A Marker of Endotoxemia Is Associated With Obesity and Related Metabolic Disorders in Apparently Healthy Chinese

LIANG SUN, MSC[1]
ZHIJIE YU, MD, PHD[1,7]
XINGWANG YE, PHD[2]
SHURONG ZOU, MD, MSC[3]
HUAIXING LI, PHD[1]
DANXIA YU, MSC[1]
HONGYU WU, MSC[1]
YAN CHEN, MD, PHD[1]
JOEL DORE, PHD[4]
KARINE CLÉMENT, MD, PHD[5]
FRANK B. HU, MD, PHD[6]
XU LIN, MD, PHD[1]

OBJECTIVE — Elevated lipopolysaccharide-binding protein (LBP), a marker of subclinical endotoxemia, may be involved in the pathogenesis of obesity and metabolic risk. We aimed to investigate the association between plasma LBP and metabolic disorders in apparently healthy Chinese.

RESEARCH DESIGN AND METHODS — A population-based study including 559 overweight/obese (BMI $\geq$24.0 kg/m^2) and 500 normal-weight (18.0 $\leq$ BMI $<$24.0 kg/m^2) subjects aged 35–54 years was conducted in Shanghai, China. Fasting plasma glucose, lipid profile, LBP, high-sensitivity C-reactive protein, interleukin-6, high-molecular-weight (HMW) adiponectin, leptin, hepatic enzymes, and body composition were measured. Metabolic syndrome was defined by the updated National Cholesterol Education Program Adult Treatment Panel III criterion for Asian Americans.

RESULTS — LBP levels were significantly higher in overweight/obese individuals than in normal-weight individuals (geometric mean 27.6 [95% CI 25.2–30.3] vs. 10.0 [9.1–11.1] μg/ml; $P < 0.001$). After multiple adjustments including BMI, the odds ratios were 3.54 (95% CI 2.05–6.09) and 5.53 (95% CI 2.64–11.59) for metabolic syndrome and type 2 diabetes, respectively, comparing the highest with the lowest LBP quartile. Further adjustments for inflammatory markers almost abolished the significant association of LBP with metabolic syndrome but not that with type 2 diabetes, and controlling for adipokines and hepatic enzymes did not substantially alter the results.

CONCLUSIONS — Elevated circulating LBP was associated with obesity, metabolic syndrome, and type 2 diabetes in apparently healthy Chinese. These findings suggested a role of lipopolysaccharide via initiation of innate immune mechanism(s) in metabolic disorders. Prospective studies are needed to confirm these results.

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Obesity, a major risk factor for metabolic disorders, is reaching epidemic proportions worldwide with 1.6 billion overweight/obese adults in 2005 (1). An unhealthy diet and lifestyle-associated obesogenic environment, along with genetic predisposition, are the main recognized drivers of the global epidemic of obesity and associated metabolic disorders. However, increasing evidence supports the fact that chronic low-grade inflammation is one of the key mechanisms underlying the pathogenesis of obesity-related metabolic disorders (2). However, the agents responsible for initiating and sustaining this low-grade inflammatory signal have yet to be identified.

A link between chronic infection and atherosclerosis has been postulated, in which lipopolysaccharide (LPS) derived from various Gram-negative bacteria might play a pivotal role (3). Animal studies (4,5) and human evidence (6) suggested that subclinical endotoxemia, indicated by low to moderately elevated LPS, may be involved in the pathogenesis of metabolic disorders. Lowering LPS concentrations through antibiotic (4) or rosiglitazone therapy (6) could improve metabolic outcomes. LPS is a potential factor in triggering the innate immune response and modulating the inflammatory cascade via stimulation of the nuclear factor-κB pathway and transcription of proinflammatory genes (7). However, the short half-life of LPS (8) and the difficulty of removing interference in blood (9) limit the utility of LPS testing in clinical or research settings.

Lipopolysaccharide-binding protein (LBP), an acute-phase protein synthesized in liver, initiates recognition and monomerization of LPS and amplifies host responses to LPS (10). Having a relatively long half-life, LBP delivers LPS to membrane and soluble forms of CD14 and consequently interacts with Toll-like receptor 4, triggering a downstream signaling cascade that leads to the upregulation of proinflammatory cytokines (7,10). Therefore, the presence of LBP could reflect an "effective" LPS level and innate immune response triggered by LPS (11,12).

Limited data have suggested that elevated LBP levels were observed in patients with nonalcoholic fatty liver disease (11) and were associated with unfavorable effects on metabolic traits in glucose-intolerant men (13) or with the increased prevalence of coronary artery disease

From the [1]Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Graduate School of the Chinese Academy of Sciences, Shanghai, China; the [2]Department of Health Sciences, Northeastern University, Boston, Massachusetts; the [3]Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China; the [4]Institut National de la Recherche Agronomique, Micalis, Unité Mixte de Recherche 1319, Jouy en Josas, France; the [5]Institut National de la Santé et de la Recherche Médicale, Nutriomique, U872 team 7, Paris, France, the Université Pierre et Marie Curie-Paris 6, Centre de Recherche des Cordeliers, Paris, France, and the Assistance Publique-Hôpitaux de Paris, Pitié-Salpêtrière Hospital, Nutrition and Endocrinology Department, Paris, France; [6]Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, Massachusetts; and the [7]SIBS-Novo Nordisk Translational Research Centre for PreDiabetes, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China.
Corresponding author: Xu Lin, xlin@sibs.ac.cn.
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(12). However, the role of LBP on obesity-related metabolic disorders has not been explored. Therefore, we aimed to investigate whether plasma LBP concentrations were associated with metabolic disorders such as metabolic syndrome, insulin resistance, and type 2 diabetes and also to what extent the associations were explained by adiposity, adipokines, hepatic enzymes, and inflammation in apparently healthy Chinese.

