Inflammation and its echo in atherosclerosis

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Chapter 6

High-Density Lipoprotein Attenuates Inflammation and Coagulation Response upon Endotoxin Challenge in Humans

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Abstract

Objective: Low high-density lipoprotein (HDL) cholesterol is a strong independent cardiovascular risk factor, which has been attributed to its role in reverse cholesterol transport (RCT). Whereas HDL also has potent anti-inflammatory effects, the relevance of this property remains to be established in humans. In the present study, we evaluated whether there is a relation between HDL and sensitivity towards a low-dose endotoxin challenge.

Methods and Results: Thirteen healthy men with genetically determined isolated low HDL-cholesterol (averaging 0.7±0.1 mmol/L) and 14 age-, and body weight-matched healthy men with normal/high HDL-cholesterol levels (1.9±0.4 mmol/L) were challenged with low dose endotoxin i.v. (1 ng/kg bodyweight). The incidence and severity of endotoxin-associated clinical symptoms was increased in the low HDL-group. Accordingly, both the inflammatory response (TNFα, IL-1β, IL-6, IL-8 and MCP-1) as well as thrombin generation (prothrombin activation fragments F1+2) were significantly increased in the low HDL-group upon endotoxin challenge.

Conclusions: Low HDL in healthy males is associated with increased sensitivity towards inflammatory stimuli as reflected by enhanced inflammatory and coagulation responses upon endotoxin challenge. These anti-inflammatory effects of HDL in humans may lend further support to HDL increasing interventions particularly in pro-inflammatory conditions, such as acute coronary syndromes.
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Introduction

Following the first report on the potential anti-atherogenic effect of high-density lipoprotein (HDL) almost sixty years ago, HDL is now generally acknowledged as a potent anti-atherogenic mediator. The impact of isolated low HDL-cholesterol on atherogenesis was recently underscored by the finding that carotid intima-media thickness (IMT) in patients with genetically-determined low apolipoproteinA-I (apoA-I) was comparable to that in patients with familial hypercholesterolemia. In line, HDL increasing drugs now are prime candidates for combined use with statins in high risk subjects.

Traditionally, the protective effect of HDL was considered to be confined to its role in the reverse cholesterol transport (RCT) pathway. However, recent evidence supports a wide array of anti-atherogenic effects by HDL, comprising anti-oxidative, anti-thrombotic as well as anti-inflammatory effects. The latter has attracted special interest, since inflammation has been acknowledged to underlie atherosclerotic lesion formation. At the same time, HDL-cholesterol consistently shows an inverse relation with systemic markers of inflammation. Interestingly, HDL-increasing compounds (e.g. reconstituted HDL) have recently been shown to attenuate systemic inflammation in humans as well as vessel wall inflammation in experimental animal models. However, it remains to be established whether HDL also exerts anti-inflammatory effects in the human setting. In the present study, we evaluated the impact of plasma HDL-cholesterol level on the sensitivity towards a low dose endotoxin challenge in subjects with genetically determined low- versus normal/high-HDL-cholesterol levels.
Methods

Study participants

Study subjects were recruited from a study designed to identify genes that control HDL-cholesterol levels. Healthy male subjects with plasma HDL-cholesterol levels below the 10th percentile (low HDL-group, n=13) and healthy male subjects with plasma HDL-cholesterol levels above the 90th percentile (high HDL-group, n=7) matched for age and sex were recruited from families in which an autosomal dominant phenotypic trait for low- or high HDL-cholesterol was established in at least 3 first degree relatives. Subjects in the low HDL-group with known genetic causes for low HDL-cholesterol were excluded from the study, including carriers of the apoA-I (L178P) mutation. Subjects with secondary dyslipidemias, such as familial combined hyperlipidemia, were excluded. We also excluded low HDL as part of the metabolic syndrome or secondary to hypertriglyceridemia. Unaffected healthy male relatives with normal (40 to 60th percentile) HDL-cholesterol levels, matched for age and gender were also recruited from families included in the database (n=7). Since the primary objective was to evaluate increased sensitivity towards inflammatory challenge in individuals with low HDL-cholesterol, data from subjects with normal and high HDL levels (n=14) were combined in the analyses.

