Association of Lipopolysaccharide-Binding Protein and Coronary Artery Disease in Men

Philipp M. Lepper, MD,*† Christian Schumann, MD,† Kathy Triantafilou, PhD,‡ F. Maximilian Rasche, MD,† Tibor Schuster, MS,§ Hedwig Frank,† E. Marion Schneider, PhD,‖ Martha Triantafilou, PhD,‡ Maximilian von Eynatten, MD#

Bern, Switzerland; Ulm and Munich, Germany; and Brighton, United Kingdom

Objectives
In this study we tested the hypothesis that lipopolysaccharide-binding protein (LBP) might be able to be used as a biomarker for coronary artery disease (CAD).

Background
The mechanisms by which the innate immune recognition of pathogens could lead to atherosclerosis remain unclear. Lipopolysaccharide-binding protein is the first protein to encounter lipopolysaccharide and to deliver it to its cellular targets, toll-like receptors; therefore, its presence might be a reliable biomarker that indicates activation of innate immune responses.

Methods
A total of 247 men undergoing elective coronary angiography were studied, and the extent of coronary atherosclerosis was assessed by 2 established scores: “extent score” and “severity score.” Levels of LBP, markers of inflammation, and traditional risk factors for CAD were assessed.

Results
Serum LBP concentration was significantly increased in 172 patients with angiographically confirmed CAD compared with 75 individuals without coronary atherosclerosis (20.6 ± 8.7 pg/ml vs. 17.1 ± 6.0 pg/ml, respectively; \( p = 0.002 \)). Moreover in multivariable logistic regression analyses, adjusted for established cardiovascular risk factors and markers of systemic inflammation, LBP was a significant and independent predictor of prevalent CAD (\( p < 0.05 \) in all models).

Conclusions
Lipopolysaccharide-binding protein might serve as a novel marker for CAD in men. The present results underlie the potential importance of innate immune mechanisms for CAD. Further studies are warranted to bolster the data and to identify pathogenetic links between innate immune system activation and atherosclerosis.

Atherosclerosis can be regarded as a chronic inflammatory response limited to the vascular bed (1). New insights into the pathogenesis of atherosclerosis clearly indicate that multiple factors, such as hypertension, high plasma concentrations of low-density lipoprotein (LDL) cholesterol, diabetes mellitus, and infection, influence the development and progression of atherosclerosis (2).

Although the inflammatory nature of the disease is widely accepted, what initiates and maintains this inflammatory state remains unclear. Microbial infections seem to promote myocardial infarction (3). Epidemiologic evidence has suggested a link between microbial infection and atherosclerosis (4–6). In particular, it is possible that lipopolysaccharide (LPS) from bacteria such as *Chlamydia pneumonia* (7–9), *Helicobacter pylori* (10,11), or *Porphyromonas gingivalis* (6,12) may be triggering the inflammatory response that leads to atherogenesis. The question that remains is how these microbial pathogens trigger this inflammatory disease. The recent discovery of toll-like receptors (TLRs), which are the key microbial sensors of the innate immune system (13), in atherosclerotic lesions (14) provides a mechanistic link between infection, innate immune recognition, inflammation, and atherosclerosis.

The detection of microbial infection and the initiation of the innate immune response rely on TLRs, which recognize microbial “signatures.” Ligation of TLRs results in the recruitment of an adaptor protein, myeloid differentiation factor 88 (MyD88), which finally leads to activation of nuclear factor \( \kappa \)B and the production of proinflammatory cytokines. A link between TLRs and atherosclerosis has been suggested in studies where expression of TLR-1, -2,
Serum samples were obtained before angiography and intraprocedural coronary angiography at the University Hospital, Heidelberg, were studied. A total of 247 male patients, ages 31 to 83 years (mean age 61.1 ± 10.1 years), who were undergoing elective coronary angiography, were included. Many of the steps involved in the interaction of lipopolysaccharides (LPS) with monocytes and endothelial cells have been revealed in the last 2 decades: LPS binds to lipopolysaccharide-binding protein (LBP) (19,20), and LPS/LBP complexes are delivered to CD14 (21) which in turn interacts with TLRs. The detection of LPS stimulates the synthesis of interleukins, growth factors, and cytokines with the potential to exert proinflammatory and proatherogenic activities (22,23). Therefore it seems that the mammalian “LPS-sensing” apparatus consists of different receptors, membrane bound and soluble, that cooperate to induce activation leading to the secretion of proinflammatory cytokines (24).

