Bacterial Endotoxin Activity in Human Serum Is Associated With Dyslipidemia, Insulin Resistance, Obesity, and Chronic Inflammation

MARIANN I. LASSENIUS, MSC[1,2]
KIRSI H. PIETILÄINEN, MD, DMSC[3]
KATI KAARTINEN, MD, DMSC[2,4]
PIRKKO J. PUSSINEN, PHD[5]
JAANA SYRJÄNEN, MD, DMSC[4]
CAROL FORSBLOM, MD, DMSC[1,2]
ILKKA PÖRSTI, MD, DMSC[4]
AILA RISSANEN, MD, DMSC[3]
JAAKKO KAPRIO, MD, DMSC[6,7]
JUKKA MUSTONEN, MD, DMSC[4]
PER-HENRIK GROOP, MD, DMSC[1,2,8]
MARKKU LEHTO, PHD[1,2]
ON BEHALF OF THE FINNDIANE STUDY GROUP*

OBJECTIVE—To investigate whether bacterial lipopolysaccharide (LPS) activity in human serum is associated with the components of the metabolic syndrome (MetS) in type 1 diabetic patients with various degrees of kidney disease and patients with IgA glomerulonephritis (IgAGN).

RESEARCH DESIGN AND METHODS—Serum LPS activity was determined with the Limulus Amoebocyte Lysate chromogenic end point assay in type 1 diabetic patients with a normal albumin excretion rate (n = 587), microalbuminuria (n = 144), macroalbuminuria (n = 173); patients with IgAGN (n = 98); and in nondiabetic control subjects (n = 345). The relationships of the LPS/HDL ratio and MetS-associated variables were evaluated with Pearson correlation.

RESULTS—The MetS was more prevalent in type 1 diabetic patients (48%) than in patients with IgAGN (15%). Diabetic patients with macroalbuminuria had a significantly higher serum LPS/HDL ratio than patients with IgAGN. In the normoalbuminuric type 1 diabetic group, patients in the highest LPS/HDL quartile were diagnosed as having the MetS three times more frequently than patients in the lowest quartile (69 vs. 22%; $P < 0.001$). High LPS activity was associated with higher serum triglyceride concentration, earlier onset of diabetes, increased diastolic blood pressure, and elevated urinary excretion of monocyte chemoattractant protein-1.

CONCLUSIONS—High serum LPS activity is strongly associated with the components of the MetS. Diabetic patients with kidney disease seem to be more susceptible to metabolic endotoxemia than patients with IgAGN. Bacterial endotoxins may thus play an important role in the development of the metabolic and vascular abnormalities commonly seen in obesity and diabetes-related diseases.

Diabetes Care 34:1809–1815, 2011

From the [1]Folkhälsan Institute of Genetics, Folkhälsan Research Center, Biomedicum Helsinki, Helsinki, Finland; the [2]Division of Nephrology, Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland; the [3]Obesity Research Unit, Division of Internal Medicine, Department of Medicine and Department of Psychiatry, Helsinki University Central Hospital, Helsinki, Finland; the [4]Department of Internal Medicine, Tampere University Hospital, and the University of Tampere Medical School, Tampere, Finland; the [5]Institute of Dentistry, University of Helsinki, Helsinki, Finland; the [6]Department of Public Health, Hjelt Institute, and the Centre of Excellence in Complex Disease Genetics, Institute for Molecular Genetics, University of Helsinki, Helsinki, Finland; the [7]National Institute for Health and Welfare, Helsinki, Finland; and the [8]Baker International Diabetes Institute, Heart and Diabetes Institute, Melbourne, Victoria, Australia.

Corresponding author: Markku Lehto, markku.lehto@helsinki.fi.

Received 22 November 2010 and accepted 28 April 2011.
DOI: 10.2337/dc10-2197
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc10-2197/-/DC1.

*A complete list of participating centers in the FinnDiane Collection can be found in the Supplementary Data.

In patients with type 1 diabetes, long duration of the disease and poor glycemic control increase the risk for micro- and macrovascular complications. A significant proportion of type 1 diabetic patients who develop these complications exhibit clinical features commonly seen in subjects with the metabolic syndrome (MetS), including dyslipidemia, insulin resistance, obesity, hypertension, and chronic inflammation (1,2). Vascular diseases are also common in patients with IgA glomerulonephritis (IgAGN), and those with a progressive form of the disease run a significantly elevated risk of developing different cardiovascular sequela (3). Whether the MetS is independently associated with the progression of IgAGN is currently unclear, but at least several related factors, including hyperuricemia, hypertriglyceridemia, weight, insulin resistance, and inflammation, seem to associate with the development of kidney dysfunction in IgAGN (4,5).

Many of these clinical subphenotypes likely emerge from the complex interactions between a genetic background, lifestyle, and environmental factors. Recent studies have highlighted the role of the innate immune system in the developmental process of these metabolic abnormalities (6). Our living environment is inhabited with commensal and pathogenic bacteria, which have both beneficial and detrimental effects on human health. If the immune defense functions properly, these microorganisms rarely cause any serious infections in healthy individuals. The situation is different in immunocompromised patients—local infections, use of antibiotics, and even diet may lead to the colonization of opportunistic bacteria in various sites of the body. In diabetic patients, long duration of the disease and poor glycemic control increase the risk for urinary tract, pulmonary, oral, and skin infections (6,7).