Written informed consent was obtained from all subjects. The study protocol was approved by the institutional review board at the Academic Medical Center in Amsterdam. Subjects with cardiovascular disease and risk factors for cardiovascular disease such as impaired fasting glucose, diabetes mellitus, hypertension, hypercholesterolemia, hypertriglyceridemia, CRP levels above 5 mg/mL, elevated Lp(a) and smoking were excluded during the screening visit. Other exclusion criteria were a history of alcohol and/or drug abuse, vaccination in the previous six months, previous exposure to endotoxin experiments, use of medication such as lipid-modifying drugs (resins, statins, niacin, fibrates e.g.), non-steroidal anti-inflammatory drugs, paracetamol and anti-oxidants. All study subjects were free from signs of acute...
infection or febrile illness during the month preceding the study. One subject in the low HDL-group was excluded because he had elevated hepatic enzymes (> 2-times upper limit of normal) and was suspect of alcohol abuse.

**Study design**

Study participants were required to refrain from alcohol and caffeine-containing beverages at least 24 hours prior to the study. The incidence, time and severity of clinical symptoms associated with endotoxemia, were recorded as follows: 0 = absent, 1 = mild, 2 = moderate and 3 = severe. Other clinical parameters such as blood pressure, heart rate and body temperature were also recorded. Carotid IMT measurements were performed at baseline as previously described. In the morning of the study day at 7.30 a.m. after an overnight fast, study participants were admitted to the research unit. At 7.45 a.m. a catheter was inserted in an antecubital vein of each arm. At 8.00 a.m. (t=0), blood was drawn for baseline measurements. Subsequently, subjects received a bolus infusion of 1 ng/kg body weight of endotoxin (Escherichia coli lipopolysaccharide, catalog number 1235503, lot G2B274, United States Pharmacopeial Convention Inc, Rockville, USA) in the antecubital vein of the contralateral arm. Blood samples were collected at t = 0, 1, 2½, 4, 6 and 8 hours after endotoxin challenge. The next morning at 8.00 a.m., 24 hours after endotoxin infusion study participants returned after an overnight fast for final blood withdrawal.

**Biochemical Analysis**

Blood was collected in EDTA, citrate and heparin anticoagulated aliquots as well as serum tubes which were kept on ice and centrifuged at 1600 g for 15 minutes at 4 °Celsius, snap frozen and stored at -80 °Celsius until analysis. Plasma total cholesterol was measured with an enzymatic colorimetric procedure (CHOD-PAP, Boehringer Mannheim, Mannheim, Germany). HDL-cholesterol was determined after precipitation of apoB containing lipoproteins by MnCl₂. LDL-cholesterol was calculated using the Friedewald formula. ApoA-I and apoB were measured using...
Beckman reagents and array nephelometry (Beckman, Brea, California, USA). Triglycerides were measured using an enzymatic colorimetric method using lipase, glycerol kinase and glycerol-3-phosphate 3 oxidase. Hematology parameters were assessed by standard laboratory techniques.

Baseline lipid measurements were repeated at least three times. First, general practitioners selected patients on the basis of HDL-cholesterol values. Next, genetic field workers collected blood samples from these patients and first degree relatives which were assayed for lipid profiles. Subsequently, eligible patients were invited for a screening visit. Finally, on the study day the research physician took baseline lipid measurements. All samples were obtained after a 12 hour overnight fast.

**Analysis of the Inflammatory Response**

CRP was measured by a high-sensitivity immunoturbimetric assay (Roche Diagnostics Corporation, Basel, Switzerland), while CRP levels in excess of 10 mg/L were assayed by immunonephelometry (P800 analyzer, Roche Diagnostic Corporation). Circulating levels of tumor necrosis factor-alpha (TNFα), interleukin-1β (IL-1β), IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1) were assessed with the luminex method (Bioplex Human Cytokines 1x96 wells, catalog number X500000 FFS, Bio-Rad Laboratories Inc, CA, USA). LPS Binding protein (LBP) was measured with a commercially available ELISA (Human LBP ELISA, Cell Sciences Inc, catalog number HK 315, Canton, MA, USA).

