Journal of the American College of Cardiology
© 2007 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 50, No. 1, 2007
ISSN 0735-1097/07/$32.00
doi:10.1016/j.jacc.2007.02.070

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. (J Am Coll Cardiol 2007;50:25–31) © 2007 by the American College of Cardiology Foundation

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 κB and the production of proinflammatory cytokines. A link between TLRs and atherosclerosis has been suggested in studies where expression of TLR-1, -2,

From the *Department of Intensive Care Medicine, University Hospital of Bern (Inselspital), Bern, Switzerland; †Department of Internal Medicine II, University of Ulm, Ulm, Germany; ‡Infection and Immunity Group, University of Sussex, Brighton, United Kingdom; §Institute of Medical Statistics and Epidemiology, Technical University of Munich, Munich, Germany; ‖Section of Experimental Anaesthesiology, University of Ulm, Ulm, Germany; and the #Department of Nephrology, Technical University of Munich, Munich, Germany. The first three authors contributed equally to this work.

Manuscript received November 27, 2006; revised manuscript received January 11, 2007, accepted February 5, 2007.

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

and -4 was found in atherosclerotic lesions (14,15) as well as in studies where loss of TLR-4 or MyD88 reduced disease severity in atherosclerosis (16,17).

Thus it is emerging that LPS from various gram-negative bacteria might lead to the initiation of events that lead to the recruitment of inflammatory cells into atherosclerotic lesions (9,11,18). 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

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 glucose (mmol/l) × fasting serum insulin (μU/ml)/22.5.

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. Cochran-Armitage trend test for proportions was used to test for linear trend across categories. For logistic regression analysis CAD-positive and -negative patients were included, but not fibromyalgia and sepsis patients. Values of $p < 0.05$ were considered to be statistically significant, except for bivariate correlation analyses in which Bonferroni correction for multiple testing was used, which led to an alpha of 0.003 (performed using the Statistica software package [StatSoft, Tulsa, Oklahoma]).

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%

Table 1 Clinical Characteristics of Patients With and Without Coronary Artery Disease (CAD)

Factor	CAD (+) (n = 172)	CAD (−) (n = 75)
Age (yrs)	62.2 ± 9.5*	59.0 ± 11.1
BMI (kg/m^2)	27.8 ± 3.4	27.0 ± 3.5
Total cholesterol (mmol/l)	5.42 ± 1.24	5.16 ± 1.08
HDL (mmol/l)	0.95 (0.83–1.16)†	1.03 (0.90–1.28)
LDL (mmol/l)	3.75 ± 1.03*	3.42 ± 0.85
VLDL (mmol/l)	0.49 (0.36–0.79)	0.48 (0.26–0.78)
Triglycerides (mmol/l)	1.44 (1.12–2.10)	1.30 (0.82–2.03)
Atherogenic index	5.38 (4.23–6.75)‡	4.74 (3.83–5.51)
Fasting plasma glucose (mmol/l)	5.67 (4.90–6.55)†	5.83 (5.45–6.56)
HOMA-IR	4.88 (3.65–6.84)‡	2.49 (1.62–4.84)
History of diabetes, n (%)	26 (15.1)	9 (12.0)
Current smoker, n (%)	114 (66.3)‡	30 (40.0)
Smoking status (pack-yrs)	15.0 (0–30.0)‡	11.9 (0–21.3)
Interleukin-6 (pg/ml)	2.33 (1.55–4.72)	2.52 (1.48–4.07)
hsCRP (mg/dl)	1.50 (0.80–3.75)	1.55 (0.70–3.08)
LBP (pg/ml)	19.2 (14.9–24.6)‡	17.5 (12.5–20.2)

Results are mean ± SE for parametrically distributed data or median (interquartile range) for nonparametrically distributed data. *$p < 0.05$ versus CAD (−) subjects (unpaired *t* test); †$p < 0.05$; ‡$p < 0.005$ versus CAD (−) subjects (Mann-Whitney *U* test).
BMI = body mass index; HDL = high-density lipoprotein; HOMA-IR = homeostasis model assessment insulin resistance; hsCRP = high-sensitivity C-reactive protein; LBP = lipopolysaccharide-binding protein; LDL= low-density lipoprotein; VLDL = very low-density lipoprotein.

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).

