Exploring the effects of six weeks of resistance training on the fecal microbiome of older adult males

by

Johnathon H. Moore

A Dissertation Submitted to the Graduate Faculty of

Auburn University

In partial fulfillment of the Requirements for the

Degree of Doctor of Philosophy

Auburn, Alabama

August 7, 2021

Keywords: Microbiome, Skeletal Muscle, Aging, Resistance Training

Approved by:

Michael D. Roberts, Chair, Associate Professor, School of Kinesiology

Andrew D. Fruge, Co-Chair, Assistant Professor, Department of Nutrition, Dietetics, and Hospitality Management

Michael D. Brown, Professor, School of Kinesiology

James R. McDonald, Michael D. Brown, Associate Clinical Professor, School of Kinesiology

ABSTRACT

Recent advances in molecular biology have enabled researchers to study the gut microbiome. However, only limited evidence has examined whether resistance training alters the gut microbiome, and no studies in this regard have been performed in an older population. Therefore, the purpose of this study was to determine if 6 weeks of resistance training in older males: i) altered bacterial species suggestive of enhanced gut microbiome diversity, and/or ii) altered taxonomic units associated with gut health chosen *a priori* through an extensive literature search. Fecal samples were collected prior to and following a 6-week resistance training intervention (2x/week) in 16 older Caucasian males (65±9 years old, 28.1±3.1 kg/m^2) with minimal prior training experience. After training concluded, DNA was isolated from pre-and post-training fecal samples, and taxa were quantified using sequencing to amplify the variable region 4 (V4) of the 16S ribosomal RNA gene. Training significantly increased whole-body lean/soft tissue mass (determined by dual energy x-ray absorptiometry) as well as leg extensor strength ($p<0.05$). Markers of microbiome diversity as well as select bacteria chosen for analysis *a priori* were not significantly altered with training. However, MetaCYC pathway analysis indicated metabolic capacity of the microbiome to produce mucin increased; blood analysis indicated serum Zonulin was significantly decreased after training ($p<0.05$), further suggesting intestinal barrier integrity was improved. In conclusion, this study adds to the limited literature examining how resistance training affects the gut microbiome. Interestingly, our data suggest that resistance training in older Caucasians may improve intestinal barrier integrity, and warrant further in-depth research in this area.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my committee.

No more dedicated, willing, and understanding group of gentlemen could be found the world over.

Dr Roberts: your support, friendship, and mentorship in the face of a rambunctious student have led to this moment. No man could better foster direction in me than you. Thank you.

Dr Fruge: your willingness to mentor, teach and listen has better opened the world of research to me. Without your guidance, I would not have persevered to this point. Thank you.

Dr Brown: your willingness to believe in me will echo on in the students I teach. Your words of wisdom will not be forgotten. Thank you.

Dr McDonald: your continual support and belief in my slow but steady progress was a powerful force. Your teachings made my journey possible. Thank you.

Gentleman, thank you, it has been an incalculable honor to have been mentored, taught, and befriended by each of you.

Further, I would like to thank the Auburn University School of Kinesiology for opening its doors and providing a space to reach my full potential.

To the rowdy, boisterous and ingenious gentlemen, and ladies of the MASL family, past and present - thank you for your friendship. All of you will go on to achieve greatness.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

CSA,	cross-sectional area
DXA,	dual energy x-ray absorptiometry
LPS,	lipopolysaccharide
LSTM,	lean/soft tissue mass
pQCT,	peripheral quantitative computed tomography
rRNA,	ribosomal RNA
SCFA,	short-chain fatty acid

CHAPTER I: INTRODUCTION

Recent advances in sequencing techniques and metabolomics have enabled researchers to study the gut microbiome. The microbiome is an integral part of the human body, and it can affect everything from caloric absorption to immune function (59). Both genetic and metabolic diversity are present, as there are millions of genes and gene functions within the microbiome such that the gut microbiota reflect characteristics of their host including diet and lifestyle factors (35). Several groups have suggested the gut microbiome can affect key aspects of host fitness, such as development, fertility, and lifespan (45). Gut microbial differences are observed between children born vaginally or Cesarean section (31). Further, gut microbial adaptations occur with initiation and duration of breast-feeding (90), and dietary habits well into adulthood can continue to alter the gut microbiome. Thus, the environment can profoundly alter the composition of the gut microbiota (111).

Significant alterations in the human gut microbiome that are not quickly resolved result in dysbiosis, which can have detrimental effects on the host (123). Dysbiosis can also be induced through administration of antibiotics (43), which often induce compositional changes that can be permanent. In young children and infants, the use of antibiotics increase the likelihood of maladaptive bacterial species that can result in necrotizing enterocolitis (43), one of the leading causes of death for neonates (67). Given the widespread and ubiquitous use of antibiotics across the globe, there has been a subsequent rise in antibiotic-induced dysbiosis (127). Antibiotics have been observed to affect neuronal transmission within the gut, including a distorted glial network, and altered cholinergic, tachykinergic and nitrergic neuronal transmission (11). The increase in antibiotic-resistant bacterial infections across the world suggests greater disposition

to opportunistic infections and dysbiosis (40). Conversely, several supplemental therapies represent a robust measure against dysbiosis and antibiotic resistance (44). The use of prebiotics, probiotics and postbiotics has grown in recent years, as the need from clinicians and the desire of consumers to find novel, and less damaging modalities of gut health maintenance continue.

Prebiotics include several types of dietary fibers that are not broken down by pancreatic enzymes. Numerous bacteria flourish on these fibers, producing several metabolites including short chain fatty acids (SCFAs). The use of fibers, specifically high soluble fibers, alter different regions of the host's intestinal tract, directly and indirectly altering gut barrier integrity and functioning. This has the capacity to alter cholesterol and bile acid reabsorption (41). In human and animal models, Gram-negative bacteria produce lipopolysaccharides (LPS). Interestingly, the impact of LPS-induced inflammation is reduced by SCFAs (25). Several bacteria thrive on the mucosa and are found predominantly near the epithelium. Encroachment by pathogens has proinflammatory effects (6). These include cell pattern recognition receptors via gut associated lymphoid system. Involved receptors include toll-like receptors as well as nucleotide-binding oligomerization domain-contain protein receptors. Further, the release and regulation of protein metabolites and immunoregulating peptides play a role in gut microbiome regulation and augmentation (98, 104).

Several changes in the gut microbiome have been observed throughout middle and older age, most notably a reduction in diversity and susceptibility to pathogenic infections (128), maladaptive disease states such as Irritable Bowel Disease (34), and potentially with degenerative brain diseases such as Alzheimer's disease (109). Interestingly, the age-related degeneration of muscle tissue (i.e., sarcopenia) is accompanied by changes in microbiota, which has generated interest in the gut-muscle axis (114). Further, the microbiome can be acutely and

chronically altered via exercise (71). Rodent studies have explored the relationship between muscle and microbiota, finding that dysbiosis and gnotobiotic are associated with decreased muscle fiber size, physical performance, glucose metabolism, and neuromuscular communication (11, 59, 86). Given that resistance training enhances several of these characteristics in older populations (17, 55, 106), it remains plausible that these adaptations are mitigated, in part, through training-induced changes in the gut microbiome. However, to our knowledge, only two human studies examining longitudinal gut microbiome changes with resistance training exist (7, 20), and both studies were carried out in college-aged individuals. Therefore, the purpose of this study was to determine if 6 weeks of resistance training in older adult participants: i) improved microbiome diversity of fecal samples, ii) altered taxonomic units associated with gut health chosen *a priori* through an extensive literature search, and iii) affected overall metabolic function of host microbiota and metabolism specific to energy harvest and gut health. We hypothesized that six weeks of resistance training would favorably alter the gut microbiome of older participants. Specifically, we hypothesized that resistance training would improve microbiome diversity and positively affect certain taxonomic units associated with positive metabolic outcomes.