**Paraoxonase-1 activity:** Serum paraoxonase-1 (PON-1) activity was measured as previously described.

**Analysis of the Procoagulant Response**

Coagulation activation was assessed by measuring plasma levels of prothrombin fragment 1+2 (F$_{1+2}$) as a marker of in vivo thrombin generation (ELISA; Dade-Behring, Marburg GmbH, Germany). ELISAs were used to measure markers of endogenous fibrinolysis, i.e. plasma levels of tissue-type plasminogen activator (tPA; Asserachrom
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tPA; Diagnostic Stago, Asnieres-sur-Seine, France), plasminogen activator inhibitor type-1 (PAI-1) antigen (Monozyme, Charlottelund, Denmark) and the fibrin split product D-dimer (Asserachrom, D-Di, Diagnostic Stago, Asnieres-sur-Seine, France).

Statistical Analysis
Results are expressed as mean ± standard deviation (SD). Differences between the low HDL-group versus the normal/high HDL-group were tested by analysis of variance (ANOVA) for repeated measures. Linear regression analysis was used to evaluate correlations between HDL-cholesterol as well as apoA-I levels versus inflammation parameters and coagulation parameters. The SPSS software package for Windows was used for statistical analysis (SPSS Inc., version 12.0, Chicago, Illinois, USA).

Results

Baseline Characteristics
The demographic and biochemical characteristics of study participants in the low HDL-group and the normal/high HDL-group are listed in table 1. The two groups were carefully matched for age and body mass index (BMI), lipids and lipoproteins except for plasma HDL-cholesterol and apolipoproteinA-I (apoA-I) levels.

Clinical Symptoms
Endotoxin infusion caused typical endotoxin-induced symptoms.8 Clinical symptoms such as backache, chills, headache, myalgia, nausea and vomiting occurred frequently, earlier and more intensive in the low HDL-group (table 2). In the low HDL-group, heart rate increased from 69 beats per minute (bpm) at baseline to 80 bpm at 4 hours versus 67 to 76 bpm in the normal/high HDL-group (P = .03, between groups). Blood pressure did not change throughout the experiment in both groups.
Table 1. Demographic and Biochemical Characteristics of the Study Subjects

<table>
<thead>
<tr>
<th></th>
<th>Low HDL-group</th>
<th>Normal/high HDL-group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=13</td>
<td>n=14</td>
</tr>
<tr>
<td>Age, years</td>
<td>35.4 ± 2.5</td>
<td>35.1 ± 5.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.9 ± 1.4</td>
<td>24.7 ± 1.5</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>125 ± 6</td>
<td>125 ± 9</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>36.6 ± 0.8</td>
<td>36.7 ± 0.7</td>
</tr>
<tr>
<td>Alcohol use, (%)</td>
<td>6/13 (46)</td>
<td>6/14 (43)</td>
</tr>
<tr>
<td>Smoking (previous), (%)</td>
<td>9/13 (69)</td>
<td>10/14 (71)</td>
</tr>
<tr>
<td>Fam history CVD, (%)</td>
<td>6/13 (46)</td>
<td>1/14 (7)</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>0.7 ± 0.1</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>2.7 ± 0.6</td>
<td>2.7 ± 0.8</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.1 ± 0.5</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.0 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>1.4 ± 1.1</td>
<td>1.3 ± 1.4</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>5.3 ± 0.5</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>Carotid IMT, mm</td>
<td>0.57 ± 0.07</td>
<td>0.52 ± 0.06</td>
</tr>
</tbody>
</table>

ApoA-I indicates apolipoproteinA-I; apoB indicates apolipoproteinB; BMI indicates body mass index; BP indicates blood pressure; CVD indicates cardiovascular disease, HDL indicates high-density lipoprotein; hsCRP indicates high sensitive C-reactive protein; IMT indicates intima-media thickness; LDL indicates low-density lipoprotein. Data are expressed as mean±SD. *P = .02 by chi-square (χ²) test; †P < .001 and ‡P < .001 by independent student’s t-test.
### Effect of HDL on endotoxin-mediated inflammation

