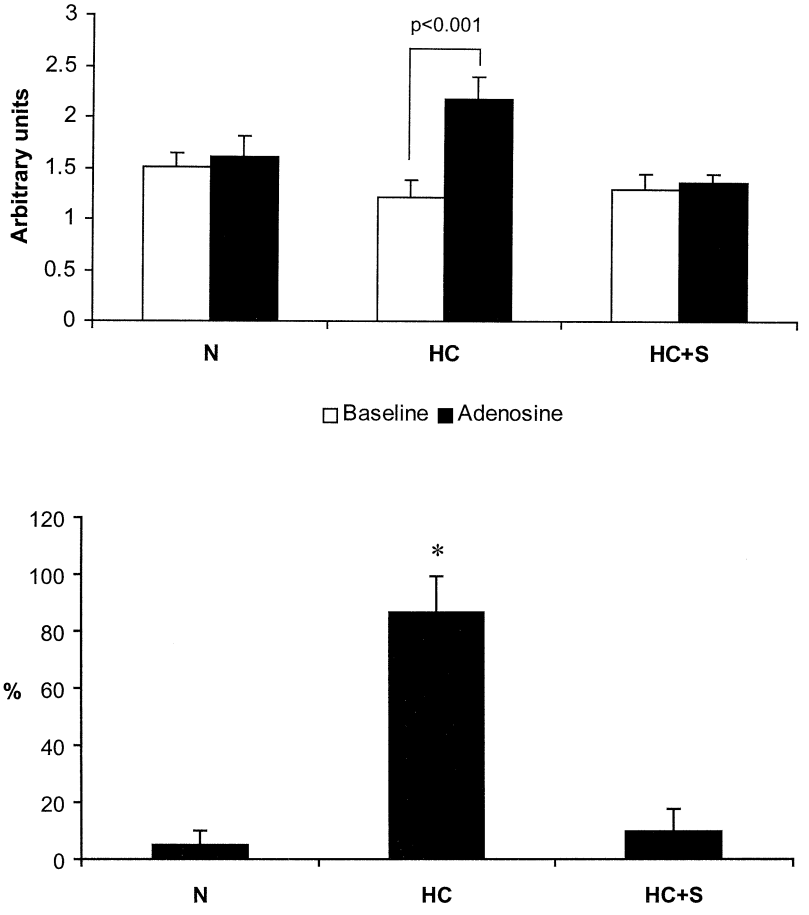

Figure 3. (Upper) Anterior wall microvascular permeability index at baseline and in response to intravenous adenosine in the three experimental groups. **(Lower)** Relative change of anterior wall microvascular permeability index in response to intravenous adenosine in the three experimental groups (compared to baseline). HC = hypercholesterolemic pigs; HC+S = hypercholesterolemic pigs receiving simvastatin; N = normocholesterolemic pigs. *$p < 0.001$ vs. N and HC+S.

normalization of MP in animals treated with simvastatin is the result of preservation of endothelial function. In this context, our results are in accordance with a previous clinical study reporting a reduction of episodes of transient myocardial ischemia with pravastatin in patients with coronary artery disease and normal to moderately elevated serum cholesterol (35). These investigators speculated that the anti-ischemic effect of pravastatin in their study was mainly mediated by a normalization of coronary endothelial function, because an angiographic study in the same patient population demonstrated only less progression but not regression of preexisting coronary atherosclerosis with pravastatin therapy (36). Our current study extends these previous observations and demonstrates that the beneficial effects of simvastatin upon MP are not only independent of lipid lowering, but also occur very early in the course of atherogenesis, even before obvious morphologic alterations of the vessel wall are present (28).

Effect of simvastatin on coronary microvascular permeability. The demonstrated changes of coronary microvascular permeability among the different experimental groups further support the hypothesis that simvastatin exerts its benefits upon MP primarily by its endothelial-protective actions. Increased microvascular permeability is a characteristic feature of endothelial dysfunction and is considered a major pathogenetic mechanism in atherogenesis (10). In accordance with our previous findings (11), we found that coronary microvascular permeability, though normal under basal conditions, significantly increased during simulated cardiac stress in hypercholesterolemic pigs. On the other hand, this abnormal alteration of vascular permeability was prevented in animals fed a high-cholesterol diet and simultaneously treated with simvastatin. Again, this effect of simvastatin was independent of any change in plasma lipid levels. These results are in line with a recently published study demonstrating a normalization of increased microvascular permeability with simvastatin therapy in hypercholesterolemic men (37). Although the relationship between endothelial dysfunction and functionally increased vascular permeability is established, other factors that might influence vessel wall permeability must also be considered. In an earlier study we have demonstrated that experimental hypercholesterolemia is associated with ultrastructural changes of the internal elastic lamina in epicardial coronary arteries

