Association of Lipopolysaccharide-Binding Protein With Aging-Related Adiposity Change and Prediabetes Among African Ancestry Men

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OBJECTIVE

Cross-sectional studies suggest that lipopolysaccharide-binding protein (LBP) may be associated with obesity and metabolic disorders. However, prospective studies examining LBP are lacking. This prospective study investigated the association between LBP and metabolic abnormalities in 580 African ancestry men (mean age, 59.1 ± 10.5 years).

RESEARCH DESIGN AND METHODS

We measured fasting serum LBP at baseline. Changes in adiposity and glucose homeostasis as well as case subjects with new type 2 diabetes and impaired fasting glucose (IFG) were assessed at a follow-up visit ~6 years later. Baseline LBP values were tested across quartiles for linear trend with metabolic measures. Multivariable logistic regression was used to determine the odds of new cases of IFG or diabetes per 1-SD greater baseline LBP.

RESULTS

LBP was significantly associated with baseline BMI, waist circumference, whole-body and trunk fat, skeletal muscle density, fasting serum insulin, and HOMA-insulin resistance (IR) (all $P < 0.01$). Greater baseline LBP was significantly associated with longitudinal increases in the percentage of trunk fat ($P = 0.025$) and HOMA-IR ($P = 0.034$), but only borderline so with a decrease in skeletal muscle density ($P = 0.057$). In men with normal glucose, baseline LBP was associated with increased odds of having IFG at follow-up after adjustment for age, baseline trunk fat, and lifestyle factors (odds ratio per 1-SD LBP: 1.51; 95% CI 1.02–2.21). This association was attenuated after additional adjustment for change in trunk fat ($P = 0.067$).

CONCLUSIONS

LBP may be a marker of prediabetes. Some of this association appears to be mediated through increased central and ectopic skeletal muscle adiposity.

Diabetes and obesity are associated with low-level, chronic inflammation (1). In recent years, the gut microbiota have come to be recognized as a contributor to this inflammation (2,3). Gram-negative bacteria contain lipopolysaccharide (LPS) in their outer membranes (4), and through their life cycles the bacteria can shed LPS

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into the circulation (5). LPS in the circulation can initiate an immune response and promote the release of inflammatory cytokines (4,5). The gut microbiota can therefore be a generator of LPS and a potential contributor to low-level inflammation.

LPS-binding protein (LBP) is produced primarily by the liver and helps mediate the LPS-induced inflammatory response (5,6). When LBP is found at low levels in the serum, it binds LPS and forms complexes with CD14, which then associates with Toll-like receptor 4 (TLR4) on the macrophage and initiates the inflammatory cytokine response (7). However, when found at higher levels, LBP helps attenuate the immune response by transferring LPS to lipoproteins for clearance (8,9).

LBP levels are greater in the presence of cytokines such as interleukin (IL)-6 and IL-1 (5), and although LBP is produced at constitutively low levels during normal physiological states, levels rise rapidly during infection (5).There are several difficulties in accurately measuring LPS in serum using the *Limulus* amebocyte lysate test, including potential interference by LPS inhibitors (10). LBP is strongly correlated with LPS levels ($r \geq 0.6$) in human serum (11) and increases in response to greater LPS in mouse models (12). Therefore, owing to its crucial role in modulating the immune response and its known correlation with LPS, LBP is generally considered to a reasonable surrogate biomarker for assessing LPS-induced inflammation in humans (13,14).

LBP levels are higher among individuals who are obese, have diabetes, or who have metabolic syndrome or glucose intolerance (13–18). Although cross-sectional studies have shown that LBP levels are associated with anthropometric and metabolic measurements (11,15,18), few longitudinal studies have investigated LBP in relation to obesity- and diabetes-related measures (13). To our best knowledge, no longitudinal studies have been conducted among African ancestry men, a population group disproportionally affected by type 2 diabetes (19,20), and thus, in particular, there is need for such study. In the current study, we tested whether baseline LBP is associated with changes in overall, central, and skeletal muscle adiposity, glucose homeostasis, and new cases of prediabetes and type 2 diabetes in a cohort of middle-aged and elderly African ancestry men.

