




# Biomarkers of leaky gut are related to inflammation and reduced physical function in older adults with cardiometabolic disease and mobility limitations

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**Abstract** Intestinal barrier dysfunction is hypothesized to be a contributing determinant of two prominent characteristics of aging: inflammation and decline in physical function. A relationship between microbial

translocation (MT), or their biomarkers (lipopolysaccharide binding protein-1 [LBP-1], soluble cluster of differentiation [sCD]-14), and physical function has been reported in healthy older adults, rats, and invertebrates. However, it is not known whether the existence of comorbidities, or clinical interventions intended to reduce comorbidities through weight loss or exercise, alters this connection. We measured inflammation, MT, and physical function in 288 overweight/obese older patients with cardiometabolic disease and self-reported mobility limitations who were enrolled in a weight loss and lifestyle intervention study. At baseline, inflammatory cytokines and LBP-1 were positively correlated after adjustment for age, gender, and body mass index. A higher LBP-1 was significantly associated with poorer physical functional after covariate adjustment. Further, even when IL-6 levels were included in the models, 400-m walk time ( $p = 0.003$ ), short physical performance battery ( $p = 0.07$ ), and IL-8 ( $p < 0.001$ ) remained positively associated with LBP-1. Lifestyle interventions improved body mass and some functional measures; however, MT and inflammation were unchanged. MT is reliably related to inflammation, and to poorer physical function in older adults with comorbid conditions. Intestinal barrier function did not appear to improve as a result of intervention assignment, suggesting alternative strategies are needed to target this pro-inflammatory pathway in aging.

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## Introduction

Decline in physical function is a characteristic of aging that can begin as early as the fifth decade of life (Hall et al. 2016). In addition to its importance in maintaining independence, physical function in the elderly can predict future cardiovascular disease, mobility disability, and mortality (Newman et al. 2006). Chronic, sterile, and low-grade inflammation indicated by circulating C-reactive protein (CRP), and interleukin 6 (IL-6) levels, is another characteristic of aging. This phenomenon has been termed inflammaging (Saffrey 2014). However, the basis of this increased inflammatory burden is unclear (Hollander and Tamawski 1985; Franceschi et al. 2007). Possible sources of circulating inflammatory stimuli include self-debris, such as macromolecules that result from cellular breakdown, and live or dead bacterial products sourced from microbiota (Franceschi et al. 2007).

The gastrointestinal (GI) tract is the largest area of the human body that interacts with the environment. The human GI tract is estimated to contain  $> 10^{14}$  microorganisms (Thursby and Juge 2017). These microorganisms are essential for digestion and absorption and are part of the natural barrier against pathogens. Although the GI tract has constant contact with these microorganisms, significant bacterial invasion of the human body rarely happens as long as the GI barrier is intact. Microbial translocation (MT) is the passage of both viable and nonviable microbial products, such as lipopolysaccharide (LPS), across an anatomically intact intestinal barrier and is considered a normal process (D'Ettorre et al. 2012). The barrier is comprised of physical (mucus and the epithelial cells), biochemical (bile salts, enzymes, antibacterial proteins), immunological (IgA and immune cells), and microbial components (the microbiota) (Chassaing et al. 2014). Any defect in these aforementioned barrier elements can result in elevated immune activation and inflammation and/or infection. Intestinal barrier dysfunction is commonly observed in aging vertebrates and invertebrates, and is associated with multiple markers of aging, including systemic metabolic dysfunction (Rera et al. 2012; Ghosh et al. 2015; Kavanagh et al. 2016; Wilson et al. 2018). A current hypothesis is that decreasing intestinal barrier function with aging can cause increased MT into the systemic blood circulation that subsequently causes systemic inflammation (inflammaging) and significant clinical outcomes (e.g., metabolic syndrome, decreased physical function, and mortality).

In a cross-sectional study of 59 older healthy adults, MT burden, measured by LPS-binding protein (LBP), was inversely correlated with physical function, measured by short physical performance battery (SPPB) and grip strength. Correlations between LBP and physical function remained significant even after adjusting for each inflammatory biomarker (Stehle Jr. et al. 2012). Although this study shed light on the association between MT and physical function in the elderly, it was a small study in participants without common comorbidities that typify normal aging. It is not clear if the association remains in older adults with comorbidities and likely higher inflammatory burdens. We aimed to examine the relationship between MT and physical function in overweight or obese older adults who had either cardiovascular disease (CVD) or cardiometabolic dysfunction and evidence of self-reported limitations in mobility. Furthermore, it is unclear whether common behavioral interventions that improve mobility may do so through some effect on MT burden. With the known association between inflammation and physical performance (Brinkley et al. 2009), we hypothesized that increased MT may cause chronic inflammation and subsequent declines in physical function with aging.

