

## A Novel Mechanism for the Beneficial Vascular Effects of High-Density Lipoprotein Cholesterol: Enhanced Vasorelaxation and Increased Endothelial Nitric Oxide Synthase Expression

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### Abstract and Introduction

#### Abstract

**Background:** Low levels of high-density lipoprotein (HDL) cholesterol increase the risk of coronary artery disease (CAD), and recent clinical studies suggest that interventions in low-HDL patients are beneficial. The purpose of this study was to examine the effect of increased HDL levels on endothelium-dependent vasodilation.

**Methods:** We studied patients with CAD with a low-density lipoprotein (LDL) level of <100 mg/dL. Patients with an HDL level of <36 mg/dL were treated with niacin (n = 11), and patients with an HDL level of >36 mg/dL were followed as controls (n = 10). Baseline and 3-month follow-up studies of flow-mediated dilation (FMD) and blood lipid levels were obtained.

**Results:** HDL levels increased from  $30.1 \pm 1.2$  to  $40.5 \pm 1.2$  mg/dL in the niacin-treated patients ( $P < .001$ ) but remained unchanged in the control patients. At baseline, FMD was impaired in both the treated ( $6.5\% \pm 1\%$ ) and the control ( $7.3\% \pm 1\%$ ) patients compared with 10 healthy subjects ( $16\% \pm 2\%$ ,  $P < .01$ ). After 3 months, FMD improved in the niacin-treated patients ( $11.8\% \pm 1\%$ ,  $P = .001$ ) but remained unchanged in the control patients ( $6.2\% \pm 1\%$ ). Exposure of cultured human vascular endothelial cells to HDL in vitro enhanced expression of endothelial nitric oxide synthase (eNOS), as shown by immunoblotting.

**Conclusions:** In patients with CAD and well-controlled LDL levels, elevation of HDL with niacin improves endothelial function. HDL increases eNOS protein expression in cultured vascular endothelial cells. Taken together, these observations suggest that HDL-mediated increases in eNOS expression may contribute to the observed enhancement in vasorelaxation and thus support a previously unrecognized mechanism for the beneficial cardiovascular effects of HDL.

#### Introduction

Atherosclerosis is a diffuse disease process that results in anatomic and physiologic abnormalities of blood vessels. The vascular endothelium plays an integral role in atherogenesis by regulating vasomotor tone, thrombosis, and platelet function.<sup>[1,2]</sup> One of the central mediators of these endothelium-dependent effects is nitric oxide, which is produced in endothelial cells by endothelial nitric oxide synthase (eNOS). Endothelial dysfunction results in part from diminished nitric oxide production and is associated with a loss of the atheroprotective effects of normal endothelium.<sup>[3]</sup> Impaired endothelium-dependent vasorelaxation can be detected in coronary arteries before the development of angiographically significant atherosclerotic plaque,<sup>[4-6]</sup> and this predicts the long-term risk of cardiac events.<sup>[7,8]</sup> Endothelial dysfunction also occurs in the peripheral circulation, and this provides another means of assessing vasomotor function. Brachial artery imaging with high-resolution ultrasound during reactive hyperemia is a widely used and accurate method of determining endothelium-dependent vasomotion.<sup>[9-11]</sup> Abnormalities in peripheral endothelial function detected by this noninvasive method correlate with the presence of coronary arterial endothelial dysfunction<sup>[12]</sup> and thus may also be a marker of future cardiovascular events.<sup>[13]</sup>

Large clinical trials using 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) in patients with elevated low-density lipoprotein (LDL) cholesterol levels have demonstrated marked improvement in clinical outcomes.<sup>[14-18]</sup> Thus, therapies that decrease LDL are the cornerstone of present-day primary and secondary prevention of atherosclerosis. Despite the important beneficial effects of these medications, however, the majority of cardiovascular events are not prevented, even in statin-treated patients. On the basis of this observation, much attention is currently focused on the identification of additional interventions that can further reduce cardiovascular risk in statin-treated patients.

One such potential therapeutic target in patients with CAD is a low level of high-density lipoprotein (HDL) cholesterol. Low HDL levels predict an elevated risk of coronary artery disease (CAD) independent of LDL levels,<sup>[19,20]</sup> and low HDL is commonly observed in men with early atherosclerosis.<sup>[21]</sup> Recent clinical trials suggest that interventions targeted at raising HDL levels in low-HDL patients reduce the risk of cardiovascular events.<sup>[15,22,23]</sup> The mechanisms that mediate the protective effects of HDL are not well understood. HDL enhances reverse cholesterol transport, promoting the efflux of cholesterol from atherosclerotic plaque, which may stabilize the plaque and make it less prone to rupture.<sup>[24]</sup> In vitro, HDL attenuates the oxidation of LDL and inhibits endothelial cell expression of inflammatory cell adhesion molecules.<sup>[25]</sup>