Lipopolysaccharides (LPS) are unique glycolipids in the cell wall of gram-negative bacteria. LPS molecules, also known as bacterial endotoxins, may trigger acute and

chronic inflammation, leading to immune cell activation and cytokine release. HDL cholesterol is one of the most important factors involved in the elimination of LPS molecules from circulation. In healthy subjects, LPS is mainly bound to HDL, whereas in patients with sepsis, LPS is redistributed toward LDL and VLDL lipoproteins (8). High LPS activity combined with low HDL levels increases the risk for cardiovascular disease (9).

LPS infusion in mammals leads to the appearance of factors known to be associated with the MetS: elevated levels of proinflammatory markers, dyslipidemia, fasting hyperglycemia, insulin resistance, and obesity (10–12). In some research applications, bacterial endotoxins have been used to induce acute kidney injury in animals. Monocyte chemoattractant protein-1 (MCP1) is a potential marker for progressive renal disease because its expression and excretion are also regulated by bacterial endotoxins (13,14).

We have recently shown that high serum levels of LPS activity are associated with the development and progression of kidney disease in Finnish type 1 diabetic patients. Most of the patients with high serum LPS activity exhibited features of dyslipidemia and insulin resistance (15). These metabolic abnormalities are frequently observed in diabetic patients with micro- or macrovascular complications (2). To our knowledge, no previous studies have explored the potential links between bacterial endotoxins and the MetS in subjects with impaired kidney function. Therefore, we wanted to investigate whether serum LPS activity is associated with metabolic risk factors in three distinct study populations: type 1 diabetic patients with various degrees of kidney disease, patients with IgAGN, and nondiabetic subjects.

RESEARCH DESIGN AND METHODS

Finnish Diabetic Nephropathy Study

A total of 911 patients with type 1 diabetes and 225 nondiabetic control (NDC) subjects were selected from the Finnish Diabetic Nephropathy Study (FinnDiane). In contrast to our previous baseline study (15), all blood samples and clinical measurements were obtained from diabetic patients during their prospective visit. Type 1 diabetes was defined as an onset of diabetes before the age of 40 years and permanent insulin treatment initiated within 1 year of diagnosis. The diabetic patients were divided into three groups by albumin excretion rate (AER) in two of three urine collections: 594 with normal AER (<30 mg/24 h), 144 with microalbuminuria (30–300 mg/24 h), and 173 with macroalbuminuria (≥300 mg/24 h; Table 1). The NDC subjects from the FinnDiane study were divided into two groups by absence or presence of overweight, defined as a BMI cutoff of 25 kg/m^2 (see DATA ANALYSIS AND STATISTICAL METHODS).

IgAGN study

The cohort with IgAGN was investigated in the Department of Internal Medicine in Tampere University Hospital (Tampere, Finland) and consisted of 223 patients living in the Pirkanmaa Health District in Finland (total population ~440,000) who had been diagnosed with the disease during an 11-year period (1980–1990). IgAGN was defined as a glomerulonephritis with IgA as the sole or main glomerular immunofluorescence finding in a renal biopsy specimen. A total of 98 patients without diabetes were reinvestigated after a median (interquartile range [IQR]) of 16.5 (14–20) years from the original renal biopsy (Table 2). The median age during the prospective visit was 51 (41–62) years. The median for the estimated glomerular filtration rate (eGFR) was 72 (13–128) mL/min/1.73 m^2 (16), and 26.5% of the patients presented with eGFR <60 mL/min/1.73 m^2. The median amount of proteinuria was 0.17 (0.1–0.52) g.

TwinFat study

NDC subjects of the TwinFat study were selected from two population-based prospective studies, FinnTwin16 (FT16) and FinnTwin12 (FT12), each consisting of five consecutive birth cohorts of Finnish twins (17). The participants were enrolled according to their responses to questions on weight and height in the last follow-up questionnaires at the age of 22 to 27 years, with the aim to cover the full BMI range of both normal-weight and obese subjects. The BMI range of the subjects participating in metabolic studies was 16.3 to 48.6 kg/m^2. Among the 242 subjects, 92 twins (46 men and 46 women, 46 full pairs) were monozygotic (MZ) and 150 twins (86 men and 64 women, 75 full pairs) were same-sex dizygotic (DZ). To provide

