Increased Cerebral CO₂ Reactivity After Heparin-Mediated Extracorporal LDL Precipitation (HELP) in Patients With Coronary Heart Disease and Hyperlipidemia

Thomas K. Pfefferkorn, MD; Hans-Peter Knüppel; Beate R. Jaeger, MD; Joachim Thiery, MD; Gerhard F. Hamann, MD

Background and Purpose—There is experimental and clinical evidence that hypercholesterolemia leads to an impairment of endothelial function in coronary and cerebral arteries. Using transcranial Doppler sonography, we examined CO₂ reactivity as a marker of cerebral vasoreactivity in patients with coronary heart disease and hyperlipidemia before and after drastic lowering of LDL cholesterol, lipoprotein(a) [Lp(a)], and fibrinogen levels by heparin-mediated extracorporal LDL precipitation (HELP).

Methods—CO₂ reactivity was determined in 13 patients with coronary artery disease and hyperlipidemia undergoing regular HELP therapy. Middle cerebral artery mean blood flow velocity (MFV) was detected by transcranial Doppler. CO₂ reactivity was calculated as the percent change of MFV during hypercapnia, induced by ventilation of carbogene (5% CO₂, 95% O₂), to normocapnia. Patients with extracranial or intracranial stenoses were excluded. Other parameters such as blood viscosity, heart rate, and blood pressure were measured to control hemorheologic and systemic influences on CO₂ reactivity.

Results—A single HELP treatment reduced total cholesterol, LDL cholesterol, Lp(a), triglycerides, and fibrinogen levels by >50% (P<0.001). Blood viscosity significantly decreased from 1.24±0.04 to 1.07±0.02 mPa (P<0.001). Blood pressure, heart rate, and MFV did not change significantly. CO₂ reactivity increased from 22%±21% to 36%±18% (P<0.05).

Conclusions—Fast and drastic removal of LDL cholesterol, Lp(a), and fibrinogen from plasma results in an improvement of cerebrovascular reactivity in patients with coronary heart disease and hyperlipidemia. The clinical use of HELP in patients with impaired cerebrovascular reactivity might be promising. (Stroke. 1999;30:1802-1806.)

Key Words: cerebrovascular reactivity • endothelium • hypercholesterolemia • lipoproteins, LDL • ultrasonography

Hypercholesterolemia is a risk factor for atherosclerotic disease. Lowering elevated LDL cholesterol levels is effective in primary and secondary prevention of myocardial infarction.¹⁻³ Pravastatin, an HMG-CoA reductase inhibitor (statin) was recently shown to reduce the risk of stroke in patients with coronary artery disease and moderate hypercholesterolemia.⁴ Meta-analysis of clinical trials also suggests that treatment with statins is effective in the prevention of ischemic stroke.⁵ If pharmacological measures are not sufficient, heparin-mediated extracorporal LDL precipitation (HELP) offers an invasive but highly efficient method to reduce serum LDL cholesterol.⁶ In addition, plasma fibrinogen and lipoprotein(a) [Lp(a)], both independent risk factors for coronary heart disease and stroke,⁷⁻⁹ are substantially reduced by HELP. With this method, fibrinogen, LDL cholesterol, and Lp(a) levels can be lowered by 60% to 75% per single treatment.¹⁰⁻¹¹ Acute effects of HELP apheresis on hemorheology are the reduction of red cell aggregability and plasma viscosity.¹²

Animal and in vitro studies have shown that experimental hypercholesterolemia enhances coronary vasoconstriction and vascular resistance.¹²⁻¹⁵ Recently, it was demonstrated that a single LDL apheresis shows a beneficial effect on the acetylcholine-induced endothelial-dependent vasoreactivity in coronary and peripheral arteries.¹⁶⁻¹⁸ However, the effects of LDL apheresis on the vasoreactivity of cerebral arteries are still unknown.