RESEARCH DESIGN AND METHODS

The Gut Microbiota and Obesity Study was a population-based case-control study among noninstitutionalized residents aged 35–54 years in Shanghai, China. This study investigated the effects of gut microbiota and environmental factors on obesity and related metabolic disorders. Two urban districts (Luwan and Zhabei) were chosen to represent people with high and low socioeconomic status in urban Shanghai. Participants were enrolled through their response to an advertisement. The fieldwork was conducted simultaneously in both districts from November 2007 through January 2008. Five hundred pairs of age- and sex-matched subjects (overweight/obesity) and control subjects (normal-weight) were planned to be recruited. This sample size presumably could provide 80% power to detect a 25% difference in gut microbiota composition between groups (14). Overweight/obesity and normal weight were defined as BMI $\geq$24.0 kg/m^2 and 18.0 $\leq$ BMI $<$24.0 kg/m^2, respectively, which was modified from the recommendation by the Working Group on Obesity in China (15).

Eligible candidates were adult residents who have lived in Shanghai for at least 10 years. Exclusion criteria included *1*) diarrhea for 3 consecutive days within the previous 3 months, *2*) heavy alcohol consumption ($\geq$40 g/day ethanol for men and $\geq$20 g/day for women), *3*) diagnosed diabetes, taking oral antidiabetic agents or insulin, cancer, coronary heart disease, myocardial infarction, stroke, or severe kidney or liver diseases, *4*) infectious diseases including tuberculosis, AIDS, and hepatitis, *5*) severe psychological disorders or physical disabilities, *6*) antibiotics used for 3 consecutive days within the previous 3 months, *7*) gastrointestinal surgery within 1 year, or *8*) pregnancy or lactation in women. The protocol was approved by the Institutional Review Board of the Institute for Nutritional Sciences and written informed consent was obtained from all participants. A total of 1,059 eligible participants (559 overweight/obese and 500 normal-weight) were successfully recruited.

Of these individuals, 960 (90.7%) completed a dual-energy X-ray absorptiometry (DEXA) scan. Baseline characteristics (BMI, waist circumference, family history of chronic diseases, smoking, alcohol drinking, and educational attainment) were similar between those with and without DEXA scans. However, individuals who did not participate in the DEXA scan were younger (43.0 vs. 46.2 years; $P < 0.001$) and tended to be male (57.6 vs. 36.7%, $P < 0.001$) compared with those who had the scan.

Data collection

Home interviews were conducted by trained physicians or public health workers from the local Centers for Disease Control and Prevention and community clinics. Information on demographic variables, health status, and behavior was collected using a standardized questionnaire. Smoking was defined as never, current, and former. Alcohol drinking was defined as "yes" or "no." Physical activity data were collected by using the International Physical Activity Questionnaire (short last 7-day format, http://www.ipaq.ki.se/scoring.pdf), and the level for each individual was calculated as a sum of MET-minute/week score and then classified as low and high by sex-specific total MET median. Educational attainment was categorized into three groups (0 to 9, 10 to 12, and $\geq$13 years of education). Family history of chronic diseases was positive if a parent or sibling had coronary heart disease, stroke, or type 2 diabetes.

After a home interview, all participants had a physical examination after overnight fasting. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, with the participants in light indoor clothing without shoes. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was obtained at the midpoint between the lowest rib and the iliac crest to the nearest 0.1 cm, after inhalation and exhalation. Blood pressure was measured by using an electronic blood pressure monitor (Omron HEM-705CP; OMRON Healthcare, Vernon Hills, IL) on the right arm of the participant in a comfortable sitting position after at least a 5-min rest. Three measurements were taken and the mean of the last two measurements was used for the analyses. Whole-body densitometry was conducted with the participant in light clothing and without carrying any metal objects by using a Hologic DXA (QDR-4500; Hologic, Waltham, MA).

Laboratory methods

Fasting peripheral venous EDTA blood samples were collected and centrifuged at 4°C and 3,000 rpm for 15 min. After being frozen, the samples were shipped in dry ice to the Institute for Nutritional Sciences and stored at −80°C until analyses. Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyltransferase (GGT) were measured enzymatically on an automatic analyzer (Hitachi 7080, Tokyo, Japan) with reagents purchased from Wako Pure Chemical Industries (Osaka, Japan). Plasma high-sensitivity (hs) C-reactive protein (CRP) was measured by a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Indianapolis, IN). A1C was quantified from resolved erythrocytes with an automated immunoassay (Roche Diagnostics). Insulin was measured with a completely homologous radioimmunoassay (Linco Research, St. Charles, MO), which had $<$0.2% cross-reactivity with proinsulin. The insulin resistance index (homeostasis model assessment of insulin resistance [HOMA-IR]) was calculated using updated homeostasis model assessment methods (http://www.dtu.ox.ac.uk/).