High levels of LDL and its oxidized derivatives are one of the best studied causes of endothelial dysfunction.<sup>[26]</sup> Lowering LDL levels with medical therapy improves, but does not normalize, endothelial function.<sup>[27-30]</sup> Although statins are effective in reducing total cholesterol and LDL levels, they exert only a modest positive effect on HDL.<sup>[14-16,29,30]</sup> In contrast, niacin, which also may be used to treat lipid abnormalities, raises HDL levels substantially. We therefore undertook the present study to examine the effect of niacin-induced increases in HDL on peripheral endothelium-dependent vasodilation in patients with CAD, low HDL levels, and well-controlled LDL levels. We also examined the effect of HDL on the expression of eNOS in cultured human vascular endothelial cells.

## Methods

### Patients and Study Design

Stable patients with CAD (defined as having >50% coronary artery narrowing in one or more vessel on coronary angiogram and no changes in cardiovascular symptoms or medical regimens within the preceding 6 months) were recruited from the Preventive Cardiology Center at Tufts-New England Medical Center (Boston, Mass). Patients with fasting LDL levels of <100 mg/dL were enrolled. Patients with an HDL level of  $\leq$ 36 mg/dL were placed in the niacin treatment group, whereas those with an HDL level of >36 mg/dL served as controls and were not treated with niacin. Exclusion criteria included a history of diabetes, gout, active liver disease, or renal insufficiency. In addition, women of child-bearing age were excluded from the study.

Niacin treatment (Niaspan, Kos Pharmaceuticals, Miami, Fla) was initiated at 375 mg per night and titrated to a maximum tolerated dose (not exceeding 1500 mg). All patients in the study were on daily aspirin therapy. Patients in the niacin-treated group were instructed to take their aspirin one-half hour before the nightly dose of niacin. Medication compliance was evaluated after 6 weeks by telephone. Control patients received no additional therapy. Patients were followed for 3 months without alterations in their medical regimens, physical activities, or diets. Assessment of left ventricular ejection fraction (LVEF [in percent]) was made for all patients with standard 2-dimensional transthoracic echocardiography. At the conclusion of the 3-month study period, fasting lipid profiles were again obtained for each patient. The protocol was approved by the Human Investigation Review Committee at the New England Medical Center.

### Vascular Studies

At baseline and after 3 months, brachial artery ultrasound imaging was performed according to previously described methods.<sup>[31,32]</sup> Longitudinal brachial artery ultrasound imaging with a high-resolution 7.5-MHz vascular probe (HDI 5000, Advanced Technology Laboratories, Bothell, Wash) was achieved with patients in a fully recumbent position after an overnight fast. Niacin therapy and vasoactive medications were withheld for at least 36 hours before vascular testing.

After a 10-minute equilibration period, baseline 2-dimensional images of the right brachial artery were obtained 2 cm above the antecubital fossa. A blood pressure cuff (Hokanson, Bellevue, Wash), placed proximal to the imaging transducer on the upper arm, was inflated to suprasystolic pressure. After exactly 5 minutes of vascular occlusion, the blood pressure cuff was deflated and the brachial artery was imaged continuously for 1 minute. Baseline resting brachial artery images were again established 10 minutes later. Patients were then administered sublingual nitroglycerin (400- $\mu$ g tablet), and the brachial artery was imaged after 5 minutes.

Endothelium-dependent vasodilation was determined by the maximal change in brachial artery diameter after 60 seconds of reactive hyperemia and was expressed as the percent change in flow-mediated dilation (FMD).

Endothelium-independent vasodilation was determined by the maximum diameter change 5 minutes after the

administration of nitroglycerin. Brachial artery diameters were measured with ultrasonic calipers by 2 independent, blinded observers on the basis of recorded super-VHS videotapes. Maximal brachial arterial diameter was calculated within a 5-cm segment of the vessel as the mean of 5 evenly spaced measurements of the distance from the near to the far arterial wall along a line perpendicular to the long axis of the artery. Mean intraobserver and interobserver variabilities of brachial reactivity measurements on normal volunteers in our laboratory were 1.8% and 2.8%, respectively.

## Lipoprotein Isolation

LDL and HDL were isolated from the plasma of healthy volunteers by sequential ultracentrifugation.<sup>[33,34]</sup> Protease inhibitors and EDTA (ethylenediaminetetraacetic acid) (1 mg/mL) were added to the plasma immediately after phlebotomy, and all of the fractions were maintained at 4°C during the entire procedure to prevent apolipoprotein oxidation and degradation. Sterile solutions and equipment were used for the entire procedure.