Lipopolysaccharide-binding protein not only is a serum protein but also intercalates into cell membranes and assumes a transmembrane configuration (25). Thus, LBP is present as a cell-surface protein even under serum-free conditions and in this configuration is involved in cell signaling (26).

Because LBP is the first protein to encounter LPS and to deliver it to its cellular targets, it seems to be the first step in this proinflammatory cascade (23); therefore, its presence might be a reliable biomarker that indicates activation of innate immune responses. It might qualify as an excellent marker for activation of innate immunity.

Owing to the economic burden and often devastating consequences for the individual caused by the sequelae of atherosclerosis, it is highly desirable to have reliable biomarkers for the development and/or progression of atherosclerosis. In the past, several markers have been discussed, and C-reactive protein (CRP) has been proposed as a reliable marker for atherosclerosis (27). Because LBP is similar to CRP, both being acute-phase proteins, we tested the hypothesis that LBP might be able to be used as a biomarker for coronary artery disease (CAD).

Methods

Patients undergoing coronary angiography. A total of 247 male patients, ages 31 to 83 years (mean age 61.1 ± 10.1 years), who were undergoing elective coronary angiography at the University Hospital, Heidelberg, were studied. Serum samples were obtained before angiography and frozen at −70°C until analyses were performed. The design and methods of the study have been reported previously (28,29).

Examinations of angiograms were performed by 2 independent investigators, who were blinded to risk factors and LBP serum levels. The extent of angiographically documented CAD was quantified as follows: 1) the “severity score” (SS) as 1-, 2-, or 3-vessel disease, defined as stenosis of more than 50% of the luminal diameter in 1, 2, or 3 coronary arteries or their major branches (30); and 2) the “extent score” (ES), in which atherosclerotic wall irregularities in 10 defined segments of the coronary arteries are taken into account. The ES has been shown to correlate better with risk factors of CAD than other scores (31). The ES was 0 to 100 points, and prevalent CAD was defined as ES >0. Coronary artery disease was found in 172 patients (47 had 1-vessel, 57 had 2-vessel, and 68 had 3-vessel disease).

Control subjects. The control group consisted of: 1) 20 patients with fibromyalgia, a noninflammatory disorder of bones and joints (32,33); and 2) 20 septic patients without suspected or known CAD. Diagnosis of primary fibromyalgia was accomplished according to the American College of Rheumatology criteria (34). Criteria for inclusion in the trial were the presence of a “jump sign” in at least half of the 18 tender points examined by applying ≤4 kg pressure with the thumb and the absence of depression as assessed by Beck's Depression Inventory (<19 points). None of the patients belonged to a group with depression or so-called somatization. All septic patients fulfilled the sepsis criteria of the 2001 International Sepsis Definitions Conference (35).

None of the study participants, except the septic patients, had any of the following disorders, associated with an acute-phase reaction: febrile acute infection or acute state of a chronic infection or an inflammatory disease; underlying hematologic or malignant diseases, severe liver and renal disorders, and surgery within the previous 4 weeks. Current medication and sociodemographic characteristics were also recorded.

Participation was voluntary, and written informed consent was obtained from each subject upon entry into the study. The study was approved by the ethics committee of the University of Heidelberg (for patients with fibromyalgia and sepsis the study was approved by the University of Ulm ethics committee).