## Methods

### Study design

This was a secondary analysis of a randomized clinical trial: The Cooperative Lifestyle Intervention Program (CLIP; NCT00119795). CLIP was a randomized controlled trial of 288 ambulatory, older (60–79 years), community-dwelling adults from three NC counties conducted between January 2005 and April 2010. The detailed study design and trial outcomes have been previously reported (Rejeski et al. 2011; Beavers et al. 2013; Beavers, Beavers et al. 2014).

### Eligibility criteria

Subjects were enrolled if they were ambulatory, overweight or obese, community-dwelling older adults who had either CVD or cardiometabolic dysfunction and evidence of self-reported limitations in mobility. Subjects had to meet the criteria: (1) age 60 to 79 years; (2) having less than 60 min/week of moderate, structured PA; (3) having a body mass index (BMI), calculated as

weight in kilograms divided by height in meters squared, greater than 28; (4) having evidence of a myocardial infarction, percutaneous transluminal angioplasty, chronic stable angina, or cardiovascular surgery in the past 6 months or an Adult Treatment Panel III diagnosis of the metabolic syndrome; (5) having a self-reported mobility limitation; and (6) willingness to sign an informed consent and a HIPPA authorization form.

Subjects were excluded if they had (1) a baseline BMI of 40 or higher; (2) bipolar disorder, depression, or schizophrenia; (3) unstable angina, symptomatic congestive heart failure, or exercise-induced complex ventricular arrhythmias; (4) resting blood pressure greater than 160/100 mmHg; (5) diagnosis of systemic diseases that precluded participants from safely participating in the interventions; (6) a fasting blood glucose level higher than 140 mg/dl (7.77 mmol/l), type 1 diabetes mellitus (DM), or type 2 DM with insulin therapy; (7) active treatment for cancer; (8) clinically significant visual or hearing impairment; (9) dementia, delirium, or impaired cognitive function; (10) participation in another medical intervention study; (11) having more than 21 alcoholic drinks per week; (12) inability to walk unassisted; and (13) inability to speak or read English.

#### Intervention arms

*Successful aging (SA)* This group received scripted educational lessons on health tailored for older adults. The SA group met weekly for the first 2 months, monthly through month 6, and then bimonthly until the end of the study. Participants in this arm did not receive a progressive, supervised program of physical activity (PA) or diet for weight loss (WL); however, both PA and nutrition for aging were addressed as separate and distinct topics.

*Physical activity* The goal of the PA intervention was to gradually increase or shape PA in a home-based environment to more than 30 min of moderately intense activity on most days of the week for a total of more than 150 min/week. Participants walked at a moderate intensity of “somewhat hard” as assessed by the Borg Rating of Perceived Exertion Scale (Borg 1982) and written self-monitoring logs were used to document. The first 6 months was considered as an intensive phase and the activities include counseling sessions: three 90-min group sessions and one 30-min individual session

each month. Months 6–12 consisted of a transition phase that was followed by a maintenance phase out to 18 months. This report is restricted to data from the 6-month intensive phase of the intervention.

*Weight loss and physical activity (WL + PA)* This group involved the PA plus dietary weight loss. The goal of WL was to reduce caloric intake to produce weight loss of 0.3 kg/week for the first 6 months for a total loss in mass of 7–10%. Participants were assigned a daily calorie intake goal based on baseline weight (1200–1500 kcal for participants < 250/114 lbs/kg, and 1500–1800 kcal for participants ≥ 250/114 lbs/kg). Participants were given food tracking booklets and at the end of each week, an average calorie intake was calculated. In addition to this weight loss intervention, PA sessions were given as described above.

#### Measurements

Participants were assessed pre-randomization and returned at 6 months. Demographics, medical history, and comorbidities were collected by self-report, and height and weight were measured at each visit. The 400-m walk test was used to assess mobility (Simonsick et al. 2001). Physical activity measured using the Lifecorder-EX accelerometer (New-Lifestyles Inc., Lees Summit, MO) was used to assess physical activity. Participants were asked to wear the accelerometer for 7 days (Rejeski et al. 2011). Short physical performance battery (SPPB) (Guralnik et al. 1994) which is composed of balance, gait speed, and chair speed was assessed at baseline and 6-month follow-up visit. Intensity levels 3–9 were classified as moderate to vigorous for this study of the elderly population. Physical activity classified as minutes of moderate to vigorous activity (AccelMVPA) and total physical activity (AccelPAEE) was analyzed.

