Microbial translocation and skeletal muscle in young and old vervet monkeys

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Abstract Intestinal barrier dysfunction leads to microbial translocation (MT) and inflammation in vertebrate and invertebrate animal models. Age is recently recognized as a factor leading to MT, and in some human and animal model studies, MT was associated with physical function. We evaluated sarcopenia, inflammation, MT biomarkers, and muscle insulin sensitivity in healthy female vervet monkeys (6–27 years old). Monkeys were fed consistent diets and had large and varied environments to facilitate physical activity, and stable social conditions. Aging led to sarcopenia as indicated by reduced walking speeds and muscle mass, but general metabolic health was similar in older monkeys ($n = 25$) as compared to younger ones ($n = 26$). When older monkeys were physically active, their MT burden approximated that in young monkeys; however, when older monkeys were sedentary, MT burden was dramatically increased. MT levels were positively associated with inflammatory burden and negatively associated with skeletal muscle insulin sensitivity. Time spent being active was positively associated with insulin sensitivity as expected, but this relationship was specifically modified by the individual monkey's MT, not

inflammatory burden. Our data supports clinical observations that MT interacts with physical function as a factor in healthy aging.

Keywords Endotoxemia · Sarcopenia · Insulin sensitivity · Non-human primate · Intestinal barrier function

Introduction

Microbial translocation (MT) is the process by which intestinal microbial products, such as lipopolysaccharide (LPS), pass across the intestinal mucosa to the submucosa and into the portal circulation. There, inflammatory responses can be initiated and circulated to peripheral tissues such as skeletal muscle. Normally, these bacterial products are efficiently cleared by local immune defenses in the intestinal wall, lymph nodes, and liver. However, increased MT occurs in aged rodents (Ma et al. 1992) and invertebrate models (Rera et al. 2012) compared to younger groups. For example, fruit flies with impaired intestinal barrier function have reduced physical activity, and their loss of intestinal barrier function predicted imminent death (Rera et al., 2012). Recent studies have documented that aged people also have greater MT (Ghosh et al., 2014), and that even in healthy older people, MT specifically relates to physical function indices (Stehle et al., 2012). Function of the intestinal barrier declines with aging—an effect that is evolutionarily conserved across species and

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suggests that aging effects, not diet, may primarily drive intestinal leakiness and MT.

MT contributes to systemic inflammation. Microbial products in the portal circulation travel directly to the liver, where most hepatic cells express members of the toll-like innate immune receptor family (TLRs). Binding of bacterial products to TLRs incites a signaling cascade that terminates with increased expression of a range of inflammatory cytokines, driven by nuclear factor kappa B. Soluble CD14 (sCD14) is a glycoprotein that is along with lipopolysaccharide-binding protein 1 (LBP-1), is secreted from the liver as a result of TLR activation, and circulates to help bind bacterial products. LBP-1 and sCD14 are both used as biomarkers to estimate an individual's MT burden and are related to inflammatory cytokine levels (Stehle et al., 2012; Reiner et al., 2013).

Advancing age and age-related comorbidities are major risk factors for developing sarcopenia, which is defined as the loss of muscle mass and function (Cruz-Jentoft et al., 2010). Although inflammatory cytokines have deleterious effects on multiple organs (Balzan et al., 2007), they are particularly important in sarcopenia and frailty development. However, the source of inflammation in geriatric patients is not well-characterized to date. Changes in body composition, often as greater visceral adiposity, may be a common source of systemic inflammation in older patients (Levine and Crimmins, 2012); however, inflammation persists in lean individuals and can be absent from overweight individuals. These inconsistencies suggest "inflamm-aging" is not yet fully understood or fully explained by altered fat deposition, and that other causes (e.g., intestinal barrier dysfunction leading to low-grade endotoxemia) should be investigated.