Laboratory methods. Venous blood was drawn in CAD and fibromyalgia patients after an overnight fasting period under standardized conditions. In septic patients, blood was drawn at various times of the day. Within 30 min after venipuncture, the remaining blood was centrifuged at 3,000 g for 10 min, immediately aliquoted and frozen at −70°C until further analysis. No specimen was inadvertently thawed during storage.

LBP. Serum was stored at −70°C and thawed on 1 occasion to perform tests. The LBP was determined from

---

**Abbreviations and Acronyms**

- **BMI** = body mass index
- **ES** = extent score
- **FPG** = fasting plasma glucose
- **HDL** = high-density lipoprotein
- **HOMA** = homeostasis model assessment
- **LBP** = lipopolysaccharide-binding protein
- **LDL** = low-density lipoprotein
- **LPS** = lipopolysaccharide
- **TLR** = toll-like receptor
- **VLDL** = very low-density lipoprotein
- **BMI** = body mass index
- **ES** = extent score
- **FPG** = fasting plasma glucose
- **HDL** = high-density lipoprotein
- **HOMA** = homeostasis model assessment
- **LBP** = lipopolysaccharide-binding protein
- **LDL** = low-density lipoprotein
- **LPS** = lipopolysaccharide
- **TLR** = toll-like receptor
- **VLDL** = very low-density lipoprotein
serum samples and controls using standardized enzyme-linked immunosorbent assay (ELISA) methods (Hycult Biotechnology, Uden, the Netherlands), and serum from 4 normal control subjects was used for interassay variation. Both the intra- and the interassay coefficients of variation were <5.0%.

**Cytokine assays.** The following markers of inflammation and hemostasis were determined by ELISA in patients’ sera: interleukin-6 (Quantikine, R&D Systems, Wiesbaden, Germany) and plasma concentrations of CRP in a highly sensitive assay (hsCRP; Dade Behring, Cupertino, California).

**Analysis of lipids/lipoproteins.** Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were determined enzymatically with a Synchron LX-20 (Beckman Coulter, Munich, Germany). The LDL and very low-density lipoprotein (VLDL) were separated by ultracentrifugation in a Beckman LM-8 ultracentrifuge in 100-µl volumes with a VT-51.2 rotor (Beckman Coulter). The atherogenic index was calculated using the following formula: (total cholesterol - HDL cholesterol)/HDL cholesterol.

**Assessment of insulin resistance.** Serum insulin immunoreactivity was determined from frozen serum by ELISA (CIS Bio International, Gif-Sur-Yvette, France). Plasma glucose was measured by a glucose-oxidase method. The degree of insulin resistance was estimated by homeostasis model assessment (HOMA) according to the method described by Matthews et al. (36). In particular, an insulin resistance score (HOMA-IR) was computed with the following formula: (fasting serum insulin (µU/ml)/22.5) × fasting serum glucose (mmol/l).

**Statistical analysis.** Statistical analyses were performed using SPSS software version 11.0 (SPSS Inc., Chicago, Illinois). Because serum levels of LBP showed a skewed distribution, Spearman correlation coefficients were used to describe the association between LBP and the continuous variables of interest. After adjustment for age and body mass index (BMI), Partial Spearman correlation coefficients were calculated for LBP, hsCRP, interleukin-6, fasting plasma glucose (FPG), HOMA-IR, pack-years smoking, ES, SS, and blood lipids, such as HDL, LDL, VLDL, plasma triglycerides, and the atherogenic index. Comparison between 2 sets of patients were performed by t test or Mann-Whitney U test. In multivariable logistic regression analyses, the independent association of LBP serum levels with the likelihood of coronary atherosclerosis was evaluated. The models, fitted for prevalent CAD as dependent variable, included age and BMI (model 1), additionally hsCRP and interleukin-6 (model 2), and LDL cholesterol, pack-years, history of diabetes and/or hypertension, and serum adiponectin (model 3). For calculation of the odds ratios (ORs), patients were categorized in quartiles based on percentiles of the serum LBP concentrations. Interquartile cutoff points of LBP were 14.3 pg/ml (25th percentile), 18.4 pg/ml (50th percentile), and 23.4 pg/ml (75th percentile): category 1 <14.3 pg/ml; 14.3 pg/ml ≤ category 2 <18.4 pg/ml; 18.4 pg/ml ≤ category 3 <23.4 pg/ml; and category 4 ≥23.4 pg/ml.