The density of the plasma was adjusted to 1.019 g/mL by the addition of KBr solution. The top fraction, containing very low density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL), was removed after centrifugation in a Beckman Ti50.2 rotor (Palo Alto, Calif) at 41,000 rpm and 4°C for 24 hours. The density of the infranatant was then increased to 1.063 g/mL, and LDL ( $d = 1.019$  to  $1.063$  g/mL) was isolated from the top of the tube after centrifugation at 41,000 rpm for 24 hours. The density of the remaining fraction was then increased to 1.210 g/mL to isolate HDL ( $d = 1.090$  to  $1.210$  g/mL) by a 63-hour centrifugation at 41,000 rpm. The infranatant was then used as lipoprotein-deficient serum (LPDS). Finally, LDL and HDL were washed in a Ti50.2 rotor at 41,000 rpm and 4°C, with LDL at a density of 1.070 g/mL for 24 hours and HDL at a density of 1.210 g/mL for 63 hours. LDL, HDL, and LPDS fractions were dialyzed against sterile phosphate-buffered saline (PBS). The protein concentration of the fractions was measured, and the apolipoprotein composition was analyzed by polyacrylamide gel electrophoresis followed by Coomassie blue staining.

## Cell Culture

EA.hy926 cells, which were the kind gift of Dr Cora-Jean Edgell (University of North Carolina), are immortalized human aortic endothelial cells (HAECs). HAECs were obtained from Clontech (Walkersville, Md). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) in 10% fetal bovine serum (FBS) on 6-well plates until they achieved 50% to 80% confluence, at which time they were rinsed with PBS and serum depleted for 18 hours in DMEM containing 0.5% FBS. Lipoprotein fractions (50 µg/mL HDL and/or 10 µg/mL LDL) or vehicle controls were then added to the cultures. LPDS was used as a negative control to show that the effect of HDL and LDL was due to lipoproteins and not contamination by other plasma proteins of lipoprotein fractions. At 24 hours after the addition of lipoproteins, the cells were harvested in a triton-based lysis buffer containing 1 mmol/L phenylmethylsulfonyl fluoride and other protease inhibitors. After the removal of insoluble components, the protein concentration of each lysate was measured, and 20 µg of cell lysate was used for Western blot analysis.

## Immunoblotting

Cell lysates were electrophoresed on polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were blocked with 5% nonfat dry milk in PBS containing 0.05% Tween at room temperature for 1 hour. Membranes were then incubated with anti-eNOS monoclonal antibody (Transduction Laboratories, Lexington, Ky) at a dilution of 1:1000 followed by incubation with horseradish peroxidase-conjugated anti-mouse IgG and developed with enhanced chemiluminescence (Amersham, Arlington Heights, Ill). The blots were then stripped and the procedure was repeated with mouse monoclonal anti-β-actin antibody (Sigma Chemical Co, St Louis, Mo) at a dilution of 1:2000. The densities of eNOS and β-actin bands were measured by densitometry, and the ratio of eNOS to β-actin was calculated.

## Statistical Analysis

Where appropriate, data are expressed as mean ± SEM. A paired Student *t* test was used to analyze blood lipid values at the beginning and end of the study. FMD among groups was compared with a 1-way analysis of variance. Subsequently, the Student-Newman-Keuls method was used for multiple pairwise comparisons. A linear regression model was also used. In all analyses, a *P* value of  $\leq .05$  was considered significant.

## Results

### In Vivo Studies

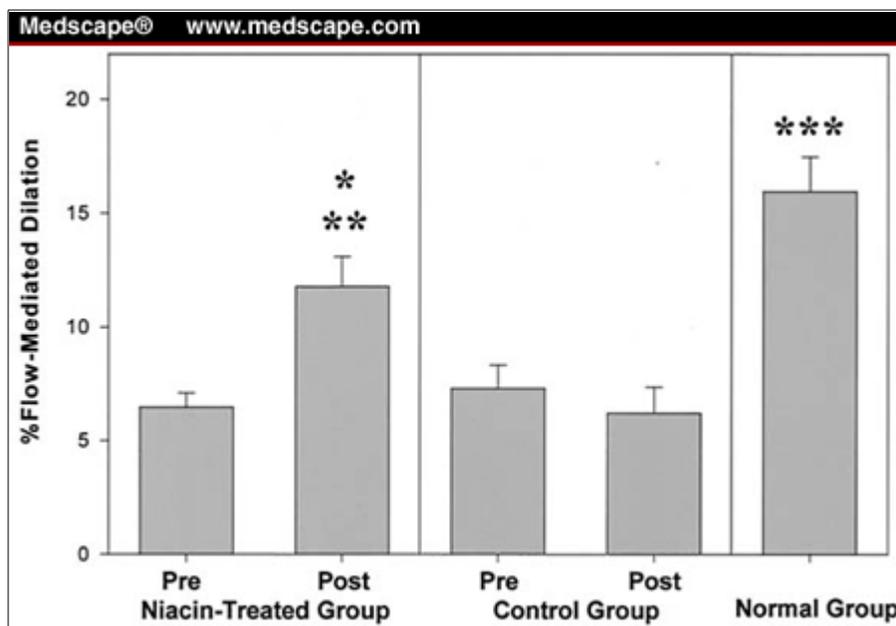
**Patient Characteristics.** Twenty-one patients (17 men) with CAD and an LDL level of  $<100$  mg/dL were followed for 3 months (10 control patients with an HDL level of  $>36$  mg/dL, 11 niacin-treated patients with an HDL level of  $\leq 36$  mg/dL). Baseline characteristics of the study patients are given in [Table 1](#).