Blood samples were collected in the early morning after a 12-h fast. The 6-month blood samples were collected after at least 24 h after the last acute bout of exercise. All blood was collected, processed, divided into aliquots, and stored in the Biological Specimen Repository at Wake Forest School of Medicine at – 80 °C. Adiponectin, high-sensitivity IL-6 (hsIL-6), IL6Sr, IL-8, and sTNFR1 were measured.

*Biomarkers of microbial translocation (MT)* All analyses were performed by ELISA. We focused on the two

plasma biomarkers: LBP-1 and soluble CD14 (sCD14). LBP-1 (Hycult Biotech, Netherlands) and sCD14 (R&D Systems, Minneapolis, MN) were measured in duplicate to improve accuracy of measures. LBP-1 is a soluble protein produced by hepatocytes and intestinal epithelial cells, which binds LPS and promotes immune responses (Muta and Takeshige 2001). sCD14 is a glycoprotein secreted from the liver as a result of toll-like innate immune receptor (TLR) family activation, allowing interaction with the antigen bound to LBP-1, and circulates to help bind bacterial products. sCD14 is a co-receptor for toll-like receptor 4. LBP-1 assay has an average coefficient of variation of 8.97% (standard error 1.58) and sCD14 has an average coefficient of variation of 11.8% (standard error 2.86).

### Statistical analysis

Means and standard deviations (SDs) were presented for continuous baseline characteristics. Counts and percentages were presented for discrete baseline characteristics. Six-month mean changes and SDs in BMI, physical function, inflammatory markers, and MT markers were calculated across the three randomization groups and compared using analysis of variance.

Baseline associations of MT markers and inflammatory markers and physical function after controlling for age, gender, and BMI were calculated using linear regression models. The outcome measures are inflammatory markers and physical function and MT markers are the covariates of interest. The distributions of outcome measures were checked and transformations were performed if needed to satisfy the conditional normality assumption. Adiponectin, total activity energy expenditure, moderate to vigorous activity energy expenditure, IL-6, IL-8, and 400-m walk time were log-transformed. The associations relating MT markers to inflammatory markers and physical function were also performed using linear regression models, with additional adjustment for group randomization at 6 months. We also performed the sensitivity analysis for those measures with outliers, by removing outlying values that were defined as 3 SDs away from the mean value.

To study the effects of study interventions on 6-month change in markers of microbial translocation, analysis of covariance was used with adjustment of baseline outcome measure, age, gender, and BMI. The overall test for randomization groups with 2 degrees of freedom (df) was presented. Further, analysis of

covariance was also used to examine the associations between baseline LBP-1 and 6-month changes in physical function after adjusting for baseline physical function, age, gender, BMI, and randomization groups.

### Results

We analyzed data of 288 ambulatory, overweight or obese, community-dwelling participants aged 60–79 who had either CVD or cardiometabolic dysfunction and evidence of self-reported limitations in mobility. Baseline characteristics are demonstrated in Table 1. The mean age was 67, 67% were female, 82% were white, and the mean BMI was 32.9 kg/m<sup>2</sup>. Study outcomes relating to changes in body composition, physical function, and biomarker changes have been previously reported (Rejeski et al. 2011; Beavers et al. 2013; Beavers, Beavers et al. 2014). Collectively, these reports describe weight loss, improved IL-6 levels, and physical function with WT + PA and are trial outcomes relevant to the current report.

At baseline, the MT biomarker LBP-1 was associated with BMI ( $r = 0.143$ ,  $p = 0.019$ ) but not with age ( $p = 0.57$ ). At baseline, serum LBP-1 level was associated with log-transformed IL-6 and log-transformed IL-8 both before and after controlling for age, gender, and BMI (Table 2; Fig. 1). Baseline serum LBP-1 levels were also associated with log-transformed total energy expenditure and SPPB, as well as log-transformed 400-m walk time. Serum sCD14 was associated with log-transformed IL-6 and log-transformed IL-8 after controlling for age, gender, and BMI (Table 2). In all analyses, LBP-1 associations were stronger than those with sCD14. The result for the sensitivity analysis after removing outliers was similar (Supplementary Table 1).

