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High-Density Lipoprotein Stimulates Myocardial Perfusion In Vivo

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Background—Several clinical studies have demonstrated a close association between plasma HDL cholesterol levels and endothelium-dependent vasodilation in peripheral arteries. In isolated arteries, HDL has been shown to mediate vasodilation via NO release. In vivo, administration of reconstituted HDL restored abnormal endothelial function of the brachial artery in hypercholesterolemic patients. However, no data are currently available on the effect of HDL on myocardial perfusion.

Methods and Results—In this study, administration of human HDL enhanced incorporation of the perfusion tracer ^{99m}Tc -methoxyisobutylisonitrile (^{99m}Tc -MIBI) into the murine heart in vivo by $\approx 18\%$. This increase was completely abolished in mice deficient for endothelial NO synthase. Because we have recently identified sphingosine 1-phosphate (S1P) as an important vasoactive component contained in HDL, we measured myocardial perfusion after administration of S1P in vivo. We observed an $\approx 25\%$ decrease in myocardial MIBI uptake, which was abolished in mice deficient for the S1P receptor S1P_3 . In $\text{S1P}_3^{-/-}$ mice, the stimulatory effect of HDL on myocardial perfusion was preserved.

Conclusions—HDL increased myocardial perfusion under basal conditions in vivo via NO-dependent mechanisms, whereas S1P inhibited myocardial perfusion through the S1P_3 receptor. Thus, HDL may reduce coronary risk via direct NO-mediated vasodilatory effects on the coronary circulation. (*Circulation*. 2004;110:3355-3359.)

Key Words: radioisotopes ■ microcirculation ■ blood flow ■ lipoproteins ■ perfusion

A number of epidemiological and clinical studies clearly show an inverse relationship between HDL levels and the risk of cardiovascular disease and clinical events. HDL is an independent risk factor in cardiovascular disease, and raising HDL levels alone resulted in a significant risk reduction of major cardiovascular events in patients with coronary disease whose primary lipid abnormality was a low HDL cholesterol level.¹ However, the mechanisms by which HDL exerts its powerful protective effects are still not clear. Among its numerous potential antiatherogenic effects, HDL cholesterol levels are directly associated with flow-mediated vasodilation in clinical patients in vivo,² and several experimental studies have shown that HDL directly induces vasodilation through activation of endothelial NO synthase (eNOS) and NO release in isolated arteries ex vivo.³ Recently, intravenous administration of reconstituted HDL was shown to acutely restore abnormal endothelial function in the brachial artery of hypercholesterolemic patients.⁴ In our study we provide evidence that intravenous administration of HDL acutely stimulates myocardial perfusion in vivo in the murine heart via eNOS activation, and we identify the HDL compo-

nent sphingosine 1-phosphate (S1P) and its receptor S1P_3 as functional opponents of this effect.

Methods

Animals

Myocardial perfusion was measured in eNOS-deficient ($e\text{NOS}^{-/-}$) mice ($n=13$; aged 18 ± 1 weeks; weight, 29.3 ± 0.24 g)⁵ as well as in mice deficient for the lysophospholipid receptor S1P_3 ($\text{S1P}_3^{-/-}$) ($n=25$; aged 24 ± 12 weeks; weight, 24.1 ± 3.6 g) and their wild-type (WT) littermates ($n=24$; aged 24 ± 11 weeks; weight, 25.2 ± 4.8 g).^{6,7} Heart weight was measured after excision for each individual mouse. The mean heart weights were identical in the WT and $\text{S1P}_3^{-/-}$ mice (0.129 ± 0.019 and 0.123 ± 0.025 g, respectively) and slightly heavier in the larger $e\text{NOS}^{-/-}$ mice (0.146 ± 0.012 g). However, the relative heart weight was the same in all 3 groups. Furthermore, within the groups from each strain that underwent different experimental interventions, all animals had the same heart weight. The studies were approved by the federal animal rights committee and were performed in accordance with institutional guidelines for health and care of experimental animals.