RESEARCH DESIGN AND METHODS

Study Population

Between 1997 and 2003, 3,170 previously unscreened men were recruited for a population-based prostate cancer screening study on the Caribbean island of Tobago, Trinidad and Tobago (21). To be eligible, men had to be ambulatory, noninstitutionalized, and not terminally ill. Recruitment for the survey was accomplished by flyers, public service announcements, and posters, informing health care workers at local hospital and health centers, and word of mouth. Approximately 60% of all age-eligible men on the island participated, and participation was similar across the island parishes. All men were invited to participate in a follow-up clinic examination between 2004 and 2007, and 2,031 men (70% of survivors) and 451 new participants completed the visit. Men were invited to complete a dual-energy X-ray absorptiometry (DXA) whole-body scan and a peripheral quantitative computed tomography (pQCT) scan of the lower leg. This visit represented the baseline for the current study. Between 2010 and 2013, we invited these men to return for repeat clinical examinations and DXA and pQCT scans. The baseline and follow-up visits followed the same procedures for questionnaire interviews, biospecimen collection, and DXA and pQCT scans. A total of 1,611 men completed the follow-up assessment (82% of survivors). On the basis of power calculations (see STATISTICAL ANALYSES below), we randomly selected 580 of these men for the current study of LBP. The Institutional Review Boards of the University of Pittsburgh and the Tobago Ministry of Health and Social Services approved this study. All participants provided written informed consent before data collection.

pQCT Scan

A pQCT scan of the calf was performed using the Stratec XCT-2000 to evaluate skeletal muscle fat and muscle cross-sectional areas. Scans were obtained at 66% of the calf length, proximal to the terminal end of the calf. This site was chosen because it is the region of the lower leg with the largest circumference with very little variability across individuals (22). Different tissues in the anayses were separated according to different density thresholds, using the "soft tissue" algorithm. On the basis of this calibration, fat, muscle, and cortical bone are measured with mineral equivalent densities of 0, 80, and 1,200 mg/cm^3, respectively. Therefore, changes in muscle tissue to fat-like tissue will be detected as a shift in mineral equivalent density of the muscle from 80 to 0 mg/cm^3. Images of the cross-sectional area of skeletal muscle and fat were analyzed using Stratec 5.5D analysis software (Orthometrix, Inc., White Plains, NY). To maintain consistency, all images were analyzed by a single investigator. We assessed intramuscular fat using measures of calf muscle density (mg/cm^3). Muscle density is a valid measure of fat accumulation within the skeletal muscle and reflects the fat content such that greater intramuscular fat is associated with lower muscle density (23).

DXA Absorptiometry Measures

DXA measurement of total body and trunk fat was made using a Hologic QDR 4500 W densitometer (Hologic Inc., Bedford, MA). Scans were analyzed with QDR 8.26a software.

Anthropometric Measurements

Standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body weight was recorded to the nearest 0.1 kg without shoes on a balance beam scale. BMI was calculated from body weight and standing height (kg/m^2). Waist circumference was measured at the narrowest point of the waist using an inelastic fiberglass tape. If there was no narrowest point, waist circumference was measured at the umbilicus.

Inflammation and Metabolic Variables

All biochemical assays in fasting serum samples were performed in the Heinz Nutrition Laboratory at the University of Pittsburgh. Fasting serum glucose was measured using an enzymatic procedure; the coefficient of variation percentage (CV%) between runs was 1.8%. Insulin was measured using a radioimmunoassay procedure developed by Linco Research, Inc.; the CV% between runs was 2.1%. The degree of insulin resistance (IR) was estimated by HOMA according to the method described by

Matthews et al. (24). In previous studies, HOMA-IR has correlated reasonably well with insulin clamp techniques (25). Baseline fasting serum LBP was measured using a Human LBP ELISA kit (Cell Sciences, Canton, MA) according to the manufacturer's protocol. Manufacturer reported inter- and intra-assay CV% were 9.8–17.8% and 6.1%, respectively.

Other Measures

Information on lifestyle habits (current smoking [yes/no], walking more than twice in the past week [yes/no], watching 14 or more hours of television per week [yes/no], and current intake of alcohol of more than 3 drinks per week [yes/no]), history of medical conditions, and medication use were assessed using standardized interviewer-administered questionnaires. Men were asked to bring all prescription medications taken in the past 30 days to their clinic visit. Participants also rated their overall health status compared with men their own age. Type 2 diabetes was defined as currently taking an antidiabetic medication, regardless of fasting serum glucose level, or having a fasting serum glucose of $\geq$126 mg/dL. Impaired fasting glucose (IFG), also known as prediabetes, was defined as a fasting serum glucose level of 100–125 mg/dL without being on any antidiabetic medication. Hypertension was defined as a systolic blood pressure of $\geq$140 mmHg and/or diastolic blood pressure of $\geq$90 mmHg and/or currently taking antihypertensive medication. High triglycerides was defined as a fasting serum triglycerides $\geq$150 mg/dL. Low HDL-cholesterol (HDL-c) was defined as a fasting serum HDL-c $<$40 mg/dL.