The average age was similar for the control and treated patients ( $63 \pm 3$  versus  $63 \pm 4$  years, respectively,  $P =$  not significant [NS]). LVEF percentage was also similar in both groups. Two control group patients had diabetes mellitus (noninsulin dependent). Two control and 9 treated patients were former tobacco users; none had used tobacco within 1 year. Stable doses of statins were part of the preexisting medical regimen in all of the control patients (mean dose the equivalent of 17 mg/d simvastatin) and in 9 of the 11 niacin-treated patients (mean dose the equivalent of 18 mg/d simvastatin). All patients in the study took aspirin. The maximum tolerated nightly dose of niacin (Niaspan) for the 11 treated patients varied: 500 mg (2 patients), 750 mg (1 patient), 1000 mg (6 patients), and 1500 mg (2 patients). Niacin was well tolerated, with one report of facial flushing and one report of gastrointestinal distress at the 1500-mg dose. Niacin therapy was not discontinued by any patient during the study. The normal control group consisted of 10 healthy subjects (6 males) with an average age of  $32 \pm 1$  years and normal LVEF.

**Lipid Analysis.** Baseline HDL levels were  $42.3 \pm 1.6$  mg/dL in the control patients compared with  $30.5 \pm 1.2$  mg/dL in the treated patients ( $P < .0002$ ) (Table II).

Baseline LDL, total cholesterol, and triglyceride levels were similar in the 2 groups and did not change during follow-up. After 3 months of niacin therapy, HDL levels in the treated patients increased significantly to  $40.5 \pm 1.2$  mg/dL ( $P < .001$ ). HDL levels in control patients remained unchanged after 3 months.

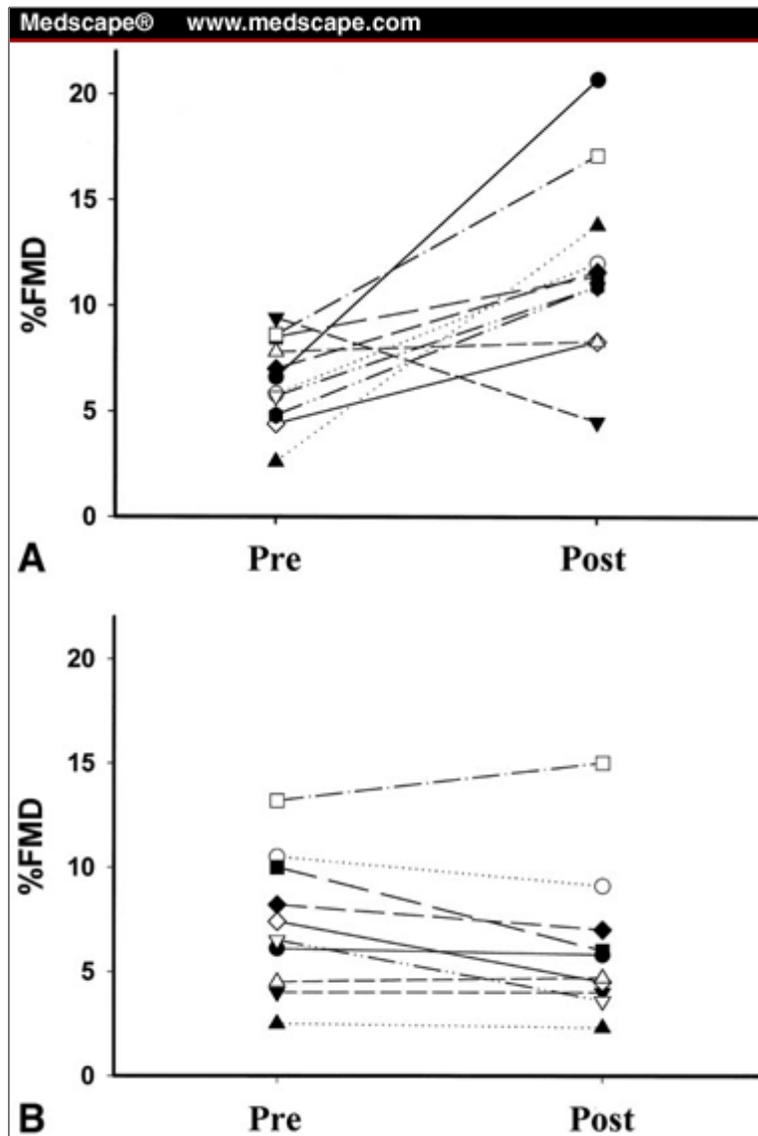
**Vascular Studies.** As shown in Figure 1 and Table III, at baseline, before receiving niacin, FMD was impaired in the treatment group ( $6.5\% \pm 1\%$  versus  $16\% \pm 2\%$  in the normal healthy group,  $P < .01$ ).



