Lipopolysaccharide-Binding Protein, a Surrogate Marker of Microbial Translocation, Is Associated With Physical Function in Healthy Older Adults

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Background. Physical function declines, and markers of inflammation increase with advancing age, even in healthy persons. Microbial translocation (MT) is the systemic exposure to mucosal surface microbes/microbial products without overt bacteremia and has been described in a number of pathologic conditions. We hypothesized that markers of MT, soluble CD14 (sCD14) and lipopolysaccharide (LPS) binding protein (LBP), may be a source of chronic inflammation in older persons and be associated with poorer physical function.

Methods. We assessed cross-sectional relationships among two plasma biomarkers of MT (sCD14 and LBP), physical function (hand grip strength, short physical performance battery [SPPB], gait speed, walking distance, and disability questionnaire), and biomarkers of inflammation (C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), TNF-α soluble receptor 1 [TNFsR1]) in 59 older (60–89 years), healthy (no evidence of acute or chronic illness) men and women.

Results. LBP was inversely correlated with SPPB score and grip strength ($p = .02$ and $p < .01$, respectively) and positively correlated with CRP ($p = 0.04$) after adjusting for age, gender, and body mass index. sCD14 correlated with IL-6 ($p = .01$), TNF-α ($p = .05$), and TNFsR1 ($p < .0001$). Furthermore, the correlations between LBP and SPPB and grip strength remained significant after adjusting for each inflammatory biomarker.

Conclusions. In healthy older individuals, LBP, a surrogate marker of MT, is associated with worse physical function and inflammation. Additional study is needed to determine whether MT is a marker for or a cause of inflammation and the associated functional impairments.

Key Words: Microbial translocation—Inflammation—Physical function—Microbiome.

AGE-ASSOCIATED increases in inflammatory biomarkers are associated with poor physical function (1–4). However, the mechanisms that initiate this increased inflammatory burden are not well understood. A previously unconsidered source of inflammatory initiation is mucosal surface microbes, primarily the gastrointestinal (GI) microbiome, through a process of microbial translocation (MT), the movement of microbes and/or microbial products across mucosal barriers without overt bacteremia. MT occurs in inflammatory bowel disease (IBD (5)), graft versus host disease (6), invasive GI surgery (7), sepsis (8), HIV infection (9,10), and obesity (11–13). Direct evidence of microbes include bacterial 16s rRNA and lipopolysaccharide (LPS) from gram-negative bacterial cell walls. Two endogenously produced reactive biomarkers formed in response to MT are soluble CD14 (sCD14), secreted by the liver and intestinal monocytes in response to LPS and other bacterial substances, and LPS-binding protein (LBP), a soluble acute-phase protein produced by hepatocytes (14,15) and intestinal epithelial cells (16,17), which binds LPS and promotes immune responses by presenting the LPS to CD14 (18). It is plausible that aging could increase MT due to age-related changes in GI function (19), immune responses (20), or both.

Associations among MT and inflammatory biomarkers have not been previously explored in older persons, nor has there been any prior investigation linking MT to physical function. Therefore, we evaluated the relationship of the

MT markers sCD14 and LBP and biomarkers of inflammation and physical function in 60- to 89-year-old participants of the HEALTHY study, a population selected to be free from any overt clinical disease and not using any prescription medications.

METHODS

Participants

The HEALTHY study was a cross-sectional study that enrolled nonsmoking older persons who reported no acute or chronic medical disorders, including hypertension or diabetes; who were taking no medications except aspirin for primary prevention; and who could discontinue aspirin at least 10 days prior to testing. The purpose of the study was to provide a comparison group for studies of age-related health conditions in order to understand the relative contributions of age and disease. Participants were recruited primarily by direct mail from community-dwelling persons aged ≥60 years. Eligibility was based on a medical history, physical examination by a board-certified internal medicine physician, medical record review, and multiple screening examinations including normal rest and exercise electrocardiogram (ECG) and echocardiogram; pulmonary function test; and chemistry and electrolyte panel. Participants who regularly participated in vigorous exercise >120 minutes per week or competitive sports were excluded. All participants provided written informed consent, and the protocol was approved by the Wake Forest University School of Medicine Institutional Review Board.

Protocol

Participants reported in the morning following an overnight fast, and any medications were held for 10 days. Blood samples were drawn in the supine, resting position and specimens were spun in a refrigerated centrifuge and plasma and serum were aliquoted and stored in 0.5 mL samples at −80°C. Plasma samples were available from 59 participants for analysis.