Plasma LBP levels were determined by a sandwich ELISA (USCN Life Science & Technology, Missouri City, TX). Plasma samples were diluted at least 200 times and assayed according to the manufacturer's instructions. The assay has a sensitivity of 0.2 ng/ml and a measurable concentration range of 0.78–50 ng/ml. The intra-assay and interassay coefficients of variation were $<$5 and $<$10%, respectively. Enzyme immunoassays were used to measure plasma interleukin (IL)-6, leptin (R&D Systems, Minneapolis, MN), and high-molecular-weight (HMW)-adiponectin (Millipore, St. Charles, MO). The interassay coefficients of variation were 9.6 and 6.5% for IL-6 at 0.49 and 5.65 pg/ml, 5.4 and 3.5% for leptin at 65.7 and 581 pg/ml, and 8.1 and 3.8% for HMW-adiponectin at 21.23 and 61.50 ng/ml, respectively. Hepatitis B surface antigen (HBsAg) was detected by a Murex HBsAg ELISA kit (Murex Biotech, Dartford, Kent, U.K.).

Definition of diseases

Metabolic syndrome was defined according to the updated National Cholesterol Education Program Adult Treatment Panel III criteria for Asian-Americans (16), including at least three of the following components: *1*) waist circumferences ≥90 cm in men or ≥80 cm in women, *2*) triglycerides ≥1.7 mmol/l, *3*) HDL cholesterol <1.03 mmol/l in men or <1.30 mmol/l in women, *4*) blood pressure ≥130/85 mmHg or current use of antihypertensive medications, and *5*) fasting plasma glucose ≥5.6 mmol/l. Type 2 diabetes was defined as fasting plasma glucose ≥7.0 mmol/l or 2-h postload plasma glucose ≥11.1 mmol/l during an oral glucose tolerance test. The selection procedure for the oral glucose tolerance test is described in supplementary Fig. A1 (available in an online appendix at http://care.diabetesjournals.org/cgi/content/full/dc10-0340/DC1). Elevated hepatic enzymes were defined as the highest quartiles of one or more hepatic enzymes: AST ≥30 IU/l, ALT ≥33 IU/l, or GGT ≥42 IU/l.

Statistical analyses

Log transformations were performed for LBP, triglycerides, insulin, HOMA-IR, inflammatory makers, adipokines, and hepatic enzymes to approximate normality. ANCOVA for continuous variables and logistic regression models for categorical variables were applied for the comparison across obesity status. Multiple comparison corrections were performed using the Benjamini and Hochberg procedure (17). Partial Spearman correlation coefficients between LBP and various parameters were calculated by adjustment for age, sex, and BMI. Individuals with presumably acute inflammation (hs-CRP >10 mg/l) were excluded ($n = 18$, 1.7%), leaving 1,041 subjects in the analysis. Of these, 942 subjects completed a DEXA scan.

Participants were classified into four groups according to their LBP quartiles in the whole sample. Multivariate logistic regression models were used to estimate the odds ratios (ORs) for metabolic syndrome, type 2 diabetes, and insulin resistance. Potential confounding variables include age, sex, lifestyle factors, education level, family history of chronic diseases, and BMI (or total body fat). In addition, we adjusted for inflammatory markers, adipokines, and elevated hepatic enzymes. Data management and statistical analyses were performed using Stata 9.2 (StataCorp, College Station, TX). Statistical tests were two-sided and $P < 0.05$ was considered statistically significant.

RESULTS

General characteristics

The prevalence of metabolic syndrome and newly defined type 2 diabetes was 42.0% ($n = 445$) and 13.8% ($n = 146$), respectively. The mean BMI values were 28.0 ± 2.7 kg/m^2 in overweight/obese subjects and 21.0 ± 1.4 kg/m^2 in normal-weight subjects ($P < 0.001$) (Table 1).

Compared with normal-weight subjects, overweight/obese individuals were more likely to have lower educational attainment and higher prevalence of metabolic syndrome and type 2 diabetes (all $P < 0.01$) (Table 1). They also had higher values for waist circumference, blood pressure, glucose, A1C, insulin, HOMA-IR, total cholesterol, LDL cholesterol, and triglycerides and lower HDL cholesterol concentration (all $P < 0.05$). Meanwhile, they exhibited higher levels of total body fat mass/percentage and trunk fat mass (all $P < 0.001$), hepatic enzymes (AST, ALT, and GGT), inflammatory markers (hs-CRP and IL-6) and leptin, accompanied by lower concentrations of HMW-adiponectin (all $P < 0.001$). Importantly, plasma LBP levels were significantly higher in overweight/obese participants than in normal-weight participants (geometric mean 27.6 [95% CI 25.2–30.3] vs. 10.0 [9.1–11.1] μg/ml; $P < 0.001$). In addition, LBP levels were higher in subjects with impaired fasting glucose than in those with normal fasting glucose (16.7 [15.0–18.6] vs. 13.8 [12.2–15.6] μg/ml; $P = 0.041$) and higher in subjects with impaired glucose tolerance than in those with normal glucose tolerance (31.2 [24.3–40.1] vs. 14.4 [12.8–16.3] μg/ml; $P < 0.001$), after adjustment for age and sex.

Correlation between LBP concentrations and metabolic parameters

LBP was positively correlated with BMI, waist circumference (all $P < 0.001$) (supplementary Table A1, available in an online appendix), blood pressure, total cholesterol, LDL cholesterol, triglycerides, glucose, insulin, HOMA-IR, hepatic enzymes, and leptin and negatively correlated with HDL cholesterol and HMW-adiponectin (all $P < 0.05$, data not shown), after adjustment for age and sex. Plasma LBP was also highly correlated with inflammatory markers (hs-CRP $r = 0.93$ and IL-6 $r = 0.50$, both $P < 0.001$) after adjustment for age and sex. These correlation coefficients were attenuated but remained statistically significant after further adjustment for BMI (supplementary Table A1). When the analyses were stratified by obesity status, significant correlations with the metabolic traits were more pronounced among overweight/obese subjects than among normal-weight individuals. In addition, LBP was strongly correlated with total body fat mass/percentage and trunk fat mass in sex-stratified correlation models (all $P < 0.001$) (data not show).