Statistical Analyses

We estimated that to have the power to detect a univariate correlation of 0.12 (β = 0.8, α = 0.05) between LBP and changes in adiposity or glucose homeostasis, we needed to assay at least 500 samples. Owing to assay requirements and formatting, we measured LBP in a final number of 580 men. We then categorized baseline serum LBP into quartiles (n = 145 each) and tested its association with baseline cross-sectional and longitudinal changes in metabolic measures using a test of linear trend. Cross-sectional models were first adjusted for baseline age and then were additionally adjusted for smoking, walking, history of cancer, perceived health status, and alcohol intake. Longitudinal models were first adjusted for baseline age and baseline metabolic measures and then additionally adjusted for smoking, walking, history of cancer, perceived health status, alcohol intake, and low HDL-c status. We used multivariable logistic regression to test for an association of LBP with new cases of IFG or type 2 diabetes identified at follow-up. Models for IFG were run in only those with normal glucose at baseline, whereas models for type 2 diabetes were run in individuals without diabetes at baseline only. Odds ratios were expressed per 1-SD greater serum LBP. Statistical significance was based on an α = 0.05, and analyses were performed using SAS 9.3 software (SAS Institute, Inc., Cary, NC).

RESULTS

General Baseline Characteristics

Baseline characteristics for the 580 African ancestry men overall and according to quartiles of LBP are reported in Table 1. The men were an average $\pm$ SD age of 59.1 $\pm$ 10.5 years. Only 8% of men were current smokers, 9% had moderate alcohol intake, 38% watched more than 14 h of television in a week, and 88% typically walked for exercise. Approximately 5% of men had a history of cancer, and 6% had a history of any cardiovascular disease. Prevalence of IFG, type 2 diabetes, high triglycerides, low HDL-c, and hypertension were high, at 23%, 21%, 19%, 22%, and 53%, respectively. Greater LBP quartile was associated with greater baseline age, having diabetes, low HDL-c status, and taking antidiabetic medication ($P < 0.05$ for all). However, having a history of cancer decreased with increasing quartiles of LBP (P = 0.04).

Association of LBP with Adiposity and Metabolic Measurements at Baseline and Follow-up

Average follow-up time was 6.0 years (range 4.6–8.5). Greater baseline LBP was associated with greater baseline BMI, waist circumference, whole-body and trunk fat percentage, and fasting insulin, and HOMA-IR and inversely associated with skeletal muscle density independent of age and other covariates including smoking, walking, history of cancer, health status, alcohol intake, and low HDL-c status (all $P < 0.05$) (Table 2).

Baseline LBP was associated with an increase in trunk fat (P = 0.025) and HOMA-IR (P = 0.034) at follow-up after adjustment for all significant covariates (Table 3). LBP was also associated with a decrease in calf skeletal muscle density in minimally adjusted models (P = 0.048) (Table 3). However, the association was attenuated after additional adjustment for smoking, walking, history of cancer, health status, alcohol intake, and low HDL-c status (P = 0.057).

Associations of LBP with New Cases of IFG and Type 2 Diabetes

At the follow-up visit, 13.5% of normoglycemic men from baseline had developed IFG and 8.2% of men free of diabetes at baseline had developed type 2 diabetes (Table 4). Baseline LBP levels were positively associated with new cases of IFG, independent of age, trunk fat percentage, physical activity, health status, and low HDL-c status. Each 1-SD greater baseline LBP was associated with an $\sim$51% increased risk of IFG (95% CI 1.02–2.21). The association was slightly attenuated and of borderline significance after accounting for change in trunk fat (P = 0.067), BMI (P = 0.046), or skeletal muscle density (P = 0.052). Baseline LBP was not significantly associated with new cases of diabetes.

CONCLUSIONS

To our knowledge, our study is the first to examine LBP and longitudinal changes in adiposity, glucose homeostasis, and diabetes risk in a population of African ancestry men, who have a high risk of developing type 2 diabetes. We found that greater LBP levels are associated with increasing central and skeletal muscle adiposity and IR as well as with an increased risk for developing IFG. Adjusting for changes in adiposity attenuated the association between LBP and IFG, suggesting that increased adiposity may play a causal role in the LBP association. These data provide further evidence for a potential link between LBP and age-associated increases in adiposity and impaired glucose metabolism.

