High-Density Lipoprotein Stimulates Myocardial Perfusion In Vivo
Bodo Levkau, Sven Hermann, Gregor Theilmeier, Markus van der Giet, Jerold Chun, Otmar Schober and Michael Schäfers

Circulation 2004, 110:3355-3359: originally published online November 15, 2004
doi: 10.1161/01.CIR.0000147827.43912.AE
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 75214
Copyright © 2004 American Heart Association. All rights reserved. Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/21/3355

Subscriptions: Information about subscribing to Circulation is online at http://circ.ahajournals.org/subscriptions/
Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail: journalpermissions@lww.com
Reprints: Information about reprints can be found online at http://www.lww.com/reprints
High-Density Lipoprotein Stimulates Myocardial Perfusion In Vivo

Bodo Levkau, MD*; Sven Hermann, MD*; Gregor Theilmeier, MD; Markus van der Giet, MD; Jerold Chun, MD, PhD; Otmar Schober, MD; Michael Schäfers, MD

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 99mTc-methoxyisobutylisonitrile (99mTc-MIBI) into the murine heart in vivo by ~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 ~25% decrease in myocardial MIBI uptake, which was abolished in mice deficient for the S1P receptor S1P₃. In S1P₃⁻/⁻ 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 S1P₃ receptor. Thus, HDL may reduce coronary risk via direct NO-mediated vasodilatory effects on the coronary circulation.

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. 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, 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.

Recently, intravenous administration of reconstituted HDL was shown to acutely restore abnormal endothelial function in the brachial artery of hypercholesterolemic patients. 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 component sphingosine 1-phosphate (S1P) and its receptor S1P₃, as functional opponents of this effect.

Methods

Animals
Myocardial perfusion was measured in eNOS-deficient (eNOS⁻/⁻) mice (n=13; aged 18±1 weeks; weight, 29.3±0.24 g) as well as in mice deficient for the lysophospholipid receptor S1P₃ (S1P₃⁻/⁻) (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). Heart weight was measured after excision for each individual mouse. The mean heart weights were identical in the WT and S1P₃⁻/⁻ mice (0.129±0.019 and 0.123±0.025 g, respectively) and slightly heavier in the larger eNOS⁻/⁻ 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

Received January 28, 2004; de novo received April 29, 2004; revision received June 30, 2004; accepted July 6, 2004.

From the Institute of Pathophysiology, Center of Internal Medicine, University Hospital Essen, Essen, Germany (B.L.); Departments of Cardiology and Angiology (B.L.), Nuclear Medicine (S.H., O.S., M.S.), and Anesthesiology (G.T.), University Hospital Münster, Münster, Germany; Medizinische Klinik IV, Universitätshospital Benjamin Franklin, Freie Universität Berlin, Berlin, Germany (M.v.d.G.); and Department of Molecular Biology, The Scripps Research Institute, La Jolla, Calif (J.C.).

*The first 2 authors contributed equally to this work.
Correspondence to Bodo Levkau, MD, Institute of Pathophysiology, University Hospital Essen, Hufelandstrasse 55, 45122 Essen, Germany. E-mail levkau@uni-essen.de
© 2004 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000147827.43912.AE

Downloaded from http://circ.ahajournals.org/ by Scripps Research Institute on February 8, 2012
use of the perfusion tracer $^{99m}$Tc-methoxyisobutylisonitrile ($^{99m}$Tc-MIBI) (2 MBq per mouse in 100 μL 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 μg/kg body wt in 50 μL 1% BSA/PBS), HDL (2 mg/kg body wt in 50 μL 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±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±0.39% ID/g. Intravenous administration of HDL (2 mg/kg body wt) enhanced myocardial $^{99m}$Tc-MIBI uptake by 18% (17.01±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-
tered HDL in mice deficient for eNOS. Basal levels of myocardial perfusion in eNOS−/− mice were similar compared with WT mice (15.64±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±1.31% ID/g) (Figure 1B).

We recently identified several bioactive lysophospholipids in HDL that are responsible for ≈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 μg/kg body wt as a bolus injection). In contrast to its vasodilatory effect in precontracted arteries ex vivo,14 S1P decreased 99mTc-MIBI uptake in vivo by 25% in WT mice (10.79±0.55% ID/g versus 14.47±0.39% ID/g; P=0.009) and 34% in eNOS−/− mice (10.31±1.03% ID/g versus 15.64±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 Gq, Gi/o, and G12/13.15–17 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, S1P1,14 (the other receptor, S1P3, is embryonically lethal6,7). To test the role of the S1P receptor in mediating the effects of native and HDL-associated S1P, respectively, on myocardial perfusion, we measured 99mTc-MIBI incorporation after administration of both agents in S1P3-deficient mice. Baseline perfusion in S1P3−/− mice was not different from WT controls (14.45±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±1.01% ID/g) (Figure 1C). In contrast, the stimulatory effect of HDL on myocardial perfusion was preserved in S1P3-deficient mice (17.70±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 99mTc-MIBI Uptake

In our study we provide the first evidence that raising HDL plasma levels directly and acutely increases basal myocardial perfusion by ≈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 arteries3,11,18 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 μg/mL is in the range of the EC50 value for HDL-induced vasodilation we have determined in isolated arteries (8.6±0.5 μg/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 phosphatidylycholine.

We recently identified S1P as one of several bioactive lysophospholipids in HDL that are responsible for ≈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 S1P1 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 Because S1P 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.

Acknowledgments

This study was supported by the Interdisziplinäres Zentrum für Klinische Forschung Münster (IZKF, BMBF-01KS 9604, project grants B10 and ZPG4 to Dr Schäfers and A11 to Dr Lekvau). eNOS+/− mice were a courtesy of Axel Gödecke, Institut für Herz- und Kreislauffysiologie, Heinrich-Heine Universität, Düsseldorf, Germany. We gratefully acknowledge the technical assistance of K. Kloke, S. Mersmann, S. Schröer, K. Parusel, and C. Bätza.

References