Table 1—*Clinical characteristics of type 1 diabetic patients*

Variable	Normal AER	Microalbuminuria	Macroalbuminuria
N (male/female)	587 (257/330)	144 (65/79)	173 (110/63)*
Age (years)	44 (36–53)	46 (37–55)	48 (40–56)†
Age at onset (years)	17 (10–25)	11 (6–19)*	11 (7–16)*
Duration of diabetes (years)	28 ± 12	33 ± 11*	35 ± 9*
HbA_{1c} (%)	7.7 ± 1.3	7.8 ± 1.8	7.8 ± 2.0
BMI (kg/m^2)	25.6 ± 4.2	26.4 ± 4.2†	27.0 ± 4.9*
Blood pressure (mmHg)			
Systolic	136 ± 17	141 ± 19†	146 ± 18*
Diastolic	78 ± 9	78 ± 10	80 ± 11†
Cholesterol (mmol/L)	4.7 ± 0.8	4.9 ± 0.8†	4.8 ± 1.1
Triglycerides (mmol/L)	0.90 (0.69–1.21)	0.95 (0.72–1.51)†	1.35 (0.93–2.03)*
HDL cholesterol (mmol/L)			
Male	1.5 ± 0.4	1.4 ± 0.4	1.3 ± 0.4†
Female	1.7 ± 0.4	1.8 ± 0.5†	1.5 ± 0.4*
apoA1 (mg/dL)	146 ± 32	152 ± 34	143 ± 33
apoB (mg/dL)	75 ± 19	78 ± 18	85 ± 25*
LPS (EU/mL)	57 (50–69)	56 (47–72)	67 (52–96)*
LPS/HDL ratio	37 (29–50)	35 (27–52)	50 (34–77)*
CRP (mg/L)	1.0 (0.3–2.5)	1.0 (0.5–2.5)	1.1 (0.6–2.9)
eGFR (mL/min/1.73 m^2)	102 (90–111)	96 (81–108)*	57 (35–84)*
Medication (%)			
Lipid-lowering	22	35*	56*
Antihypertensive	31	79*	98*
Smoking (%)	17	20	26*
Retinopathy (%)	20	55*	78*
Coronary heart disease (%)	6	9	15*

Continuous data are presented as the median (interquartile range) or as mean ± SD. All variables are compared with normoalbuminuric patients: *$P \leq 0.001$, †$P \leq 0.05$.

Table 2—*Clinical characteristics of nondiabetic study subjects*

Variable	NDC-lean	NDC-ow	NDC-all	IgAGN
N (male/female)	219 (96/123)	126 (81/45)*	345 (177/168)	98 (61/37)
Age (years)	33 ± 10	33 ± 9	33 ± 10	52 ± 13*
BMI (kg/m^2)	22.2 ± 1.7	28.2 ± 2.8*	24.3 ± 3.6	26.5 ± 4.3*
Waist (cm)				
Male	85 (80–88)	97 (92–102)†	89 (84–96)	91 (87–100)
Female	76 (72–81)	90 (86–98)*	79 (74–85)	80 (71–87)
Blood pressure (mmHg)				
Systolic	123 ± 14	130 ± 13*	126 ± 14	148 ± 21*
Diastolic	75 ± 8	80 ± 8	77 ± 8	90 ± 11*
Glucose (mmol/L)	4.8 (4.5–5.1)	5.0 (4.7–5.4)*	4.9 (4.6–5.2)	4.7 (4.4–5.1)†
Insulin (μU/mL)	4.2 (2.9–5.5)	5.7 (4.4–10.0)*	4.8 (3.3–6.7)	7.7 (6.1–10.7)*
HOMA-IR	1.0 (0.8–1.5)	1.3 (0.8–2.0)†	1.1 (0.8–1.6)	1.6 (1.1–2.2)*
Cholesterol (mmol/L)	4.5 ± 0.9	4.9 ± 0.9*	4.7 ± 0.9	5.3 ± 1.1*
Triglycerides (mmol/L)	0.8 (0.7–1.1)	1.1 (0.8–1.4)	0.9 (0.7–1.2)	1.2 (0.8–1.6)*
HDL cholesterol (mmol/L)				
Male	1.5 ± 0.3	1.4 ± 0.3†	1.4 ± 0.3	1.3 ± 0.3†
Female	1.8 ± 0.3	1.6 ± 0.4†	1.7 ± 0.4	1.8 ± 0.4
LPS (EU/mL)	60 (44–80)	62 (49–82)	61 (44–79)	49 (40–55)*
LPS/HDL ratio	37 (28–51)	45 (35–62)*	41 (30–54)	34 (26–43)*
CRP (mg/L)	0.7 (0.5–1.5)	1.2 (0.7–3.3)†	0.9 (0.5–2.0)	1.7 (0.8–2.9)*
Medication (%)				
Lipid-lowering	1	0	1	19*
Antihypertensive	2	4	3	56*
Smoking (%)	14	21	16	15

Continuous values are presented as median (interquartile range) or as mean ± SD. NDC-lean (BMI ≤ 25 kg/m^2) and NDC-ow (BMI >25 kg/m^2) are compared with each other; NDC-all (NDC-lean + NDC-ow) and IgAGN groups are compared with each other. *$P \leq 0.001$, †$P \leq 0.05$.

control subjects for unrelated individuals, one sibling was randomly selected from each twin pair for the current study. These were further divided into lean and overweight subjects by a BMI cutoff of 25 kg/m^2 (see DATA ANALYSIS AND STATISTICAL METHODS).

All study protocols were approved by the local ethics committees and were in accordance with the Declaration of Helsinki. All study subjects gave their written informed consent to their participation in the study.