Chronic inflammation is believed to be a risk factor for obesity and IR (26,27). LPS is derived from the outer membrane of gram-negative bacteria (4,5), and although low levels of LPS can be found in the circulation of healthy individuals, higher levels can produce inflammatory responses (5). LBP is expressed at constitutively low

Table 1—General characteristics of 580 African ancestry men overall and by quartile of LBP

Trait	Overall	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* value*
LBP (μg/mL)	22.3 (8.4)	12.6 (3.8–16.8)	19.2 (16.8–21.3)	23.9 (21.3–27.1)	33.4 (27.2–57.4)	N/A
Age (years)	59.1 (10.5)	57.2 (10.6)	59.5 (10.2)	59.2 (10.1)	60.4 (10.9)	0.0188
Lifestyle (%)						
Current smoking	7.6	4.9	10.3	6.2	8.3	0.5233
Watch ≥14 h television/week	37.8	37.5	38.2	41.7	34.3	0.7332
Walk ≥3×/week	87.6	88.9	91.7	85.5	84.0	0.0920
Drink ≥4 drinks/week	8.8	6.3	9.7	9.7	9.0	0.4272
Comorbidities (%)						
Any cancer	4.8	6.9	5.5	5.5	1.4	0.0408
Any cardiovascular disease	5.9	6.3	3.5	5.5	8.3	0.3435
Hypertension	52.6	46.5	54.5	57.2	52.1	0.2965
High triglycerides	19.0	17.9	16.1	20.0	22.3	0.2458
Low HDL-c	21.9	18.6	19.6	20.0	29.5	0.0343
Good/excellent health	93.1	93.4	95.9	93.1	89.5	0.1054
IFG	23.1	27.8	20.0	22.8	21.5	0.3087
Diabetes	20.9	18.8	15.9	20.0	29.2	0.0201
Medications (%)						
Antidiabetic	14.3	11.1	12.4	13.8	20.1	0.0304
Antihypertensive	25.2	20.8	25.5	24.8	29.2	0.1333

Values are presented as mean (range), mean (SD), or as indicated. N/A, not applicable. **P* value was determined using a test for linear trend across quartiles of LBP.

levels and increases in the presence of inflammatory cytokines such as IL-6 and IL-1 (5). LBP functions by binding to LPS, which accelerates LPS binding to CD14 and subsequent presentation to macrophage TLR4 receptors (7). Importantly, Cani et al. (28) showed that chronic infusion of LPS caused weight gain in mice similar to that of a high-fat diet, thereby linking metabolic LPS from the gut microbiome to systemic inflammation and weight gain.

Table 2—Association of LBP with baseline anthropomorphic and metabolic measures in African ancestry men

		LBP quartile (μg/mL)				
Anthropometric and metabolic measures	Overall Mean (SD)	12.6 (3.8–16.8)	19.2 (16.8–21.3)	23.9 (21.3–27.1)	33.4 (27.2–57.4)	*P* value for trend
BMI (kg/m^2)						
Age adjusted	26.2 (4.8)	25.5 (0.4)	25.5 (0.4)	26.3 (0.4)	27.3 (0.4)	0.0001
Multivariable adjusted*		25.6 (0.4)	25.7 (0.4)	26.2 (0.4)	27.2 (0.4)	0.0015
Waist circumference (cm)						
Age adjusted	92.4 (10.8)	91.2 (0.9)	90.4 (0.9)	92.5 (0.9)	95.7 (0.9)	0.0001
Multivariable adjusted*		91.5 (0.9)	90.7 (0.9)	92.3(0.9)	94.8 (0.9)	0.0043
Whole-body fat (%)						
Age adjusted	20.5 (5.5)	19.6 (0.4)	19.7 (0.4)	20.5 (0.4)	22.3 (0.4)	0.0001
Multivariable adjusted*		19.7 (0.4)	19.9 (0.4)	20.5 (0.4)	22.0 (0.4)	0.0002
Amount of whole-body fat in trunk (%)						
Age adjusted	48.6 (5.3)	48.2 (0.4)	47.5 (0.4)	49.3 (0.4)	49.6 (0.4)	0.0034
Multivariable adjusted*		48.2 (0.4)	47.6 (0.4)	49.3 (0.4)	49.3 (0.4)	0.0146
Calf muscle density (mg/cm^3)						
Age adjusted	73.5 (4.3)	74.2 (0.3)	74.1 (0.3)	73.5 (0.3)	72.3 (0.3)	0.0001
Multivariable adjusted*		74.1 (0.3)	74.0 (0.3)	73.6 (0.3)	72.5 (0.3)	0.0008
Glucose† (mg/dL)						
Age adjusted	92.2 (12.0)	92.4 (1.1)	91.5 (1.1)	91.4 (1.1)	93.7 (1.2)	0.5031
Multivariable adjusted*		92.2 (1.1)	91.5 (1.1)	91.3 (1.2)	93.3 (1.2)	0.5508
Insulin† (mg/dL)						
Age adjusted	12.2 (6.5)	11.0 (0.6)	11.8 (0.6)	12.6 (0.6)	13.7 (0.6)	0.0012
Multivariable adjusted*		10.8 (0.6)	12.0 (0.6)	12.6 (0.6)	13.1 (0.6)	0.0063
HOMA-IR†						
Age adjusted	2.8 (1.6)	2.5 (0.1)	2.7 (0.1)	2.9 (0.1)	3.1 (0.2)	0.0017
Multivariable adjusted*		2.5 (0.1)	2.8 (0.1)	2.9 (0.1)	3.0 (0.2)	0.0076