CHAPTER II: LITERATURE REVIEW

The following sections of this literature review will discuss studies that have used various techniques to elucidate how aging, exercise and various diseases affect the gut microbiome. Additionally, mechanistic rodent research using antibiotics, germ-free mice, and probiotics will be discussed. While there is emerging evidence of a muscle-microbiome connection, this evidence is sparse. Likewise, there is very little evidence examining how resistance training affects the gut microbiome, and whether resultant alterations are associated with resistance training adaptations. Thus, the culmination of this review will address these literature gaps and lead into to the purpose(s) of my dissertation question.

Gut microbiome characteristics

Merriam-Webster's Dictionary defines the microbiome as, "*A community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment and especially the collection of microorganisms living in or on the human body.*" When querying PubMed.gov with the search term "gut microbiome" it is evident that this area of research has exponentially blossomed, with 0-10 publications per year being published from the years 2000-2007, and 5,500-8,500 publications per year being published between 2018-2020. A healthy person is inhabited with trillions of microbes (19). Indeed, while this includes fungi, viruses, and archaea, the microbiome (when healthy) includes roughly 1000-2000 different bacterial taxa. It has been estimated that genome size of microbiota surpasses the human genome by 150 times, and that there are 10 times more bacterial cells than all human cells (99). Of these, a majority cannot be cultivated using traditional and current techniques (102). However, the availability of

culture-independent sequencing and metagenomic testing now available, which is quickly becoming cheaper, has allowed for a more complete analysis of the microbiome. One type of sequencing allows for the unique sequence identification of 16S ribosomal RNA (rRNA) of different species obtained from fecal samples (83). Indeed, the use of 16S rRNA sequencing has rapidly increased our comprehension of the gut microbiome (120). Further, the capacity to examine large swaths of the microbiome via sequencing has allowed for associations and correlations to develop in relation to microbiome changes and states of disease and maladaptation (15, 74). In this regard, sequencing endeavors have revealed that *Bacteroidetes* and *Firmicutes* account for nearly 99% of all the species in the microbiome (51). Further, it has been demonstrated that *Bacteroidetes* tends to be inversely proportional to *Firmicutes* (51).

While extensive, the microbiome does not directly interact with most cell types given that it is confined to the gastrointestinal tract. However, metabolites produced by the microbiome are candidates for microbiome-to-cell communication. Short-chain fatty acids (SCFAs) are the main metabolites produced by the microbiota through the anaerobic fermentation of indigestible fibers and resistant starch, and the most abundant microbiome-derived SCFAs include acetate, butyrate, and propionate (37, 70, 108). Acetate acts, in part, by binding to the G-protein coupled receptors GPR41 and GPR43 (FFAR2, FFAR3). These receptors are expressed in the colon, small intestine, and insulin-sensitive tissues including the liver, pancreas, and skeletal muscle (56, 88, 112). Additionally, SCFAs produced by bacteria, such as *Faecalibacterium*, are capable of entering into circulation where they can act as ligands for muscle cells and other tissues (27, 28). SCFA receptors have also been shown to play an important role in altering glucose metabolism and uptake, along with playing a role in insulin sensitivity (57). Further, mitochondrial biogenesis is modulated, in part, by SCFAs (50). Low-grade inflammation has been associated

with the reduction of SCFA-producing bacteria in the gut (118). Butyrate may be involved in the regulatory pathways responsible for increased ATP synthesis and improving metabolic efficiency (26). Butyrate can also inhibit histone deacetylase, which implicates its role in genetic regulation across a variety of cell types (122). Given the broad range of tissues affected, this shows the wide-ranging role SCFAs may exert within the body. Also notable, gram-negative bacteria produce lipopolysaccharides (LPS), and LPS can permeate the mucosal lining to interact with and adversely affect various cell types via inflammatory signaling. Interestingly, the impact of LPS-induced inflammation is reduced by SCFAs (25). The collective evidence presented above shows that, in addition to the compositional complexity of the microbiome, one must consider the flux of metabolites produced by different bacteria and how these may affect target tissues.

Muscle-microbiome axis

Skeletal muscle plays major roles in stability, locomotion, and force production, with further roles in endocrine function (52). Exercise, whether it be resistance or endurance training, can have long-lasting impacts on skeletal muscle physiology (48). Indeed, many exercise-induced adaptations occur due to localized signals (e.g., mTORC1 activation during resistance exercise or AMPK activation during endurance exercise). However, a bi-directional communication from the microbiome and endocrine system has emerged, and bacteria are capable of producing metabolites as a means of interaction between skeletal muscle and the gut microbiome (105). While the muscle-microbiome axis is indeed complex and still being elucidated, several rodent studies have established interesting relationships. For instance, the expression of the peroxisome proliferator-activated receptor (PPAR)-gamma transcription factor is altered in skeletal muscle via butyrate, a SCFA produced by numerous genera as discussed

above (72). Further, germ-free mice have been shown to present larger muscle fibers that are fewer in number with an overall reduced skeletal muscle weight compared to mice with intact microbiomes (59). Another study administered antibiotic treatments to healthy mice to assess the effects of microbiome alteration on various performance indices (86). The authors showed that the mice preformed more poorly on skeletal muscle endurance tests. However, the effects of the antibiotics on the endurance capacity of the mice were completely abated via reseeding the microbiome. Captui et al. (11) found the neuromuscular function in juvenile mice was hindered with the onset of dysbiosis. Specifically, C57Bl/6 juvenile male mice were administered antibiotic treatment for 14 days, and tissue was collected and analyzed using immunohistochemistry and western blots. The findings of the research suggested a decrease in myenteric plexus neurons, alterations in the glial network, and decrements in various neurotransmitters. *L. Plantarum*, when supplemented in healthy young mice, increases muscle weights, swim time and grip strength (14). Another study showed a decrease in LPS and systemic inflammation along with increased muscle mass when providing the prebiotic oligofructose to mice (9). Correlations have also been found with antibiotic use and muscle wasting in mouse models (73), and fecal transfers to mice exposed to antibiotics have been shown to promote muscle hypertrophy (86). Thus, ample evidence suggests skeletal muscle form and function are seemingly affected by alterations in the composition and metabolites produced by the gut microbiome.

Aging and the microbiome

Sarcopenia is an accelerated loss of muscle mass, and is typically viewed as an age-related health condition that primarily affects the elderly (84). Sarcopenia is exacerbated through

acute and chronic stressors such low-grade inflammation, inactivity, poor diet, and poor endocrine function (79). There is strong evidence for the overlap of sarcopenia and physical fragility (82). Further, anabolic resistance (i.e., the inability of sarcopenic muscle to hypertrophy) is heavily associated with insulin resistance and chronic low-grade inflammation (46). In general, older individuals tend to have a decrease in nutrient intake and absorption, and this can play a role in the development of sarcopenia (89, 121). Physical activity has been viewed as a countermeasure to stave off sarcopenia (62). In particular, strength training has used to combat sarcopenia given that it typically promotes myofiber hypertrophy, enhancements in insulin sensitivity, improved muscle capillary density, and mitochondrial biogenesis (78).