Laboratory analyses

Blood samples were drawn after an overnight fast. Nearly all of the biochemical analyses were performed in the Helsinki University Central Hospital Laboratory (HUSLAB; www.hus.fi) and in PSHP Laboratory Centre in Tampere (www.laboratorio.fi), which are accredited hospital laboratories that belong to the Labquality Ltd. (www.labquality.fi) national quality assessment program. Serum lipids were analyzed in three distinct laboratories: FinnDiane study (Central Laboratory, Division of Cardiology, Biomedicum, Helsinki), TwinFat study (HUSLAB), and the IgAGN study (PSHP Laboratory Centre). A set of 20 blood samples was sent to each laboratory for the assessment glucose, creatinine, cholesterol, triglycerides, and HDL cholesterol. All tested variables showed strong correlations between different laboratories (Spearman correlations $r \geq 0.96$). Moreover, no significant differences were seen in analyte concentrations (data not shown). Insulin concentrations based on radioimmunoassay (LINCO Research Inc., St. Charles, MO) were transformed to match the insulin concentrations obtained with immunofluorometric assays (AutoDelfia, PerkinElmer, Beaconsfield, U.K.) (18).

FinnDiane study

Fasting glucose values were measured from whole blood with a HemoCue device (HemoCue, Espoo, Finland). Plasma insulin was analyzed with the AutoDelfia immunofluorometric assay (PerkinElmer). High-sensitive C-reactive protein (hsCRP) was measured from serum by immunoprecipitation (Thermo Scientific, Vantaa, Finland). Serum lipid and lipoprotein concentrations were determined with a Cobas Mira analyzer using automated enzymatic methods (Hoffmann La Roche, Basel, Switzerland) in the Central Laboratory (Division of Cardiology, Biomedicum, Helsinki). Except for lipids, all other clinical measurements were performed in the HUSLAB. In type 1 diabetic patients, urinary MCP1 (uMCP1) was detected by ELISA according to the manufacturer's instructions (R&D Systems, Abingdon, U.K.). The uMCP1 protein levels were normalized with urinary creatinine concentrations (uCreat).

IgAGN study

Serum insulin concentrations were determined using a human insulin–specific radioimmunoassay kit (Linco Research). Serum hsCRP values were analyzed using an immunoturbidimetric method with Cobas Integra 700 (provided by HoffmannLa Roche). Other laboratory variables were analyzed using the serum samples and spot and collection urine samples using in-house routine analytic methods in the laboratory of Tampere University Hospital (PSHP Laboratory Centre, Tampere).

TwinFat study

Plasma glucose was measured using the spectrophotometric hexokinase and glucose-6-phosphate dehydrogenase assay (Gluko-quant glucose/hexokinase, Roche Diagnostics) with an automatic analyzer (Roche Diagnostics Hitachi 917,

Hitachi Ltd, Tokyo, Japan). Serum insulin concentrations were determined with the AutoDelfia immunofluorometric assay, and hsCRP with the particle-enhanced Cobas CRP-LatexHS immunoturbidimetric assay (Roche Diagnostics, Basel, Switzerland) on a Modular automatic analyzer. Concentrations of serum total cholesterol, HDL cholesterol, and triglyceride were measured with respective enzymatic kits from Roche Diagnostics using a Hitachi Modular autoanalyzer. All assays were performed in the HUSLAB.

The eGFR was determined by the Chronic Kidney Disease Epidemiology Collaboration equation (16). In type 1 diabetic patients, insulin sensitivity was determined using the equation for the estimated glucose disposal rate (eGDR) (2).

In the nondiabetic subjects, the homeostasis model assessment was used to calculate insulin resistance (HOMA-IR) index [plasma insulin (μU/mL) × plasma glucose (mmol/L)/22.5] (19). Before the HOMA calculations, blood glucose values were transformed to plasma values with a conversion factor (blood glucose × 1.12 = plasma glucose). The MetS was assessed according to the recent joint statement (20): central obesity (waist circumference ≥94 cm in men and ≥80 cm in women), triglycerides ≥1.7 mmol/L, HDL cholesterol <1.0 mmol/L in men and <1.30 mmol/L in women, blood pressure ≥130/85 mmHg, and fasting glucose ≥6.11 mmol/L. Three of five criteria are required for the diagnosis of MetS. All diabetic patients by definition fulfilled the criteria for high blood glucose.

In IgAGN patients, waist measurements were obtained from a baseline visit 6 years before the prospective visit, when blood samples were taken for the LPS analysis. The waist criterion was considered met only if BMI was unchanged or higher at the prospective visit.

Serum LPS activity was measured with the Limulus Amoebocyte Lysate chromogenic end-point assay (Hycult Biotechnology, Uden, the Netherlands; inter- and intra-assay coefficients of variation, 4.5%; sensitivity, 1.0 EU/mL). Because HDL cholesterol is the main factor involved in neutralization of endotoxemia, we used the LPS/HDL ratio as a functional measure of the LPS activity (8,9,15).

Data analysis and statistical methods

In NDC subjects (FinnDiane control subjects + TwinFat individuals), interaction terms between MetS-associated variables and LPS activity were tested in a linear multivariate model. Because a significant interaction was found between BMI and LPS activity (data not shown), we subdivided 225 subjects from the FinnDiane and 118 from the TwinFat studies into two groups by a BMI cutoff of 25 kg/m^2. Clinical characteristics of lean NDC (NDC-lean: BMI ≤25 kg/m^2) and overweight NDC (NDC-ow: BMI >25 kg/m^2) subjects are presented in Table 2. The NDC-lean and the NDC-ow cohorts were combined into one larger cohort (NDC-all) for the evaluation of overall correlations between selected clinical variables (Supplementary Appendix 2).