**Figure 1.** Endothelium-dependent vasodilation at baseline and after 3 months. Percent FMD in niacin-treated patients improved from  $6.5\% \pm 1\%$  at baseline (*pre*) to  $11.8\% \pm 1\%$  after 3 months of therapy (*post*) (*asterisk*,  $P = .001$ ). Despite this improvement, FMD in the niacin-treated patients did not completely normalize (*triple asterisk*,  $P < .05$  vs post niacin treated patients). Control patients did not show a significant change in FMD during the 3-month study period (*pre*  $7.3\% \pm 1\%$  to *post*  $6.2\% \pm 1\%$ ,  $P =$  NS). FMD at 3 months was statistically different between the control and niacin-treated patients (*double asterisk*,  $P = .005$ ).

At follow-up, after 3 months of niacin treatment, FMD improved significantly to  $11.8\% \pm 1\%$  ( $P = .001$  vs baseline). FMD in the niacin-treated patients remained somewhat reduced compared with that in the healthy normal controls ( $16\% \pm 2\%$ ,  $P < .05$ ). Endothelium-dependent vasodilation was also impaired at baseline in the control (nontreated) patients and remained unchanged after 3 months of follow-up (FMD  $7.3\% \pm 1\%$  at entry,  $6.2\% \pm 1\%$  at 3 months,  $P =$  NS). At follow-up, FMD was significantly greater in the niacin-treated patients than in the control patients ( $P = .005$ ). Endothelium-independent vasodilation induced by nitroglycerin remained unchanged in both patient groups during the 3-month study period (control patients: baseline  $16.3\% \pm 2\%$ , 3 months  $14.4\% \pm 4\%$ ; treated patients: baseline  $15.8\% \pm 3\%$ , 3 months  $13.6\% \pm 1\%$ ;  $P =$  NS for all groups).

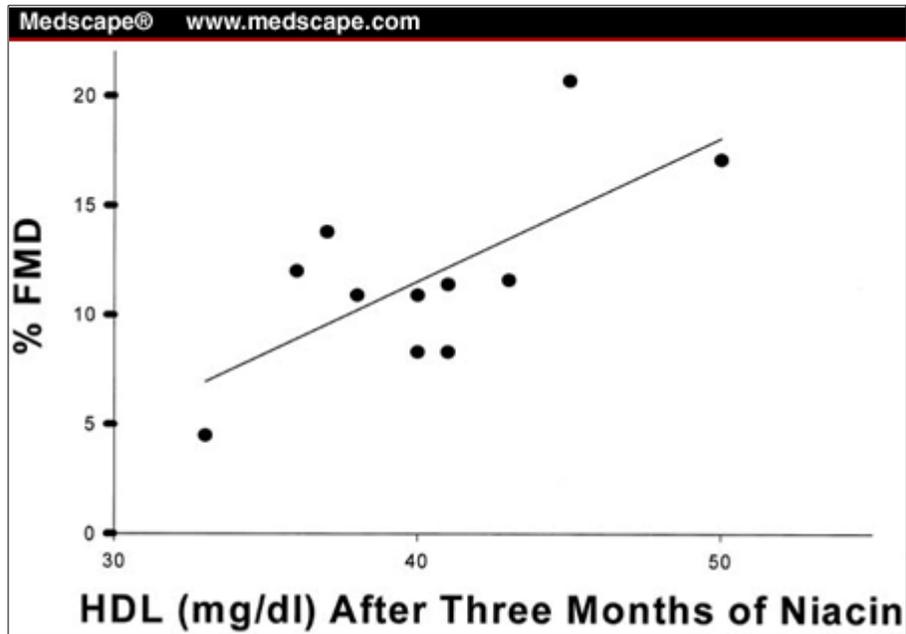
After 3 months of niacin, 10 of the 11 niacin-treated patients showed improvement in endothelium-dependent vasodilation compared with baseline values (Figure 2, A).