MT reduces insulin sensitivity; this is metabolically significant as insulin sensitivity affects muscle anabolism and thus maintenance of muscle mass (Kelly et al., 2012). Circulating concentrations of LBP-1 differed in people who were normal, insulin-resistant, or had type 2 diabetes (Moreno-Navarrete et al., 2012); however, participants also had significant differences in age and diet. In rodent experiments where age was controlled, increasing TLR-mediated inflammation in peripheral tissues by multiple methods (e.g., infusing LPS, diet-induced endotoxemia, or microbiomic alterations) all caused reduced insulin sensitivity (Cani et al., 2007, 2008). Evidence from a few clinical studies shows that MT increases with age (Reiner et al., 2013; Ghosh et al.,

2015). Thus, we hypothesized that aging monkeys would have higher MT rates than younger counterparts. This change in MT would then increase inflammation and decrease muscle insulin sensitivity. The importance of this topic pertains to understanding the sources of inflammation in aging, and what is the magnitude of the inflammatory effect on clinically relevant tissues. Clinical studies of aged people are unavoidably confounded by dietary differences which have been shown to drive disability through changes in both inflammatory status and microbiomes (Claesson et al., 2012). We have demonstrated in this monkey model that intestinal barrier dysfunction and resultant inflammation is an age-related change seen in the absence of microbiome changes (Mitchell et al., 2016). This allows us to isolate the gut in a healthy aging primate and document the co-occurrence of changes in other aging tissues. The aim of our study was to examine the interactions between inflammation and insulin sensitivity in muscle and sarcopenia in healthy monkeys that were aging under consistent environmental conditions, including diet which is known to significantly influence MT rates (Cani et al., 2008).

Methods

Animal procedures All study procedures were approved by and performed in accordance with the Wake Forest University Institutional Animal Care and Use Committee. Monkeys in this study were female and came from a multigenerational pedigreed colony of vervet monkeys (*Chlorocebus aethiops sabaues*). The age range of monkeys used in the studies described extended from 6 to 27.3 years (maximum lifespan ≈ 27 years; Table 1) which corresponds to a human age range of approximately 20–90 years. In this species, the mean age of "young" monkeys approximates that of a 25- to 30-year-old person, and the mean age of the "old" monkeys approximates that of a 60- to 65-year-old person.

Two studies are included in this report (Figure S1). In the first study, 51 vervet monkeys were assessed for physical function, MT, and inflammatory biomarkers. Vervets were housed in large indoor/outdoor corrals (173 m² in size) that contained elevated perches, platforms, and climbing structures. In subsets of this cohort, we obtained additional data on muscle mass by computer tomography (CT) image analysis or measures of

Table 1 Baseline characteristics, sarcopenia measures, and physical function observations of old ($n = 26$) and young ($n = 25$) monkeys

Variable	Units	Young	Old	<i>p</i> value
Age	years	9.4 (0.34)	21.0 (0.43)	0.003
Weight	kg	5.69 (0.18)	5.14 (0.17)	0.02
Waist	cm	36.2 (0.93)	34.9 (0.91)	0.15
Body mass index	kg/m ²	28.7 (0.88)	27.2 (0.95)	0.12
Glucose	mg/dL	69 (3.06)	80 (5.40)	0.04
Insulin	μIU/mL	12.4 (1.80)	17.3 (5.56)	0.21
Triglycerides	mg/dL	44.6 (3.93)	51.4 (4.12)	0.12
High-density lipoprotein cholesterol	mg/dL	89.4 (7.31)	75.5 (5.73)	0.07
Total plasma cholesterol	mg/dL	187 (12.84)	167 (8.55)	0.11
Muscle attenuation	HU	53.3 (0.56)	53.5 (0.67)	0.43
Hind limb fat	%BW	3.08 (0.20)	2.66 (0.37)	0.16
Thigh cross-sectional area	cm ²	95.0 (5.17)	101.4 (4.95)	0.19
Fat/lean	AU	0.23 (0.02)	0.21 (0.04)	0.37
Walk/run	Freq/h	50.2 (5.47)	52.3 (5.61)	0.40
Climbing	Freq/h	16.0 (3.04)	10.5 (1.96)	0.06
Leaping	Freq/h	13.88 (2.29)	9.27 (1.76)	0.06
Total locomotion	Freq/h	80.1 (10.37)	72.0 (8.82)	0.28