Associations of LBP concentrations with metabolic syndrome, type 2 diabetes, and insulin resistance

The risk for metabolic syndrome in the whole study sample increased progressively across the LBP quartiles ($P_{trend} < 0.001$) (Table 2) and those in the highest LBP quartile had an OR of 3.54 (95% CI 2.05–6.09) compared with those in the lowest quartile (model 2), after adjustment for age, sex, lifestyle factors, family history of chronic diseases, and BMI. Similar trends were also observed for the metabolic syndrome components. Further adjustment for inflammatory markers (model 3) abolished the significant associations for metabolic syndrome and most of its components except for hypertriglyceridemia and low HDL cholesterol.

For those with newly defined type 2 diabetes, the OR in the highest quartile was 5.53 (95% CI 2.64–11.59) compared with that in the lowest LBP quartile ($P_{trend} < 0.001$) in the multivariable model (Table 2, model 2). A positive association between LBP and insulin resistance, represented by the highest quartile of HOMA-IR, was also observed in nondiabetic participants (OR 1.90 [95% CI 1.10–3.28]). The ORs for diabetes and insulin resistance were slightly attenuated but remained statistically significant after further adjustment for hs-CRP and IL-6 (model 3).

The associations between LBP and metabolic disorders were not attenuated by additionally controlling for HMW-adiponectin, leptin, and elevated hepatic enzymes (as categorical or continuous variables) in model 2 (data not shown). Replacing BMI with total body fat mass in the multiple regression models also did not materially change the magnitude of the associations. The results remained

Table 1—*Characteristics of participants according to obese status*

	Normal weight ($18.0 \leq$ BMI <24.0 kg/m^2)	Overweight/obesity (BMI $\geq$24.0 kg/m^2)	*P* value*
n	500	559	
Age (years)†	45.8 ± 5.5	46.0 ± 5.4	0.604
BMI (kg/m^2)	21.0 ± 1.4	28.0 ± 2.7	<0.001‡
Men†	175 (35.0)	234 (41.9)	0.022‡
Physical inactivity	249 (49.8)	281 (50.3)	0.869
Education levels			0.002‡
0–9 years	114 (22.8)	175 (31.3)	
10–12 years	273 (54.6)	281 (50.3)	
>12 years	113 (22.6)	103 (18.4)	
Current smoker (yes)	117 (23.4)	158 (28.3)	0.767
Alcohol drinker (yes)	183 (36.6)	204 (36.5)	0.245
Family history of chronic diseases	200 (40.0)	222 (39.7)	0.939
Newly defined type 2 diabetes	36 (7.2)	110 (19.7)	<0.001‡
Metabolic syndrome	51 (10.2)	394 (70.5)	<0.001‡
Waist circumference (cm)	75.9 ± 6.1	93.2 ± 8.2	<0.001‡
Systolic blood pressure (mmHg)	118.4 ± 15.3	130.9 ± 17.6	<0.001‡
Diastolic blood pressure (mmHg)	74.7 ± 9.8	84.0 ± 11.6	<0.001‡
Glucose (mmol/l)	5.81 ± 1.13	6.30 ± 1.54	<0.001‡
A1C (%)	5.58 ± 0.62	5.79 ± 0.78	<0.001‡
Insulin (μU/ml)	7.3 (7.0−7.7)	11.3 (10.9−11.8)	<0.001‡
HOMA-IR	0.85 (0.82−0.89)	1.33 (1.27−1.38)	<0.001‡
Total cholesterol (mmol/l)	5.16 ± 1.14	5.33 ± 1.18	0.014‡
LDL cholesterol (mmol/l)	3.14 ± 0.93	3.41 ± 0.99	<0.001‡
HDL cholesterol (mmol/l)	1.53 ± 0.44	1.23 ± 0.34	<0.001‡
Triglycerides (mmol/l)	1.01 (0.96−1.06)	1.61 (1.53−1.69)	<0.001‡
DEXA scan	456 (91.2)	504 (90.2)	0.697
Total body fat mass (kg) (n = 960)			
Men	11.8 ± 3.3	20.7 ± 4.3	<0.001‡
Women	15.7 ± 2.8	25.7 ± 5.1	<0.001‡
Total body fat (%) (n = 960)			
Men	19.0 ± 4.3	25.6 ± 3.4	<0.001‡
Women	29.4 ± 3.8	36.1 ± 3.6	<0.001‡
Trunk fat mass (kg) (n = 960)			
Men	6.3 ± 2.1	12.1 ± 2.7	<0.001‡
Women	7.7 ± 1.8	13.7 ± 3.0	<0.001‡
HBsAg carriage	44 (8.8)	50 (8.9)	0.916
Elevated hepatic enzymes	141 (28.2)	294 (52.6)	<0.001‡
AST (IU/l)	23.6 (22.9–24.3)	26.3 (25.4–27.2)	<0.001‡
ALT (IU/l)	19.1 (18.2–20.0)	27.8 (26.5–29.2)	<0.001‡
GGT (IU/l)	22.5 (21.3–23.8)	33.1 (31.2–35.0)	<0.001‡
hs-CRP (mg/l)	0.61 (0.57–0.66)	1.38 (1.28–1.49)	<0.001‡
IL-6 (pg/ml)	1.19 (1.13–1.26)	1.67 (1.59–1.76)	<0.001‡
HMW-adiponectin (μg/ml)	3.16 (2.93–3.41)	1.88 (1.73–2.04)	<0.001‡
Leptin (ng/ml)	3.99 (3.70–4.31)	8.94 (8.40–9.51)	<0.001‡
LBP (μg/ml)	10.0 (9.1–11.1)	27.6 (25.2–30.3)	<0.001‡

Data are arithmetic mean ± SD, *n* (%), or geometric mean (95% CI). n = 1,059. Percentages may not sum to 100 because of rounding. *P value was calculated after adjustment for age and sex; P value for body fat comparison was not adjusted for sex. †Data not adjusted for itself. ‡Benjamini and Hochberg–corrected statistical significance (17).

similar after exclusion of HBsAg-positive subjects (n = 91, 8.7%) or subjects with $18.0 \leq$ BMI <18.5 kg/m^2 (n = 14, 1.3%) (data not shown).