Body Mass Index

Body mass (kg) was measured on a standard calibrated scale, and height (cm) was measured using a stadiometer.

Physical Function

Short physical performance battery.—Physical function was assessed using the *Short physical performance battery* (SPPB) (21–23). A score (0-12) is calculated based on (a) the time (s) needed to walk 4 m at a normal pace; (b) the time needed to stand up and sit down 5 times as quickly as possible from a chair with the arms folded across the chest; and (c) a timed balance test that measures the ability to maintain balance with feet together in three positions (side by side, semitandem, and tandem) with 12 being the best score.

Gait speed.—Participants were asked to walk at their usual pace over a 4-m course. Two trials were performed, and the faster of the two walks was used to compute gait speed.

Six-minute walk distance.—Walking distance (ft) was determined by measuring how far the participants walked in 6 minutes at normal pace.

Hand grip strength.—Grip strength in both hands was measured using an adjustable, hydraulic grip strength dynamometer (Jamar Hydraulic Hand Dynamometer, Model No. BK-7498, Fred Sammons, Inc., Burr Ridge, IL). If a participant reported wrist or hand pain, or had undergone surgery of the upper extremity in the past 3 months, the affected hand was not tested, and results of the other hand were used. The two trials for each hand were averaged with the larger score being between the two hands used in the analysis.

Self-reported disability questionnaire.—The Pepper Assessment Tool for Disability (PAT-D) is a 19-item self-report disability questionnaire to assess functional difficulty (24–26).

Maximal oxygen uptake (VO$_2$).—Exercise capacity was assessed by measuring maximal VO$_2$ (VO$_2$max). VO$_2$max was measured with a modified Bruce protocol during a progressive exercise test on a motorized treadmill according to standards established by the American Heart Association and as previously described in detail (27,28). Expired gases were measured with a Med Graphics (Minneapolis, MN) CardiO2 system following calibration for VO$_2$ and VCO$_2$. Patients were encouraged to give a maximal, exhaustive effort as judged by respiratory exchange ratio >1.05, rate of perceived exertion >15, and >90% age-predicted maximal heart rate. Blood pressure, heart rate, and ECG were continuously monitored. VO$_2$ was assessed by averaging the final three 15-second averages, ventilatory threshold by the Wasserman method, and VE/VCO$_2$ slope as previously described in detail in older patients in our laboratory (27,28).

Inflammatory Cytokines and MT Markers

Inflammatory biomarker concentrations were measured from plasma collected after an overnight fast. Fasting plasma concentrations of IL-6 (Intra-assay CV: 8.6%; Inter-assay CV, low: 0.98%; high: 0.70%), TNF-α (Intra-assay CV: 6.5%; Inter-assay CV, low: 0.17%; high: 2.2%), TNF-α soluble receptor 1 (TNFsR1; Intra-assay CV: 8.8%; Inter-assay CV, low: 3.0%; high: 4.4%), and sCD14 (Intra-assay CV: 5.7%; Inter-assay CV, low: 2.5%; high: 8.4%) were determined by high-sensitivity Quantikine immunoassay kits (R&D Systems, Minneapolis, MN). CRP was measured using an automated immunoanalyzer (Immulite; Diagnostics Products Corp.). Finally, plasma LBP (Intra-assay CV: 4.2%; Inter-assay CV, low: 0.22%;

high: 5.0%) was determined with an ELISA kit (Cell Sciences, Canton, MA).

Statistics

Means and standard deviations were calculated for the continuous variables: age, BMI, SPPB, grip strength, gait speed, 6-minute walk distance, PAT-D MT biomarkers (LBP and sCD14), and inflammatory biomarkers (IL-6, CRP, TNFsR1, and TNF-α). Proportions were calculated for categorical variables such as gender and race. Distributions of all the biomarkers were skewed; so, logarithm transformations were applied before further analyses. Unadjusted and partial (adjusted for age, gender, and BMI) Pearson correlations were obtained among all the biomarkers. Multiple regression models were used to examine associations between physical performance or disability measures and MT biomarkers (individual and combined, respectively) in a stepwise fashion: first an unadjusted model was fitted (Model 1); then age, gender, BMI, and relative VO$_2$max were adjusted in a second model (Model 2); finally, a series of models were fitted by adding other individual inflammatory biomarkers to Model 2. Because there were only three African American participants, race was not included for all the adjusted analyses, but the conclusions did not change even when adjusting for race in addition to other covariates. All analyses were performed using SAS 9.2 (Cary, NC). A p-value of .05 was considered as statistical significant.