Means with standard errors of the mean presented in parentheses are shown

muscle insulin sensitivity (Figure S1). In the second study, a separate additional cohort of old vervet monkeys that were either corral-housed (as described above) or sedentary in cage housing (0.58 m² in size) was assessed for MT and inflammatory biomarker concentrations. All monkeys were fed a low-fat, high-carbohydrate primate laboratory chow (Diet 5038, Lab Diet, Purina, St. Louis, MO) supplemented with fresh produce five times per week for the duration of the study. All vervets had ad libitum access to food and water and were maintained in stable environmental, dietary, and social conditions. Complete details of sample sizes for both studies and variables measured are presented as Supplementary Data (Figure S1).

For blood collections, monkeys were sedated with ketamine hydrochloride (15 mg/kg intramuscularly) to enable femoral venipuncture. Plasma was processed and stored at −80 °C. All analyses were measured by ELISA in plasma and endpoints included the following: LBP-1 (Hycult Biotech, Netherlands), C-reactive protein (CRP; ALPCO Inc., Salem, NH), interleukin 6 (IL-6), and sCD14 (R&D Systems, Minneapolis, MN). Fasting insulin, glucose, lipids, and lipoprotein concentrations were measured as previously described (Kavanagh et al., 2007b).

Physical activity assessments Observations of activity were made while each monkey was voluntarily moving in the outdoor portion of corral housing units. All assessments were collected at comparable times of day during the same season. Four 15-min observation periods per monkey were conducted to record instances of walking/running, climbing, and leaping (all expressed as frequency per hour). Walking speed was calculated as distance / time based on the time it took to pass between landmarks of known distance. Five estimates of walking speed were obtained per monkey. Any motivated walking (e.g., during feeding time or negative social interactions) was excluded. Activity assessments were conducted as previously described (Choi et al., 2012).

Body composition Waist circumference was measured for each monkey by placing a measuring tape around the abdomen at the level of the umbilicus and the circumference recorded in centimeters. Thigh circumference was measured at the midpoint between the hip and knee on both legs and averaged. Body mass index (BMI) was calculated using the crown-rump length (sitting height). Previously obtained CT scans were available from 27 monkeys (Table S1 and Figure S1). Resultant images were reconstructed with TeraRecon Aquarius Intuition

software (Foster City, CA). The quadriceps midpoint was calculated as the images slice that fell mid-distance between the sacroiliac junction and the patella. On the midpoint slice, muscle attenuation values were obtained from three regions of interest placed over the quadriceps musculature of each leg, and all six Hounsfield unit (HU) values were averaged for a final estimate. The images were further analyzed for composition by applying HU thresholds to isolate lean tissue (-5 to 135 HU) and fat tissue (-140 to -40 HU) as previously described (Kavanagh et al., 2007b). Lean tissue area at the midpoint slice was recorded as cross-sectional area. The regional tissue composition of the entire hind limb mass was calculated by isolating only images distal to the femoral head to the toes. The resulting tissue volumes were corrected for muscle density of 1.055 g/cm^3 or fat density of 0.918 g/cm^3 and expressed as percentage of total body weight.

Insulin sensitivity Basal- and insulin-stimulated skeletal muscle biopsies were collected from 20 monkeys (Table S1 and Figure S1) in a procedure previously described (Kavanagh et al., 2007b). Muscle biopsies were taken from the *vastus lateralis* muscle, and then regular insulin was infused into a peripheral vein at $40 \text{ U/m}^2/\text{min}$ for 15 min. This amount was predicted to increase the average circulating insulin concentration to $100 \text{ } \mu\text{U/mL}$ (DeFronzo et al., 1979). A second muscle biopsy sample was collected adjacent to the site of the first biopsy during the final minute of insulin infusion, frozen in liquid nitrogen, and stored at -80°C until analysis. Protein was extracted from biopsy samples and analyzed for total and phosphorylated (activated) Akt levels by ELISA, according to the manufacturer's recommendations (Biosource, Camarillo, CA). Akt activation is the last committed step in the insulin signaling pathway before the glucose transporter gets translocated to the cell membrane. In our laboratory, Akt activation has been a reliable indicator of insulin resistance in muscle (Kavanagh et al., 2007b). Data are reported as the induction of total protein activated with insulin.