Despite the intervention arms of the trial being successful in achieving weight loss, improving physical function, and reducing IL-6 (Rejeski et al. 2011; Beavers et al. 2013), there were no significant differences in changes in MT markers by intervention groups after 6 months of study (Table 3). The analyses of MT and inflammatory markers and physical function at 6 months demonstrate that there remained a significant association between LBP-1 and log-transformed IL-6 and log-transformed IL-8 (Fig. 2). Also, serum sCD14 was associated with log-transformed serum IL-6 after controlling for age, gender, BMI, and randomization groups. However,

**Table 1** Summary statistics describing the clinical characteristics, physical function, inflammatory markers, and markers of microbial translocation for all study participants ( $n = 288$ ) prior to randomization

Variables	Baseline ( $n = 288$ )
Age (mean (SD))	67.0 (4.8)
Female ( $n$ (%))	193 (67)
White ( $n$ (%))	236 (82)
BMI ( $\text{kg}/\text{m}^2$ (SD))	32.9 (3.8)
400-m walk time (seconds (SD))	353 (75)
AccelMVPA (minutes (SD))	115 (77)
AccelPAEE (minutes (SD))	1288 (597)
Adiponectin (ng/ml (SD))	7764 (5820)
IL-6 (pg/ml (SD))	2.7 (1.9)
IL-8 (pg/ml (SD))	3.9 (6.9)
IL_6sR (pg/ml (SD))	40,651 (12,127)
SPPB (SD)	10 (1.5)
LBP (ng/ml (SD))	38 (13)
CD14 (ng/l (SD))	2.4 (1.0)

**Abbreviations:** *SD*, standard deviation; *BMI*, body mass index; *AccelMVPA*, moderate to vigorous physical activity energy expenditure; *AccelPAEE*, total physical activity energy expenditure; *IL*, interleukin; *SPPB*, short physical performance battery; *LBP-1*, lipopolysaccharide-binding protein 1; *sCD14*, soluble cluster of differentiation 14

there was no significant association with biomarkers of MT and physical function at 6 months (Table 4). The result for the sensitivity analysis after removing outliers was similar except that the association between sCD14 and log-transformed IL-8 became significant ( $p = 0.014$ ; Supplementary Table 2). Further, baseline LBP-1 levels did not predict changes in physical function over the 6-month period.

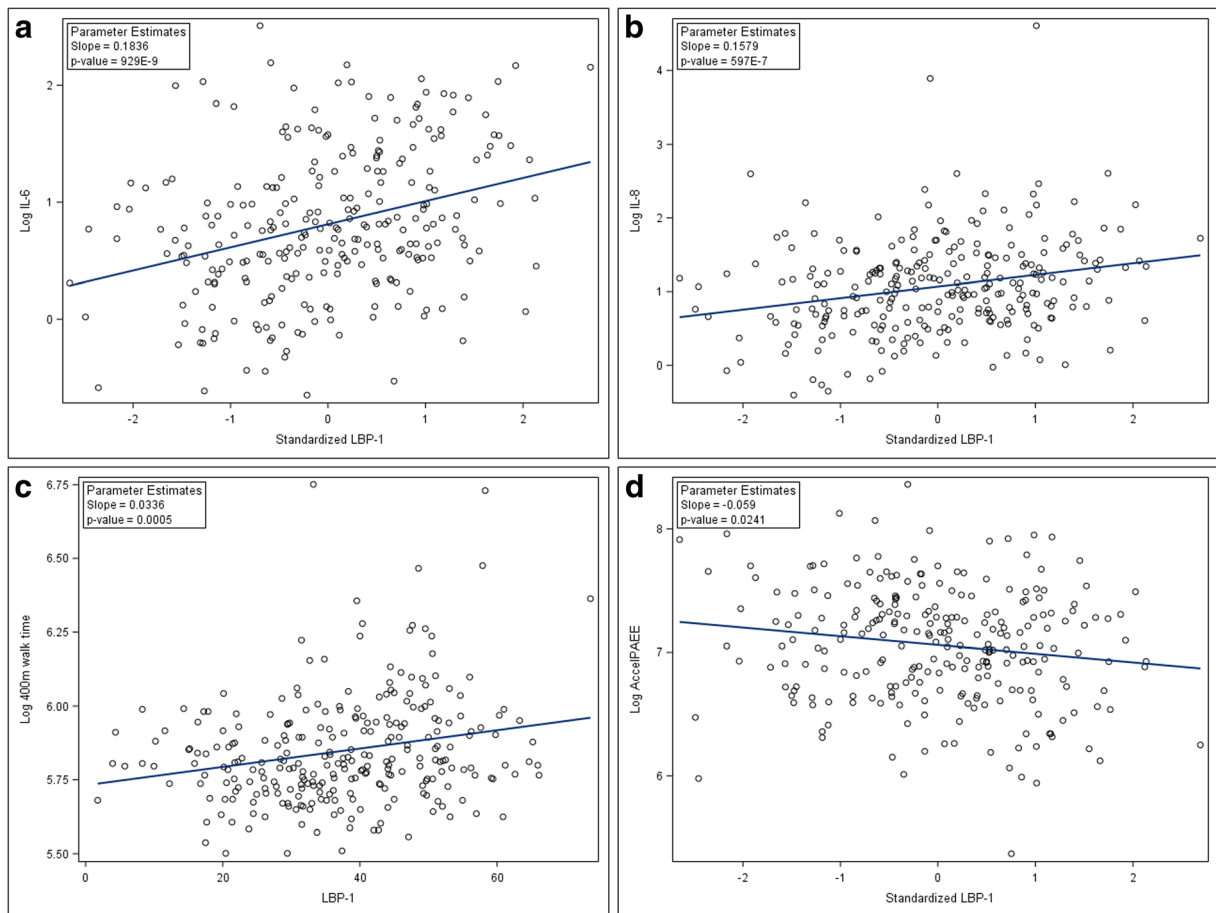
**Table 2** Baseline associations of MT markers with inflammatory markers and physical function after controlling for age, gender, and BMI