We further conducted joint classification analyses to examine whether obesity, trunk fat mass, HMW-adiponectin, and elevated hepatic enzymes modified the associations of LBP with metabolic syndrome and type 2 diabetes (Fig. 1). No significant interactions were observed between LBP and these factors ($P > 0.05$ for all interaction tests).

CONCLUSIONS — Our data showed significant associations between elevated LBP concentrations and the risk for metabolic syndrome, insulin resistance, and type 2 diabetes independent of conventional cardiovascular risk factors in an apparently healthy Chinese population. Further adjustment for inflammatory factors, but not for adipokines and elevated hepatic enzymes, substantially attenuated the associations for metabolic syndrome and most of its components, suggesting that chronic inflammation may mediate the effects of innate immune response induced by LPS-LBP.

In a previous human study, Ghanim et al. (18) reported that increased plasma LPS and LBP and also expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells (MNCs) could be induced by consuming a meal with a high-fat, high-carbohydrate content, but not with isocaloric fruit and fiber, implying a potential role of the LPS-LBP pathway in postprandial inflammation and related metabolic disorders. In addition, positive correlations between LBP and metabolic traits such as BMI, diastolic blood pressure, fasting glucose, insulin, and triglycerides were observed in 60 men with glucose intolerance (13). Moreover, a higher LBP level was associated with increased prevalence of coronary artery disease independent of established cardiovascular risk factors in 247 male patients (12). With a relatively large sample size of apparently healthy men and women, our study provides more convincing evidence about the relationship between LBP and metabolic abnormalities.

In recent years, the effects of microbiota on health have attracted increasing attention, and low-grade endotoxemia or LPS was found to link to various metabolic consequences. However, most studies have been performed in mice and few in human populations. Studies in mice demonstrated that two- to threefold increased circulating LPS induced by a high-fat diet or LPS infusion led to increased levels of fasting glucose and insulin and body weight gain (4,5). The occurrences of a metabolic response could be counteracted by a CD14 mutant (5) or improved by changing gut microbiota (4). In humans, a high LPS concentration was found in individuals with type

Table 2—*ORs (95% CI) for metabolic syndrome, type 2 diabetes, and insulin resistance according to quartiles of LBP*

	Quartile of LBP				
	Q1 (LBP ≤6.5 μg/ml)	Q2 (6.5 < LBP ≤15.8 μg/ml)	Q3 (15.8 < LBP ≤42.0 μg/ml)	Q4 (LBP >42.0 μg/ml)	P_{trend}
Metabolic syndrome (n = 1,041)	35/260	91/260	132/261	176/260	
Model 1	1	3.45 (2.22–5.37)	6.25 (4.05–9.65)	12.90 (8.28–20.10)	<0.001
Model 2	1	2.78 (1.64–4.72)	3.04 (1.81–5.12)	3.54 (2.05–6.09)	<0.001
Model 3	1	2.56 (1.50–4.38)	2.38 (1.36–4.15)	1.80 (0.84–3.88)	0.053
Central obesity	59/260	110/260	164/261	204/260	
Model 1	1	2.50 (1.71–3.66)	5.92 (4.01–8.72)	12.80 (8.42–19.47)	<0.001
Model 2	1	1.64 (0.85–3.16)	2.54 (1.30–4.97)	2.53 (1.18–5.43)	0.005
Model 3	1	1.49 (0.77–2.90)	1.88 (0.90–3.92)	1.09 (0.35–3.35)	0.304
Elevated blood pressure	66/260	89/260	119/261	157/260	
Model 1	1	1.51 (1.02–2.23)	2.24 (1.53–3.28)	4.19 (2.85–6.15)	<0.001
Model 2	1	1.16 (0.76–1.76)	1.32 (0.87–2.00)	1.73 (1.11–2.69)	0.013
Model 3	1	1.09 (0.71–1.67)	1.08 (0.69–1.68)	0.94 (0.50–1.75)	0.982
Hypertriglyceridemia	25/260	70/260	102/261	130/260	
Model 1	1	3.61 (2.18–5.99)	5.82 (3.56–9.52)	9.34 (5.72–15.25)	<0.001
Model 2	1	3.11 (1.85–5.24)	4.05 (2.43–6.76)	4.98 (2.94–8.43)	<0.001
Model 3	1	3.04 (1.80–5.12)	3.77 (2.23–6.38)	4.10 (2.14–7.85)	<0.001
Low HDL cholesterol	65/260	82/260	116/261	118/260	
Model 1	1	1.43 (0.97–2.11)	2.64 (1.81–3.86)	2.77 (1.89–4.04)	<0.001
Model 2	1	1.17 (0.78–1.75)	1.72 (1.15–2.58)	1.39 (0.90–2.15)	0.056
Model 3	1	1.18 (0.78–1.76)	1.76 (1.15–2.68)	1.54 (0.87–2.72)	0.027
Hyperglycemia	140/260	157/260	174/261	188/260	
Model 1	1	1.28 (0.90–1.82)	1.64 (1.14–2.35)	2.14 (1.48–3.09)	<0.001
Model 2	1	1.18 (0.82–1.69)	1.34 (0.92–1.97)	1.49 (0.99–2.27)	0.047
Model 3	1	1.08 (0.75–1.56)	1.00 (0.66–1.52)	0.60 (0.32–1.13)	0.382
Type 2 diabetes (n = 1,041)	10/260	22/260	43/261	67/260	
Model 1	1	2.29 (1.06–4.95)	4.71 (2.30–9.61)	8.30 (4.15–16.59)	<0.001
Model 2	1	1.99 (0.91–4.33)	3.53 (1.69–7.37)	5.53 (2.64–11.59)	<0.001
Model 3	1	1.93 (0.88–4.21)	3.06 (1.45–6.46)	3.23 (1.38–7.58)	0.003
	Q1 (LBP ≤5.9 μg/ml)	Q2 (5.9 < LBP ≤12.7 μg/ml)	Q3 (12.7 < LBP ≤37.8 μg/ml)	Q4 (LBP >37.8 μg/ml)	P_{trend}
Insulin resistance (n = 899)	27/224	38/225	72/225	87/225	
Model 1	1	1.49 (0.87–2.54)	3.48 (2.13–5.70)	4.64 (2.85–7.55)	<0.001
Model 2	1	1.12 (0.64–1.98)	1.95 (1.14–3.32)	1.90 (1.10–3.28)	0.005
Model 3	1	1.14 (0.64–2.01)	1.97 (1.14–3.41)	1.95 (0.97–3.92)	0.014