CO₂ reactivity is a marker for cerebral vasoreactivity (CVR). Being a strong physiological stimulus, CO₂ leads to vasodilatation of the cerebral arterioles, mediated by endothelial smooth muscle cell interactions.¹⁹ Assuming a constant diameter of the middle cerebral artery (MCA), increased blood flow in the precapillary system is correlated with increased MCA mean blood flow velocity (MFV).²⁰ This
of CVR in patients with coronary artery disease and
before and after HELP apheresis. End-tidal CO2 partial pressure was
55 mm had been obtained. Blood pressure and pulse were measured
probes were bilaterally fixed over the temporal bone window when
While the patients were sitting in a comfortable position, 2-Mhz
nial Doppler studies were performed with the MultiDop X4 (DWL).
transcran-
after HELP apheresis. Informed consent for the Doppler examina-
and
exhibit hemodynamically relevant stenosis (50%) or occlusion of
brain-supplying vessels and to ensure a detectable MCA signal.
For extracorporal LDL apheresis, the system Plasmat Secura
(Braun) was used. The average treatment lasted 2 hours, 2.8 to 3.0 L
filtrated. Detailed description of this method has
status of the patients before initiation of HELP therapy, see Table 1.
All patients had extracranial and intracranial Doppler sonography to
CO2 reactivity changes and pretreatment
other parameters before and after HELP apheresis, a non parametric
test was performed (Wilcoxon matched pairs signed ranks test). To
test an association between CO2 reactivity changes and pretreatment
CO2 reactivity or LDL cholesterol, a bivariate non parametric
correlation (Spearman) was performed.

CO2 reactivity was determined 30 minutes before and 30 minutes
after HELP apheresis. Informed consent for the Doppler examina-
tions was obtained from all patients before the procedure. Transcran-
ial Doppler studies were performed with the MultiDop X4 (DWL).
While the patients were sitting in a comfortable position, 2-Mhz
probes were bilaterally fixed over the temporal bone window when
highest intensity and velocity of the MCA signal in a depth of 45 to
55 mm had been obtained. Blood pressure and pulse were measured
before and after HELP apheresis. End-tidal CO2 partial pressure was
continuously monitored in 5 of our 13 patients during the Doppler
examination with a CO2 monitor (EGM I, Heyer).

**Subjects and Methods**

Thirteen male patients (mean age 57±7.5 years) with a history of
coronary heart disease (7 patients with heart transplantation due to
ischemic cardiomyopathy) and hyperlipidemia were included in the
study. All of them were on regular HELP therapy (1- to 4-week
intervals) because of elevation of LDL cholesterol or Lp(a) (threshold
values: LDL cholesterol 180 mg/dL, Lp(a) 45 mg/dL) with
insufficient response to diet and drug therapy. Most patients received
antihypertensive therapy. All patients with a history of heart trans-
plantation received immunosuppressive therapy with cyclosporine,
azatioprine, and steroids. For detailed information on the clinical
status of the patients before initiation of HELP therapy, see Table 1.
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exclude hemodynamically relevant stenosis (>50%) or occlusion of
brain-supplying vessels and to ensure a detectable MCA signal.

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(Braun) was used. The average treatment lasted 2 hours, 2.8 to 3.0 L
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55 mm had been obtained. Blood pressure and pulse were measured
before and after HELP apheresis. End-tidal CO2 partial pressure was
continuously monitored in 5 of our 13 patients during the Doppler
examination with a CO2 monitor (EGM I, Heyer).

Bilateral MCA MFV was continuously monitored and digitally
recorded for later off line analysis. Patients breathed through a
plastic mouthpiece, and a nose clamp kept their nostrils closed. A
valve mechanism allowed for prompt switching from room air to
carbogene (5% CO2, 95% O2). The patients started by breathing
room air for 30 to 60 seconds, until a steady state in mean MCA
blood flow velocity was obtained. They were then asked to hyper-
ventilate for 30 to 60 seconds. Thereafter, patients were allowed to
breathe normally for another minute. In the next step, patients were
ventilated with carbogene for at least 1 minute, until mean MCA
blood velocity and end-tidal CO2 partial pressure remained stable.
Finally, patients breathed room air again until mean MCA blood
flow velocity had normalized.

For off line analysis, mean MCA blood flow velocities were
defined from the recorded Doppler curves at the following time
points: resting (normocapnia), during hyperventilation after a steady
state in flow velocity had been obtained (hypocapnia), and after at
least 1 minute of carbogene ventilation, when end-tidal CO2 partial
pressure and flow velocity had reached stable values (hypercapnia).

CO2 reactivity was calculated as the percent change of MCA MFV
in hypercapnia compared with normocapnia (CO2 reactivity=
(MFV Hypercapnia − MFV Normocapnia) × 100% / MFV Normocapnia).
Changes in CO2 reactivity were presented as absolute percent
differences (change in CO2 reactivity=CO2 reactivity after HELP − CO2 reactivity before HELP).

Blood viscosity was measured before and after HELP apheresis.