Aging itself is a catalyst that drives alterations in the gut microbiome. It has been reported that the microbiome robustly adapts until three years of age in humans, at which point it begins to stabilize and resemble the adult microbiome (49, 126). However, in older individuals, the rate of aberrant changes in microbiome composition accelerates roughly after 65 years of age (103, 113). In this regard, the relative proportions of *Bacteroidetes* predominate in the elderly compared to higher proportions of *Firmicutes* in young adults (129). Significant decreases in Bifidobacterial, Bacteroides, and Clostridium cluster IV have also been reported with aging (119). Until recently, the consequences of these changes over the lifespan have been understudied. However, there is now emerging evidence that microbiome dysbiosis may be a catalyst of physical frailty (69). Further, there are interesting associations between the gut microbiome and skeletal muscle aging. For instance, a review by Ticinesi et al. (115) provides evidence that the age-associated changes in the microbiome could influence muscle protein synthesis in addition to upregulating chronic inflammation via insulin resistance. Fielding et al. (39) found that the species *Barnesiella intestinihominis*, among a number of other genus and

family level bacteria, was associated with age-related muscle strength maintenance in older adults. Testosterone levels decrease with age in men, and alterations in gut microbiota may drastically alter the amounts of testosterone and other sex steroids in circulation (81). Poutahidis et al. (97) found that increasing the total number of *L. reuteri* bacterium via supplementation in rats increased testicular size and circulating testosterone in aged rats. Alpha-diversity is the total expression of bacterial species detected in a fecal sample using metagenomic testing. Interestingly, it has been reported that fecal alpha-diversity highly correlated with the Rockwood frailty index (53). As mentioned above, many of the microbiome effects on muscle may be modulated through SCFAs. In this regard, Walsh et al. showed that the administration of butyrate helped prevent an age-related reduction in muscle mass in rodents (94). While not the focus of this review, what should not be discounted is how poor dietary choices over the lifespan may adversely affect the gut microbiome. For instance, researchers have shown that young mice exposed to a high fat diet, which contributes to insulin resistance via low grade inflammation, experienced an increase in body weight along with decreased glucose tolerance and heightened circulating levels of LPS (8). This is problematic given that LPS elicits an inflammatory response and can induce skeletal muscle atrophy (33). Animal-based diets have also been shown to increase the abundance of bile-tolerant microorganisms (*Alistipes*, *Bilophila* and *Bacteroides*) while decreasing levels of Firmicutes that metabolize dietary plant polysaccharides (*Roseburia*, *Eubacterium rectale* and *Ruminococcus bromii*) (23). Notably, it has been argued that this can lead to increases in gut and systemic inflammation, which again, may adversely affect skeletal muscle.

Exercise and the microbiome

Exercise training has the potential to increase muscle mass or aerobic endurance. However, currently emerging evidence suggests the gut microbiome may also influence exercise adaptations (13). First, a number of animal models has shown that microbiome diversity increases with exercise training (29, 61, 68). Additionally, Scheinman et al. (107) found that, when isolating, culturing, and administering *Villanella atypical* from stool samples of an Olympic athlete to mice, the mice significantly increased performance on exhaustive treadmill runs. In a review titled "*Microbiota and muscle highway – two way traffic*" Hawley (47) highlights data from another study showing bacterial genus *Villanella*, which metabolizes acetate from lactate through the methylmanyl-CoA pathways, is more highly enriched in runners following a marathon. There are also interesting associations in this area of research. For instance, in premenopausal women, microbiome composition was found to correlate with aerobic fitness (125). Further, gut microbiota diversity is reduced in overweight/obese persons compared to the microbial diversity of professional athletes (16). These and other findings led Wosinska et al. (124) to conclude in a review article that athletic performance is altered by several species of bacteria, and the possibility of designer bacteria to enhance sports performance is seemingly feasible.

While the aforementioned findings have been informative, few studies have specifically explored the impact of exercise intervention studies on the microbiome in humans. Allen et al. (3) examined the effects of a six-week endurance training intervention in 18 obese and 14 lean individuals. Notably, the microbiome make-up between lean and obese individuals were different at the beginning of the study, and those differences were reduced following exercise intervention. Munukka et al. (85) observed only modest changes in overall community composition following a six-week endurance training intervention in 18 overweight women.

Cronin et al. (20) performed an eight-week combined aerobic and resistance training intervention study where 90 participants were randomized to one of three groups including exercise-only, exercise with a whey protein dietary supplement, and whey protein supplementation only. In short, the authors reported no significant changes in the microbiome due with exercise training. Byruca et al. (7) examined how either endurance training or resistance training for 8 weeks affected the gut microbiome. Interestingly, endurance exercise elicited more robust changes in the microbiome relative to resistance training, and this finding led authors to conclude that resistance training either does not affect the microbiome or does so in a more subtle manner. However, what should be noted is that the participants in this study were apparently healthy, younger adults. Thus, it remains unknown as to whether resistance training can affect the microbiome in older adult participants.

Purpose Statement

Muscle loss with aging has been associated with frailty and detrimental health outcomes and as stated above, the microbiome has been speculated to play a role in this process. Exercise in general has been shown to combat muscle loss with aging and as discussed above, may impact the gut microbiome. However, it is currently unclear as to whether resistance training affects the gut microbiome. Therefore, the purpose of this study was to determine if 6 weeks of resistance training in older adults (between ages 55-80 years old): i) improved alpha-diversity, and/or ii) altered taxonomic units associated with gut health chosen *a priori* through an extensive literature search. Moreover, we sought to determine if changes in gut microbiome markers with training were associated with various training adaptations. Fecal samples were collected approximately one week prior to and ~72 hours following the exercise intervention. Samples were processed to extract bacterial RNA and sent to a collaborator for 16S rRNA sequencing. Following

sequencing, bacteria were strategically interrogated as listed in Table 1. Notably, these bacteria were identified through a comprehensive literature review, where targets were selected based on prior literature deeming their involvement in health or muscle physiology. More details regarding training methodologies and analyses are presented in Chapter III. We hypothesized that six weeks of resistance training would favorably alter the gut microbiome of older participants. Specifically, we hypothesized that resistance training would improve alpha-diversity, taxonomic units associated with positive metabolic outcomes, and microbial metabolism specific to energy harvest and gut health.

INSERT TABLE 1 HERE

CHAPTER III: METHODS

Ethics approval

This study is a secondary analysis. The original study investigated the effects of protein supplementation with resistance training on skeletal muscle hypertrophy in older untrained individuals (60). Prior to any data collection, this study was approved by the Auburn University Institutional Review Board (IRB) (Protocol # 19-249 MR 1907), conformed to standards set by the latest revision of the Declaration of Helsinki, and was registered as a clinical trial (NCT04015479). Men and women aged 50-80 years with minimal resistance training experience, defined here as not having performed structured RT for at least three months prior, were recruited for this study. Participants were recruited via flier, email inquiry and newspaper advertisement. Interested participants were informed of the study and testing procedures either over the phone or face-to-face at the Auburn University School of Kinesiology. Eligibility criteria indicated that potential participants had to: i) be between the ages of 50-80 years old, ii) not actively be participating in structured RT for at least 3 months prior, iii) be free of metal implants, and iv) possess blood pressure readings within normal ranges, with or without medication (i.e. <140/90 SBP/DBP). Exclusion criteria included: i) individuals having a known peanut allergy, ii) individuals having a body mass index $\geq$ 35 kg/m^2, iii) individuals being exposed to medically necessary radiation in the last 6 months, or iv) individuals having a medical condition contradicting participation in a RT program, giving blood, or donating a skeletal muscle biopsy (i.e., blood clotting disorders or taking blood thinning medications). Participants deemed eligible based on the aforementioned criteria provided written and verbal consent to participate. A medical history questionnaire was obtained at the time of consenting and

participants were scheduled to return to the Auburn University School of Kinesiology to complete study procedures described below.

Study design

Participants reported to the School of Kinesiology on 16 separate occasions. Visit one (V1) included screening to determine eligibility, gathering consent and obtaining a health history. V1 also involved sending participants who consented to be in the study home with stool sample collection kits and food logs. For food logs, participants were instructed to record all food consumed over two weekdays and one weekend day. Participants were instructed to return the kit and food log prior to the first resistance-training day. Visit two (V2; PRE) included a testing battery comprised of urine specific gravity (USG) testing, height and body mass assessments, assessment of the right leg vastus lateralis (VL) muscle thickness using ultrasound, a full body dual-energy x-ray absorptiometry (DXA) scan, a peripheral quantitative computed tomography (pQCT) scan at the mid-thigh of the right leg, and a right leg strength assessment using an isokinetic dynamometer. V3 included the participant's first muscle tissue sample collection and the participant's first resistance exercise bout. V4 included the participants' second muscle biopsy. Visits five (V5) through fifteen (V15) were supervised workouts at the Auburn University School of Kinesiology. During V15 participants were provided with their second stool collection kit and food log. Visit sixteen (V16; POST) occurred roughly 72 hours following V15, and included a repeat of the V2 testing battery. Specific testing methodologies are detailed below.