Normally distributed variables are presented as mean ± SD, whereas skewed variables are presented as median with IQR. Correlations between LPS-related variables and components of the MetS were evaluated with Pearson correlation analyses. To avoid the influence of HDL on the factors in the correlation analyses, residuals from the linear regression of LPS and HDL were calculated. Residuals or LPS alone were correlated to the MetS features to avoid the effect of HDL cholesterol. Statistical differences between groups were determined with the Student *t* test or the Mann-Whitney *U* test, when appropriate. Differences between multiple groups were analyzed by ANOVA or the Kruskal-Wallis test, depending on the distribution. The association between clinical variables and serum LPS activity was assessed with multivariate linear regression analyses. A value of $P \leq 0.05$ was considered significant. All statistical analyses were done with SPSS 15.0 software (SPSS Inc., Chicago, IL).

RESULTS

The overall frequency of the MetS was 48% in type 1 diabetic patients. The MetS was more prevalent in diabetic patients with higher degree of albuminuria: macroalbuminuria (70%), microalbuminuria (47%), and normal AER (43%). Type 1 diabetic patients with macroalbuminuria had a significantly higher LPS/HDL ratio (50 [34–77] EU/mL; $P < 0.001$) compared with diabetic patients with microalbuminuria (37 [29–50] EU/mL) or normal AER (35 [27–52] EU/mL; Table 1). Of all tested variables, serum LPS activity showed the strongest correlation with serum triglyceride concentrations (all type 1 diabetic patients, $r = 0.73$, $P < 0.001$).

In type 1 diabetic patients with normal AER, serum LPS activity was assessed by number of individual components of the MetS (scores 1–5). Because of the low number of patients, those who had four or five scores were pooled into one group. A concomitant rise in the serum LPS activity and the metabolic score was observed: one score, 54 (48–62); two scores, 55 (49–63); three scores, 57 (51–70); and four and five scores, 78 (62–94). High blood pressure was the most frequent component of the MetS, followed by increased waist circumference, low HDL cholesterol, and high triglycerides (Supplementary Appendix 1).

Type 1 diabetic patients with normal AER were divided into quartiles according to their LPS/HDL ratio. The 147 patients in the highest quartile (quartile 4) were significantly younger and had developed diabetes at an earlier age compared with the 145 patients in the lowest quartile (quartile 1). The quartile 4 subjects also had significantly higher HbA_{1c}, BMI, waist circumference, serum triglycerides, apolipoprotein B (apoB), and diastolic blood pressure than subjects in quartile 1. Insulin sensitivity, measured with eGDR, decreased with increasing LPS/HDL ratio. The percentage of diabetic patients who fulfilled the MetS criteria was significantly higher in quartile 4 than in quartile 1. The eGFR and the uMCP1/uCreat ratio were also significantly higher in quartile 4 than in quartile 1 (Table 3). In diabetic patients, the overall correlation between LPS/HDL-ratio and uMCP1/uCreat was $r = 0.21$ ($P < 0.001$).

These results did not change if diabetic patients were subdivided into quartiles by LPS activity instead of by the LPS/HDL ratio (data not shown). The association between clinical outcomes and serum LPS activity was assessed with multivariate linear regression analyses. In diabetic patients with normal AER, the variables with the strongest association with serum LPS activity were serum triglycerides ($\beta = 0.69$, $P < 0.001$), age at onset of diabetes ($\beta = -0.14$, $P < 0.001$), diastolic blood pressure ($\beta = 0.10$, $P = 0.004$), and uMCP1/creatinine ratio ($\beta = 0.10$, $P = 0.003$). Clinical variables significantly associated in the univariate model (HbA_{1c}, HDL cholesterol, and waist circumference) were not significant in the multivariate analysis. Similar results were obtained if the variables were added into the model by the backward elimination method (data not shown).

The overall prevalence of the MetS was much lower in the nondiabetic cohorts than in the type 1 diabetic patients: IgAGN (15%), NDC-ow (15%), and NDC-lean (2%). The NDC-ow group had significantly higher serum LPS/HDL

Table 3—*LPS/HDL quartiles in type 1 diabetic patients with normal albumin excretion*