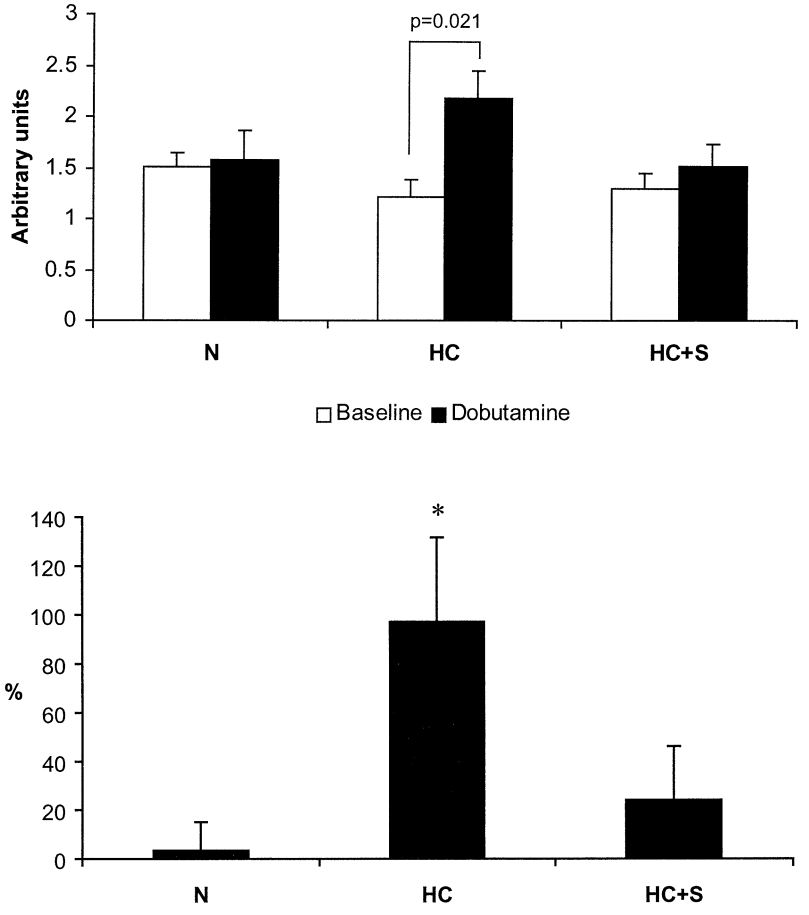

Figure 4. (Upper) Anterior wall microvascular permeability index at baseline and in response to intravenous dobutamine in the three experimental groups. **(Lower)** Relative change of anterior wall microvascular permeability index in response to intravenous dobutamine in the three experimental groups (compared to baseline). HC = hypercholesterolemic pigs; HC+S = hypercholesterolemic pigs receiving simvastatin; N = normocholesterolemic pigs. *$p < 0.05$ vs. N.

(38). Thus, these structural alterations of the vessel wall might contribute to changes in vascular permeability, and the underlying mechanism for the effect of statins on microvascular permeability might also be secondary to the modification of compartments of the vessel wall other than the endothelium.

Suggested mechanism. We recently demonstrated that adverse changes of MP and coronary microvascular permeability in hypercholesterolemia such as those observed in the present study might be mediated by an increase in oxidative stress (11). Moreover, we have shown that simvastatin may reduce oxidative stress (17). Therefore, it may be speculated that the beneficial effects exerted by simvastatin are at least partly mediated by its antioxidant effects.

Pharmacologic stress tests in pigs. The magnitude of the MP response to intravenous dobutamine in normal animals was similar to that observed in healthy humans and pigs (39,40). On the other hand, although clearly significant, the increase in MP in response to intravenous adenosine in normal animals was less than that observed in previous studies in humans using intracoronary Doppler flow measurements (41). Several EBCT studies employing venous injections of contrast media reported underestimation of absolute MP at high flow rates induced by coronary vasodilators such as adenosine (22). Reasons postulated for these observations included failure to correct for intramyocardial vascular volume, which increases in relation to myocardial blood flow, and the use of intravenous (rather than intraaortic) injections of contrast, which results in spread of the bolus (22). In the present study, changes in intramyocardial vascular volume during adenosine infusion were taken into account for calculation of MP, and thus a major confounding effect by this variable is unlikely. However, we cannot exclude the possibility that the duration of the arterial input curve (left ventricular cavity curve) exceeded coronary transit time in the high flow state induced by adenosine and led to some underestimation of absolute MP. This may account for the moderate, though significant, increase in MP in response to adenosine in normal animals. Interestingly, however, intravenous administration of contrast did not substantially decrease MP reserve to intravenous adenosine in humans when EBCT methodology similar to the current study and a correction for changes in intramyocardial vascular volume were used (22). Hence, and because the

EBCT-derived MP reserve to dobutamine was not attenuated, species variability in the response to intravenous adenosine might have contributed to the relatively moderate MP reserve observed in normal animals.