	LBP-1 (SD = 13.5) Standardized regression coefficient $\pm$ SE	$p$ value	SCD14 (SD = 0.97) Standardized regression coefficient $\pm$ SE	$p$ value
Log adiponectin	$-0.05 \pm 0.04$	0.27	$0.02 \pm 0.04$	0.64
Log IL-6	$0.18 \pm 0.04$	$< 0.001$	$0.08 \pm 0.04$	0.035
Log IL-8	$0.16 \pm 0.04$	$< 0.001$	$0.10 \pm 0.04$	0.012
IL-6sR	$1412 \pm 740$	0.06	$450 \pm 738$	0.54
Log AccelMVPA	$-0.06 \pm 0.05$	0.18	$-0.05 \pm 0.05$	0.28
Log AccelPAEE	$-0.06 \pm 0.03$	0.024	$-0.01 \pm 0.03$	0.81
SPPB	$-0.17 \pm 0.08$	0.049	$-0.13 \pm 0.09$	0.14
Log 400-m walk time	$0.03 \pm 0.01$	0.001	$0.01 \pm 0.010$	0.16

**Abbreviations:** *SE*, standard error

## Discussion

We demonstrate that there is a significant cross-sectional association between baseline biomarkers of MT (LBP-1, sCD14) and inflammatory markers including serum IL-6 and IL-8 in this study of 288 ambulatory, overweight or obese, community-dwelling participants of a clinical trial (The Cooperative Lifestyle Intervention Program, CLIP) aged 60–79 who had either CVD or cardiometabolic dysfunction and evidence of self-reported limitations in mobility. This is a novel description of the association between MT markers and physical function in a clinical population with comorbidities. Baseline LBP-1 was associated with poorer physical function, estimated by total energy expenditure, SPPB, and 400-m walk time, after controlling for age, gender, and BMI. sCD14 was also associated with 400-m walk time. This association has been reported in healthy elderly participants and we demonstrate that this biological relationship is indeed still present in less healthy adults, and MT remains associated with function even after adjustment for inflammatory biomarkers. In that prior, but smaller, study of 59 older adults (60–89 years), Stehle et al. demonstrated that LBP was inversely correlated with SPPB score and the correlation between LBP and SPPB remained significant even after adjusting for inflammatory biomarkers (Stehle Jr. et al. 2012). Our study demonstrated similar associations between markers of MT and physical function, but is novel as it is observed in a much larger number ( $n = 278$ ) of older population with significant comorbidities. Chronic inflammation induced by MT that is evidenced by elevated inflammatory markers is known to be associated with low physical function in older adults with multiple comorbidities (Brinkley et al. 2009).



**Fig. 1** Scatterplots of baseline values showing unadjusted associations of raw LBP-1 concentrations and inflammation biomarkers: IL-6 (a) and IL-8 (b), and physical function measures; 400-m walk

time (c), physical activity (d; total energy expenditure as measured by wearable accelerometers (AccelPAEE))