Baseline values are presented as mean (SE). *Multivariable models include adjustment for baseline age, smoking, walking, history of cancer, health status, alcohol intake, and low HDL-c status. †Metabolic factors are reported only in men who were nondiabetic at baseline (n = 457).

Table 3—Association of LBP with absolute changes in anthropomorphic and metabolic measures in African ancestry men

Change in anthropometric and metabolic measures	Overall Mean (SD)	LBP quartile (μg/mL) 12.6 (3.8–16.8)	19.2 (16.8–21.3)	23.9 (21.3–27.1)	33.4 (27.2–57.4)	*P* value for trend
Change in BMI (kg/m^2)						
Baseline age and BMI adjusted	−0.1 (2.3)	−0.3 (0.2)	−0.2 (0.2)	−0.2 (0.2)	0.2 (0.2)	0.0969
Multivariable adjusted*		−0.2 (0.2)	−0.2 (0.2)	−0.2 (0.2)	0.1 (0.2)	0.2465
Change in waist circumference (cm)						
Baseline age and waist adjusted	4.6 (6.8)	4.0 (0.6)	4.5 (0.6)	4.5 (0.6)	5.5 (0.6)	0.0771
Multivariable adjusted*		4.0 (0.6)	4.5 (0.6)	4.4 (0.6)	5.4 (0.6)	0.1211
Change in whole-body fat (%)						
Baseline age and whole-body fat adjusted	2.8 (3.1)	2.6 (0.3)	2.9 (0.3)	2.5 (0.3)	3.1 (0.3)	0.3764
Multivariable adjusted*		2.7 (0.3)	2.8 (0.3)	2.4 (0.3)	3.1 (0.3)	0.5218
Change in trunk fat (%)						
Baseline age and trunk fat adjusted	1.4 (2.9)	1.2 (0.2)	1.1 (0.2)	1.3 (0.2)	2.1 (0.2)	0.0039
Multivariable adjusted*		1.2 (0.2)	1.1 (0.2)	1.3 (0.2)	2.0 (0.2)	0.0251
Change in calf muscle density (mg/cm^3)						
Baseline age and muscle density adjusted	−2.5 (3.2)	−2.3 (0.3)	−2.2 (0.3)	−2.4 (0.3)	−3.1 (0.3)	0.0479
Multivariable adjusted*		−2.2 (0.3)	−2.2 (0.3)	−2.4 (0.3)	−3.0 (0.3)	0.0574
Change in fasting serum glucose† (mg/dL)						
Baseline age and glucose adjusted	1.8 (19.2)	1.0 (1.8)	0.2 (1.8)	2.5 (1.8)	3.7 (2.0)	0.2120
Multivariable adjusted*		2.0(2.5)	−2.7 (2.5)	−2.9 (2.5)	3.6 (2.6)	0.2998
Change in fasting serum insulin† (mg/dL)						
Baseline age and insulin adjusted	2.7 (9.0)	1.6 (0.9)	2.6 (0.8)	2.4 (0.9)	4.2 (0.9)	0.0530
Multivariable adjusted*		1.3 (0.8)	2.5 (0.8)	2.3 (0.8)	4.0(0.9)	0.0581
Change in HOMA-IR†						
Baseline age and HOMA-IR adjusted	0.8 (3.1)	0.4 (0.3)	0.7 (0.3)	0.9 (0.3)	1.4 (0.3)	0.0287
Multivariable adjusted*		0.3 (0.3)	0.6 (0.3)	0.7 (0.3)	1.4 (0.3)	0.0338

Absolute changes in values are presented as mean (SE). *Multivariable models include adjustment for appropriate baseline metabolic trait, and baseline age, smoking, walking, history of cancer, health status, alcohol intake, and low HDL-c status. †Metabolic factors reported only in men with follow-up data who were nondiabetic at baseline (*n* = 426).