Conclusions. In summary, this study demonstrates that simvastatin preserves MP response and coronary microvascular integrity during situations of cardiac stress in experimental hypercholesterolemia independent of lipid lowering. These results contribute to the growing evidence that statins exert their beneficial effects to a considerable part in a lipid-independent manner.

Acknowledgments

The authors thank James D. Krier for his skillful technical assistance, and the staff of the EBCT at St. Mary's Hospital in Rochester, Minnesota, for their technical assistance with the performance of the experiments.

Reprint requests and correspondence: Dr. Amir Lerman, Division of Cardiovascular Diseases, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905. E-mail: lerman.amir@mayo.edu.

REFERENCES

1. Sheperd J, Cobbe SM, Ford I, et al., for the West of Scotland Coronary Prevention Study Group. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995;333:1301–7.
2. Scandinavian Simvastatin Survival Study Group. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344: 1383–9.
3. Downs JR, Clearfield M, Weiss S, et al., for the AFCAPS/TexCAPS Research Group. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. JAMA 1998;279:1615–22.
4. Sacks FM, Pfeffer MA, Moye LA, et al., for the Cholesterol and Recurrent Events Trial Investigators. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. N Engl J Med 1996;335:1001–9.
5. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. N Engl J Med 1998;339:1349–57.
6. Aengevaeren WR. Beyond lipids—the role of the endothelium in coronary artery disease. Atherosclerosis 1999;147 Suppl 1:S11–16.
7. Lefer AM, Scalia R, Lefer DJ. Vascular effects of HMG CoA-reductase inhibitors (statins) unrelated to cholesterol lowering: new concepts for cardiovascular disease. Cardiovasc Res 2001;49:281–7.
8. Cohen RA, Zitnay KM, Haudenschild CC, Cunningham LD. Loss of selective endothelial cell vasoactive functions caused by hypercholesterolemia in pig coronary arteries. Circ Res 1988;63:903–10.
9. Lerman A, Burnett JC Jr. Intact and altered endothelium in regulation of vasomotion. Circulation 1992;86 Suppl:III12–9.
10. Wu CC, Chang SW, Chen MS, Lee YT. Early change of vascular permeability in hypercholesterolemic rabbits. Arterioscler Thromb Vasc Biol 1995;15:529–33.
11. Rodriguez-Porcel M, Lerman A, Best PJM, Krier JD, Napoli C, Lerman LO. Hypercholesterolemia impairs myocardial perfusion and permeability: role of oxidative stress and endogenous scavenging activity. J Am Coll Cardiol 2001;37:608–15.
12. Britten MB, Zeiher AM, Schachinger V. Clinical importance of coronary endothelial vasodilator dysfunction and therapeutic options. J Intern Med 1999;245:315–27.
13. Hasdai D, Gibbons RJ Holmes DR Jr., Higano ST, Lerman A. Coronary endothelial dysfunction in humans is associated with myocardial perfusion defects. Circulation 1997;96:3390–5.
14. Al Suwaidi J, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000;101:948–54.
15. Egashira K, Hirooka Y, Kai H, et al. Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia. Circulation 1994;89:2519–24.
16. Gould KL, Martucci JP, Goldberg DI, et al. Short-term cholesterol lowering decreases size and severity of perfusion abnormalities by positron emission tomography after dipyridamol in patients with coronary artery disease. A potential noninvasive marker of healing endothelium. Circulation 1994;89:1530–8.
17. Wilson SH, Simari RD, Best PJM, et al. Simvastatin preserves coronary endothelial function in hypercholesterolemia in the absence of lipid lowering. Arterioscler Thromb Vasc Biol 2001;21:122–8.
18. Rumberger JA, Feiring AJ, Lipton MJ, Higgins CB, Ell SR, Marcus ML. Use of ultrafast computed tomography to quantitate regional myocardial perfusion: a preliminary report. J Am Coll Cardiol 1987; 9:59–69.
19. Schmermund A, Lerman LO, Rumberger JA, et al. Effects of acute and chronic angiotensin receptor blockade on myocardial vascular blood volume and perfusion in a pig model of coronary microembolization. Am J Hypertens 2000;13:827–37.
20. Möhlenkamp S, Behrenbeck TR, Lerman A, et al. Coronary microvascular functional reserve: quantification of long-term changes with electron-beam CT—preliminary results in a porcine model. Radiology 2001;221:229–36.
21. Möhlenkamp S, Lerman LO, Lerman A, et al. Minimally invasive evaluation of coronary microvascular function by electron beam computed tomography. Circulation 2000;102:2411–6.
22. Bell MR, Lerman LO, Rumberger JA. Validation of minimally invasive measurement of myocardial perfusion using electron beam computed tomography and application in human volunteers. Heart 1999;81:628–35.
23. Feiring AJ, Rumberger JA, Reiter SJ, et al. Determination of left ventricular mass in dogs with rapid-acquisition cardiac computed tomographic scanning. Circulation 1985;72:1355–64.
24. Reiter SJ, Rumberger JA, Feiring AJ, Stanford W, Marcus ML. Precision of measurements of right and left ventricular volume by cine computed tomography. Circulation 1986;74:890–900.
25. Lerman LO, Siripornpitak S, Maffei NL Sheedy PF 2nd, Ritman EL. Measurement of in vivo myocardial microcirculatory function with electron beam CT. J Comput Assist Tomogr 1999;23:390–8.
26. Davignon J, Laaksonen R. Low-density lipoprotein-independent effects of statins. Curr Opin Lipidol 1999;10:543–59.
27. Hasler-Rapacz J, Kempen HJ, Princen HMG, Kudchodkar BJ, Lacko A, Rapacz J. Effects of simvastatin on plasma lipids and apolipoproteins in familial hypercholesterolemic swine. Arterioscler Thromb Vasc Biol 1996;16:137–43.
28. Hasdai D, Sangiorgi G, Spagnoli LG, et al. Coronary artery apoptosis in experimental hypercholesterolemia. Atherosclerosis 1999;142:317–25.
29. Ruffolo RR Jr. The pharmacology of dobutamine. Am J Med Sci 1987;294:244–8.
30. Embrey RP, Brooks LA, Dellsperger KC. Mechanism of coronary microvascular responses to metabolic stimulation. Cardiovasc Res 1997;35:148–57.
31. Olsson RA, Pearson JD. Cardiovascular purinoreceptors. Physiol Rev 1990;70:761–845.
32. Schiele JO, Schwabe U. Characterization of the adenosine receptor in microvascular coronary endothelial cells. Eur J Pharmacol 1994;269: 51–8.
33. Hein TW, Kuo L. cAMP-independent dilation of coronary arterioles to adenosine: role of nitric oxide, G proteins, and K_{ATP} channels. Circ Res 1999;85:634–42.
34. Smits P, Williams SB, Lipson DE, Banitt P, Rongen GA, Creager MA. Endothelial release of nitric oxide contributes to the vasodilator effect of adenosine in humans. Circulation 1995;92:2135–41.
35. Van Boven AJ, Jukema JW, Zwinderman AH, Crijns HJ, Lie KI, Bruschke AV, on behalf of the REGRESS Study Group. Reduction of