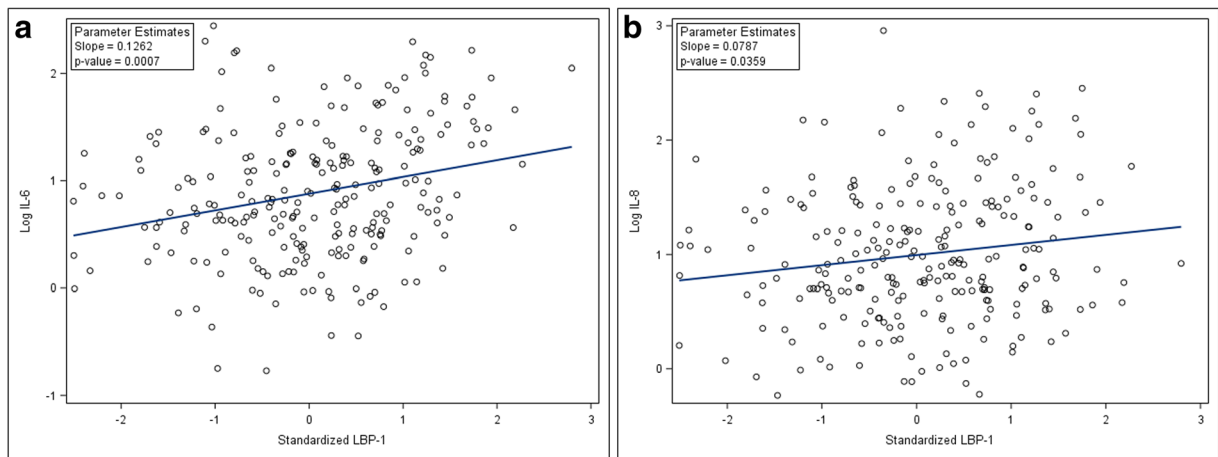
Our study indicated that diet and exercise do not reliably reduce biomarkers of MT. Two other studies have examined the results of health interventions in older adults with conflicting results (Ghosh et al. 2015; Rodriguez-Miguel et al. 2015), where structured intensive exercise in a US-based population failed to change TLR4 muscle levels, whereas low-intensity exercise in a European-based population did improve

muscle TLR4 levels. Muscle TLR4 is an endpoint that is elevated in older muscle and is presumed to be elevating in response to higher circulating LPS (Ghosh et al. 2015). Exercise interventions in aged rats and non-human primates replicated the positive effects of activity that were reported in a European study of older people (Oliveira et al. 2011; Kavanagh et al. 2016) and may reflect the generally healthier overall status of these

**Table 3** Effects of study interventions on 6-month change in markers of microbial translocation

	Successful aging ( $N = 86$ ) Raw mean (SD)	Physical activity ( $N = 86$ ) Raw mean (SD)	Weight loss and physical activity ( $N = 95$ ) Raw mean (SD)	$p$ value*
6-Month change in LBP-1	1.05 (11.50)	-0.27 (11.38)	0.93 (14.76)	0.87
6-Month change in CD14	0.11 (1.05)	-0.03 (1.00)	0.04 (0.91)	0.76

\* $p$  value (2df test) was calculated using the analysis of covariance after adjusting for baseline outcome measure, age, gender, and BMI



**Fig. 2** Scatterplots of unadjusted post-study (6-month) values showing the persistent associations of LBP-1 with inflammation: IL-6 (a), IL-8 (b)

European human and laboratory animal subjects. The lack of exercise and lifestyle effect of MT biomarkers in our US-based clinical population is consistent with that in the smaller US-based study reported by Ghosh et al. (2015).

Our study found that MT related to functional outcomes independent of IL-6, which might suggest a separate pathway exists in which TLR signaling in skeletal muscle leads to functional decline. Our prior non-human primate studies confirm MT mediates relationships between physical activity and insulin sensitivity, independent of IL-6 (Kavanagh et al. 2016). Thus, the GI barrier could be a new target for improving health in aging. The original reporting of this trial demonstrated that the weight loss plus exercise intervention was successful in reducing IL-6 concentrations (Beavers

et al. 2013). This group also had reduced leptin concentrations, so IL-6 lowering was suggested to be mediated by the reductions in fat mass. Improvements in physical function and adiposity observed in the study did not alter gut barrier function and microbial translocation. Multiple observations now report that physical function and microbial translocation associate independently of IL-6; thus, this is a novel pathway to understand.