Owing to limitations in accurately measuring LPS levels in the serum (10), not many studies have directly correlated LBP to LPS levels in the serum of humans. However, a single study by Moreno-Navarrete et al. (11) showed that LBP and LPS levels were strongly correlated with coefficients ≥0.6. In addition, LBP has been widely suggested to be a potential marker of gut-derived LPS and consequent LPS-induced inflammation (13–15,17,18). Therefore, most current human research infers that LBP variation is both correlated and caused by variation in LPS. However, it is also possible that variations in LBP in our study may be due to variations in cytokines or an acute-phase reaction unrelated to LPS (29).

Our findings that LBP was significantly and positively associated with baseline obesity measures are in line with other findings in the literature (11–13,16–18). A longitudinal study among 2,529 Chinese individuals found that higher baseline levels of LBP were correlated with an increased number of metabolic syndrome components (13). In contrast, baseline LBP measurements were not related to changes in BMI, waist circumference, whole-body fat, or fasting insulin levels in our sample. However, we found that LBP was associated with an increase in trunk fat over an average of 6 years' follow-up. Our findings raise the possibility that LPS may be more strongly associated with central adiposity, which is a stronger risk factor for type 2 diabetes, than overall adiposity (30). An association of LBP with adiposity is biologically plausible because LBP, which is primarily produced by hepatocytes, is also produced by adipocytes in response to local proinflammatory cytokines (12).

We also found that LBP was positively associated with HOMA-IR at baseline and during follow-up in individuals

Table 4—Association of baseline serum LBP with new cases of impaired fasting glucose and diabetes in African ancestry men

Model	New cases of IFG in non-IFG subjects at baseline		New cases of diabetes in subjects without diabetes at baseline	
	(*N* IFG/non-IFG: 43/275)	*P* value	(*N* diabetic/nondiabetic: 35/393)	*P* value
1. Age	1.50 (1.04–2.17)	0.0300	1.19 (0.81–1.73)	0.3753
2. Age + walking + health status + low HDL-c status	1.52 (1.03–2. 22)	0.0332	1. 22 (0.83–1.79)	0.3234
3. Model 2 + trunk fat	1.51 (1.02–2.21)	0.0376	1.16 (0.79–1.70)	0.4620
4. Model 3 + change in trunk fat	1.44 (0.98–2.12)	0.0668	1.16 (0.79–1.71)	0.4514

Odds ratios (95% CIs) are shown for 1-SD greater LBP.

without diabetes and positively associated with insulin at baseline, although this association was not confirmed by changes in insulin during follow-up. One previous study found that higher levels of serum LBP correlate with HOMA-IR (17), while another found that an association with HOMA-IR may depend on obesity status (16).

We also found, for the first time, that greater LBP levels are associated with a lower skeletal muscle density and its decrease with aging, indicative of an increase in ectopic skeletal muscle adiposity. This finding is in line with other previous data, in particular, a study using mouse models, which showed that LPS injections can lead to changes in muscle quality (31). Ectopic skeletal muscle adiposity is greater among African ancestry individuals than Caucasians (32–36) and has been shown to be an important risk factor for type 2 diabetes (37,38). Whether LBP may partly explain ethnic/racial differences in skeletal muscle adiposity is unclear. Our findings will need to be confirmed in other populations, including those of African ancestry.