**Figure 2.** Individual changes in endothelium-dependent vasodilation at baseline and after 3 months. **A**, FMD before (*pre*) and after (*post*) 3 months with niacin therapy. FMD improved in all but one niacin-treated patient. **B**, No significant interval change was observed in FMD in any of the control patients.

In one niacin-treated patient, vascular function worsened during the study period and, of note, this was accompanied by a decrease in HDL level. FMD did not change significantly in any control patient during the study period (Figure 2, *B*).

As shown in Table II, in the niacin-treated group, HDL increased significantly (by an average of 33%) during the study, whereas all other lipid measurements remained unchanged. Therefore, we examined the relationship between on-treatment HDL and endothelium-dependent vasodilation at follow-up. With a linear regression model, FMD at follow-up was highly correlated with HDL level achieved with niacin therapy ( $R = 0.7$ ;  $P < .02$ ) (Figure 3) In contrast, no significant correlation was noted between endothelium-dependent vasodilation at follow-up and either baseline HDL levels or the percent change in HDL in either the control patients or the niacin-treated patients.

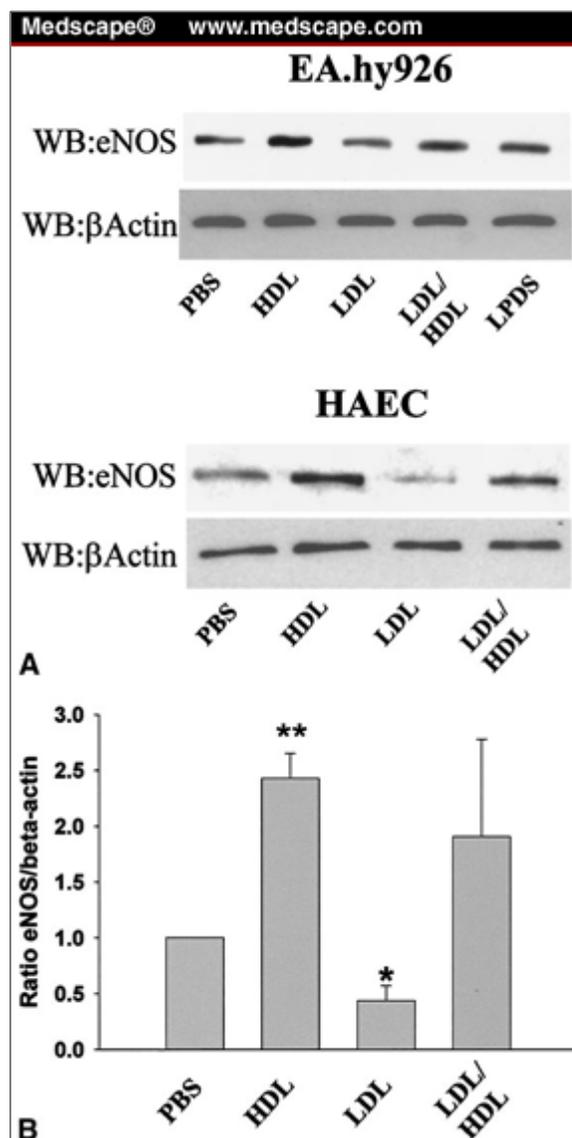


**Figure 3.** Relationship between endothelium-dependent vasodilation and HDL. After 3 months of niacin treatment, FMD correlated with HDL levels in the niacin-treated patients ( $R = 0.7$ ,  $P < .02$ ).

In addition, no correlation was noted when comparing FMD with individual patient doses of niacin.

#### In Vitro Studies

Given the central role of eNOS-dependant production of nitric oxide in mediating hyperemia-induced, endothelium-dependent vasodilation, we next examined the effects of HDL on eNOS protein expression in cultured human vascular endothelial cells. Lysates of quiescent EAhy926 cells (a human endothelial cell hybridoma) and HAECs exposed to various lipoprotein subfractions were immunoblotted for eNOS protein. As shown in Figure 4, A, HDL increased eNOS abundance in both cell types studied.



**Figure 4.** HDL increases eNOS protein abundance in cultured human endothelial cells. **A**, Quiescent EA.hy926 cells, or HAECs, were exposed to HDL, LDL, the combination, or LPDS for 24 hours, and the lysates were immunoblotted for eNOS and subsequently for  $\beta$ -actin. In both cell types, HDL increased eNOS expression, whereas LDL decreased eNOS expression. Addition of HDL to LDL-treated cells prevented the LDL-induced decrease in eNOS levels. **B**, After normalization of the intensity of the eNOS bands for  $\beta$ -actin, HDL increased eNOS expression an average of  $2.4 \pm 0.2$ -fold ( $n = 3$  independent experiments; *asterisk*,  $P < .05$  vs PBS [phosphate-buffered saline]; *double asterisk*,  $P < .001$  vs PBS). Bars represent the mean  $\pm$  SE.