RESULTS

Table 1 provides descriptive statistics for participant characteristics, body composition, physical function, inflammatory biomarkers, and MT markers. The participants were predominantly women, white, and normal to overweight. The mean (±standard deviation) age was 69.2±7.4 years. Given the selection criteria, the sample performed well across all functional assessments. The inflammatory biomarkers are comparable to prior reports for these markers in older populations (1,4), and the level of the MT markers (LBP, sCD14) in this population was comparable to levels previously reported in healthy controls (9,29). However, the LBP levels previously reported in young, healthy controls appear to be a mean of approximately 10 μg/mL, whereas the mean LBP levels in this healthy, older population are 22.4±9.4 μg/mL.

Figure 1 shows the distribution of the MT markers, sCD14 and LBP, in this healthy, older population. Similar to inflammatory biomarkers, MT markers were not normally distributed. Age was not associated with either inflammatory or MT biomarkers (data not shown). After adjusting for gender, race, and BMI, the markers with the strongest association with age are LBP ($r = 0.18$, $p = .18$) and IL-6 ($r = 0.21$, $p = .13$).

The relationships among inflammatory and MT biomarkers are shown in Table 2. LBP was most strongly correlated with CRP (0.27, $p = .04$). SCD14 was directly associated with IL-6 (0.33, $p = .01$) and TNF-α (0.26, $p = 0.05$) and inversely associated with sTNFR1 (−0.52, $p < .0001$). Partial correlations adjusting for age, gender, race, and BMI did not materially differ from unadjusted associations (data not shown).

We assessed associations between physical function and MT markers using multiple linear regression modeling (Table 3). The unadjusted associations are shown along with adjustments for age, gender, and BMI (Model 1), or age, gender, BMI, and relative VO$_2$max (Model 2). Because inflammatory biomarkers may mediate the association between MT markers and function, further adjustment for each biomarker was done in a separate step. LBP showed a strong association with both SPPB and grip strength, even after adjusting for each inflammatory biomarker.

Table 1. Descriptive Statistics of Participants

| Characteristic	n	Mean or Percent	SD	Median	Range
Age (years)	59	69.2	7.4	68.0	60–89
Women	37	63.0			
Caucasian (%)	56	95			
African American (%)	3	5.0			
BMI (kg/m^2)	59	25.9	5.0	25.2	17.1–42.4
SPPB score (0–12)	58	11.2	0.80	11.0	9–12
Grip strength (kg)	56	34.2	11.8	31.8	15.5–62.5
Gait speed, 4 m (s)	58	1.22	0.17	1.25	0.89–1.60
PAT-D (1–5)	58	1.09	0.20	1.05	1.00–2.33
Walk distance, 6-min (ft)	58	1,855.2	227.8	1861.0	1,363.0–2,478.0
VO$_2$ max (mL/kg/min)	58	25.1	7.1	23.9	14.0–50.4
IL-6 (pg/mL)	59	1.42	0.92	1.11	0.42–4.73
TNF-α (pg/mL)	59	1.90	5.6	0.79	0.52–41.9
CRP (mg/L)	59	2.29	2.74	1.10	0.30–15.0
TNFsR1 (pg/mL)	59	1,604.7	571.3	1,458.6	884.5–3,180.1
sCD14 (ng/mL)	59	2,114.6	1,316.5	1,524.8	816.6–8,570.0
LBP (ng/mL)	59	22,394.8	9,356.8	20,316.0	4,400.0–63,104.0
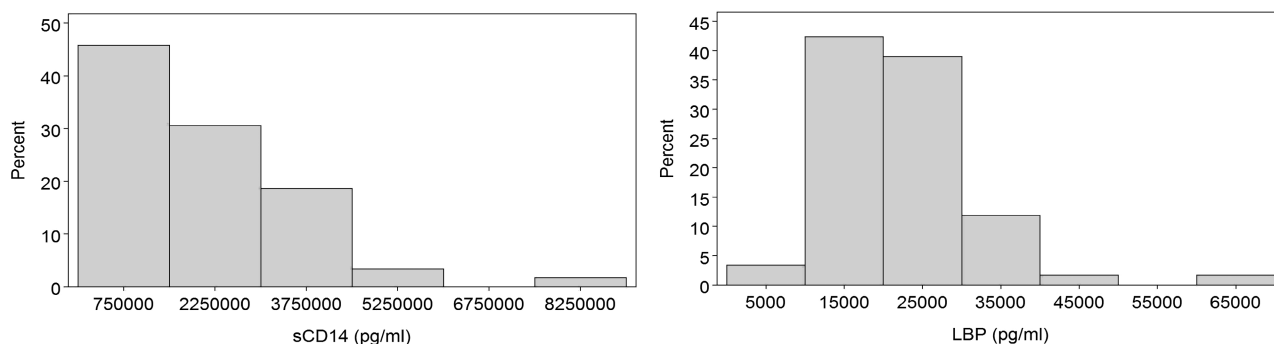
Figure 1. Histograms of the distribution of the microbial translocation markers sCD14 and lipopolysaccharide-binding protein in the healthy, older population.