#### Table 2. Effect of HDL levels on Endotoxin-Induced Clinical Symptom

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Low HDL-group</th>
<th>Normal/high HDL-group</th>
<th>(^P)</th>
<th>(^{†}P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=13</td>
<td>n=14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Backache</strong></td>
<td>5</td>
<td>2</td>
<td>0.2 ± 0.4</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td><strong>Chills</strong></td>
<td>10</td>
<td>6</td>
<td>0.4 ± 0.6</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td><strong>Fever</strong></td>
<td>10</td>
<td>8</td>
<td>37.8 ± 0.8</td>
<td>4.1 ± 2.0</td>
</tr>
<tr>
<td><strong>Headache</strong></td>
<td>10</td>
<td>6</td>
<td>0.5 ± 0.7</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td><strong>Myalgia</strong></td>
<td>5</td>
<td>2</td>
<td>0.2 ± 0.4</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td><strong>Nausea</strong></td>
<td>10</td>
<td>5</td>
<td>0.4 ± 0.6</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td><strong>Vomiting</strong></td>
<td>5</td>
<td>2</td>
<td>0.2 ± 0.6</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td><strong>Total AE</strong></td>
<td>55</td>
<td>29</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The total number of volunteers suffering from a specific symptom, mean maximum severity (0 = absent, 1= mild, 2= moderate, 3 = severe) and time of maximum severity (hours, relative to endotoxin infusion) are summarized. AE indicates adverse events. *Mean severity for fever is denoted as temperature in °C. *Fever is defined as a body temperature of > 38 °C. Data are expressed as mean±SD. \(^P\) indicates difference between the mean maximum severity by independent student's t-test between both groups, \(^{†}P\) indicates difference between the time of maximum severity by independent student's t-test between both groups.

### Lipid and Lipoprotein Response

Endotoxin challenge induced modest decreases in LDL-cholesterol from 2.7±0.6 to 2.4±0.5 mmol/L (P < .01) and from 2.7±0.8 to 2.4±0.9 mmol/L (P < .01), TG levels from 1.1±0.5 to 0.8±0.6 mmol/L (P < .01) and from 1.0 ± 0.6 to 0.6±0.3 mmol/L (P < .01) and apoB from 1.1±0.2 to 0.9±0.2 (P < .01) and from 1.0±0.2 to 0.7±0.2 mmol/L (P < .05), in the low- and normal/high HDL-group, respectively. Notably, HDL-cholesterol and apoA-I levels did not change upon endotoxin infusion over time. The lipids and lipoprotein response after endotoxin challenge was not significantly different between both groups.
Inflammatory Response

Leukocyte Response: After endotoxin infusion, early leukocytopenia and monocytopenia was comparable between both groups. The subsequent increase in leukocytes, monocytes, however, was significantly higher in the low HDL-group (figure 1A+B). In addition, endotoxin infusion significantly increased the neutrophil response in the low HDL-group (figure 1C).
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Figure 1. Effect of Inflammatory Challenge on Leukocyte Response

(A) leucocytes, (B) monocytes and (C) neutrophils curves in the low HDL-group (●) and the normal/high HDL-group (○). * indicates difference between timepoints by unpaired Student’s t test (P < .05), †P < .01 indicates difference between the low HDL-group versus the normal/high HDL-group using ANOVA repeated measures analysis. Data are expressed as means±SD.

Cytokines: Baseline cytokine levels were similar in both groups. The effects upon endotoxin infusion on each cytokine are shown in figure 2. At 1 hour post-infusion TNFα levels were significantly increase in the low HDL-group compared to the normal/high HDL-group (figure 2A). A similar effect was noted for IL-1β, IL-6, IL-8 and MCP-1 levels at 2½ hours post endotoxin infusion (figures 2B-2E).
Figure 2. Effect of Inflammatory Challenge on Inflammation and Coagulation Activation