Preliminary dose-ranging studies with 5 to 50  $\mu\text{g/mL}$  HDL demonstrated the greatest effect on eNOS abundance at 50  $\mu\text{g/mL}$  (data not shown). In contrast, LDL reduced eNOS expression, and coincubation of LDL-treated cells with HDL prevented the LDL-induced decrease in eNOS expression. LPDS had no effect on eNOS expression. When normalized to the amount of  $\beta$ -actin detected on the same membrane, HDL increased eNOS expression an average of  $2.4 \pm 0.2$  in a total of 3 repetitions of this experiment (Figure 4, B).

## Discussion

Endothelial injury impairs arterial relaxation and is a critical early pathophysiologic event in the atherosclerotic process.<sup>[1-3]</sup> Lipid lowering with statins in patients with elevated LDL improves, but does not normalize, endothelial vasomotor function.<sup>[27,35]</sup> Although the use of this class of medication markedly reduces clinical cardiovascular events, the majority of CAD events are not prevented in statin-treated patients.<sup>[14-18]</sup> Low levels of HDL increase the risk of the development of CAD, whereas high HDL levels are protective against this disease.<sup>[19,21]</sup> The results presented here demonstrate that 3 months of niacin therapy significantly improves endothelium-dependent vasodilation in CAD patients

with low HDL and well-controlled LDL levels. The importance of HDL in addition to LDL is underscored by the fact that the majority of these patients were already treated with statin therapy. In this study, niacin increased HDL without altering total cholesterol, triglyceride, or LDL levels, suggesting that the niacin-induced increase in HDL contributes to the improvement in peripheral endothelial function. It is also quite possible, however, that niacin therapy altered additional factors not measured here, such as LDL particle size, that contribute to the observed effect on endothelial function.

Recent clinical studies targeting patients with low HDL levels demonstrate that aggressive intervention with statins or gemfibrozil reduces clinical events despite only modest changes in HDL levels.<sup>[15,22,36]</sup> The extent to which these observations are due to changes in HDL specifically remains unclear. The data presented here suggest that the relationship between HDL and endothelial function is not a simple one. For example, at baseline, patients scheduled to receive niacin had lower HDL levels than did control patients, yet their endothelial function was impaired to equivalent levels. In addition, at follow-up, the 2 groups had similar HDL levels, but the niacin-treated patients had significantly improved endothelium-dependent vasorelaxation. Indeed, in our analysis, the only statistically significant relationship was between the HDL levels and FMD after 3 months of niacin therapy. These observations suggest that the vasomotor effects of HDL may not be determined by the absolute abundance of circulating HDL but rather by its ability to perform some of its functions.

The potential mechanisms that mediate the vascular protective effects of HDL are not completely understood. HDL has previously been reported to exert a number of effects that may also enhance vascular function. For example, HDL and its associated proteins attenuate the formation of the highly atherogenic oxidized LDL species previously shown to impair endothelial function.<sup>[37]</sup> In addition, HDL stabilizes prostacyclin, an important vasodilator and platelet inhibitor.<sup>[38]</sup> Furthermore, HDL enhances reverse cholesterol transport, promoting the efflux of cholesterol from atherosclerotic plaque, thereby stabilizing the plaque and making it less prone to rupture.<sup>[39]</sup> The current findings suggest a previously unrecognized mechanism for the vascular protective effects of HDL. Impaired endothelium-dependent vasorelaxation, assessed by reactive hyperemia, results from reduced bioavailability of nitric oxide and interventions that increase endothelial production of nitric oxide enhance vasorelaxation.<sup>[1,10]</sup> Thus, the current observation that increasing HDL improves vasomotion raises the hypothesis that HDL augments the ability of the endothelium to produce nitric oxide.

To further examine this hypothesis, we undertook a series of experiments investigating the effects of HDL on eNOS expression in cultured human vascular endothelial cells. The results presented here demonstrate for the first time that HDL increases the abundance of eNOS protein in these cells and, further, that HDL offsets LDL-induced inhibition of eNOS expression. Although these findings lend support to the hypothesis that the improvement in endothelial function observed in these low-HDL patients in vivo results from increased expression of eNOS, additional clinical experimentation is required to further explore this possibility. Identification of the molecular mechanisms that mediate the effect of HDL on eNOS expression also requires further study.