Measurement of Myocardial Perfusion In Vivo

Myocardial perfusion was estimated in anesthetized mice (inhalation of 2% isoflurane at a flow of 0.5 L/min oxygen per mouse) with the

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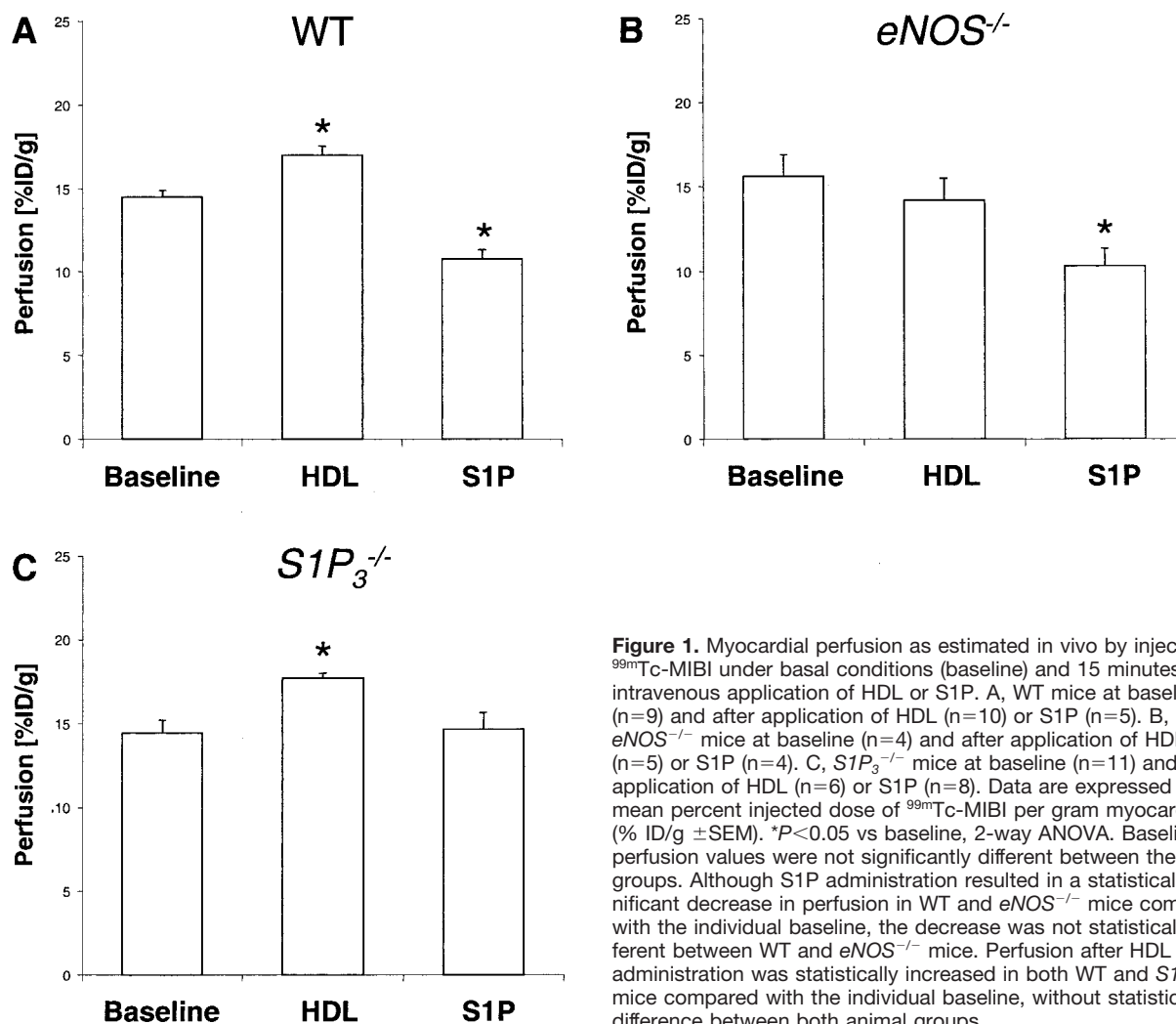


Figure 1. Myocardial perfusion as estimated in vivo by injection of ^{99m}Tc-MIBI under basal conditions (baseline) and 15 minutes after intravenous application of HDL or S1P. A, WT mice at baseline (n=9) and after application of HDL (n=10) or S1P (n=5). B, *eNOS*^{-/-} mice at baseline (n=4) and after application of HDL (n=5) or S1P (n=4). C, *S1P*₃^{-/-} mice at baseline (n=11) and after application of HDL (n=6) or S1P (n=8). Data are expressed as mean percent injected dose of ^{99m}Tc-MIBI per gram myocardium (% ID/g ± SEM). **P*<0.05 vs baseline, 2-way ANOVA. Baseline perfusion values were not significantly different between the 3 groups. Although S1P administration resulted in a statistically significant decrease in perfusion in WT and *eNOS*^{-/-} mice compared with the individual baseline, the decrease was not statistically different between WT and *eNOS*^{-/-} mice. Perfusion after HDL administration was statistically increased in both WT and *S1P*₃^{-/-} mice compared with the individual baseline, without statistical difference between both animal groups.