Variable	LPS/HDL quartiles			
	Quartile 1 <29.0	Quartile 2 29.0–37.2	Quartile 3 37.2–50.5	Quartile 4 >50.5
N (male/female)	145 (34/111)	148 (61/87)	147 (79/68)	147 (83/64)
Age (years)	48 (40–57)	47 (39–54)	44 (36–55)†	39 (31–47)*
Age at onset (years)	19 (11–28)	16 (10–25)	17 (10–26)	13 (9–21)*
Duration of diabetes (years)	29 ± 13	29 ± 12	27 ± 13	25 ± 10†
HbA_{1c} (%)	7.3 ± 1.6	7.6 ± 1.2	7.7 ± 1.2†	7.9 ± 0.9*
BMI (kg/m^2)	24.4 ± 3.0	25.5 ± 3.5†	25.7 ± 4.0†	26.9 ± 5.5*
Waist (cm)				
Male	89 ± 9	92 ± 10	93 ± 10	96 ± 11†
Female	80 ± 9	83 ± 10†	83 ± 11†	90 ± 13*
Blood pressure (mmHg)				
Systolic	137 ± 20	136 ± 17	137 ± 17	135 ± 15
Diastolic	76 ± 10	77 ± 8	78 ± 9	81 ± 9*
eGDR (mg/kg/min)	7.4 ± 2.3	7.0 ± 2.3	6.4 ± 2.3*	6.1 ± 2.3*
Cholesterol (mmol/L)	4.7 ± 0.7	4.6 ± 0.7	4.6 ± 0.8	4.8 ± 0.9
Triglycerides (mmol/L)	0.7 (0.6–0.8)	0.8 (0.7–0.9)†	1.0 (0.8–1.2)*	1.4 (1.2–1.8)*
HDL cholesterol (mmol/L)				
Male	2.1 ± 0.5	1.6 ± 0.2*	1.4 ± 0.2*	1.1 ± 0.2*
Female	2.0 ± 0.4	1.7 ± 0.2*	1.5 ± 0.3*	1.4 ± 0.3*
apoA1 (mg/dL)	161 ± 33	145 ± 31*	142 ± 29*	136 ± 29*
apoB (mg/dL)	64 ± 14	70 ± 13*	77 ± 16*	88 ± 22*
Nonesterified fatty acids (mmol/L)	227 (134–455)	191 (123–378)	200 (128–328)	240 (167–385)
CRP (mg/L)	0.7 (0.3–1.6)	0.7 (0.3–1.4)	1.0 (0.4–2.1)	1.2 (0.4–3.5)†
Serum creatinine (μmol/L)				
Male	72 ± 12	77 ± 12	78 ± 13	76 ± 11
Female	67 ± 11	64 ± 10	64 ± 12	68 ± 12
eGFR (mL/min/1.73 m^2)	97 (84–108)	100 (92–109)†	103 (93–115)†	106 (93–115)*
uMCP1/uCreat (pg/mL/μmol/L)	13 (8–23)	14 (9–23)	15 (9–25)	16 (10–30)†
Metabolic syndrome (%)	22	38†	42*	69*
Medication				
Antihypertension (%)	35	26	33	32
Lipid-lowering (%)	23	16	21	26
Smoking (%)	10	13	18†	25†

Continuous values are presented as median (interquartile range) or mean ± SD. All values compared with quartile 1. *$P \leq 0.001$, †$P \leq 0.05$.

ratios than the NDC-lean or the IgAGN cohorts (Table 2). The subjects in the NDC-ow group had also higher systolic blood pressure, fasting insulin, serum cholesterol, and serum CRP than subjects in the NDC-lean group. Correlations between serum endotoxin levels (LPS/HDL ratio, LPS, and LPS-HDL correlation residual) and individual components of the MetS are shown in Supplementary Appendix 2. In nondiabetic patients, the most significant variable associated with the serum LPS/HDL ratio was serum triglycerides (NDC-all, $r = 0.51$; IgAGN, $r = 0.50$). Regardless of the analysis mode the results remained comparable. Notably, correlations of individual MetS components were stronger overall in the NDC-ow than in the NDC-lean group. Effects of BMI and fasting insulin on serum LPS activity were explored in the nondiabetic cohorts. Subjects in the NDC-all were divided into eight groups based on a BMI cutoff of 25 kg/m^2 and quartiles of fasting insulin. As seen in Supplementary Appendix 3, BMI and insulin had a synergistic effect on the LPS/HDL ratio.

Of note, neither measurements of kidney function nor the amount of proteinuria was associated with serum LPS activity in the IgAGN cohort (data not shown). Although patients with IgAGN and diabetic patients with macroalbuminuria had a similar BMI (26.5 ± 4.3 vs. 27.0 ± 4.9 kg/m^2; $P = 0.34$), the LPS/HDL ratio was significantly higher in the diabetic group (35 [26–43] vs. 50 [34–77] EU/mL; $P < 0.001$). The high LPS/HDL ratio could not be entirely explained by impaired kidney function, because these results remained similar when the IgAGN subjects and the patients with type 1 diabetes were matched for sex and eGFR (data not shown). Of note, the differences between the groups were more pronounced in patients with higher triglyceride concentrations (Supplementary Appendix 4).

CONCLUSIONS—The current study demonstrates that serum LPS activity strongly correlates with MetS-associated features. The association of serum LPS activity with the components of the MetS is in line with results obtained from FINRISK97, a large Finnish population-based study that also demonstrated that endotoxemia is an independent risk factor for incident diabetes (21). High serum LPS activity combined with metabolic abnormalities may thus pose a potent risk for the later development of micro- and macrovascular complications and

offer a new tool to estimate the metabolic risk profile in other populations as well.

Dyslipidemia is a well-recognized risk factor for the development of macrovascular complications in type 1 diabetic patients. Lipid abnormalities may also increase the risk for microvascular complications (e.g., diabetic retinopathy and nephropathy) (22). Hypertriglyceridemia is a prognostic marker for declining kidney function in IgAGN patients (5) and is also a risk marker for coronary heart disease in these patients (3). In the current study, most diabetic patients with high serum LPS activity had elevated serum triglycerides and low HDL-cholesterol concentrations. Of all the tested clinical variables, the strongest correlation was observed between the LPS/HDL ratio and serum triglyceride concentrations. High fasting concentrations of triglycerides predict postprandial hypertriglyceridemia and the development of insulin resistance (23).