Pre- and Post-intervention Testing Battery

The testing sessions described below occurred during morning hours (05:00–09:00) following an overnight fast for all but a small subset of participants who reported to the laboratory after working hours at 17:00-18:30 following a ~4-5 hour fast.

Body Composition Assessments. During V2 and V16, participants reported to the Auburn University School of Kinesiology wearing casual sports attire (i.e. athletic shirt and shorts, tennis shoes). Participants submitted a urine sample (~5 mL) to assess USG levels using a handheld refractometer (ATAGO; Bellevue, WA, USA). Notably, all participants possessed USG values less than 1.020 indicating that they were well hydrated. Height and body mass were assessed using a digital column scale (Seca 769; Hanover, MD, USA) with mass and height being collected to the nearest 0.1 kg and 0.5 cm, respectively. Thereafter, right leg VL images were captured in the transverse plane using real-time B-mode ultrasonography (LOGIQ S7 Expert, GE Healthcare, USA) utilizing a multi-frequency linear-array transducer (3-12 MHz, GE Healthcare, USA) and subsequently analyzed for VL thickness. Participants were instructed to stand and displace bodyweight to the left leg to ensure the right leg was relaxed. Measurements were standardized by placing the transducer at the midway point between the inguinal crease and proximal border of the patella. All images were captured and analyzed by the same investigator (S.C.O.) with a 24-hr test-retest reliability using intraclass correlation coefficient ($ICC_{3,1}$), standard error of the measure (SEM), and minimal difference (MD) to be considered real of 0.991, 0.06, and 0.16 cm, respectively. Participants then underwent a full body dual-energy x-ray absorptiometry (DXA) scan (Lunar Prodigy; GE Corporation, Fairfield, CT, USA) for determination of total lean soft tissue mass (LSTM) and fat mass FM. Quality assurance testing

and calibration were performed the morning of data-collection days to ensure the scanner was operating to manufacturer specification. Scans were analyzed by the same technician using the manufacturer's standardized software. Test-retest reliability using $ICC_{3,1}$, SEM, and MD were previously determined for LSTM (0.99, 0.36, and 0.99 kg, respectively) and FM (0.99. 0.43, and 1.19 kg). Following the DXA scan, a cross-sectional image of the right thigh at 50% of the femur length was acquired using a pQCT scanner (Stratec XCT 3000, Stratec Medical, Pforzheim, Germany). Scans were acquired using a single 2.4 mm slice thickness, a voxel size of 0.4 mm and scanning speed of 20 mm/sec. All images were analyzed for total muscle cross-sectional area (mCSA, cm^2) and density (mg/cm^3) using the pQCT BoneJ plugin freely available through ImageJ analysis software (NIH, Bethesda, MD). All scans were performed and analyzed by the same investigator (K.C.Y.). Test-retest reliability using $ICC_{3,1}$, SEM, and MD was previously determined for mCSA (0.99, 0.84, and 2.32 cm^2, respectively).

Right Leg Isokinetic Strength Assessment. Participants performed maximal isokinetic right leg extensions on an isokinetic dynamometer (System 4 Pro, BioDex Medical Systems, Shirley, NY, USA). Participants were fastened to the dynamometer so that the right knee was aligned with the axis of the dynamometer. Seat height was adjusted to ensure the hip angle was approximately 90°. Prior to peak torque assessment, each participant performed a warmup consisting of submaximal to maximal isokinetic knee extensions. Participants then completed five maximal voluntary isokinetic knee extension actions at 60°/sec and 120°/sec. Sets were separated by 60 sec of rest. Participants were provided verbal encouragement during each set. The isokinetic extension resulting in the greatest peak torque value was used for analyses.

Resistance training

Participants completed supervised RT twice weekly for either ten weeks or six weeks. All training sessions were separated by at least 48 hours to allow for a period of recovery. Each RT session consisted of five exercises including seated leg press, leg extensions, lying leg curls, barbell bench press and cable pull-downs. For each exercise, participants performed three sets of 10-12 repetitions with 1 minute of rest between sets. At the end of each set, participants were asked to rate the level of difficulty where 0 = easy, 5 = moderate difficulty and 10 = hard. If values were below 7, weight was modestly added to increase exertion on the subsequent set. If values were 10, or the participant could not complete the set, weight was removed prior to the next set. Participants were encouraged to be as truthful as possible when assessing difficulty and were provided verbal encouragement and feedback during and following each set. The intent of this training method was to consistently challenge participants so that perceived exertion after each set of 10-12 repetitions was at a 7-9 rating. Training data for each participant were logged, allowing us to ensure that training effort was maximized within each training session, and participants were successfully implementing progressive overload in an individualized fashion. Notably, study personnel supervised all training throughout the study.

Food log analysis

Participants were instructed to self-report their habitual food intake for three consecutive days and return these food logs at V3 and V24 or V16 (10- and 6-week cohort, respectively). Participants were asked not to change their diet in any way. Study staff entered each food log into the Automated Self-Administered 24-Hour Dietary Assessment tool (ASA24), which uses the United States Department of Agriculture Food and Nutrient Database for Dietary Studies to provide values for 195 nutrients, nutrient ratios and other food components.

Fecal microbiome analysis

Immediately upon receipt of stool samples, stool was aliquoted and stored at -80°C until processing. Fecal microbial DNA was isolated using Zymo Research kits (Irvine, CA, USA, Cat. #D6010). DNA samples were prepared and polymerase chain reaction (PCR) using the Illumina Miseq instrument (San Diego, CA, USA) was employed to amplify variable region 4 (V4) of the 16S rRNA gene, which yielded the amplicon library for individual samples as described previously (10, 21, 58). Raw data files underwent FASTQ conversion using MiSeq reporter (58); UCLUST clustered sequences into amplicon sequence variants (previously operational taxonomic units [OTUs]) with a similarity threshold at 97%. Taxonomic assignments were issued using the Mothur classifier, and SILVA database (v 138.1) (100). ASVs with an average abundance <0.005% were not included in the final table, and remaining ASVs were grouped to summarize varying hierarchical levels.

Alpha-diversity of individual samples was measured using Observed Species, and Whole Tree Phylogeny. Beta-diversity was measured using Bray Curtis, Unweighted Unifrac, and Weighted Unifrac metrics to determine overall compositional change in the entire sample from baseline to follow-up. Further, Kruskal-Wallis one-way analysis of variance tests were performed to compare relative abundance of all OTUs, with false discovery rate (FDR) correction to determine differences between PRE and POST.

Functional genes were predicted based on MetaCYC database of metabolic pathways (12) by PICRUSt2 (phylogenetic investigation of communities by reconstruction of unobserved state 2) (32) based on 16S rRNA sequencing data (63). Longitudinal change in functional gene analysis was compared by Welch's t-test with Bonferroni correction using the software STAMP

2.1.3 (93). *A priori* selected MetaCYC pathways associated with SCFA production, mucin production, and mucin degradation were analyzed using MANOVA.

Statistical analysis

In addition to bioinformatics approaches related to microbiome metadata mentioned above, key dependent variables will include PRE and POST values of bacterial genera presented in Table 1. Secondary dependent variables will include: i) DXA LSTM changes from PRE to POST, ii) VL thickness changes from PRE to POST as determined by ultrasound, iii) pQCT-determined changes in mid-thigh muscle thickness from PRE to POST, and iv) self-reported dietary macronutrient intakes at PRE and POST. Critically, change scores in bacteria of interest will be associated with change scores in secondary dependent variables as another layer of analysis for this project.

All statistical analyses were performed using SPSS v26.0 (IBM Corp, Armonk, NY, USA). For all dependent variables over time, dependent samples t-tests were performed. Change scores (or delta scores) in key training variables were also calculated by subtracting PRE values from POST values, and these scores were associated with certain bacteria using Pearson correlations. Statistical significance was established as $p<0.05$, and relevant p-values are depicted in-text or within figures.