**Results**

Baseline characteristics of patients with and without angiographically documented CAD are given in Table 1. In 247 men undergoing elective coronary angiography, CAD was found in 172 individuals (69.6%). The individuals with CAD were older and more likely to be current smokers and had increased pack-years and LDL cholesterol levels, higher FPG and HOMA-IR, elevated atherogenic index, and lower HDL cholesterol. Serum LBP levels were significantly increased in patients with CAD compared with those without CAD (mean 20.6 ± 8.7 pg/ml, median 19.2 pg/ml vs. mean 17.1 ± 6.0 pg/ml, median 17.5 pg/ml, respectively; p = 0.002) (Fig. 1). Moreover, when CAD patients were compared with septic patients (LBP mean 64.5 ± 32.4 pg/ml, median 65.6 pg/ml) and patients with fibromyalgia (LBP mean 14.4 ± 6.0 pg/ml, median 15.0 pg/ml), it was evident that CAD patients had “intermediate” levels of LBP that were statistically significant different from the other entities (both p < 0.002) (Fig. 1).

Table 2 shows bivariate correlations between cardiovascular risk factors and angiographic scores and serum LBP. After Bonferroni correction, LBP levels were significantly positively associated with the atherogenic index, pack-years, and HOMA-IR. As expected, serum LBP levels were highly correlated with markers of systemic inflammation (interleukin-6 r = 0.375; hsCRP r = 0.497; both p < 0.001). In partial correlation analysis, adjusted for age and BMI, the associations between serum LBP and pack-years, interleukin-6, and hsCRP remained statistically significant (Table 2).

Table 3 shows multivariable logistic regression models to estimate the ORs of CAD across quartiles of LBP levels. After adjustment for age and BMI, participants in the highest compared with the lowest quartile of LBP levels had a significantly increased risk of prevalent CAD (OR 5.444; 95% confidence interval [CI] 2.018 to 14.691; p = 0.001; model 1). Further adjustment for interleukin-6 and hsCRP (OR 6.072; 95% CI 1.914 to 19.263; p = 0.002; model 2) and, finally, adjustment for established cardiovascular risk factors, such as LDL cholesterol, smoking status, history of diabetes and/or hypertension, and serum adiponectin levels, did not substantively affect this relationship (OR 5.551; 95%
CI 1.602 to 19.227; p = 0.007; model 3). Moreover in Cochran-Armitage trend test for proportions, the linear trend across LBP quartiles was also statistically significant (p = 0.001).

The ORs for the presence of CAD in each of the quartiles based on the serum LBP concentration are also shown in Figure 2. For clinical translation, cutoff points were selected. The BMI- and age-adjusted ORs for CAD in the second, third, and fourth quartiles for serum LBP were 1.593 (95% CI 0.749 to 3.387), 2.138 (95% CI 0.954 to 4.343), and 5.444 (95% CI 2.018 to 14.691), respectively, compared with the first quartile (Fig. 2).

**Discussion**

To support the role of innate immunity for the development of CAD, we investigated whether LBP is associated with the presence and extent of coronary heart disease in 247 men with clinically stable CAD who underwent elective coronary angiography.