Data analysis Data are presented as mean $\pm$ standard error of mean (SEM) for each group. Group sizes for each measure are indicated in figure and tables. Data were analyzed for normality and logarithmically transformed where necessary (IL-6, Akt-p induction with insulin) before analysis of variance (ANOVA) was performed to assess for group differences. Post hoc

determinations of group differences were done using Tukey's honestly significant difference tests. Associations were calculated by Pearson's correlation coefficients. Mediation analyses were done by assessing the significance of partial correlation models with covariates. Multiple regression models were created with backward stepwise removal of variables. Statistical analysis was performed using Statistica v10 (StatSoft Inc. Tulsa, OK). Significance was set at $\alpha \leq 0.05$ for group differences and ≤ 0.10 for trends.

Results

Monkeys naturally aging under optimal conditions (e.g., no predators, healthy diet, stable social conditions, and opportunity to freely exercise) show few and mild metabolic deteriorations with age (Table 1). Higher fasting glucose concentrations were observed in the older monkeys, although these remained in the normal reference range for vervets (Chichester et al., 2015). Older monkeys also weighed less and showed a trend towards lower HDL cholesterol concentrations (16 % lower). This difference could be biologically meaningful in the context of a cholesterol-free diet.

Old monkeys were still sarcopenic despite their overall good health, as defined by reduced muscle mass and function (Fig. 1). In multivariate regression models, age was the only significant variable predicting walking speed ($R^2 = 0.47$, $p = 0.0005$) which explained 25 % of the variance in outcomes. Trends for reduced leaping and jumping were seen in old monkeys (Table 1). However, the total time old monkeys spend walking or running (locomoting) was not different, although they were slower than their younger counterparts.

Muscle density and adiposity by CT analysis were comparable. Older, sarcopenic (but still active) monkeys had inflammatory biomarker levels comparable to young monkeys (Table 2). Microbial translocation markers were numerically higher in old active monkeys, with LPB-1 concentrations 30 % higher on average compared to young active monkeys ($p = 0.17$). Sedentary old monkeys had marked higher LBP-1 concentrations compared to age-matched monkeys that were still active (Table 3). These sedentary old monkeys were sourced from the same colony and still consumed the same healthy diet but were sedentary. Changes in LBP-1 were seen in the absence of differences in age,

Fig. 1 Older healthy monkeys are sarcopenic. Walking speed was slower (**a**; $n = 51$; $p < 0.001$), and muscle mass was reduced as measured by leg circumference (**b**; $n = 22$, $p = 0.05$) and by CT-

determined total lean tissue mass measured from both hind limbs (**c**; $n = 27$, $p = 0.04$) in old monkeys. * $p < 0.05$, *** $p < 0.001$

weight, or metabolic health biomarkers (Table 3). Active old monkeys had LBP-1 levels comparable to the young and old monkeys in the larger cohort (Table 2). In our larger active cohort, MT biomarkers were positively and significantly associated with inflammatory burden (Fig. 2). LBP-1 and sCD14 were moderately associated with CRP ($r = 0.59$, $p = 0.001$ and $r = 0.24$, $p = 0.07$, respectively).

Sarcopenia is believed to be linked to chronic sub-clinical inflammatory processes and resultant insulin resistance, leading to reduced anabolic signaling in muscle. Old monkeys were not insulin resistant, since induction of Akt phosphorylation with insulin stimulation was comparable to that in young monkeys (Table S1; $p = 0.39$). Insulin stimulation was confirmed by measurement of blood glucose, which decreased on average by 17 %, and by the average 98 % increase in muscle Akt-p levels across the 15-min insulin infusion period. Consistent with our observations from healthy active monkeys that MT is associated with inflammation (Fig. 2), LBP-1 was associated with insulin sensitivity (Fig. 3a). Monkeys with higher concentrations of LBP-1 had little to no response to insulin. The high LBP-1 concentrations in active monkeys were lower than levels measured from sedentary monkeys (Table 3), so marked

inhibition of insulin signaling in muscle tissue of old, sedentary monkeys could be expected.