**Statistical Analysis**

All data are presented as mean±SD. To compare CO2 reactivity and
other parameters before and after HELP apheresis, a non parametric
test was performed (Wilcoxon matched pairs signed ranks test). To
test an association between CO2 reactivity changes and pretreatment
CO2 reactivity or LDL cholesterol, a bivariate non parametric
correlation (Spearman) was performed.

**Results**

**Total Cholesterol, LDL Cholesterol, HDL Cholesterol, Lp(a), Triglycerides, Fibrinogen, and Plasma Viscosity**

HELP apheresis reduced total cholesterol, LDL cholesterol,
Lp(a), and triglycerides by >50% (P<0.001). HDL choles-
terol was reduced by 15% (P<0.01). For exact individual
values and means, see Table 2. Fibrinogen and plasma
viscosity were reduced by 56% and 14%, respectively
(P<0.001; see Table 3).
Blood Pressure and Heart Rate
There were no significant changes in blood pressure or heart rate. Mean blood pressure values were 147/88 mm Hg before and 141/86 mm Hg after HELP (NS). Pulse rate values were 86 and 83 bpm, respectively (NS).

MCA Mean Blood Flow Velocity
Average pretreatment MFV was 54.8±6.4 cm/s for the left and 56.4±6.8 cm/s for the right MCA. After HELP apheresis MFV was 56.7±9.9 cm/s and 57.9±9.5 cm/s, respectively. Combining both sides, average MCA MFV was 55.6±6.0 cm/s before and 57.3±9.3 cm/s after HELP apheresis. These changes were not significant.

CO₂ Reactivity
Pretreatment CO₂ reactivity calculated as the percent change of MCA MFV in hypercapnia compared with normocapnia ranged from −12% to 54%, with a mean of 22%±21%. After HELP apheresis values ranged from −2% to 68%, with a mean of 36%±18% (Table 3). The relative increase in CO₂ reactivity of 14% was significant (P<0.05) (Figure 1). Changes of CO₂ reactivity in individual patients are presented in Figure 2. Pretreatment LDL cholesterol, Lp(a), triglycerides, and fibrinogen, as well as pretreatment CO₂ reactivity, were not significantly associated with change in CO₂ reactivity. However, change of CO₂ reactivity tended to be positively associated with pretreatment LDL cholesterol and negatively associated with pretreatment CO₂ reactivity (Figures 3 and 4). Lack of significance might be due to the low number of patients. An estimated number of 28 patients would be necessary to possibly reach significance.

### TABLE 2. Lipid Status of Patients Before and After LDL Apheresis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Total Cholesterol, mg/dL</th>
<th>LDL Cholesterol, mg/dL</th>
<th>HDL Cholesterol, mg/dL</th>
<th>Lp(a), mg/dL</th>
<th>Triglycerides, mg/dL</th>
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Mean±SD 244±46 117±16 143±40 68±20 42±6 35±5 73±62 36±26 268±128 103±65

### TABLE 3. Fibrinogen, Plasma Viscosity, and CO₂ Reactivity in Patients Before and After LDL Apheresis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Fibrinogen, mg/dL</th>
<th>Viscosity, mPa</th>
<th>CO₂ Reactivity, %</th>
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<tr>
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<td>1.28</td>
</tr>
</tbody>
</table>

Mean±SD 350±99 153±45 1.24±0.04 1.07±0.02 22±21 36±18

Figure 1. CO₂ reactivity ((MFV<sub>Hypercapnia</sub>−MFV<sub>Normocapnia</sub>)×100%/MFV<sub>Normocapnia</sub>) before and after HELP therapy. Box plots with median values, 25th and 75th percentiles, and total ranges. Change in CO₂ reactivity was significant (P=0.011, Wilcoxon test).
Discussion

We showed for the first time that a single LDL apheresis with the HELP system significantly improves CO2 reactivity. This improvement tended to be more pronounced in patients with low pretreatment CO2 reactivity and elevated pretreatment LDL cholesterol levels. Significant effects might have been missed due to the small number of patients and the huge interindividual variability in the CO2 reactivity. Intraindividual reproducibility in detecting CO2 reactivity by transcranial Doppler is known to be high.21

The reduction rates of LDL cholesterol, Lp(a), triglycerides, and fibrinogen matched well the results from previous studies using the HELP system in patients with coronary heart disease.6 Although all patients had a history of hyperlipidemia, only some had pathologically elevated LDL cholesterol or Lp(a) levels at the time of the HELP apheresis. This is a consequence of repeated HELP apheresis in all and concomitant treatment with statins in some patients (with no history of heart transplantation). Previous studies have demonstrated a new steady state in LDL cholesterol and fibrinogen levels after 4 to 8 treatment sessions.23

As expected, blood viscosity was markedly reduced after HELP apheresis. This alteration had no influence on systemic hemodynamic parameters such as mean arterial blood pressure or heart rate. Interestingly, mean MCA blood flow velocity did not change after HELP apheresis.