In patients with angiographically documented CAD, we found significantly increased levels of LBP compared with angiographically CAD-negative patients. The CAD-positive patients were found to have "intermediate" levels of LBP compared with those with sepsis and fibromyalgia. Furthermore, LBP levels showed significant Bonferroni-corrected correlations with established cardiovascular risk factors, such as the atherogenic index and pack-years smoking. As we expected on the basis of its physiologic role, LBP was positively correlated with markers of inflammation, such as hsCRP and interleukin-6. When we further analyzed the data by partial correlation analysis, adjusted for age and BMI, the associations between LBP and these risk factors remained statistically significant.

In a multivariable logistic regression analysis, we showed an estimate for the partial association between LBP and the presence of CAD. In 3 statistical models, LBP was shown to be a significant and independent predictor of prevalent CAD. Moreover, the multivariable adjusted OR for CAD revealed that male patients with increased levels of LBP (>23.4 pg/ml) had a 5-fold increase in CAD prevalence.

As mentioned in the preceding, CAD might partly be triggered by subclinical infections, and some studies provide...
serologic evidence for the involvement of LPS and pathogen-associated molecular patterns with CAD (6–10). However, we want to point out that several studies found no association between infection and CAD or question this concept (11,37,38). We believe that LBP has the advantage of serving as a sensitive marker for the presence of LPS in the bloodstream, and our study might stimulate further work regarding the role of infectious diseases for CAD.

The nature and physiologic role of LBP has well been defined (19–21), and LBP has been shown to bind to lipopolysaccharides, lipoproteins, and lipopeptides (39). Among a variety of ligands, oxidized LDL leads to up-regulation of TLR-4 (15), and subsequently the activation of innate immunity leads to up-regulation of LBP synthesis in the liver, mainly via interleukin-1 and -6 (40). We propose LBP as an additional marker for CAD risk, because LBP can be regarded as a surrogate marker for activated innate immune system, which plays an important role in the pathophysiology of atherosclerosis. By comparing levels of LBP with septic patients and patients with fibromyalgia, we could show that CAD patients had “intermediate” levels of LBP which were different from critically ill patients and patients with fibromyalgia.

Among a variety of ligands, oxidized LDL leads to up-regulation of TLR-4 (15), and subsequently the activation of innate immunity leads to up-regulation of LBP synthesis in the liver, mainly via interleukin-1 and -6 (40). We propose LBP as an additional marker for CAD risk, because LBP can be regarded as a surrogate marker for activated innate immune system, which plays an important role in the pathophysiology of atherosclerosis. By comparing levels of LBP with septic patients and patients with fibromyalgia, we could show that CAD patients had “intermediate” levels of LBP which were different from critically ill patients and patients with fibromyalgia.

### Table 2
Correlation Coefficients Between Serum Concentrations of LBP and Coronary Risk Factors in Men (n = 247)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted*</th>
<th>Adjusted for Age and BMI†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r p Value</td>
<td>r p Value</td>
</tr>
<tr>
<td>Age</td>
<td>0.150</td>
<td>0.020</td>
</tr>
<tr>
<td>BMI</td>
<td>0.124</td>
<td>0.054</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.128</td>
<td>0.047</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.147</td>
<td>0.024</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.146</td>
<td>0.024</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>0.103</td>
<td>0.113</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.127</td>
<td>0.051</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>0.212</td>
<td>0.001†</td>
</tr>
<tr>
<td>Pack-years</td>
<td>0.235</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.271</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>History of diabetes</td>
<td>0.032</td>
<td>0.618</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>0.020</td>
<td>0.753</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>0.375</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>hsCRP</td>
<td>0.497</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Severity score</td>
<td>0.133</td>
<td>0.041</td>
</tr>
<tr>
<td>Extent score</td>
<td>0.088</td>
<td>0.174</td>
</tr>
</tbody>
</table>

*p value represents 2-tailed Spearman Correlation coefficients; †p value represents partial correlation analysis; ‡Bonferroni-corrected statistical significance.

Abbreviations as in Table 1.