As expected, insulin sensitivity was also positively associated with activity levels (Fig. 3b). LBP-1 levels had a weak negative relationship with activity levels ($r = -0.15$, $p = 0.17$). To explore interactions between insulin sensitivity and physical function further, we examined partial correlation coefficients with models including MT and inflammatory markers as covariates. MT specifically accounted for 13–17 % of the variability in the relationship between insulin sensitivity and physical function in healthy monkeys (Table 4). When MT is accounted for in the model, the relationship between insulin sensitivity and physical function became non-significant. In contrast, inflammatory biomarkers did not significantly influence this relationship.

Discussion

Our study is the first to combine measures of MT, physical function, and insulin sensitivity in a primate model. Our monkey model faithfully represents the spectrum of age-associated diseases in people, including sarcopenia and metabolic disorders (Kavanagh et al.,

Table 2 Circulating concentrations of selected microbial translocation and inflammatory biomarkers in active young and old

Variable	Units	N (young/old)	Young	Old	<i>p</i> value
LBP-1	μg/mL	20/22	3.03 (0.60)	3.94 (0.71)	0.17
sCD14	μg/mL	21/23	2.05 (0.10)	2.21 (0.14)	0.17
CRP	ng/mL	13/17	13.4 (4.73)	151 (2.09)	0.37
IL-6	pg/mL	11/16	0.51 (0.17)	0.64 (0.25)	0.35

Means with standard errors of the mean presented in parentheses are shown

LBP-1 lipopolysaccharide-binding protein 1, sCD14 soluble CD14, CRP C-reactive protein, IL-6 interleukin 6

Table 3 Sedentary old monkeys had greater microbial translocation levels despite comparable general health

Variable	Units	N (active/sedentary)	Active old	Sedentary old	p value
Age	years	8/16	19.9 (0.49)	20.5 (0.76)	0.58
Weight	kg	8/16	5.80 (0.35)	5.16 (0.24)	0.14
Waist	cm	8/16	39.7 (1.70)	37.0 (1.58)	0.29
Glucose	mg/dL	8/16	82 (17)	94 (21)	0.98
CRP	ng/mL	8/16	56.4 (6.87)	30.5 (5.84)	0.23
LBP-1	μg/mL	7/7	2.80 (0.71)	26.3 (6.56)	0.01
sCD14	μg/mL	7/12	0.39 (0.01)	21.0 (10.6)	0.08

Means with standard errors of the mean presented in parentheses are shown

2007a; Choi et al., 2012), and this species has comparable intestinal structure and immune function to people (Gibbons and Spencer, 2011). We demonstrate that healthy aging (optimal diet and maintained activity levels) prevents the development of metabolic disease and increases in MT, but does not prevent sarcopenia. This protection from age-associated intestinal barrier dysfunction is lost when monkeys become sedentary and biomarkers of MT increase. The MT burden of individual monkeys was related to both levels of inflammatory biomarkers and insulin sensitivity of muscle tissue. Insulin sensitivity was related to levels of physical activity, but MT and not inflammation specifically accounted for a portion of the variance in associations between activity levels and insulin sensitivity.

Intestinal “leakiness” occurs with advancing age across many species (Ma et al., 1992; Rera et al., 2012; Tran and Greenwood-Van Meerveld, 2013; Ghosh et al., 2014), but only recently has become recognized as an important mediator of healthspan, lifespan, and insulin signaling in invertebrates (Rera et al., 2012; Ulgherait et al., 2014). A “leaky” mucosa allows greater translocation of microbial antigens into the intestinal wall and portal circulation, inciting inflammation both locally and remotely. We examined the