Model 1: adjusted for age and sex. Model 2: further adjusted for smoking (current, yes or no), alcohol drinking (yes or no), physical activity (low or high by the sex-specific MET/week median), education (0–9, 10–12, and ≥13 years), family history of chronic diseases (yes or no), and BMI. Model 3: further adjusted for hsCRP and IL-6.

2 diabetes (6). The administration of rosiglitazone, a hypoglycemic agent with a potential anti-inflammatory effect (19), could reduce LPS levels (6). However, the short half-life of LPS (8) and the disadvantages associated with its assay (9) have limited its potential applications in routine clinical settings and large-scale studies. Having a relatively long half-life and reliable measurements, LBP might serve as a marker reflecting an "effective" LPS level and innate immune response triggered by LPS (11,12). Therefore, circulating LBP levels can be considered a promising early biomarker and intervention target for inflammatory conditions.

We observed a stronger correlation between LBP and inflammatory markers than that between LBP and adipokines after adjustment for BMI. Moreover, adjusting for hs-CRP and IL-6 almost eliminated the associations of LBP with metabolic syndrome and most of its traits (Table 2, model 3), whereas controlling for HMW-adiponectin and leptin had little impact on the associations. A potential mechanism is that LPS-LBP–triggered immune response activates the nuclear factor-κB pathway, stimulates formation of IL-6, IL-1, and tumor necrosis factor-α (7), and upregulates CRP synthesis in hepatocytes. However, it is noteworthy that low plasma HMW-adiponectin contributed additional risk for metabolic syndrome and type 2 diabetes under the high LBP condition (Fig. 1*E* and *F*). In fact, existing data showed that LPS increased plasma leptin levels and downregulated adiponectin receptor mRNA in healthy volunteers (20).

We also examined whether adjustment for hepatic enzymes altered the associations, because LBP is synthesized in liver and LPS could cause hepatocyte damage by inducing upregulation of tumor necrosis factor-α expression in Kupffer cells (7). Previous prospective studies also suggested that elevated he-