The improvement in HDL we observed was larger than expected from previous reports with niacin.<sup>[40]</sup> This might be related to the combined use of niacin with a statin in most of our patients. Previous reports have noted a slight improvement in HDL levels with concomitant administration of statins and nicotinic acid compared with either drug alone but not of the magnitude observed in our study.<sup>[41]</sup> No significant change was noted in LDL levels with niacin therapy; this is likely due to the low initial levels of LDL and the concomitant use of statins by most of the subjects.

## Study Limitations

Several limitations of our study should be considered. First, niacin therapy was not administered in either a randomized or a blinded fashion. This could have several implications for interpretation of the results. Although all enrolled patients had CAD and well-controlled LDL levels, by design the control patients had higher HDL levels at baseline than did the niacin-treated patients. The control patients were included in the study to demonstrate that any change observed in FMD in the niacin-treated patients resulted from the institution of niacin treatment and was not the result of other factors. The use of control patients who were matched for baseline HDL levels with the niacin-treated patients may have been preferable, but the control group included in the present study seems adequate to exclude the possibility that the changes in FMD in the niacin-treated patients resulted simply from the passage of time. The lack of blinding of the patients to their treatment assignment also seems unlikely to have biased the results of the study because the primary study end point, FMD, was an objective measurement determined by 2 independent observers who were blinded to the treatment assignments. Second, niacin itself may have the potential to act as a vasodilatory agent,<sup>[42]</sup> and this may have contributed to the improved vasomotion detected in this study. Although we cannot rule out a direct effect of niacin on vascular tone, this is unlikely to have confounded the results because niacin was withheld for at least 36 hours before the vascular studies. In addition, no correlation was found between FMD and individual patient doses of niacin. Third, although aspirin therapy has been shown to improve endothelial vasomotor function in patients with CAD,<sup>[43]</sup> this is unlikely to have played a significant role in this study because all patients were taking aspirin. Finally, although the sample size of each group is relatively small, the treated and control groups are well matched, and the major findings achieved statistical significance.

In summary, raising HDL with 3 months of niacin therapy significantly improved endothelium-dependent vasodilation in CAD patients with low initial HDL and well-controlled LDL levels. In vitro studies demonstrate that HDL enhances eNOS protein expression in human vascular endothelial cells. This is the first demonstration that raising HDL levels enhances vasorelaxation and, taken together with the in vitro findings, suggests a novel mechanism for the beneficial vascular effects of HDL. Our findings indicate that therapies aimed at raising HDL in patients who have already achieved target LDL levels can improve vascular function and thus may confer additional clinical benefits.

## Tables

**Table I. Baseline characteristics of study populations**

	Control patients	Treated patients	Healthy controls
Female/male (n)	2:8	2:9	4:6
Age (y)	63 ± 3	63 ± 4	32 ± 1
LVEF (%)	54 ± 2	48 ± 3	60 ± 1
Diabetes mellitus (n)	2	0	0
Former smokers* (n)	2	9	0
Active smokers (n)	0	0	0
Hypertension (n)	5	5	0
Peripheral vascular disease(n)	1	3	0
Statin therapy (n)	10	9	0
Aspirin therapy (n)	10	11	0

LVEF, Left ventricular ejection fraction; *statin*, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. Where appropriate, values given as mean ± SEM.

\*No tobacco use for at least 1 year.

**Table II. Lipid levels at baseline and follow-up**

	Baseline (mg/dL)	Follow-up (mg/dL)	P
Control patients			
Totalcholesterol	150 ± 7	146 ± 9	.26
HDL cholesterol	42.3 ± 1.6	39.8 ± 1.9	.73
LDL cholesterol	73.2 ± 4.7	72.2 ± 4.2	.86
Triglycerides	189 ± 41	167 ± 41	.06
Niacin-treated patients			
Totalcholesterol	137 ± 6	142 ± 10	.51
HDLcholesterol	30.5 ± 1.2	40.5 ± 1.2	<.001
LDLcholesterol	70.3 ± 4.2	64.1 ± 6.2	.13
Triglycerides	195 ± 34	176 ± 44	.55

Values given as mean ± SEM.

**Table III. Brachial artery ultrasound measurements**

	Baseline	Follow-up	P
Control patients			
Baseline diameter (mm)	4.2 ± 0.2	4.4 ± 0.2	.2
FMD diameter (mm)	4.5 ± 0.2	4.6 ± 0.2	.3
FMD (%)	7.3 ± 1.0	6.2 ± 1.1	.08
Niacin-treated patients			
Baseline diameter (mm)	4.5 ± 0.3	4.6 ± 0.2	.5
FMD diameter (mm)	4.7 ± 0.3	5.1 ± 0.2	.03
FMD (%)	6.5 ± 0.6	11.8 ± 1.3	.001

Values given as mean ± SEM.

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