use of the perfusion tracer ^{99m}Tc-methoxyisobutylisonitrile (^{99m}Tc-MIBI) (2 MBq per mouse in 100 μ L 0.9% NaCl; Cardiolite, Bristol-Myers-Squibb Medical Imaging), as recently described.⁸ Five minutes after intravenous tracer injection, mice were euthanized by cervical dislocation, and the whole heart was taken out, rinsed, dabbed dry, and weighed. The radioactivity of the whole heart was measured by a gamma counter system (Wallac 1480 Wizard), calibrated with 5% of the injected dose (100 kBq). The injected radioactivity was corrected for remaining activity in the syringes after injection and potential paravascular leakage at the injection site by counting syringes and tissue excised from the injection site. Myocardial perfusion was finally estimated by the MIBI flow, calculated as percentage of injected dose (ID) of ^{99m}Tc-MIBI measured in the whole heart divided by the heart weight (% ID/g).^{8,9} To study the effect of S1P and HDL on myocardial perfusion, MIBI flow was measured in untreated animals (baseline) and 15 minutes after intravenous injection of S1P (Sigma; 38 μ g/kg body wt in 50 μ L 1% BSA/PBS), HDL (2 mg/kg body wt in 50 μ L 0.9% saline), or vehicle, respectively. HDL (d=1.125 to 1.210 g/mL) was isolated from human plasma as described.¹⁰ There was no difference in basal MIBI flow between untreated and vehicle-treated animals.

Hemodynamics

Systolic blood pressure and heart rate were determined in isoflurane-anesthetized mice with the use of a computerized tail-cuff system (TSE 9002, Technical & Scientific Equipment GmbH).

Statistical Analysis

Perfusion values are expressed as mean \pm SEM. Two-way ANOVA was used to compare individual mean values with the use of multiple *t* tests, which were corrected by the Bonferroni method. A probability value <0.05 was considered statistically significant.

Results

We measured the whole heart uptake of the perfusion tracer ^{99m}Tc-MIBI after intravenous administration of HDL in vivo. We used this value as a surrogate marker of myocardial perfusion, although it is not an absolute measure of perfusion but rather reflects the net uptake of the tracer in the myocardium ("MIBI flow"). It is critically dependent on accurate measurements of the injected activity and the heart weight,⁸ and we meticulously controlled for both.

Basal myocardial ^{99m}Tc-MIBI uptake in the murine heart in vivo was $14.47 \pm 0.39\%$ ID/g. Intravenous administration of HDL (2 mg/kg body wt) enhanced myocardial ^{99m}Tc-MIBI uptake by 18% ($17.01 \pm 0.54\%$ ID/g; *P*=0.036) (Figure 1A). Several ex vivo studies performed in isolated arteries have shown that HDL mediates vasodilation via activation of eNOS and NO release.^{3,11,12} To test whether the increase in myocardial perfusion induced by HDL is due to NO release, we adminis-

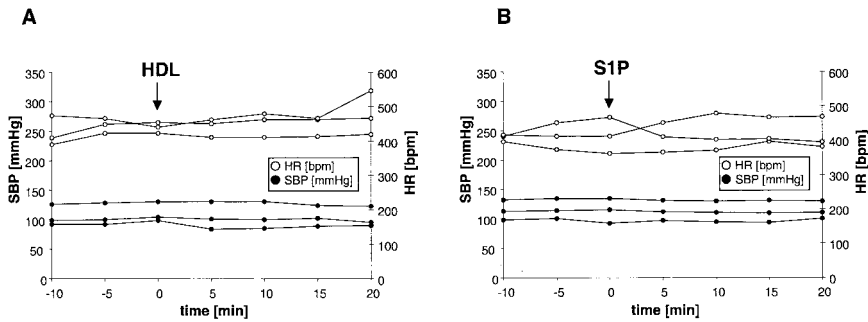


Figure 2. Systolic blood pressure (SBP) and heart rate (HR) as measured by a tail-cuff system in isoflurane-anesthetized mice. Baseline values as well as values after intravenous injection of 2 mg/kg body wt HDL (A) and 38 μ g/kg S1P (B) are shown. Arrow marks time of administration of HDL or S1P.

tered HDL in mice deficient for eNOS. Basal levels of myocardial perfusion in *eNOS*^{-/-} mice were similar compared with WT mice ($15.64 \pm 1.26\%$ ID/g), in agreement with findings that blockade of basal NO release does not affect myocardial tissue perfusion.¹³ In contrast, the increase in HDL-induced myocardial perfusion was completely abolished in eNOS-deficient mice ($14.19 \pm 1.31\%$ ID/g) (Figure 1B).