(A) TNFα, (B) IL-1β, (C) IL-6, (D) IL-8, (E) MCP-1 and (F) F1+2 curves in the low HDL-group (●) and the normal/high HDL-group (○). * indicates difference between timepoints by unpaired Student's t test (P < .05). †P value indicates difference between the low HDL-group versus the normal/high HDL-group using ANOVA repeated measures analysis. Data are expressed as means±SD.
Acute phase proteins: Although the groups had similar baseline hsCRP levels (table 1), CRP was significantly elevated in the low HDL-group compared to the normal/high HDL-group at 24 hours (43.2±6.5 mg/L versus 27.2±5.9 mg/L, P < .01). Baseline LBP levels were elevated in the low HDL-group (15.8±6.0 μg/ml) as opposed to the normal/high HDL-group (12.4±5.3 μg/ml, P = .03) and remained significantly elevated after endotoxin infusion through the 24 hour period (37.7±11.3 μg/ml versus 29.0±12.0 μg/ml, P < .01 respectively).

Paraoxonase-1 activity: Serum PON-1 activity was similar at baseline in both groups and was unaffected by endotoxin challenge (data not shown).

Coagulation Response
Starting out with similar prothrombin fragments (F 1+2) in both groups, F1+2 levels were significantly increased the low HDL-groups at timepoints 4 and 6 but returned to baseline in both groups at 24.

Fibrinolysis
Fibrinolytic markers (D-dimer, tPA and PAI-1) were similar between groups both at baseline and after endotoxin infusion, peaking at 2½ hours following endotoxin infusion and returning to normal levels at 24 hours (data not shown).

Linear Regression Analysis
Linear regression analysis revealed a significant inverse relation between HDL-cholesterol as well as apoA-I levels versus leukocytes (figure 3A), pro-inflammatory cytokines (figure 3B) as well as pro-thrombin fragments (data not shown).
Figure 3. Correlation between apoA-I levels and endotoxin-induced systemic inflammatory response in all 27 study subjects

(A) leucocytes and (B) TNFα. Lines indicate linear regression analysis between apoA-I versus leucocytes at 24 hours and TNFα at 1 hour.
Discussion

In the present study, we demonstrate that apparently healthy males with genetically
determined isolated low HDL-cholesterol levels are characterized by an increased
sensitivity towards a low dose endotoxin challenge compared to subjects with normal/
high HDL-cholesterol levels. Throughout the whole range of HDL-cholesterol levels,
there was an inverse relation between apoA-I levels and sensitivity towards this
inflammatory challenge. These findings lend further support to the relevance of HDL
as an anti-inflammatory mediator in vivo.

Study Population

In order to evaluate the interaction between HDL and inflammation, we recruited
participants from a study designed to identify genes that control HDL-cholesterol
levels, excluding subjects with known genetic causes for low HDL, such as apoA-I
mutations. In line with a primary selection on low HDL-cholesterol levels, difference
in HDL-cholesterol was more pronounced than the difference in apoA-I, implying
the abundance of smaller HDL particles in the low HDL-group. In the control group,
both subjects with normal HDL-cholesterol levels were included as well as 7 subjects
with genetically determined high HDL-cholesterol levels. Importantly, the low and
normal/high HDL-groups were carefully matched for parameters known to affect
HDL-cholesterol levels or inflammatory state such as BMI and smoking.

Clinical Parameters and Lipid Changes

Endotoxin-induced symptoms like backache, chills, body temperature rise, hearth rate
increase, headache, myalgia and nausea were increased and noted more severe in the low
HDL-group compared to the normal/high HDL-group. In line with these results, low
HDL-cholesterol level is associated with an increased mortality and severity of septic
disease. The sequential changes in lipids and (apo)lipoproteins following endotoxin
challenge were comparable between groups. Within the 24 hours observation period,
neither HDL-cholesterol nor apoA-I changed significantly upon endotoxin challenge,
which is in line with previous analyses employing a 1 ng/kg bodyweight LPS infusion
in healthy volunteers.
**Inflammatory Response**