Serum LPS activity correlated positively with serum apoB concentrations, which suggests that endotoxins are most likely transported by VLDL, LDL, or chylomicron particles. Most of the circulating endotoxins are generally believed to originate from bacteria colonizing the gut and provide a large reservoir of LPS molecules (24). Human and animal studies have both highlighted the importance of the composition of the diet for its influence on the endotoxin absorption process. Consumption of an energy-rich high-fat diet may result in increased levels of gut-derived bacterial endotoxins in circulation. Moreover, obesity-related changes in the gut microflora have been reported both in mice and humans. Alterations in the bacterial composition of the gut may also result in significant changes in the metabolic nutrient harvest (10,11). We note that a positive correlation was observed between the LPS/HDL ratio and obesity-related variables in all study groups. Could high serum LPS activity levels in type 1 diabetic patients be explained by gut-related factors other than diet? In addition to the dietary habits, administration of antimicrobial agents may disrupt the normal composition of the gut microbiota. Bacterial overgrowth may also result in intestinal dysmotility in diabetic patients. Diabetes itself may also induce ultrastructural changes in the mucosal barrier of the gut, which in turn may lead to increased permeability of the intestine.

Insulin resistance combined with obesity increases the risk for type 2 diabetes and cardiovascular disease (20). Notably, insulin resistance has also been strongly linked to the development of kidney dysfunction in patients with type 1 diabetes (2). Kidney patients may already be insulin-resistant before the onset of kidney failure (25). In vivo experiments have demonstrated that injections of bacterial endotoxins induce systemic inflammation, which is accompanied by the appearance of insulin resistance (12). In the current study, we showed that serum LPS activity is inversely correlated with insulin sensitivity both in diabetic and nondiabetic cohorts.

Elevated serum levels of inflammatory markers, such as tumor necrosis factor-α, interleukin-6, and CRP, are common in obesity and diabetes-related diseases (6). We have shown that serum levels of these markers are increased in type 1 diabetic patients with kidney disease (1). In the current study, serum LPS activity correlated positively with CRP in the three study cohorts, which suggests that the "low-grade inflammation" is explained to some extent by exposure to structural components of the gram-negative bacteria. LPS induces the expression of the MCP1 chemokine, which recruits and activates monocytes from the circulation to the site of inflammation.

Notably, previous studies have implicated a potential role for MCP1 in kidney-related diseases (13,14). We have previously shown that serum LPS activity increases along with declining kidney function (15). In the current study, the urinary MCP1/creatinine ratio increased in parallel with the serum LPS activity in diabetic patients with normal AER, which could indicate that the LPS-stimulated MCP1 excretion may be an early marker for kidney disease progression. Type 1 diabetic patients with kidney dysfunction had significantly higher levels of serum LPS activity and displayed more often pathologic features of the MetS than patients with IgAGN. This would indicate that diabetic patients are more susceptible to metabolic endotoxemia than patients with IgAGN.

Taken together, these data show that endotoxins derived from gram-negative bacteria are strongly associated with the MetS variables in vivo. These observations may have clinical implications, because high LPS activity is more often found in subjects who show signs of dyslipidemia, insulin resistance, overweight, and inflammation—factors that increase the risk for diverse micro- and macrovascular complications.

Acknowledgments—This study was supported by the Folkhälsan Research Foundation (P.-H.G.), the Wilhelm and Else Stockmann Foundation (P.-H.G., M.I.L., M.L.), the Diabetes Research Foundation (M.L.), Medicinska Understödsförening Liv och Hälsa, the Waldemar von Frenckell Foundation (M.I.L.), and Helsinki University Hospital Research Funds (A.R., K.H.P.); grants from Novo Nordisk, the Yrjö Jahnsson Foundation, the Biomedicum Helsinki Foundation, the Signe and Ane Gyllenberg Foundation, and the Finnish Foundation for Cardiovascular Research (K.H.P.); and the Academy of Finland Centre of Excellence in Complex Disease Genetics (J.K.). No other potential conflicts of interest relevant to this article were reported.

M.I.L. researched and analyzed data, wrote the manuscript, and reviewed and edited the manuscript. K.H.P. researched and analyzed data, contributed to discussion, and reviewed and edited the manuscript. K.K. researched and analyzed data and reviewed and edited the manuscript. P.J.P. contributed to the analysis plan and to the presentation and interpretation of data and reviewed and edited the manuscript. J.S. researched and analyzed data and reviewed and edited the manuscript. C.F. contributed to the analysis plan and to the presentation and interpretation of data and reviewed and edited the manuscript. I.P. and A.R. contributed to discussion and reviewed and edited the manuscript. J.K. researched and analyzed data, contributed to discussion, and reviewed and edited the manuscript. J.M. contributed to discussion and reviewed and edited the manuscript. P.-H.G. contributed to the analysis plan and to the presentation and interpretation of data and reviewed and edited the manuscript. M.L. researched and analyzed data, wrote the manuscript, and reviewed and edited the manuscript.

Parts of this study were presented in abstract form at the 46th Annual Meeting of the European Association for the Study of Diabetes, Stockholm, Sweden, 20–24 September 2010.