We recently identified several bioactive lysophospholipids in HDL that are responsible for $\approx 60\%$ of its eNOS-mediated vasodilatory effect. Among these, S1P had potent vasodilatory effects in phenylephrine-precontracted isolated aortae.¹⁴ Therefore, we measured myocardial perfusion in vivo after intravenous administration of S1P (38 μ g/kg body wt as a bolus injection). In contrast to its vasodilatory effect in precontracted arteries ex vivo,¹⁴ S1P decreased ^{99m}Tc-MIBI uptake in vivo by 25% in WT mice ($10.79 \pm 0.55\%$ ID/g versus $14.47 \pm 0.39\%$ ID/g; $P=0.009$) and 34% in *eNOS*^{-/-} mice ($10.31 \pm 1.03\%$ ID/g versus $15.64 \pm 1.26\%$ ID/g; $P=0.002$) (Figure 1A and 1B).

S1P exerts its physiological effects by activating its cognate high-affinity G protein-coupled receptors S1P₁₋₅, resulting in the activation of different subsets of heterotrimeric G proteins including G_q, G₁₀, and G_{12/13}.¹⁵⁻¹⁷ We have previously shown that S1P-mediated eNOS-dependent vasodilation ex vivo is completely abolished in precontracted aortae from mice deficient for 1 of the 2 major S1P receptors in endothelial cells, S1P₃¹⁴ (the other receptor, S1P₁, is embryonically lethal^{6,7}). To test the role of the S1P₃ receptor in mediating the effects of native and HDL-associated S1P, respectively, on myocardial perfusion, we measured ^{99m}Tc-MIBI incorporation after administration of both agents in S1P₃-deficient mice. Baseline perfusion in S1P₃^{-/-} mice was not different from WT controls ($14.45 \pm 0.79\%$ ID/g) (Figure 1C). However, the decrease in myocardial perfusion observed in WT mice after S1P administration was completely abolished in S1P₃-deficient mice ($14.65 \pm 1.01\%$ ID/g) (Figure 1C). In contrast, the stimulatory effect of HDL on myocardial perfusion was preserved in S1P₃-deficient mice ($17.70 \pm 0.26\%$ ID/g [$P=0.012$], which corresponds to a 23% increase in myocardial perfusion) (Figure 1C). To exclude systemic hemodynamic effects of the intravenous application of S1P and HDL on myocardial perfusion, we measured heart rate and systolic blood pressure after administration of the substances. We observed no alterations through S1P (Figure 2A) or HDL (Figure 2B).

Discussion

Effects of HDL and S1P on Myocardial Perfusion as Estimated by ^{99m}Tc-MIBI Uptake

In our study we provide the first evidence that raising HDL plasma levels directly and acutely increases basal myocardial perfusion by $\approx 20\%$ in mice. This effect was completely dependent on NO release as it was abolished in eNOS-deficient mice. This is in agreement with all ex vivo studies that have shown NO-dependent vasodilation by HDL in isolated arteries^{3,11,18} as well as the in vivo study by Spieker and coworkers,⁴ which has shown improvement of both acetylcholine- and flow-mediated dilation in peripheral arteries by administration of reconstituted HDL. The HDL dose we used (a bolus injection of 2 mg/kg body wt) is comparable to the one used by Spieker and coworkers (an infusion of 80 mg/kg body wt per hour over 4 hours). The resulting plasma HDL concentration of ≈ 25 μ g/mL is in the range of the EC₅₀ value for HDL-induced vasodilation we have determined in isolated arteries (8.6 ± 0.5 μ g/mL).¹⁴ However, there is a difference in the HDL composition between both studies in that we used native HDL, whereas Spieker and coworkers used reconstituted HDL containing only apolipoprotein A1 and phosphatidylcholine.