Early leukocytopenia, monocytopenia and decreased neutrophil count were comparable between groups (Figure 1). The magnitude of the “early” leukocyte margination has been shown to closely reflect the dose of endotoxin infused. Hence, a similar degree of early leukocytopenia implies comparable exposure to endotoxin in both the low- as well as normal/high HDL-group. In contrast, endotoxin challenge elicited augmented leukocytosis, monocytosis and increased neutrophil count in the low HDL-group at later time points (Figure 1). Similarly, the increase of pro-inflammatory cytokines as well as acute phase reactants was also elevated in the low HDL-group compared to the normal/high HDL-group. Linear regression analysis revealed a strong inverse relation between HDL-cholesterol as well as apoA-I levels versus leukocyte response, pro-inflammatory cytokines and plasma CRP levels, supporting an anti-inflammatory effect of HDL throughout a wide concentration range (Figure 3).

**Mechanism of HDLs’ anti-inflammatory effects**

Theoretically, the increased in vivo anti-inflammatory effect of HDL in the normal/high HDL-group could be adjudicated solely to increased scavenging of endotoxin, thereby minimizing the amount of endotoxin available to elicit inflammatory activation. However, several other facts must be taken into account. First, the scavenging of endotoxin by HDL does not equal HDL-mediated neutralization of endotoxin bioactivity. Endotoxin triggers the inflammatory cascade by binding of the TLR4 receptor on monocytes and endothelial cells which occurs within seconds, whereas HDL requires several minutes to scavenge endotoxin within its lipid moiety. Even after endotoxin sequestration, HDL needs hours to fully neutralize the bio-activity of ‘trapped’ endotoxin. Second, endotoxin elicits rapid sequestration of leukocytes and monocytes, the magnitude of which closely mirrors the dose of endotoxin exposure. Since leukocyte and monocyte margination were identical in low- and normal/high HDL-group, decreased exposure towards endotoxin is less likely. Combined, these data imply that HDL scavenging cannot fully account for the differences in inflammatory and coagulation responses observed in the low- versus normal/high HDL-group. In
this respect, HDL may also have direct anti-inflammatory effects, independently from endotoxin scavenging.\textsuperscript{6,15} First, the HDL-particle harbors protective enzymes, such as PON-1 which has been shown to inhibit monocyte migration.\textsuperscript{16} However, in the present study, PON-1 activity was similar in both groups. Other moieties within HDL have also been implicated to exert anti-inflammatory effects. Thus, apoA-I itself has a direct inhibitory effect on several pro-inflammatory loops.\textsuperscript{17} In fact, the potent, inverse relation between apoA-I and endotoxin-induced systemic response may highlight a role for apoA-I as anti-inflammatory mediator in vivo.

Procoagulant Response
Endotoxin infusion resulted in a significantly larger increase in thrombin generation in the low HDL-group as compared to the normal/high HDL-group, implying that HDL may also attenuate coagulation activation.\textsuperscript{18} Mechanistically, HDL may attenuate coagulation either indirectly by modulating the inflammatory cascade via its associated proteins,\textsuperscript{19} but also by directly affecting the coagulation system. Previous in vitro studies have already shown that HDL enhances the activity of the important physiological anticoagulant protein C pathway.\textsuperscript{20} In line with these findings, population-based studies have shown an inverse relationship between HDL-cholesterol levels and tissue factor pathway inhibitor (TFPI) levels.\textsuperscript{21} Furthermore, HDL have been shown to inhibit the expression of tissue factor by endothelial cells.\textsuperscript{22} An inverse relation between HDL and coagulation has been supported by clinical observations. Indeed, low levels of HDL have been associated with increased risk for venous thrombosis.\textsuperscript{23} HDL may also be entangled with arterial thrombosis in the setting of acute coronary syndromes. In the MIRACL study, it was shown that HDL-cholesterol levels at the time of acute coronary syndromes had strong prognostic impact on short-term clinical outcome, the latter being driven by arterial thrombosis on ruptured plaques.

Summary
In the present study we provide evidence that endogenous HDL attenuates the inflammatory and coagulation response towards low-dose endotoxin challenge. These
findings provide a further impetus for implementation of HDL-increasing strategies in the acute, often inflammatory setting of e.g. myocardial infarction and acute coronary syndrome.25

Acknowledgments

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