Table 2. Correlation (Pearson's) Matrix Showing the Relationships Among Inflammatory Biomarkers and MT Markers

	Log sCD14	Log IL-6	Log CRP	Log TNF-α	Log TNFsR1
Log LBP	0.19	0.04	**0.27**	0.05	−0.11
p-Value	0.15	0.77	**0.04**	0.69	0.41
Log sCD14		**0.33**	0.21	**0.26**	**−0.52**
p-Value		**0.01**	0.10	**0.05**	**<0.0001**
Log IL-6			**0.31**	0.24	−0.03
p-Value			**0.02**	0.06	0.81
Log CRP				0.13	0.09
p-Value				0.33	0.52
Log TNF-α					−0.21
p-Value					0.11

Bold values represent significant correlations at a *p* value of .05 or less.

Furthermore, in the unadjusted models, LBP showed significant associations across all measures of physical function (data not shown) including gait speed (−0.10, *p* = .05), 6-minute walk distance (−170.4, *p* = .02), and PAT-D (0.13, *p* = .05). Although sCD14 associated with several inflammatory biomarkers, it did not associate with any of the physical function measurements in the unadjusted or adjusted models. When both MT markers were combined (data not shown), the pattern of associations changed little, suggesting independent contributions to function.

DISCUSSION

We believe that this is the first report of an association between markers of MT and physical function in older adults. MT markers were associated with physical function independently of age, gender, BMI, aerobic fitness level (VO$_2$), and inflammatory biomarkers. MT (increased plasma LBP and sCD14) was associated with poorer physical function across several measures (SPPB and grip strength). This association is particularly notable given the very truncated distribution of physical function in this sample (all SPPB scores >8). Moreover, the population was free of clinically manifest disease; so, the associations are unlikely to be due to confounding medical comorbidities. As expected, after adjusting for age, gender, and BMI,

MT markers were correlated with several inflammatory biomarkers (IL-6, TNF-α, CRP, and TNFsR1). An inverse correlation of sCD14 with TNFsR1 appears to be contrary to the other results (−0.53, *p* < .0001) and requires further exploration. Finally, there were no significant age-associated differences perhaps due to the restricted age range and modest sample size.

The mechanisms linking LBP to physical function also require further elucidation. There are limited data suggesting both direct and indirect mechanisms. MT could be responsible for physical function changes indirectly through an inflammatory intermediary (1). Because increased inflammation is strongly correlated with decreased physical function, it could be a likely explanation for the observed results. However, because LBP maintains its significant association with physical function after adjusting for inflammatory biomarkers, it suggests that alternative pathways may play a role. For example, LPS can directly induce muscle catabolism through TLR4 by the activation of ubiquitin–proteasome and autophagy-lysosome pathways (30). Furthermore, work by Frost and Lang (31–33) support several other ways in which MT products, including LPS and peptidoglycan, can directly impact myocytes. In their work, they show that endotoxin can affect muscle/myocytes directly by inhibiting translation through the stimulation of nitric oxide synthase activity (31). In addition, the myocyte can directly recognize and respond to LPS by producing cytokines (TNF-α) which work in an autocrine loop on the myocyte by altering nuclear factor kappaB and mTor signaling ultimately resulting in altered muscle growth (32,33). Directly measuring LPS in populations may aid in confirming this as a possible mechanism, but difficulties with the assay, the need to collect samples under LPS-free conditions, and high variability of LPS throughout the day (eg, similar to blood glucose) may mean LBP is a better marker of MT burden (eg, analogous to blood glucose and glycated hemoglobin [HgbA1C]).