In our sample, greater LBP was associated with an increase in IR and increased odds of newly generating an IFG over 6 years of follow-up. However, LBP was not significantly associated with new cases of type 2 diabetes. TLR4 activation on insulin target cells by LPS can lead to activation of the Jun N-terminal kinase and inhibitor of κB kinase pathways, both of which can inhibit insulin's action by blocking phosphorylation of insulin receptor substrates proteins and increasing insulin receptor substrates degradation. Furthermore, the Jun N-terminal kinase/inhibitor of κB kinase pathways lead to activation of nuclear factor-κB, which increases production of proinflammatory cytokines and further inhibits insulin signaling (1). Skeletal muscle TLR4 expression is known to be higher in individuals who are obese or who have type 2 diabetes and to correlate with IR (39). LBP is a marker of circulating LPS and facilitates the binding of LPS to TLR4; thus, LBP may be an important biomarker for predicting the development of IR due to LPS-induced inflammation, and therefore, prediabetes. Lack of a significant association with new cases of type 2 diabetes in our population may be due to lack of statistical power, although a recent publication by Zhou et al. (40) suggests that LBP measurements alone are not sufficient to predict type 2 diabetes. Alternately, LBP may be an important marker of only the early metabolic disturbances seen in prediabetes. Future studies are needed to more definitively test this hypothesis.

A strength of our study lies in its longitudinal design. Only one other longitudinal study has been conducted to date, and it was in a Chinese population (13). In addition, the availability of DXA and CT measures allowed us to more accurately describe general and regional body fat distribution associated with LBP, compared with previous studies.

Our study also has some limitations. Our sample included middle-aged and elderly African ancestry men, and thus, our findings may not apply to younger men, women, or other ethnic groups. Also, dietary data were limited in our study and because dietary influences can affect gut bacteria or may affect diabetes risk through other means, having information on food intake could allow for us to have a more holistic picture of how lifestyle might affect LBP. We currently do not have liver function or disease information for our sample. LBP is primarily a hepatically produced protein, and the liver is a site for ectopic fat development; thus, this information may be an important factor to investigate in future studies. Finally, we measured LBP only at baseline and, therefore, cannot examine whether change in LBP is a stronger correlate of metabolic changes than a single LBP measurement.

In conclusion, the current study shows that greater serum LBP concentrations are associated with increases in trunk and skeletal muscle adiposity and IR with aging among African ancestry men. The association with IFG and early changes in IR suggest that LBP may be more informative among individuals with normal serum glucose. Further research is needed to better understand the mechanisms underlying the relationship between LBP, adiposity, and IR and prediabetes.

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References

1. Lontchi-Yimagou E, Sobngwi E, Matsha TE, Kengne AP. Diabetes mellitus and inflammation. Curr Diab Rep 2013;13:435–444
2. Manco M, Putignani L, Bottazzo GF. Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. Endocr Rev 2010;31:817–844
3. Kelly CJ, Colgan SP, Frank DN. Of microbes and meals: the health consequences of dietary endotoxemia. Nutr Clin Pract 2012;27:215–225
4. Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. Annu Rev Biochem 2002;71:635–700
5. Van Amersfoort ES, Van Berkel TJ, Kuiper J. Receptors, mediators, and mechanisms involved in bacterial sepsis and septic shock. Clin Microbiol Rev 2003;16:379–414
6. Grube BJ, Cochane CG, Ye RD, et al. Lipopolysaccharide binding protein expression in primary human hepatocytes and HepG2 hepatoma cells. J Biol Chem 1994;269:8477–8482
7. Ding PH, Jin LJ. The role of lipopolysaccharide-binding protein in innate immunity: a revisit and its relevance to oral/periodontal health. J Periodontal Res 2014;49:1–9
8. Wurfel MM, Kunitake ST, Lichenstein H, Kane JP, Wright SD. Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. J Exp Med 1994;180:1025–1035
9. Lamping N, Dettmer R, Schröder NW, et al. LPS-binding protein protects mice from septic shock caused by LPS or gram-negative bacteria. J Clin Invest 1998;101:2065–2071
10. Novitsky TJ. Limitations of the Limulus amebocyte lysate test in demonstrating circulating lipopolysaccharides. Ann N Y Acad Sci 1998;851:416–421
11. Moreno-Navarrete JM, Ortega F, Serino M, et al. Circulating lipopolysaccharide-binding protein (LBP) as a marker of obesity-related insulin resistance. Int J Obes 2012;36:1442–1449