interaction between systemic inflammation and insulin resistance on muscle, because level of insulin resistance modifies important relationships such as those between obesity and sarcopenia (Levine and Crimmins, 2012). Muscle anabolism and regeneration are both impaired in aging (Fry et al., 2011; Vasilaki and Jackson, 2013). Insulin resistance also impairs important anabolic signaling in muscle tissue, thus promoting sarcopenia (Timmerman et al., 2010). We propose that intestinally derived bacterial products reduce effectiveness of insulin signaling and responses to insulin’s anabolic signaling. Exercise training increases both insulin sensitivity and muscle mass in the elderly (Ogawa et al., 2010), and the third National Health and Nutrition Examination Survey reported that skeletal muscle index was related to insulin sensitivity, even after adjusting for obesity measures and age (Srikanthan and Karlamangla, 2011). Maintaining insulin sensitivity by eliminating chronic low-grade inflammation (which may be the result of MT) could support muscle repair and regeneration, thus aiding the maintenance of muscle mass and function in aging. Invertebrate studies support this direct link between intestinal barrier function and physical function, muscle mass, and myocyte apoptosis (Rera et al., 2012; Ulgherait et al., 2014).

Fig. 2 Microbial translocation biomarkers lipopolysaccharide-binding protein (LBP-1, $n = 42$; **a**) and soluble CD14 (sCD14, $n = 44$; **b**) positively associate with inflammatory burden as estimated by C-reactive protein concentrations in plasma from healthy, active monkeys

Fig. 3 **a** Greater microbial translocation as measured by LBP-1 concentrations in the circulation of young and old monkeys is associated with reduced insulin signaling effectiveness in skeletal

muscle. **b** Better insulin sensitivity is associated with greater levels of spontaneous activity in all monkeys ($N = 20$)

Stehle et al. reported that greater MT was associated with worsening physical function in older people and that this relationship was also independent of inflammatory cytokines (Stehle et al., 2012), consistent with results from the current study. To date, only two exercise intervention trials in older people have been published with opposing results on muscle toll-like receptor 4 (TLR4) levels, which like LBP-1 reflects MT burden (Ghosh et al., 2014; Rodriguez-Miguelez et al., 2015). Ghosh et al. found that an intense exercise program did not reduce muscle levels of TLR4 in a US population of sedentary, normal-weight, aged individuals. However, Rodriguez-Miguelez et al. reported that TLR4 levels dropped 50 % in overweight, older participants following a low-intensity vibration-platform-based exercise intervention. In the study by Ghosh et al. (Ghosh et al., 2014), circulating LBP-1 concentrations were close to double those in young people, as were muscle TLR4 receptor levels. In our study of vervet monkeys, increased LBP-1 levels per se were not associated with age, but the combination of being old and sedentary resulted in ninefold greater LBP-1 concentrations. The participants in the clinical studies just cited were sedentary based on exercise frequency, but spontaneous movement also may have been lower in older participants. The clinical trial by Rodriguez-Miguelez et al. (Rodriguez-Miguelez et al., 2015) demonstrated that low-intensity exercise halves the expression of TLR4 in muscle, which would also be expected to lower circulating LBP-1 concentrations. Possible explanations for these opposite outcomes include differences between US and Mediterranean diets, baseline physical activity levels that differ by culture (Hallal et al., 2012), or reductions in total daily activity among older people in high-intensity exercise trials (Washburn et al., 2014). In obese rodents, exercise reduced MT which supports

clinical results reported by Rodriguez-Miguelez et al. (Oliveira et al., 2011) and is consistent with the differences in LBP-1 from active and sedentary old monkeys.

In the study by Stehle et al., the specific relationship between LBP-1 and a composite measure of physical function (the short physical performance battery) was unaltered by adjustment for IL-6 and CRP (Stehle et al., 2012), as observed in healthy monkeys in our study. Even the strength of the unadjusted relationship (regression coefficient for people was -0.59) was similar to what we report in Table 4. If better composite measures of physical function were validated in monkeys, the strength of the association with LBP-1 levels may improve.