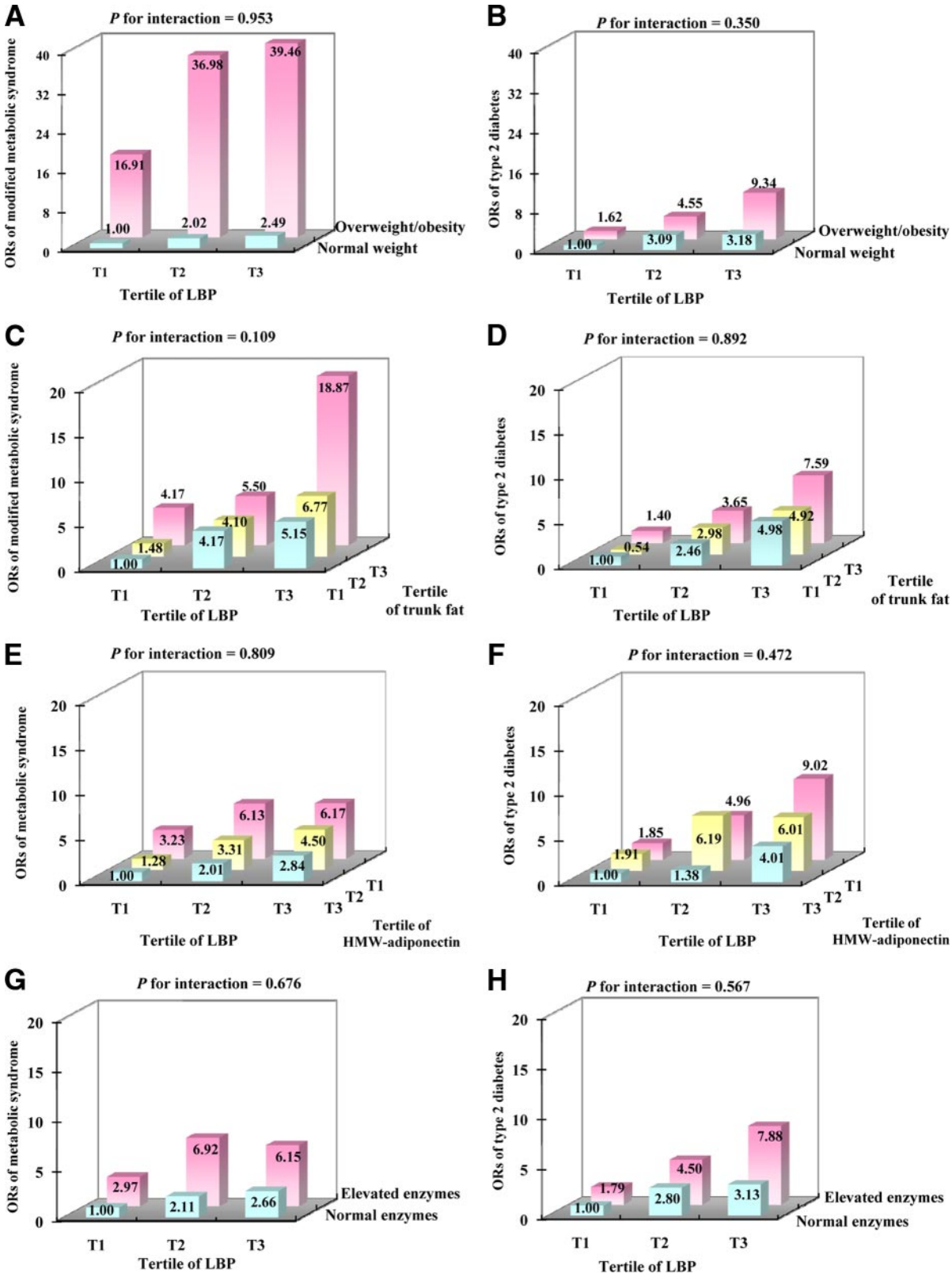

Figure 1—*ORs for metabolic disorders according to joint classification of LBP and obesity status* (A *and* B)*, trunk fat (sex and obesity-stratified tertile [T],* C *and* D)*, HMW-adiponectin* (E *and* F)*, and hepatic enzymes* (G *and* H). A–D*: Modified metabolic syndrome was defined as having two or more components of metabolic syndrome without central obesity. Adjusted for age, sex, smoking, alcohol drinking, physical activity, education, and family history of chronic diseases.* E–H*: Adjusted for age, sex, smoking, alcohol drinking, physical activity, education, family history of chronic diseases, and BMI.*

patic enzymes predicted risk of metabolic syndrome and type 2 diabetes (21). However, no material change was observed when we controlled for elevated hepatic enzymes or excluded individuals having hepatitis B infection. Collectively, our findings indicate that the inflammatory cascade initiated by LPS-LBP triggered an innate immune response, which is a major mechanism linking LBP to the pathogenesis of metabolic disorders.

Interestingly, unlike the case for metabolic syndrome risk, controlling for inflammatory markers in current study did not alter the significant associations of LBP with dyslipidemia, insulin resistance, and type 2 diabetes (Table 2). The minor effect of inflammation on the association between LBP and dyslipidemia might be explained by a sequence identity shared between LBP and lipid transfer proteins that could modify lipid homeostasis during subclinical endotoxemia (22). Previous studies demonstrated that hyperinsulinemia might affect immune competence, including functions of neutrophils (13) and hepatic Kupffer cells (23), which subsequently influenced LPS clearance and LBP synthesis. Nevertheless, endotoxemia might induce insulin resistance, whereas CD14 mutant mice showed hypersensitivity to insulin (5). MNCs were also suggested to be a target of insulin action and involved in the interaction between inflammation and insulin resistance (24). The increased suppressor of cytokine signaling-3 expression in MNCs, accompanied by increased LPS and LBP concentrations after a high-fat, high-carbohydrate meal, might also interfere with insulin signaling and play a role in insulin resistance (18,24). Our data suggested that LBP might exert its influence directly on insulin action in addition to stimulating a downstream pathway generating inflammatory cytokines. Moreover, the LBP gene was recently found to be genetically susceptible to type 2 diabetes in Japanese (25), which may provide one possible reason that the conventionally inflammatory cytokine showed little impact on the association between LBP and type 2 diabetes.

To our knowledge, this is the first study that systematically investigated the associations of LBP concentrations with the risk of obesity-related metabolic disorders. Admittedly, the cross-sectional nature of this study does not allow for a causal inference. Nonetheless, we tried to eliminate potential effects of acute inflammation and other potential confounders by applying strict exclusion criteria and also included a relatively large sample size with both sexes. Certainly, these results should be examined prospectively and in different populations to establish the causal relationship between LBP, inflammation, and metabolic outcomes.

Our study indicates that LBP is significantly associated with obesity-related metabolic disorders in apparently healthy Chinese. These findings indicate the role of LPS-initiated innate immune mechanisms in metabolic diseases. Future prospective studies are needed to clarify whether LBP predicts future risk of metabolic disorders.

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L.S. contributed to the study design, researched data, contributed to discussion, and wrote the manuscript. Z.Y. contributed to the study design, researched data, contributed to discussion, and reviewed/edited the manuscript. X.Y., S.Z., and H.L. contributed to the study design, researched data, and reviewed/edited the manuscript. D.Y. and H.W. researched data and reviewed/edited the manuscript. Y.C. contributed to the study design and reviewed/edited the manuscript. J.D. reviewed/edited the manuscript. K.C. wrote the manuscript. F.B.H. and X.L. contributed to the conception and design of the study, contributed to discussion, reviewed/edited the manuscript.