This is in contrast to the investigation of Izumi et al,24 who found a small but significant increase in MCA blood flow velocity after pharmacological defibrination with the venom batroxobin. The latter study was performed on normal subjects with a mean age of 32 years and no history of vascular disease. There are 2 explanations for the discrepancy with our results. First, there was considerable variance regarding pretreatment MCA blood flow velocities in our patients. Thus, our patient number might have been too small to detect significant changes on flow velocities. Second, there might be specific limitations of hemorheologic effects on cerebral blood flow in our patients. These limitations include older age, reduced cardiac output due to ischemic heart disease, generalized vascular alterations due to atherosclerosis, and denervation of the graft in the transplanted patients.

Despite constant blood flow velocities, a significant increase in CO2 reactivity as a marker of CVR was observed. Izumi et al24 also observed increased CO2 reactivity after defibrination with batroxobin. However, in their (healthy) subjects, this increase was, as pointed out before, associated with increased cerebral blood flow. The independent increase in CO2 reactivity in our patients suggests a direct influence of HELP apheresis on CVR.

HELP apheresis in our patients seemed to have a stronger effect on CO2 reactivity if pretreatment values were low. A low initial CO2 reactivity may represent a state of endothelial dysfunction. In this context the effect of HELP apheresis could be explained as the normalization of disturbed endothelial function, as it was recently demonstrated for peripheral and coronary arteries.16–18 Patients with high initial CO2 reactivity and normal endothelial function may have no range for further improvement.

Considering a direct influence of HELP apheresis on endothelial function and CVR, several mechanisms may play a role. In experimental long-lasting hypercholesterolemia in pigs, vasocon-
strictive effects of endothelin-1 are pronounced while vasorelaxing effects of endothelium-derived relaxing factor/nitric oxide are reduced. This imbalance leads to increased vascular tone.13

Regarding short-term effects, Andrews and coworkers13 demonstrated inhibition of endothelium-dependent relaxation after exposure to native but not to chemically modified LDL cholesterol in an in vitro model of rabbit aorta. The authors proposed that a receptor-dependent mechanism mediated the inhibition of endothelium-dependent relaxation. Kugiyama and coworkers,14 however, showed that inhibition of vascular relaxation only occurs after exposure to oxidized, but not to native, LDL cholesterol. These controversial results could be explained by insufficient protection of native LDL against oxidation in the study by Andrews et al, with subsequent intraexperimental development of oxidized LDL being the main factor for inhibited arterial relaxation. In a more recent study, Hein and Kuo25 investigated the influence of native and oxidized LDL on porcine coronary arterioles. They found an inhibition of nitric oxide–mediated vasorelaxation after 1 hour exposure to both forms of LDL. Oxidized LDL produced more severe inhibition than native LDL. The inhibitory effects of both LDLs were prevented by administration of a cell-permeable superoxide scavenger, suggesting a role of superoxide anions in LDL-mediated inhibition of vasorelaxation. Galle and coworkers15 used bovine arterial endothelial cells to investigate short-term effects of both forms of LDL. They showed that oxidized LDL, and to a lesser degree native LDL, inactivated endothelium-derived relaxing factor after its release from the endothelium, which suggests a further pathway of LDL-mediated vasomotor impairment.

The discussed mechanisms of acute impairment of arterial relaxation by LDL cholesterol may help to explain the observed increase in cerebrovascular reactivity detected in our patients as early as 30 minutes after HELP apheresis. One could speculate that reduction of LDL cholesterol acutely leads to normalization of CVR. As early as 2 hours after HELP apheresis and thus leads to normalization of CVR.

We conclude that HELP apheresis in patients with ischemic heart disease and hyperlipidemia directly affects CVR by normalizing endothelial function. In cases of compromised cerebral perfusion, endothelium-dependent CVR improvement may have beneficial effects on cerebral microcirculation.

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References
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