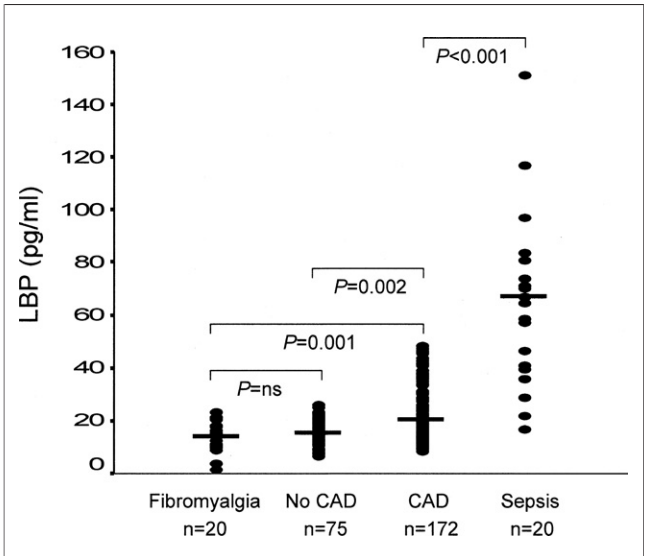

Figure 1 Comparison of Serum LBP Levels

Patients with and without incident coronary artery disease (CAD) and control subjects with fibromyalgia and sepsis. Data are presented as individual values, and the **central line in each column** represents the median. p values by Mann-Whitney *U* test. LBP = lipopolysaccharide-binding protein.

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

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

Variable	Unadjusted*		Adjusted for Age and BMI†	
	r	p Value	r	p Value
Age	0.150	0.020	—	—
BMI	0.124	0.054	—	—
Total cholesterol	0.128	0.047	0.124	0.056
LDL cholesterol	0.147	0.024	0.135	0.039
HDL cholesterol	−0.146	0.024	−0.120	0.065
VLDL cholesterol	0.103	0.113	0.084	0.200
Triglycerides	0.127	0.051	0.131	0.044
Atherogenic index	0.212	0.001‡	0.165	0.011
Pack-years	0.235	<0.001‡	0.207	0.001‡
HOMA-IR	0.271	<0.001‡	0.178	0.012
History of diabetes	0.032	0.618	0.030	0.644
History of hypertension	0.020	0.753	0.041	0.532
Interleukin-6	0.375	<0.001‡	0.287	<0.001‡
hsCRP	0.497	<0.001‡	0.453	<0.001‡
Severity score	0.133	0.041	0.080	0.219
Extent score	0.088	0.174	0.038	0.587

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

Abbreviations as in Table 1.

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

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

Variable	Model 1			Model 2			Model 3		
	Exp(B)	95% CI Exp(B)	p	Exp(B)	95% CI Exp(B)	p	Exp(B)	95% CI Exp(B)	p
LBP			0.003*			0.017*			0.046*
Quartile 1	1.000			1.000			1.000		
Quartile 2	1.539	0.749–3.387	0.385	1.448	0.640–3.276	0.374	1.535	0.610–3.862	0.363
Quartile 3	2.138	0.954–4.343	0.156	1.980	0.876–4.476	0.101	2.083	0.849–5.110	0.109
Quartile 4	5.444	2.018–14.691	0.001*	6.072	1.914–19.263	0.002*	5.551	1.602–19.227	0.007*
Age	1.027	0.998–1.057	0.068	1.035	1.004–1.068	0.026*	1.044	1.009–1.080	0.014*
BMI	1.096	0.999–1.203	0.053	1.098	0.993–1.215	0.069	1.039	0.931–1.160	0.497
hsCRP†				0.638	0.286–1.422	0.271	0.435	0.177–1.066	0.069
Interleukin-6†				1.088	0.385–3.078	0.874	0.609	0.194–1.913	0.396
LDL cholesterol							1.011	1.000–1.022	0.057
Pack-years							1.025	1.005–1.046	0.013*
History of diabetes							0.994	0.378–2.618	0.991
History of hypertension							3.158	1.545–6.457	0.002*
Adiponectin†							0.225	0.066–0.769	0.017*
c index			0.652			0.673			0.767

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.