CHAPTER 4: MANUSCRIPT TO BE SUBMITTED TO A SCIENTIFIC JOURNAL

Exploring the effects of six weeks of resistance training on the fecal microbiome of older adult males

Johnathon H. Moore[1], Kristen S. Smith[2], Dongquan Chen[3], Donald A. Lamb[2], Morgan A. Smith[1], Shelby C. Osburn[1], Bradley A. Ruple[1], Casey Morrow[3], Kevin W. Huggins[2], James D. McDonald[1], Michael D. Brown[1], Kaelin C. Young[1,4], Michael D. Roberts[1,4], Andrew D. Frugé[1,*]

Affiliations: [1]School of Kinesiology, Auburn University, Auburn, AL 36849, USA; [2] Department of Nutrition, Dietetics and Hospitality Management, Auburn University, Auburn, AL 36849, USA; [3]University of Alabama at Birmingham, Birmingham, AL, USA; [4]Edward Via College of Osteopathic Medicine Auburn, AL 36832, USA

*Address correspondence to:
Andrew D. Frugé, PhD
Department of Nutrition, Dietetics and Hospitality Management
Auburn University
e-mail: fruge@auburn.edu

Abstract

Recent advances in molecular biology have enabled researchers to study the gut microbiome. However, only limited evidence has examined whether resistance training alters the gut microbiome, and no studies in this regard have been performed in an older population. Therefore, the purpose of this study was to determine if 6 weeks of resistance training in older males: i) altered bacterial species suggestive of enhanced gut microbiome diversity, and/or ii) altered taxonomic units associated with gut health chosen *a priori* through an extensive literature search. Fecal samples were collected prior to and following a 6-week resistance training intervention (2x/week) in 16 older Caucasian males (65±9 years old, 28.1±3.1 kg/m^2) with minimal prior training experience. After training concluded, DNA was isolated from pre-and post-training fecal samples, and taxa were quantified using sequencing to amplify the variable region 4 (V4) of the 16S ribosomal RNA gene. Training significantly increased whole-body lean/soft tissue mass (determined by dual energy x-ray absorptiometry) as well as leg extensor strength ($p<0.05$). Markers of microbiome diversity as well as select bacteria chosen for analysis *a priori* were not significantly altered with training. However, MetaCYC pathway analysis indicated metabolic capacity of the microbiome to produce mucin increased; blood analysis indicated serum Zonulin was significantly decreased after training ($p<0.05$), further suggesting intestinal barrier integrity was improved. In conclusion, this study adds to the limited literature examining how resistance training affects the gut microbiome. Interestingly, although microbiome diversity and several bacterial species were not altered, our data suggest that resistance training in older Caucasians may improve intestinal barrier integrity, and warrant further in-depth research in this area.

Keywords: resistance training, gut microbiome, aging, intestinal barrier integrity

Introduction

Recent advances in sequencing techniques and metabolomics have enabled researchers to study the gut microbiome. The microbiome is an integral part of the human body, and it can affect everything from caloric absorption to immune function (59). Both genetic and metabolic diversity are present, as there are millions of genes and gene functions within the microbiome such that the gut microbiota reflect characteristics of their host including diet and lifestyle factors (35). Several groups have suggested the gut microbiome can affect key aspects of host fitness, such as development, fertility, and lifespan (45). Gut microbial differences are observed between children born vaginally or Cesarean section (31). Further, gut microbial adaptations occur with initiation and duration of breast-feeding (90), and dietary habits well into adulthood can continue to alter the gut microbiome. Thus, the environment can profoundly alter the composition of the gut microbiota (111).

Significant alterations in the human gut microbiome that are not quickly resolved result in dysbiosis, which can have detrimental effects on the host (123). Dysbiosis can also be induced through administration of antibiotics (43), which often induce compositional changes that can be permanent. In young children and infants, the use of antibiotics increase the likelihood of maladaptive bacterial species that can result in necrotizing enterocolitis (43), one of the leading causes of death for neonates (67). Given the widespread and ubiquitous use of antibiotics across the globe, there has been a subsequent rise in antibiotic-induced dysbiosis (127). Antibiotics have been observed to affect neuronal transmission within the gut, including a distorted glial network, and altered cholinergic, tachykinergic and nitrergic neuronal transmission (11). The increase in antibiotic-resistant bacterial infections across the world suggests greater disposition to opportunistic infections and dysbiosis (40). Conversely, several supplemental therapies represent a robust measure against dysbiosis and antibiotic resistance (44). The use of prebiotics,

probiotics and postbiotics has grown in recent years, as the need from clinicians and the desire of consumers to find novel, and less damaging modalities of gut health maintenance continue.

Prebiotics include several types of dietary fibers that are not broken down by pancreatic enzymes. Numerous bacteria flourish on these fibers, producing several metabolites including short chain fatty acids (SCFAs). The use of fibers, specifically high soluble fibers, alter different regions of the host's intestinal tract, directly and indirectly altering gut barrier integrity and functioning. This has the capacity to alter cholesterol and bile acid reabsorption (41). In human and animal models, Gram-negative bacteria produce lipopolysaccharides (LPS). Interestingly, the impact of LPS-induced inflammation is reduced by SCFAs (25). Several bacteria thrive on the mucosa and are found predominantly near the epithelium. Encroachment by pathogens has proinflammatory effects (6). These include cell pattern recognition receptors via gut associated lymphoid system. Involved receptors include toll-like receptors as well as nucleotide-binding oligomerization domain-contain protein receptors. Further, the release and regulation of protein metabolites and immunoregulating peptides play a role in gut microbiome regulation and augmentation (98, 104).

Several changes in the gut microbiome have been observed throughout middle and older age, most notably a reduction in diversity and susceptibility to pathogenic infections (128), maladaptive disease states such as Irritable Bowel Disease (34), and potentially with degenerative brain diseases such as Alzheimer's disease (109). Interestingly, the age-related degeneration of muscle tissue (i.e., sarcopenia) is accompanied by changes in microbiota, which has generated interest in the gut-muscle axis (114). Further, the microbiome can be acutely and chronically altered via exercise (71). Rodent studies have explored the relationship between muscle and microbiota, finding that dysbiosis and gnotobiotic are associated with decreased

muscle fiber size, physical performance, glucose metabolism, and neuromuscular communication (11, 59, 86). Given that resistance training enhances several of these characteristics in older populations (17, 55, 106), it remains plausible that these adaptations are mitigated, in part, through training-induced changes in the gut microbiome. However, to our knowledge, only two human studies examining longitudinal gut microbiome changes with resistance training exist (7, 20), and both studies were carried out in college-aged individuals. Therefore, the purpose of this study was to determine if 6 weeks of resistance training in older adult participants: i) improved microbiome diversity as determined through the analysis of pre- and post-training fecal samples, ii) altered taxonomic units associated with gut health chosen *a priori*, and iii) affected overall metabolic function of host microbiota and metabolism specific to energy harvest and gut health. We hypothesized that six weeks of resistance training would favorably alter the gut microbiome of older participants. Specifically, we hypothesized that resistance training would improve microbiome diversity and positively affect certain taxonomic units associated with metabolic outcomes.