transient myocardial ischemia with pravastatin in addition to the conventional treatment in patients with angina pectoris. Circulation 1996;94:1503–5.
36. Jukema JW, Bruschke AVG, van Boven AJ, et al., on behalf of the REGRESS Study Group. Effects of lipid lowering by pravastatin on progression and regression of coronary artery disease in symptomatic men with normal to moderately elevated serum cholesterol levels. Circulation 1995;91:2528–40.
37. Dell' Omo G, Bandinelli S, Penno G, Pedrinelli R, Mariani M. Simvastatin, capillary permeability, and acetylcholine-mediated vasomotion in atherosclerotic, hypercholesterolemic men. Clin Pharmacol Ther 2000;68:427–34.
38. Kwon HM, Sangiorgi G, Spagnoli LG, et al. Experimental hypercholesterolemia induces ultrastructural changes in the internal elastic lamina of porcine coronary arteries. Atherosclerosis 1998; 139:283–9.
39. Leppo JA. Comparison of pharmacologic stress agents. J Nucl Cardiol 1996;3:S22–S26.
40. Chen C, Li L, Cheng LL, et al. Incremental doses of dobutamine induce a biphasic response in dysfunctional left ventricular regions subtending coronary stenoses. Circulation 1995;92:756–66.
41. Wilson RF, Wyche K, Christensen BV, Zimmer S, Laxson DD. Effects of adenosine on human coronary arterial circulation. Circulation 1990;82:1595–606.