Preservation of insulin sensitivity is associated with health- and lifespan in multiple animal models and in humans (Atzmon et al., 2004; Brown-Borg and Bartke, 2012; Ulgherait et al., 2014). Our data suggest that becoming sedentary leads to greater MT and endotoxemia, which in rodent models initiates reduced

Table 4 Mediation analyses

Covariates	R value	p value
None	0.52	0.02
CRP	0.50	0.03
IL-6	0.53	0.02
CRP + IL-6	0.50	0.03
LBP-1	0.45	0.06
sCD14	0.47	0.04
LBP-1 + sCD14	0.43	0.08

Inflammatory processes did not significantly influence the relationship between physical function and insulin sensitivity; however, microbial translocation covariates reduced the association to non-significance

insulin signaling effectiveness and metabolic disease (Cani et al., 2007; Amar et al., 2011). This effect of endotoxin and insulin signaling has been replicated in human skeletal muscle myocytes and was reversible with blockade of LPS binding (Liang et al., 2013). Both being sedentary and insulin resistant contribute to reduced muscle anabolic signaling and permit loss of muscle mass, which sets the stage for development of frailty and its clinical consequences. These data implicate MT as a proximal cause of insulin resistance in muscle, even in healthy individuals, which may be a modifiable pathway (Ma et al., 1992).

Factors known to increase MT include diet and age, and these factors interact to predict health outcomes (Claesson et al., 2012; Reiner et al., 2013; Ghosh et al., 2015). Time and diet factors, along with changes in the microbiome, typically shift concomitantly in people, making it difficult to identify why intestinal leakiness increases with aging. We have demonstrated in this monkey model that becoming aged does not result in a changed microbiome (Mitchell et al., 2016), consistent with the stability of diet and physical environmental influences. This is a strength of our study that is unique to the non-human primate model. We have repeatedly assessed the fecal microbiome in young and old monkeys from this colony and found no differences. This is not unexpected as human studies indicate high stability of individual microbiomes, and microbiome shifts occur slowly in response to changes in diet and environment (Claesson et al., 2011; Claesson et al., 2012). Another strength is our thorough measurement of movement in terms of speed, type, and duration enabling us to observe that old monkeys are similarly active as young monkeys in these ideal conditions however tend to restrict their activity to slow walking. Thus, we can demonstrate that despite functional changes, aging healthy monkeys show mild metabolic perturbations. We also found that removing normal physical activity increased LBP-1, which in active monkeys influences both insulin sensitivity and the inflammatory burden.

Our study also has some limitations. These include the fact that we assessed only female monkeys in a cross-sectional manner, and not all endpoints were available for all monkeys. We also did not measure LPS levels; however, LBP-1 is an accepted and reliable measure of integrated LPS exposure. We also were limited in our inability to fully characterize local mucosal immunological responses, as immunosenescence is present in aged vervet monkeys and is an important

phenotype of older people (Haberthur et al., 2010). Immune suppression within mesenteric adipose tissue, and resultant loss of remodeling capacity, has been proposed as an age-related change that results in decreased intestinal barrier function and insulin resistance in rodent models (Wernstedt Asterholm et al., 2014; Bapat et al., 2015). It is possible that waning mucosal immunity and consequential gut leakiness with aging may be offset with moderate exercise as has been seen in rodents (Oliveira et al., 2011), which has implications for clinical geriatricians and physicians managing older patients.

We conclude that monkeys aging under ideal conditions with respect to activity, diet, and social stability still become sarcopenic despite good metabolic health and normal MT burden. MT is related to inflammation and insulin sensitivity and markedly increases in old monkeys that are sedentary. MT specifically modifies the relationship between physical activity and skeletal muscle insulin sensitivity, as is also demonstrated in people, and highlights MT as an important pathway contributing to maintaining muscle function. Reduced physical activity augments MT, which could lead to a vicious cycle propagating sarcopenia in normal aging through reduced insulin sensitivity of muscle. As aged people are at risk for sarcopenia and accumulating comorbidities that reduce the ability to be physically active, steps to maintain intestinal barrier function in the elderly may be an avenue to limit loss of muscle mass and function.

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Compliance with ethical standards All study procedures were approved by and performed in accordance with the Wake Forest University Institutional Animal Care and Use Committee.

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