Materials and Methods

Ethics approval

This study is a secondary analysis, where 16 males that completed 6 weeks of resistance training were analyzed. The original study investigated the effects of peanut protein supplementation with resistance training on skeletal muscle hypertrophy in older untrained individuals (60). Nine of these subjects received the peanut protein supplement, and seven of these subjects received no supplement. Prior to any data collection, this study was approved by the Auburn University Institutional Review Board (IRB) (Protocol # 19-249 MR 1907), conformed to standards set by the latest revision of the Declaration of Helsinki, and was registered as a clinical trial

(NCT04015479). Men and women aged 50-80 years with minimal resistance training experience, defined here as not having performed structured RT for at least three months prior, were recruited for this study. Participants were recruited via flier, email inquiry and newspaper advertisement. Interested participants were informed of the study and testing procedures either over the phone or face-to-face at the Auburn University School of Kinesiology. Eligibility criteria indicated that potential participants had to: i) be between the ages of 50-80 years old, ii) not actively be participating in structured RT for at least 3 months prior, iii) be free of metal implants, and iv) possess blood pressure readings within normal ranges, with or without medication (i.e. <140/90 SBP/DBP). Exclusion criteria included: i) individuals having a known peanut allergy, ii) individuals having a body mass index $\geq$ 35 kg/m^2, iii) individuals being exposed to medically necessary radiation in the last 6 months, or iv) individuals having a medical condition contradicting participation in a RT program, giving blood, or donating a skeletal muscle biopsy (i.e., blood clotting disorders or taking blood thinning medications). Participants deemed eligible based on the aforementioned criteria provided written and verbal consent to participate. A medical history questionnaire was obtained at the time of consenting and participants were scheduled to return to the Auburn University School of Kinesiology to complete study procedures described below.

Study design

Participants reported to the School of Kinesiology on 16 separate occasions. Visit one (V1) included screening to determine eligibility, gathering consent and obtaining a health history. V1 also involved sending participants who consented to be in the study home with stool sample collection kits and food logs. For food logs, participants were instructed to record all food

consumed over two weekdays and one weekend day. Participants were instructed to return the kit and food log prior to the first resistance-training day. Visit two (V2; PRE) included a testing battery comprised of urine specific gravity (USG) testing, height and body mass assessments, assessment of the right leg vastus lateralis (VL) muscle thickness using ultrasound, a full body dual-energy x-ray absorptiometry (DXA) scan, a peripheral quantitative computed tomography (pQCT) scan at the mid-thigh of the right leg, and a right leg strength assessment using an isokinetic dynamometer. V3 included the participant's first muscle tissue sample collection, blood collection for serum analysis, and the participant's first resistance exercise bout. V4 included the participants' second muscle biopsy. Visits five (V5) through fifteen (V15) were supervised workouts at the Auburn University School of Kinesiology. During V15 participants were provided with their second stool collection kit and food log. Visit sixteen (V16; POST) occurred roughly 72 hours following V15, and included a repeat of the V2 testing battery in addition to a second blood draw and third muscle biopsy. Specific testing methodologies are detailed below.

Pre- and Post-intervention testing battery

The testing sessions described below occurred during morning hours (05:00–09:00) following an overnight fast for all but a small subset of participants who reported to the laboratory after working hours at 17:00-18:30 following a ~4-5 hour fast.

Body Composition Assessments. During V2 and V16, participants reported to the Auburn University School of Kinesiology wearing casual sports attire (i.e. athletic shirt and shorts, tennis shoes). Participants submitted a urine sample (~5 mL) to assess USG levels using a handheld refractometer (ATAGO; Bellevue, WA, USA). Notably, all participants possessed USG values less than 1.020 indicating that they were well hydrated. Height and body mass were assessed

using a digital column scale (Seca 769; Hanover, MD, USA) with mass and height being collected to the nearest 0.1 kg and 0.5 cm, respectively. Thereafter, right leg VL images were captured in the transverse plane using real-time B-mode ultrasonography (LOGIQ S7 Expert, GE Healthcare, USA) utilizing a multi-frequency linear-array transducer (3-12 MHz, GE Healthcare, USA) and subsequently analyzed for VL thickness. Participants were instructed to stand and displace bodyweight to the left leg to ensure the right leg was relaxed. Measurements were standardized by placing the transducer at the midway point between the inguinal crease and proximal border of the patella. All images were captured and analyzed by the same investigator (S.C.O.) with a 24-hr test-retest reliability using intraclass correlation coefficient ($ICC_{3,1}$), standard error of the measure (SEM), and minimal difference (MD) to be considered real of 0.991, 0.06, and 0.16 cm, respectively. Participants then underwent a full body dual-energy x-ray absorptiometry (DXA) scan (Lunar Prodigy; GE Corporation, Fairfield, CT, USA) for determination of total lean soft tissue mass (LSTM) and fat mass FM. Quality assurance testing and calibration were performed the morning of data-collection days to ensure the scanner was operating to manufacturer specification. Scans were analyzed by the same technician using the manufacturer's standardized software. Test-retest reliability using $ICC_{3,1}$, SEM, and MD were previously determined for LSTM (0.99, 0.36, and 0.99 kg, respectively) and fat mass (0.99. 0.43, and 1.19 kg). Following the DXA scan, a cross-sectional image of the right thigh at 50% of the femur length was acquired using a pQCT scanner (Stratec XCT 3000, Stratec Medical, Pforzheim, Germany). Scans were acquired using a single 2.4 mm slice thickness, a voxel size of 0.4 mm and scanning speed of 20 mm/sec. All images were analyzed for total muscle cross-sectional area (mCSA, cm^2) and density (mg/cm^3) using the pQCT BoneJ plugin freely available through ImageJ analysis software (NIH, Bethesda, MD). All scans were performed and analyzed

by the same investigator (K.C.Y.). Test-retest reliability using $ICC_{3,1}$, SEM, and MD was previously determined for mCSA (0.99, 0.84, and 2.32 cm^2, respectively). Following pQCT scans, right leg vastus lateralis (VL) images were captured in the transverse plane using real-time B-mode ultrasonography (LOGIQ S7 Expert, GE Healthcare, USA) utilizing a multi-frequency linear-array transducer (3-12 MHz, GE Healthcare, USA). Participants stood and displaced bodyweight to the left leg to ensure the right leg was relaxed. Measurements obtained at the midway point between the inguinal crease and proximal border of the patella. All images were captured and analyzed by the same investigator (S.C.O.) with a 24-hr test-retest reliability using $ICC_{3,1}$, SEM, MD to be considered real of 0.991, 0.06, and 0.16 cm, respectively. Images were analyzed for VL thickness using associated software.

Right Leg Isokinetic Strength Assessment. Participants performed maximal isokinetic right leg extensions on an isokinetic dynamometer (System 4 Pro, BioDex Medical Systems, Shirley, NY, USA). Participants were fastened to the dynamometer so that the right knee was aligned with the axis of the dynamometer. Seat height was adjusted to ensure the hip angle was approximately 90°. Prior to peak torque assessment, each participant performed a warmup consisting of submaximal to maximal isokinetic knee extensions. Participants then completed five maximal voluntary isokinetic knee extension actions at 60°/sec and 120°/sec. Sets were separated by 60 sec of rest. Participants were provided verbal encouragement during each set. The isokinetic extension resulting in the greatest peak torque value was used for analyses.

Resistance training

Participants completed supervised RT twice weekly for either ten weeks or six weeks. All training sessions were separated by at least 48 hours to allow for a period of recovery. Each RT

session consisted of five exercises including seated leg press, leg extensions, lying leg curls, barbell bench press and cable pull-downs. For each exercise, participants performed three sets of 10-12 repetitions with 1 minute of rest between sets. At the end of each set, participants were asked to rate the level of difficulty where 0 = easy, 5 = moderate difficulty and 10 = hard. If values were below 7, weight was modestly added to increase exertion on the subsequent set. If values were 10, or the participant could not complete the set, weight was removed prior to the next set. Participants were encouraged to be as truthful as possible when assessing difficulty and were provided verbal encouragement and feedback during and following each set. The intent of this training method was to consistently challenge participants so that perceived exertion after each set of 10-12 repetitions was at a 7-9 rating. Training data for each participant were logged, allowing us to ensure that training effort was maximized within each training session, and participants were successfully implementing progressive overload in an individualized fashion. Notably, study personnel supervised all training throughout the study.

Food log analysis

Participants were instructed to self-report their habitual food intake for three consecutive days and return these food logs at V3 and V24 or V16 (10- and 6-week cohort, respectively). Participants were asked not to change their diet in any way. Study staff entered each food log into the Automated Self-Administered 24-Hour Dietary Assessment tool (ASA24), which uses the United States Department of Agriculture Food and Nutrient Database for Dietary Studies to provide values for 195 nutrients, nutrient ratios and other food components.