Cross-talk that occurs between the microbiome and its host could occur in a variety of scenarios. For example, the

Table 3. Association Between MT Markers and Physical Function Measures with Adjustment for Different Covariates

	SPPB				Grip Strength			
	Regression coefficient	SE	Change in 1 SD	p-Value	Regression coefficient	SE	Change in 1 SD	p-Value
Log LBP								
Unadjusted	−0.76	0.24	−0.39	**<0.01**	−3.89	3.77	−0.14	0.31
Model 1	−0.59	0.24	−0.31	**0.02**	−6.46	1.96	−0.23	**<0.01**
Model 2	−0.58	0.24	−0.33	**0.02**	−6.39	2.01	−0.23	**<0.01**
Model 2 + TNFsR1	−0.56	0.25	−0.29	**0.03**	−6.54	2.04	−0.24	**<0.01**
Model 2 + TNF-α	−0.57	0.24	−0.29	**0.02**	−6.45	2.02	−0.23	**<0.01**
Model 2 + IL-6	−0.64	0.24	−0.33	**0.01**	−6.16	2.05	−0.22	**<0.01**
Model 2 + CRP	−0.55	0.25	−0.28	**0.04**	−5.56	2.06	−0.20	**0.01**
Log sCD14								
Unadjusted	−0.35	0.22	−0.21	0.11	−4.13	3.77	−0.15	0.28
Model 1	−0.30	0.21	−0.18	0.16	0.08	2.14	0.003	0.97
Model 2	−0.31	0.23	−0.18	0.19	0.02	2.38	0.02	0.81
Model 2 + TNFsR1	−0.30	0.27	−0.17	0.27	0.49	2.81	0.02	0.86
Model 2 + TNF-α	−0.28	0.24	−0.16	0.25	0.20	2.54	0.01	0.94
Model 2 + IL-6	−0.24	0.24	−0.14	0.34	−0.09	2.46	0.00	0.97
Model 2 + CRP	−0.27	0.24	−0.16	0.26	2.05	2.36	0.07	0.39

Bold values represent significant associations at a p value of .05 or less.

microbiome's role in food digestion will ultimately cause downstream effects to the host stemming from the harvested nutrients and vitamins as well as from caloric extraction (34,35). On the other hand, as previously described (9–13), microbial components can be directly responsible for the microbiome–host cross-talk. Such direct cross-talk could stem from direct mucosal damage or permeability changes or mucosal immunology changes that occur in response to a variety of factors including diet and the microbiome. Examples of such changes have been reported in aging humans and animal models (36,37). Another potential mechanism of MT involves the active transport of microbial products, such as LPS, through lipid transport pathways (38,39), which is supported by recent reports suggesting an endotoxemia–obesity link (11–13). However, regardless of the translocation mechanism, the GI tract is likely one of the principal sources of microbial products because of its massive bacterial load compared with other anatomical sites. At the same time, the GI microbiome is also under the most constant selective pressure stemming from diet changes, disease states, and medications, all important influences in the older population.

Limitations

The primary limitation of this study is the use of endogenously produced markers of MT. Endogenous biomarkers of MT (LBP and sCD14) have the advantage that they are easily measured and reflect the host's response to microbial products. However, their level should be interpreted with caution because they are also acute phase reactant proteins which can be directly influenced by various inflammatory factors such as IL-1, IL-6, IL-22,

and TNF-α (14,15). We attempted to account for any such influences in this study by adjusting for measured inflammatory biomarkers in our linear regression models. In addition, levels of these biomarkers depend on the ability of the individual to produce responsive proteins which may vary among individuals or change with age as liver function changes (40). This healthy, older population is ideal to assess an association with age because there is little interference from chronic conditions making it less likely that associations will be confounded by illness or treatment. At the same time, this limits the applicability of these findings to the majority of older adults. The study's sample size was modest, and a number of statistical comparisons were made. This means that the Type I and Type II error rates are relatively high; thus, these results should be interpreted cautiously. The 95% confidence intervals from the models provide information regarding the range of probable effect sizes that are consistent with the presented data. Finally, because this was a cross-sectional study with no interventions, only a correlation can be stated and therefore the association may or may not be causally related. For these reasons, it will be important to measure MT markers (sCD14, LBP, LPS, 16s rRNA, etc.) in more diverse populations.

In conclusion, this study has shown for the first time, increased concentrations of endogenous markers of MT (sCD14 and LBP) to be associated with impaired physical function in an older population. This suggests that MT is a mechanism by which the microbiome can affect the host and may play a previously unrecognized role, resulting in adverse consequences including impaired physical function.

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