12. Moreno-Navarrete JM, Escoté X, Ortega F, et al. A role for adipocyte-derived lipopolysaccharide-binding protein in inflammation- and obesity-associated adipose tissue dysfunction. Diabetologia 2013;56:2524–2537
13. Liu X, Lu L, Yao P, et al. Lipopolysaccharide binding protein, obesity status and incidence of metabolic syndrome: a prospective study among middle-aged and older Chinese. Diabetologia 2014;57:1834–1841
14. Ruiz AG, Casafont F, Crespo J, et al. Lipopolysaccharide-binding protein plasma levels and liver TNF-alpha 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
15. Gonzalez-Quintela A, Alonso M, Campos J, Vizcaino L, Loidi L, Gude F. Determinants of serum concentrations of lipopolysaccharide-binding protein (LBP) in the adult population: the role of obesity. PLoS One 2013;8:e54600
16. Serrano M, Moreno-Navarrete JM, Puig J, et al. Serum lipopolysaccharide-binding protein as a marker of atherosclerosis. Atherosclerosis 2013;230:223–227
17. Kheirandish-Gozal L, Peris E, Wang Y, et al. Lipopolysaccharide-binding protein plasma levels in children: effects of obstructive sleep apnea and obesity. J Clin Endocrinol Metab 2014; 99:656–663
18. Sun L, Yu Z, Ye X, et al. A marker of endotoxemia is associated with obesity and related metabolic disorders in apparently healthy Chinese. Diabetes Care 2010;33:1925–1932
19. Hennis A, Wu SY, Nemesure B, Li X, Leske MC; Barbados Eye Study Group. Diabetes in a Caribbean population: epidemiological profile and implications. Int J Epidemiol 2002;31:234–239
20. Brancati FL, Kao WH, Folsom AR, Watson RL, Szklo M. Incident type 2 diabetes mellitus in African American and white adults: the Atherosclerosis Risk in Communities Study. JAMA 2000;283:2253–2259
21. Bunker CH, Patrick AL, Konety BR, et al. High prevalence of screening-detected prostate cancer among Afro-Caribbeans: the Tobago Prostate Cancer Survey. Cancer Epidemiol Biomarkers Prev 2002;11:726–729
22. Simonsick EM, Maffeo CE, Rogers SK, et al. Methodology and feasibility of a home-based examination in disabled older women: the Women's Health and Aging Study. J Gerontol A Biol Sci Med Sci 1997;52:M264–M274
23. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R. Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. J Appl Physiol (1985) 2000;89:104–110
24. 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
25. Bonora E, Targher G, Alberiche M, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000;23:57–63
26. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annu Rev Physiol 2010;72:219–246
27. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol 2011;29:415–445
28. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–1772
29. Blairon L, Wittebole X, Laterre PF. Lipopolysaccharide-binding protein serum levels in patients with severe sepsis due to gram-positive and fungal infections. J Infect Dis 2003;187:287–291
30. van Greevenbroek MM, Schalkwijk CG, Stehouwer CD. Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences. Neth J Med 2013;71:174–187
31. Doyle A, Zhang G, Abdel Fattah EA, Eissa NT, Li YP. Toll-like receptor 4 mediates lipopolysaccharide-induced muscle catabolism via coordinate activation of ubiquitin-proteasome and autophagy-lysosome pathways. FASEB J 2011; 25:99–110
32. Miljkovic I, Cauley JA, Petit MA, et al.; Osteoporotic Fractures in Men Research Group; Tobago Health Studies Research Group. Greater adipose tissue infiltration in skeletal muscle among older men of African ancestry. J Clin Endocrinol Metab 2009;94:2735–2742
33. Albu JB, Kovera AJ, Allen L, et al. Independent association of insulin resistance with larger amounts of intermuscular adipose tissue and a greater acute insulin response to glucose in African American than in white nondiabetic women. Am J Clin Nutr 2005;82:1210–1217
34. Gallagher D, Kuznia P, Heshka S, et al. Adipose tissue in muscle: a novel depot similar in size to visceral adipose tissue. Am J Clin Nutr 2005;81:903–910
35. Visser M, Kritchevsky SB, Goodpaster BH, et al. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. J Am Geriatr Soc 2002; 50:897–904
36. Ryan AS, Nicklas BJ, Berman DM. Racial differences in insulin resistance and mid-thigh fat deposition in postmenopausal women. Obes Res 2002;10:336–344
37. Goodpaster BH, Krishnaswami S, Resnick H, et al. Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. Diabetes Care 2003;26:372–379
38. Goodpaster BH, Thaete FL, Kelley DE. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. Am J Clin Nutr 2000;71:885–892
39. Reyna SM, Ghosh S, Tantiwong P, et al. Elevated Toll-like receptor 4 expression and signaling in muscle from insulin-resistant subjects. Diabetes 2008;57:2595–2602
40. Zhou H, Hu J, Zhu Q, et al. Lipopolysaccharide-binding protein cannot independently predict type 2 diabetes mellitus: a nested case-control study. J Diabetes 6 Mar 2015 [Epub ahead of print]. DOI: 10.1111/1753-0407.12281