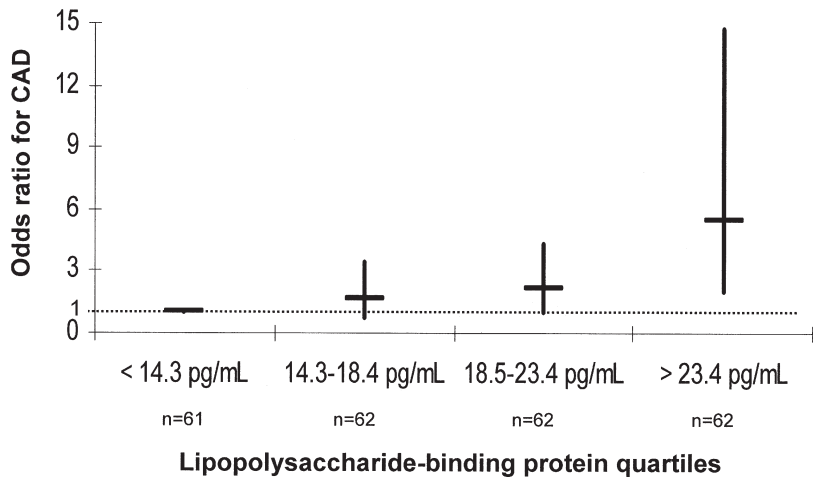

Figure 2 Odds Ratio for CAD

The second, third, and fourth quartiles compared with the first quartile of lipopolysaccharide-binding protein, adjusted for age and body mass index. **Vertical bars** indicate 95% confidence intervals. CAD = coronary artery disease.

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

1. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868–74.
2. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
3. Lusis AJ. Atherosclerosis. Nature 2000;407:233–41.
4. Kol A, Sukhova GK, Lichtman AH, Libby P. Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor-alpha and matrix metalloproteinase expression. Circulation 1998;98:300–7.
5. Kol A, Bourcier T, Lichtman AH, Libby P. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. J Clin Invest 1999;103:571–7.
6. Gibson FC 3rd, Yumoto H, Takahashi Y, Chou HH, Genco CA. Innate immune signaling and *Porphyromonas gingivalis*-accelerated atherosclerosis. J Dent Res 2006;85:106–21.
7. Grayston JT. Background and current knowledge of *Chlamydia pneumoniae* and atherosclerosis. J Infect Dis 2000;181 Suppl 3: S402–10.
8. Kuo CC, Grayston JT, Campbell LA, Goo YA, Wissler RW, Benditt EP. *Chlamydia pneumoniae* (TWAR) in coronary arteries of young adults (15–34 years old). Proc Natl Acad Sci U S A 1995;92:6911–4.
9. Schumacher A, Seljeflot I, Lerkerod AB, Sommervoll L, Otterstad JE, Arnesen H. Positive Chlamydia pneumoniae serology is associated with elevated levels of tumor necrosis factor alpha in patients with coronary heart disease. Atherosclerosis 2002;164:153–60.
10. Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? Lancet 1997;350:430–6.
11. Danesh J, Wong Y, Ward M, Muir J. Chronic infection with *Helicobacter pylori*, *Chlamydia pneumoniae*, or cytomegalovirus: population based study of coronary heart disease. Heart 1999;81:245–7.