The exact reasons for why GI barrier dysfunction occurs in aging are unknown but at least in invertebrate models, the barrier loss is a transition marker that indicates imminent decline and death (Rera et al. 2012; Tricoire and Rera 2015; Dambroise et al. 2016). This “second phase of aging” is independent of chronological age and characterized by intestinal immunodeficiency which may be comparable with the older unhealthy

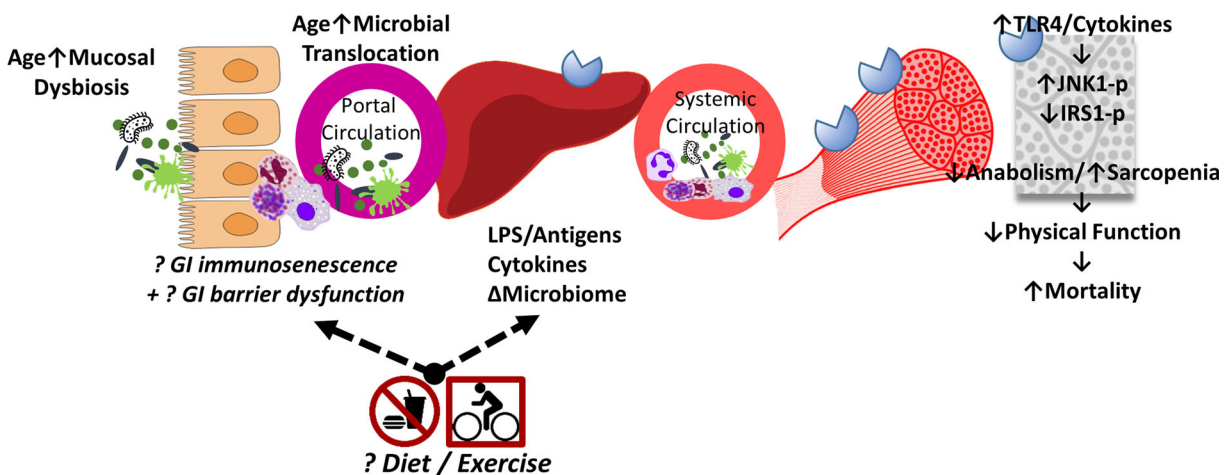
**Table 4** Association between MT markers and inflammatory markers and physical function after controlling for age, gender, BMI, and randomization groups at 6 months (post-intervention)

	LBP-1 (SD = 13.5) Standardized regression coefficient ± SE	<i>p</i> value	SCD14 (SD = 0.97) Standardized regression coefficient ± SE	<i>p</i> value
Log adiponectin	− 0.02 ± 0.04	0.68	0.02 ± 0.04	0.60
Log IL-6	0.13 ± 0.04	0.001	0.08 ± 0.04	0.040
Log IL-8	0.08 ± 0.04	0.046	0.08 ± 0.04	0.051
IL-6sR	1022 ± 789	0.20	626 ± 776	0.42
Log AccelMVPA	0.01 ± 0.05	0.78	− 0.03 ± 0.05	0.51
Log AccelPAEE	0.02 ± 0.03	0.45	− 0.01 ± 0.03	0.86
SPPB	0.03 ± 0.10	0.73	0.10 ± 0.10	0.30
Log 400-m walk time	0.01 ± 0.01	0.43	0.01 ± 0.01	0.47

adult, whereby inflammation is high and unresponsive to lifestyle interventions typically known to improve health outcomes. The gastrointestinal system is the largest immune organ in the human body with organized gut-associated lymphoid tissue, and lymphocytes in lamina propria and surface (Pabst et al. 2008). Relevant to the immune changes seen with age and the effects of lifestyle interventions, immune profiling of master cyclists (Duggal et al. 2018) reports that many cell populations were equivalent to those of younger adults. However, there were cell types that did not show differences after a lifetime of physical activity and included circulatory B cell populations, which have key roles in local mucosal immunity. We outline the interactions of elements in these pathways in Fig. 3, noting that the effect of diet and exercise may not alter the immunological competency at the mucosal surface.

With aging, intestinal epithelial cells produce more pro-inflammatory cytokines such as IL-6, TNF- $\alpha$ , and IL-1 $\beta$ ; they in turn increase intestinal epithelial tight junction permeability (Man et al. 2014). Further, the role of gut microbiome in human health recently has

received much deserved attention. The collective genome size of gut microbiome is 150-fold (Wu et al. 2013) of the human genome, and the role in human health is beginning to be understood. Aging in general has not been associated with changes in the fecal microbiome that are generalizable (Jeffery et al. 2016; Anand et al. 2017), but the dysbiosis seen with aging is still an active area of investigation. Age-related differences in microbiome measured from the intestinal mucosal surface and in circulation suggest aging does change the microbiome that translocates across intestinal wall (Maffei et al. 2017; Buford et al. 2018; Wilson et al. 2018). While these niche microbiomes are different in older people, the microbial populations in the intestinal lumen, which are excreted, may be comparable (Lyra et al. 2012; Wilson et al. 2018). A different circulatory microbiome in aged people (Buford et al. 2018) is highly suggestive that alterations in the mucosal-associated microbiomes exist with aging, as described in animal models (Wilson et al. 2018). It is unknown if the immunological decline in the aging host dictates the composition of the mucosal microbiome and its passage