Fecal microbiome analysis

Immediately upon receipt of stool samples by Auburn University staff (K.S.S. and J.H.M.), stool was aliquoted and stored at -80°C until processing. Fecal microbial DNA was isolated by Auburn University staff (K.S.S. and J.H.M.) using Zymo Research kits (Irvine, CA, USA, Cat. #D6010). Samples were then shipped to the University of Alabama Birmingham for DNA PCR analysis. DNA samples were prepared and polymerase chain reaction (PCR) using the Illumina Miseq instrument (San Diego, CA, USA) was employed to amplify variable region 4 (V4) of the 16S rRNA gene, which yielded the amplicon library for individual samples as described previously (10, 21, 58). Raw data files underwent FASTQ conversion using MiSeq reporter (58); UCLUST clustered sequences into amplicon sequence variants (previously operational taxonomic units [OTUs]) with a similarity threshold at 97%. Taxonomic assignments were issued using the Mothur classifier, and SILVA database (v 138.1) (100). ASVs with an average abundance <0.005% were not included in the final table, and remaining ASVs were grouped to summarize varying hierarchical levels.

The following bioinformatics methods were performed by Auburn University researchers (A.D.F. and J.H.M.). Microbiome diversity of individual samples was measured using Observed Species, Whole Tree Phylogeny, Shannon Index and Simpson Index. Beta-diversity was measured using Bray Curtis, Unweighted Unifrac, and Weighted Unifrac metrics to determine overall compositional change in the entire sample from baseline to follow-up. Further, Kruskal-Wallis one-way analysis of variance tests were performed to compare relative abundance of all OTUs, with false discovery rate (FDR) correction to determine differences between PRE and POST. Functional genes were predicted based on MetaCYC database of metabolic pathways (12) by PICRUSt2 (phylogenetic investigation of communities by reconstruction of unobserved state 2) (32) based on 16S rRNA sequencing data (63). Longitudinal change in functional gene

analysis was compared by Welch's t-test with Bonferroni correction using the software STAMP 2.1.3 (93). *A priori* selected MetaCYC pathways associated with SCFA production (*L-glutamate degradation V [via hydroxyglutarate]; L-lysine fermentation to acetate and butanoate; Bifidobacterium shunt; hexitol fermentation to lactate, formate, ethanol and acetate; pyruvate fermentation to acetate and lactate II; acetylene degradation; 4-aminobutanoate degradation V; acetyl-CoA fermentation to butanoate II; pyruvate fermentation to butanoate; succinate fermentation to butanoate; pyruvate fermentation to propanoate I*), mucin production (*GDP-mannose biosynthesis*), and mucin degradation (*D-galactarate degradation I; superpathway of hexuronide and hexuronate degradation; D-galacturonate degradation I; D-glucarate degradation I; superpathway of D-glucarate and D-galactarate degradation; lactose and galactose degradation I; galactose degradation I [Leloir pathway]*)were analyzed using MANOVA. Moreover, select bacterial species were interrogated based on an extensive literature review, where targets were selected based on their involvement in health or muscle physiology

INSERT TABLE 1 HERE

Serum assays

Venous blood was drawn from the antecubital vein, and samples were collected into a 5 mL serum separator tube (BD Vacutainer, Franklin Lakes, NJ, USA). Approximately 30 minutes following collection, tubes were centrifuged at 3,500 *g* for 5 minutes at room temperature. Aliquots were then placed in 1.7 mL polypropylene tubes and stored at -80ºC until batch-processing. Serum Zonulin was analyzed using a commercially available antibody-based colorimetric kit (Abcam, Cambridge, MA, USA; cat #: ab219048). Serum LPS was also

analyzed using a commercially available antibody-based colorimetric kit (Mybiosource, San Diego, CA, USA; cat #: MBS9716036). Coefficient of variation values for all duplicates were 4.2% for Zonulin, and 18.0% for LPS.

Statistical analysis

In addition to bioinformatics approaches related to microbiome metadata mentioned above, key dependent variables included PRE and POST values of bacterial genera presented in Table 1. Secondary dependent variables included PRE and POST values for DXA LSTM, VL thickness measures assessed via ultrasound, pQCT-determined mid-thigh muscle thickness, knee extensor peak torque, and self-reported dietary macronutrient intakes. All statistical analyses were performed using SPSS v26.0 (IBM Corp, Armonk, NY, USA). For all dependent variables over time, dependent samples t-tests were performed. Statistical significance was established as $p<0.05$, and relevant p-values are depicted in-text or within figures.

Results

Participant characteristics and general training adaptations

The 16 men that were analyzed for this study were 65±9 years old (age range 51-78 years old), all Caucasian, and had a study entry body mass index of 28.1±3.1 kg/m^2.

Table 2 presents training adaptations in participants. One variable not presented in this table is total training volume throughout the 6-week study, which was 96,277±27,619 kg. In short, DXA LSTM, VL thickness and leg extensor peak torque significantly increased ($p<0.05$), DXA fat mass significantly decreased ($p=0.034$), and mid-thigh mCSA determined by pQCT showed no change ($p=0.154$). Two-way ANOVAs indicated changes in these variables with

training did not differ between participants in the peanut supplement group (n=9) and non-supplement group (n=7) (interaction p-values were >0.10 for all variables).

INSERT TABLE 2 HERE

Data from self-reported food recalls

Table 3 presents 3-day food recall data prior to study initiation and during the last week of training. In short, self-reported protein and fiber intakes significantly increased ($p<0.05$), and calorie, carbohydrate, and fat intakes showed no significant changes. Two-way ANOVAs indicated changes in protein and fiber intakes were greater in the peanut supplemented participants ($p<0.05$) given that both of these metrics were bolstered through contents in the nutritional supplement.

INSERT TABLE 3 HERE

Changes in microbiome diversity metrics with resistance training

Metrics of microbiome diversity are presented in Table 4. In short, none of these metrics significantly changed with training. Additionally, independent samples t-tests indicated change scores in these variables did not differ between participants in the peanut supplement group (n=9) and non-supplement group (n=7) (p-values were >0.10 for all variables).

INSERT TABLE 4 HERE

Microbiome markers of interest identified through systematic literature review

Of the 17 microbiome markers selected in Table 1, only 11 of the original 17 selected microbiome markers had an abundance that could be detected and filtered. Results of these markers are presented in Table 5. None of these markers were significantly affected by training.

Taxa not detectable included *Bacillus subtilis*, *Lactobacillus rhamnoses*, *Clostridium difficile*, *Lactobacillus plantarum*, *Bifidobacterium breve*, and *Lactobacillus acidophilus*. Independent samples t-tests indicated change scores in these variables did not differ between participants in the peanut supplement group (n=9) and non-supplement group (n=7) (p-values were >0.10 for all variables).

INSERT TABLE 5 HERE

MetaCYC pathway changes

MetaCYC pathways associated with SCFA production, mucin production, and mucin degradation were chosen *a priori* to be interrogated herein. The reason why we chose to examine these functional pathways was that SCFAs produced by the microbiome can aid in reducing systemic inflammation, and inflammation can be detrimental to muscle mass maintenance. Additionally, mucin production aids in maintaining a healthy intestinal barrier, and intestinal barrier integrity is instrumental for preventing the intrusion of pathogens (or bacterial-borne compounds such as LPS) from the gut into circulation. Results are presented in Table 6. While short-chain fatty acid and mucin degradation did not change with training, mucin biosynthesis increased with training (p=0.047). Independent samples t-tests indicated change scores in these variables did not differ between participants in the peanut supplement group (n=9) and non-supplement group (n=7) (p-values were >0.10 for all variables).