The authors thank Dr. Lena Thorn, Folkhälsan Institute of Genetics, for critical review of the article, and Dr. Janne Pitkäniemi (Hjelt Institute, University of Helsinki) for statistical review of the manuscript. Laboratory technicians and nurses M. Parkkonen, A.-R. Salonen, A. Sandelin, T. Soppela, and J. Tuomikangas at the Folkhälsan Institute of Genetics are acknowledged for their skilful technical assistance. The authors also acknowledge the physicians and nurses at each study center (Supplementary Appendix 5).

References

1. Saraheimo M, Teppo AM, Forsblom C, Fagerudd J, Groop PH. Diabetic nephropathy is associated with low-grade

inflammation in type 1 diabetic patients. Diabetologia 2003;46:1402–1407
2. Thorn LM, Forsblom C, Fagerudd J, et al.; FinnDiane Study Group. Metabolic syndrome in type 1 diabetes: association with diabetic nephropathy and glycemic control (the FinnDiane study). Diabetes Care 2005;28:2019–2024
3. Myllymäki J, Syrjänen J, Helin H, Pasternack A, Kattainen A, Mustonen J. Vascular diseases and their risk factors in IgA nephropathy. Nephrol Dial Transplant 2006; 21:1876–1882
4. Kaartinen K, Syrjänen J, Pörsti I, et al. Inflammatory markers and the progression of IgA glomerulonephritis. Nephrol Dial Transplant 2008;23:1285–1290
5. Syrjänen J, Mustonen J, Pasternack A. Hypertriglyceridaemia and hyperuricaemia are risk factors for progression of IgA nephropathy. Nephrol Dial Transplant 2000; 15:34–42
6. King GL. The role of inflammatory cytokines in diabetes and its complications. J Periodontol 2008;79(Suppl.):1527–1534
7. Benfield T, Jensen JS, Nordestgaard BG. Influence of diabetes and hyperglycaemia on infectious disease hospitalisation and outcome. Diabetologia 2007;50:549–554
8. Wendel M, Paul R, Heller AR. Lipoproteins in inflammation and sepsis. II. Clinical aspects. Intensive Care Med 2007;33:25–35
9. Pussinen PJ, Tuomisto K, Jousilahti P, Havulinna AS, Sundvall J, Salomaa V. Endotoxemia, immune response to periodontal pathogens, and systemic inflammation associate with incident cardiovascular disease events. Arterioscler Thromb Vasc Biol 2007;27:1433–1439
10. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–1772
11. Laugerette F, Vors C, Peretti N, Michalski MC. Complex links between dietary lipids, endogenous endotoxins and metabolic inflammation. Biochimie 2011;93:39–45
12. Mehta NN, McGillicuddy FC, Anderson PD, et al. Experimental endotoxemia induces adipose inflammation and insulin resistance in humans. Diabetes 2010;59:172–181
13. Wang Y, Rangan GK, Goodwin B, Tay YC, Harris DC. Lipopolysaccharide-induced MCP-1 gene expression in rat tubular epithelial cells is nuclear factor-kappaB dependent. Kidney Int 2000;57:2011–2022
14. Banba N, Nakamura T, Matsumura M, Kuroda H, Hattori Y, Kasai K. Possible relationship of monocyte chemoattractant protein-1 with diabetic nephropathy. Kidney Int 2000;58:684–690
15. Nymark M, Pussinen PJ, Tuomainen AM, Forsblom C, Groop PH, Lehto M; FinnDiane Study Group. Serum lipopolysaccharide activity is associated with the progression of kidney disease in Finnish patients with type 1 diabetes. Diabetes Care 2009;32: 1689–1693
16. Levey AS, Stevens LA, Schmid CH, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–612
17. Kaprio J. Twin studies in Finland 2006. Twin Res Hum Genet 2006;9:772–777
18. Lundgren VM, Isomaa B, Lyssenko V, et al.; Botnia Study Group. GAD antibody positivity predicts type 2 diabetes in an adult population. Diabetes 2010;59:416–422
19. 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;28:412–419
20. Alberti KG, Eckel RH, Grundy SM, et al.; International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009; 120:1640–1645
21. Pussinen PJ, Havulinna AS, Lehto M, Sundvall J, Salomaa V. Endotoxemia is associated with an increased risk of incident diabetes. Diabetes Care 2011;34: 392–397
22. Tolonen N, Forsblom C, Thorn L, et al.; FinnDiane Study Group. Relationship between lipid profiles and kidney function in patients with type 1 diabetes. Diabetologia 2008;51:12–20
23. Dekker MJ, Wright AJ, Mazurak VC, et al. Fasting triacylglycerol status, but not polyunsaturated/saturated fatty acid ratio, influences the postprandial response to a series of oral fat tolerance tests. J Nutr Biochem 2009;20:694–704
24. Ghoshal S, Witta J, Zhong J, de Villiers W, Eckhardt E. Chylomicrons promote intestinal absorption of lipopolysaccharides. J Lipid Res 2009;50:90–97
25. Fliser D, Pacini G, Engelleiter R, et al. Insulin resistance and hyperinsulinemia are already present in patients with incipient renal disease. Kidney Int 1998;53:1343–1347