**Fig. 3** Overall model that is supported by this, and prior studies. Unknown interactions are indicated by italics, question marks, and broken arrows. Aging leads to altered microbiome on the mucosal surface. It is unknown if the microbiome interferes with the barrier function (immune and physical components), or if the aging host's reduction in gastrointestinal (GI) barrier function facilitates an altered surface microbiome, or some combination of both processes. Increased microbial translocation with aging is consistently observed, which travels via the portal circulation to incite inflammation in the liver via toll-like receptor (TLR) binding, and results in increased cytokines, and altered bacteria/bacterial products such as lipopolysaccharide (LPS)-binding protein. In the systemic circulation, inflammatory cytokines such as IL-6 are generally accepted to be elevated in aged adults. TLR4 and inflammatory

signaling both cause reductions in insulin's anabolic actions via c-Jun kinase (JNK1) activation, which inhibits insulin receptor signaling via insulin receptor substrate (IRS) 1 activation. Global inflammation and insulin resistance permit sarcopenia and reduced physical function, known to influence mortality risk. It is unknown if the benefits of dietary modification (restriction, fasting, or quality) or exercise are able to modulate the age-related changes at the primary steps within this pathway. To date, benefits of lifestyle interventions are seen in laboratory animal models and small clinical trials, but larger clinical trials fail to show these interventions can reliably reduce microbial translocation. Efforts to understand how the mucosal barrier and normal surface microbiome are needed to halt this microbial contribution to inflammaging



across the intestinal barrier, or if the changed microbiome (different species, greater abundance) is able to evade or overwhelm the intestinal barrier. Microbes are metabolically active, and microbial metabolite profiles have been related to both positive and negative health span outcomes (Wang et al. 2011; Lewis et al. 2018). The microbiome composition, its local activity, and products all may have a biological effect on the competency of the intestinal barrier.

Our study has a few limitations. First, the study population was an elderly population who are overweight or obese with significant comorbidities and mobility limitation. Since there might be an association between comorbidities (e.g., cardiovascular disease) and MT, we cannot rule out any confounding effects of the participants' comorbidities. Also, we used endogenous biomarkers of MT (LBP-1, sCD14) that are acute phase proteins. LPS is a direct marker of endotoxemia and has been measured for that purpose. However, the variability of LPS throughout the day and the need to collect the sample under LPS-free conditions make LPS a difficult marker to measure in human subjects to assess MT. For these reasons, LBP-1 was considered a preferred biomarker of MT burden.

The relationships of MT and specific physical functional outcomes are few. Our current study is the largest associative study performed to date and thus is significant in that the relationship between MT and physical function is present, independent of inflammatory biomarkers, and is poorly responsive to well-accepted health intervention strategies. Our study also focused on an unhealthy population, and it is known that obese and type II diabetes subjects have higher MT biomarker levels (Cani et al. 2007; Amar et al. 2008), and accompanying inflammatory outcomes are negatively correlated with muscle insulin sensitivity (Shi et al. 2006; Schaap et al. 2009; Liang et al. 2013; Kavanagh et al. 2016; Grosicki et al. 2018). In human myotubes, LPS can induce inflammatory response which in turn reduces the anabolic actions of insulin signaling (Liang et al. 2013) which is an important sarcopenia pathway. In a study of 178 elderly subjects, it was demonstrated that fecal microbial composition is correlated with measures of frailty and markers of inflammation (Claesson et al. 2012), and gut microbial diversity is inversely associated with frailty (Jackson et al. 2016) but causes and effects are undetermined. We believe that targeting intestinal immunocompetence to reduce MT would benefit insulin sensitivity, reduce inflammation, and overall

be positive for muscular health in aging. Interventions known to aid the mucosal barrier function are few. Sevelamer reduces MT in dialysis patients but was not effective in HIV enteropathy (Sandler et al. 2014). Oral immunoglobulins have reported variable effectiveness (Asmuth et al. 2013; Wilson et al. 2018), and metformin has also reported to reduce MT (independent of its actions on the microbiome) (Moreno-Navarrete et al. 2011; Spruss et al. 2012). As ours and others' studies show that diet and exercise are not universally beneficial for restoring intestinal barrier competency, strategies such as these should be pursued for reducing MT and inflammatory burden in older adults.

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