### Table 3
Multivariable Logistic Regression Predicting the Likelihood of CAD According to Quartiles of LBP Levels, Markers of Systemic Inflammation, and Established Cardiovascular Risk Factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp(B)</td>
<td>95% CI</td>
<td>Exp(B)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>1.000</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1.539</td>
<td>0.749–3.387</td>
<td>1.448</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>2.138</td>
<td>0.954–4.343</td>
<td>1.980</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>5.444</td>
<td>2.018–14.691</td>
<td>6.072</td>
</tr>
<tr>
<td>Age</td>
<td>1.027</td>
<td>0.998–1.057</td>
<td>1.035</td>
</tr>
<tr>
<td>BMI</td>
<td>1.096</td>
<td>0.999–1.203</td>
<td>1.098</td>
</tr>
<tr>
<td>hsCRP†</td>
<td></td>
<td></td>
<td>0.638</td>
</tr>
<tr>
<td>Interleukin-6†</td>
<td></td>
<td></td>
<td>1.088</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td></td>
<td></td>
<td>1.011</td>
</tr>
<tr>
<td>Pack-years</td>
<td></td>
<td></td>
<td>1.025</td>
</tr>
<tr>
<td>History of diabetes</td>
<td></td>
<td></td>
<td>0.994</td>
</tr>
<tr>
<td>History of hypertension</td>
<td></td>
<td></td>
<td>3.158</td>
</tr>
<tr>
<td>Adiponectin†</td>
<td></td>
<td></td>
<td>0.225</td>
</tr>
<tr>
<td>c index</td>
<td>0.652</td>
<td></td>
<td>0.673</td>
</tr>
</tbody>
</table>

LBP quartiles: category 1 <14.3 pg/ml; 14.3 pg/ml < category 2 <18.4 pg/ml; 18.4 pg/ml < category 3 <23.4 pg/ml; and category 4 ≥23.4 pg/ml. *Statistical significance (p < 0.05); †log-transformed variables.

Abbreviations as in Table 1.
from patients that can be regarded to be in a noninflammatory state. Furthermore, within the CAD group, LBP levels increased with the severity of coronary heart disease, possibly reflecting a more widespread inflammation in those patients. Because LBP serum levels showed some overlap between groups, which might be caused by an imprecision of the currently used test, automation of the test procedure is highly desirable to reduce this imprecision of the marker. With a lower imprecision and further reduction of analytic noise, it can be assumed that an even better separation between groups is possible.

Interestingly, in multivariable analysis, LBP was independent of interleukin-6 and hsCRP levels in predicting the prevalence of CAD. Of course, elevated hsCRP is a strong predictor of future cardiovascular risk in patients with established CAD, with or without a previous myocardial infarction. Blake and Ridker (41) have shown that elevated hsCRP can predict risk of adverse cardiovascular events, including death, acute myocardial infarction, and need for revascularization procedures, in patients with acute coronary syndromes. Lipopolysaccharide-binding protein has not been thoroughly investigated regarding its potency in being a cardiovascular risk factor yet. Nevertheless, on the basis of the present data and theoretic considerations LBP might find its mechanistic role in coronary plaque development.

In conclusion, the present study is the first to report that elevated levels of circulating LBP represent a strong and independent predictor of the presence of CAD in men. The results indicate that the measurement of serum LBP may be a useful risk marker for CAD that needs further investigation in prospective trials. Novel drugs might use targets of the innate immune system, such as LBP, to offer a pathogenetically orientated treatment to lower cardiovascular risk.

Acknowledgments

The authors are indebted to Dr. T. Stratz (Rheumaklinik Bad Säckingen, Germany) for the provision of fibromyalgia patients and their data, and thank Dr. J. Zweigner (Charité Berlin, Germany) for helpful discussion.

Reprint requests and correspondence: Dr. Philipp M. Lepper, Department of Intensive Care Medicine, Inselspital, University of Bern, CH-3010 Bern, Switzerland. E-mail: philipp.lepper@insel.ch.

REFERENCES