We recently identified S1P as one of several bioactive lysophospholipids in HDL that are responsible for $\approx 60\%$ of the vasodilatory effect of HDL in phenylephrine-precontracted isolated aortae ex vivo.¹⁴ Therefore, we tested the effect of S1P on myocardial perfusion. In contrast to its vasodilatory effect in precontracted isolated arteries, intravenous administration of S1P reduced myocardial perfusion in vivo. This effect was completely mediated by the S1P₃ receptor as it was abolished in S1P₃^{-/-} mice. It is extremely complex to compare the effects of S1P in vitro and in vivo, especially because S1P is well known to induce both vasoconstriction and vasodilation in different settings. We have previously shown that S1P has NO-dependent vasodilative effects in vivo as intra-arterial administration of S1P in rats decreased mean arterial blood pressure.¹⁴ However, to detect the vasodilative effect of S1P, we had to raise mean arterial blood pressure initially by infusion of endothelin. In isolated arteries, S1P also had opposing effects dependent on the initial arterial tone: Whereas S1P had a vasodilative effect on arteries precontracted with phenylephrine, its effect on native, noncontracted arteries was exactly the opposite.¹⁴ This biological behavior of S1P resembles the action of vasodilators such as diadenosine polyphosphates and sug-

gests that it has a dual function: It contracts arteries under basal conditions, whereas it dilates arteries with increased arterial tone.^{14,19–22} This appears to be mediated by at least 2 different pathways: one affecting endothelial cells^{14,21} and another affecting vascular smooth muscle cells^{19,20,22}; these may differ among individual vascular beds.^{19,20} In support of our study, SIP has recently been shown to induce coronary vasoconstriction in blood-perfused sinoatrial node and papillary muscle preparations¹² and in isolated rat coronary arteries.²⁰ Therefore, although SIP is a physiological component of HDL and substantially reduced myocardial perfusion in our study, it does not appear to diminish the original stimulatory effect of HDL on perfusion because there was no further increase in perfusion by HDL in *SIP₃^{-/-}* mice.

^{99m}Tc-MIBI as an In Vivo Myocardial Perfusion Tracer: Application, Validity, and Limitations

^{99m}Tc-MIBI is a myocardial perfusion tracer with a myocardial uptake that is not proportional to absolute myocardial blood flow. Although its uptake correlates linearly to microsphere blood flow up to ≈ 2 to 3 mL/min per gram tissue, it is disproportionately lower under high blood flow (so-called roll-off).²³ This has been elegantly shown for other flow tracers such as ¹³NH₃.²⁴ In rats, the myocardial blood flow at rest is higher (3.5 mL/min per gram)²⁵ than the critical threshold for linear ^{99m}Tc-MIBI uptake.²³ Thus, relatively small differences in tracer uptake under conditions of high blood flow would correspond to rather dramatic changes in real myocardial blood flow. This has been confirmed in vivo in mice, in which a 12% to 14% decline in myocardial ¹³NH₃ uptake after clonidine administration correlated extremely well with the expected 50% reduction in blood flow predicted from the 50% decrease in heart rate.⁹ Similar studies have been performed with ^{99m}Tc-MIBI as well, in which case the formula $Y = 1.31 \times X \times (1 - e^{-143/X})$ has been used to calculate relative differences in blood flow from relative changes in ^{99m}Tc-MIBI uptake.²³ Although it is tempting to use this formula to calculate perfusion (because this would result in rather dramatic changes in myocardial blood flow in our study), there are several critical issues that preclude this because they must be considered in advance: (1) knowledge of the absolute perfusion value at baseline (as yet unknown in mice); (2) possible differences in basal perfusion among species (the aforementioned formula was established for canine myocardium); and (3) the necessity of a constant fractional cardiac output (which is likely to be the case in our study because blood pressure and heart rate remain constant, but nevertheless it was not directly measured in other organs). Therefore, the methodology used in this study as a measure of myocardial perfusion can reflect the directional changes of myocardial blood flow without being able to absolutely quantify their magnitude.

In summary, we suggest that HDL is a major intrinsic coronary vasodilator and have identified 1 mechanism by which it increases basal myocardial perfusion. Possible mechanisms for the eNOS-dependent coronary vasodilation by HDL may involve eNOS activation by HDL via the endothelial scavenger receptor B1, as shown for peripheral arteries ex

vivo.^{3,26,27} Because SIP may have opposite vasoactive effects dependent on the underlying arterial tone, its effect as an HDL constituent on myocardial perfusion (inhibitory under basal conditions as measured here) may be very different under conditions of increased arterial tone. In conclusion, we identify HDL as a direct and potent stimulator of myocardial perfusion in vivo. This may represent a novel cardioprotective function of HDL in addition to and/or as a part of its antiatherogenic effect.

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