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References

1. World Health Organization. Obesity and overweight: Fact Sheet No. 311 [article online], 2006. Available from http://www.who.int/mediacentre/factsheets/fs311/en/print.html/. Accessed 26 October 2007
2. Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 2005;111:1448–1454
3. Wiedermann CJ, Kiechl S, Dunzendorfer S, Schratzberger P, Egger G, Oberhollenzer F, Willeit J. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. J Am Coll Cardiol 1999;34:1975–1981
4. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008;57:1470–1481
5. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–1772
6. Creely SJ, McTernan PG, Kusminski CM, Fisher M, Da Silva NF, Khanolkar M, Evans M, Harte AL, Kumar S. Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Physiol Endocrinol Metab 2007;292:E740–E747
7. Siebler J, Galle PR, Weber MM. The gut-liver-axis: endotoxemia, inflammation, insulin resistance and NASH. J Hepatol 2008;48:1032–1034
8. Munford RS. Detoxifying endotoxin: time, place and person. J Endotoxin Res 2005;11:69–84
9. Novitsky TJ. Limitations of the *Limulus* amebocyte lysate test in demonstrating circulating lipopolysaccharides. Ann NY Acad Sci 1998;851:416–421
10. Weiss J. Bactericidal/permeability-increasing protein (BPI) and lipopolysaccharide-binding protein (LBP): structure, function and regulation in host defence against Gram-negative bacteria. Biochem Soc Trans 2003;31:785–790
11. Ruiz AG, Casafont F, Crespo J, Cayón A, Mayorga M, Estebanez A, Fernadez-Escalante JC, Pons-Romero F. Lipopolysaccharide-binding protein plasma levels and liver TNF-α gene expression in obese patients: evidence for the potential role of endotoxin in the pathogenesis of non-alcoholic steatohepatitis. Obes Surg 2007;17:1374–1380
12. Lepper PM, Schumann C, Triantafilou K, Rasche FM, Schuster T, Frank H, Schneider EM, Triantafilou M, von Eynatten M. Asso-

ciation of lipopolysaccharide-binding protein and coronary artery disease in men. J Am Coll Cardiol 2007;50:25–31
13. Gubern C, López-Bermejo A, Biarnés J, Vendrell J, Ricart W, Fernández-Real JM. Natural antibiotics and insulin sensitivity: the role of bactericidal/permeability-increasing protein. Diabetes 2006; 55:216–224
14. Li M, Wang B, Zhang M, Rantalainen M, Wang S, Zhou H, Zhang Y, Shen J, Pang X, Zhang M, Wei H, Chen Y, Lu H, Zuo J, Su M, Qiu Y, Jia W, Xiao C, Smith LM, Yang S, Holmes E, Tang H, Zhao G, Nicholson JK, Li L, Zhao L. Symbiotic gut microbes modulate human metabolic phenotypes. Proc Natl Acad Sci USA 2008;105:2117–2122
15. Wu Y. Overweight and obesity in China. BMJ 2006;333:362–363
16. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005;112:e285–e290
17. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res 2001;125:279–284
18. Ghanim H, Abuaysheh S, Sia CL, Korzeniewski K, Chaudhuri A, Fernandez-Real JM, Dandona P. Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. Diabetes Care 2009;32:2281–2287
19. Mohanty P, Aljada A, Ghanim H, Hofmeyer D, Tripathy D, Syed T, Al-Haddad W, Dhindsa S, Dandona P. Evidence for a potent antiinflammatory effect of rosiglitazone. J Clin Endocrinol Metab 2004;89:2728–2735
20. Anderson PD, Mehta NN, Wolfe ML, Hinkle CC, Pruscino L, Comiskey LL, Tabita-Martinez J, Sellers KF, Rickels MR, Ahima RS, Reilly MP. Innate immunity modulates adipokines in humans. J Clin Endocrinol Metab 2007;92:2272–2279
21. Nannipieri M, Gonzales C, Baldi S, Posadas R, Williams K, Haffner SM, Stern MP, Ferrannini E. Liver enzymes, the metabolic syndrome, and incident diabetes: the Mexico City Diabetes study. Diabetes Care 2005;28:1757–1762
22. Hudgins LC, Parker TS, Levine DM, Gordon BR, Saal SD, Jiang XC, Seidman CE, Tremaroli JD, Lai J, Rubin AL. A single intravenous dose of endotoxin rapidly alters serum lipoproteins and lipid transfer proteins in normal volunteers. J Lipid Res 2003;44:1489–1498
23. Cornell RP, McClellan CC. Modulation of hepatic reticuloendothelial system phagocytosis by pancreatic hormones. J Reticuloendothel Soc 1982;32:397–407
24. Ghanim H, Aljada A, Daoud N, Deopurkar R, Chaudhuri A, Dandona P. Role of inflammatory mediators in the suppression of insulin receptor phosphorylation in circulating mononuclear cells of obese subjects. Diabetologia 2007;50: 278–285
25. Takeuchi F, Yanai K, Inomata H, Kuzuya N, Kajio H, Honjo S, Takeda N, Kaburagi Y, Yasuda K, Shirasawa S, Sasazuki T, Kato N. Search of type 2 diabetes susceptibility gene on chromosome 20q. Biochem Biophys Res Commun 2007;357: 1100–1106