12. Malek R, Fisher JG, Caleca A, et al. Inactivation of the *Porphyromonas gingivalis* fimA gene blocks periodontal damage in gnotobiotic rats. J Bacteriol 1994;176:1052–9.
13. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335–76.
14. Edfeldt K, Swedenborg J, Hansson GK, Yan ZQ. Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. Circulation 2002;105:1158–61.
15. Xu XH, Shah PK, Faure E, et al. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. Circulation 2001;104:3103–8.
16. Michelsen KS, Doherty TM, Shah PK, Arditi M. TLR signaling: an emerging bridge from innate immunity to atherogenesis. J Immunol 2004;173:5901–7.
17. Michelsen KS, Wong MH, Shah PK, et al. Lack of toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. Proc Natl Acad Sci U S A 2004;101:10679–84.
18. Miyamoto T, Yumoto H, Takahashi Y, Davey M, Gibson FC 3rd, Genco CA. Pathogen-accelerated atherosclerosis occurs early after exposure and can be prevented via immunization. Infect Immun 2006;74:1376–80.
19. Heumann D, Gallay P, Barras C, et al. Control of lipopolysaccharide (LPS) binding and LPS-induced tumor necrosis factor secretion in human peripheral blood monocytes. J Immunol 1992;148:3505–12.
20. Tobias PS, Soldau K, Ulevitch RJ. Isolation of a lipopolysaccharide-binding acute phase reactant from rabbit serum. J Exp Med 1986;164: 777–93.
21. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990;249:1431–3.
22. Mattila KJ, Valtonen VV, Nieminen MS, Asikainen S. Role of infection as a risk factor for atherosclerosis, myocardial infarction, and stroke. Clin Infect Dis 1998;26:719–34.
23. Schumann RR, Rietschel ET, Loppnow H. The role of CD14 and lipopolysaccharide-binding protein (LBP) in the activation of different cell types by endotoxin. Med Microbiol Immunol (Berl) 1994;183: 279–97.
24. Triantafilou M, Triantafilou K. The dynamics of LPS recognition: complex orchestration of multiple receptors. J Endotoxin Res 2005; 11:5–11.
25. Gutsmann T, Haberer N, Carroll SF, Seydel U, Wiese A. Interaction between lipopolysaccharide (LPS), LPS-binding protein (LBP), and planar membranes. Biol Chem 2001;382:425–34.
26. Gutsmann T, Muller M, Carroll SF, MacKenzie RC, Wiese A, Seydel U. Dual role of lipopolysaccharide (LPS)-binding protein in neutralization of LPS and enhancement of LPS-induced activation of mononuclear cells. Infect Immun 2001;69:6942–50.
27. Koenig W, Lowel H, Baumert J, Meisinger C. C-Reactive protein modulates risk prediction based on the Framingham score: implications for future risk assessment: results from a large cohort study in southern Germany. Circulation 2004;109:1349–53.
28. von Eynatten M, Schneider JG, Humpert PM, et al. Serum adiponectin levels are an independent predictor of the extent of coronary artery disease in men. J Am Coll Cardiol 2006;47:2124–6.
29. Dugi KA, Brandauer K, Schmidt N, et al. Low hepatic lipase activity is a novel risk factor for coronary artery disease. Circulation 2001;104: 3057–62.
30. Wolk R, Berger P, Lennon RJ, Brilakis ES, Somers VK. Body mass index: a risk factor for unstable angina and myocardial infarction in patients with angiographically confirmed coronary artery disease. Circulation 2003;108:2206–11.
31. Sullivan DR, Marwick TH, Freedman SB. A new method of scoring coronary angiograms to reflect extent of coronary atherosclerosis and improve correlation with major risk factors. Am Heart J 1990;119: 1262–7.
32. Clark S, Tindall E, Bennett RM. A double blind crossover trial of prednisone versus placebo in the treatment of fibrositis. J Rheumatol 1985;12:980–3.
33. Nampiaparampil DE, Shmerling RH. A review of fibromyalgia. Am J Manag Care 2004;10:794–800.
34. Wolfe F, Smythe HA, Yunus MB, et al. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990;33:160–72.
35. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 2003;31:1250–6.
36. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
37. Koenig W, Rothenbacher D, Hoffmeister A, et al. Infection with *Helicobacter pylori* is not a major independent risk factor for stable coronary heart disease: lack of a role of cytotoxin-associated protein A–positive strains and absence of a systemic inflammatory response. Circulation 1999;100:2326–31.
38. Brenner H, Berg G, Frohlich M, Boeing H, Koenig W. Chronic infection with *Helicobacter pylori* does not provoke major systemic inflammation in healthy adults: results from a large population-based study. Atherosclerosis 1999;147:399–403.
39. Schroder NW, Heine H, Alexander C, et al. Lipopolysaccharide binding protein binds to triacylated and diacylated lipopeptides and mediates innate immune responses. J Immunol 2004;173:2683–91.
40. Nanbo A, Nishimura H, Muta T, Nagasawa S. Lipopolysaccharide stimulates HepG2 human hepatoma cells in the presence of lipopolysaccharide-binding protein via CD14. Eur J Biochem 1999; 260:183–91.
41. Blake GJ, Ridker PM. C-Reactive protein and other inflammatory risk markers in acute coronary syndromes. J Am Coll Cardiol 2003;41:37S–42S.