INSERT TABLE 6 HERE

Serum Zonulin and lipopolysaccharide changes

We opted to assay select serum markers of gut integrity given that MetaCYC pathway analysis indicated mucin biosynthesis was predicted to increase with resistance training, and mucus is vital for intestinal barrier integrity. Zonulin (a.k.a. Haptoglobin) was the first assayed serum marker, and this is a tight junction protein involved with gut epithelial cell barrier function (36). LPS, the second serum marker, is considered an endotoxin produced by Gram-negative bacteria and high circulating levels largely reflect an impaired mucosal barrier (1). Zonulin levels decreased with training ($p=0.046$, Fig. 1a), and LPS levels did not change with training ($p=0.301$).

INSERT FIUGRE 1 HERE

Discussion

To our knowledge, this is the third study to examine how resistance training affects fecal microbiome markers, and is the first study to do so in an older population. The 6-week training program was effective in in promoting select phenotype changes that it increased whole-body LSTM, VL thickness, and knee extensor strength. Although most microbiome markers were unaffected with training, MetaCYC pathway analysis predicted that mucin biosynthesis capacity was increased. This finding was strengthened with follow-up analysis showing that serum Zonulin was down regulated. Hence, while resistance training-induced changes in the gut microbiome were not striking, the modest changes that did occur may have partially improved gut epithelial cell barrier function. However, we are cautious in this interpretation given the limited evidence unveiled in this study. Major findings as well as limitations in these data are discussed in greater detail below.

As mentioned previously, several human studies have examined how endurance training affects the gut microbiome. Allen et al. (3) examined the effects of a six-week endurance training intervention in 18 obese and 14 lean individuals. The authors found that the microbiota were different between lean and obese individuals at the beginning of the study, and those differences were reduced following exercise intervention. Munukka et al. (85) observed only modest changes in overall community composition following a six-week endurance training intervention in 18 overweight women. Cronin et al. (20) performed an eight-week combined aerobic and resistance training intervention study where 90 participants were randomized to one of three groups including exercise-only, exercise with a whey protein dietary supplement, and whey protein supplementation only. In short, the authors reported no significant changes in the microbiome with exercise training. Byruca et al. (7) examined how either endurance training or resistance training for 8 weeks affected the gut microbiome in healthy, younger adults. Interestingly, endurance exercise elicited more robust changes in the microbiome relative to resistance training, and this finding led authors to conclude that resistance training either does not affect the microbiome or does so in a more subtle manner. Our data largely agree with the data by Cronin and colleagues as well as the data by Byruca and colleagues in that microbiome diversity nor individual bacteria interrogated were not altered with resistance training. The reason as to why resistance training does not impact the microbiome versus endurance training is difficult to answer. However, this may largely be due to the stress imposed on the gastrointestinal system with endurance versus resistance training. In this regard, it has been reported that 30-50% of endurance athletes complain of gastrointestinal stress during exercise, and sources of such stress can be due to mechanical perturbations, increases in core temperature, and reductions in visceral blood flow (24). Moreover, it has been estimated that an exercise bout

lasting greater than 2 hours at 60% VO_2max appears to be the threshold whereby significant gastrointestinal perturbations manifest, irrespective of fitness status (18). Hence, we posit that resistance exercise bouts likely do not meet a gut-stress threshold, that this is likely why resistance training does not robustly affect the gut microbiome.

While the studies by Cronin and colleagues as well as Byruca and colleagues were the first to interrogate how resistance exercise alters the gut microbiome, the current data add unique insight literature in given that: a) this is the first study performed in an older population, and b) Byruca and colleagues only presented markers of microbiome diversity, whereas we added additional insight with MetaCYC pathway analysis. With regard to the later, we discovered that bacteria involved with mucin biosynthesis were altered to the point of up regulating this pathway. Mucins are O-glycosylated molecules that are produced by intestinal goblet cells, have gel-like properties, coat the intestinal lumen to generate a mucus later, and generate a bacteria-free zone at the epithelial surface (54). Mucus turnover is very high in mammals, with estimates in mice suggesting a turnover rate of 1-2 hours (91). Until recently, the mucus layer was thought to be a simple lubricant for assisting in the progression of the food bolus through the gut (91). However, it has been recently demonstrated in rodents that fiber deficiency leads to a deterioration of the gut mucosal layer, and eventual dysbiosis ensues (30). Additionally, research in mice has shown that chronic exposure to stress reduced the expression of mucin-2 mRNA and the number of goblet cells (42). Interestingly, aging in rodents has been shown to reduce mucosal layer thickness. To this end, Sovran et al. (110) showed that 19-month old mice displayed a ~6-fold reduction in mucosal layer thickness compared to 10-week old littermates. In explaining the significance of these findings, the authors hypothesized that the age-associated reduction in mucus thickness may be one of a few determining factors that drives the prevalence

of age-associated cholitis (110), especially since mucus is needed to prevent epithelial cell contact with pathogenic bacteria.

Given the MetaCYC pathway analysis findings of upregulated mucin production along with the literature discussed above, we became interested in determining whether markers of intestinal barrier integrity were altered with training. Interestingly, while serum LPS levels were not significantly altered, we discovered serum Zonulin levels were significantly down regulated. Zonulin is a protein that is critical for the formation of tight junctions between intestinal epithelial cells. It is generally recognized that higher serum Zonulin levels indicate potential "gut leakiness" due to increased intestinal permeability (2). Hence, these findings lend credence to resistance training improving intestinal barrier integrity, and this may be due to the modest but seemingly meaningful alterations in gut bacteria responsible for mucin production. One topic that needs to be discussed is the cross-reactivity of certain commercial assays that are designed to assess serum Zonulin/Haptoglobin samples. It has been shown that certain kits detect both Haptoglobin and complement C3 (2), and this has dampened enthusiasm in using commercial assays for serum Zonulin detection. However, we used a kit by Abcam, and results of this kit have been validated through the use of proteomics (80). Thus, this provides us with more confidence that our findings were meaningful. Aside from assay logistics, our hypothesis that resistance training improves intestinal barrier integrity in older populations needs to be further validated through additional experimentation. In particular, time course studies where multiple blood draws and fecal samples are collected and more extensively analyzed for intestinal integrity biomarkers will provide valuable insight.

Experimental considerations

This study has various limitations. First, only 16 older Caucasian males were studied. Thus, we are uncertain as to whether these findings extrapolate to older females or persons of other ethnicities. While our n-size is limited, our n-size is line with n-sizes from Byruca et al. (7) who examined the effects of resistance training on the gut micobiome; biome analysis was only performed on n=9 at PRE and n=15 at POST. Additionally, other studies that have examined the effects of endurance training have used similar n-sizes to show changes in the gut microbiome do occur in overweight, untrained participants; Manukka et al. (85) and Allen et al. (3) both examined n=18 participants. However, this does not rule out that the inclusion of more participants may have altered our findings, and this needs to be considered in the context of the study. One notable limitation is the length of training only being 6 weeks in duration. Indeed, the training program was effective in increasing whole-body LSTM, VL thickness, and lower body strength. However, these changes were modest given that training was relatively short (1.2% DXA LSTM, 5.2% VL thickness, 15.3% in leg extensor strength). Hence, it is unknown if longer-term resistance training (e.g., years or decades) elicits more notable shifts in the gut microbiome. Our data are also limited with regard to the number of bacterial species identified (~160). In this regard, there are over 1,000 bacterial species in the gut microbiome (19), and replicating our approach with advanced interrogation techniques (e.g., metagenomics) is warranted. Finally, analyzing the gut microbiome via stool sampling may adequately represent bacterial colonization of the large intestine, and this too must be considered when interpreting these data.

Conclusions

This study continues to expand upon the current scientific knowledge regarding the human gut microbiome, and how it is affected by resistance training. Despite the limitations of this study, our novel observation regarding the potential upregulation in mucin biosynthesis and improvement in intestinal barrier integrity warrants further research.

Declarations

Ethics approval and consent to participate

All procedures described herein were approved by the Auburn University IRB (protocol #19-249 MR 1907).

Consent for publication

Not applicable

Availability of data and material

All raw data can be obtained by emailing the corresponding author (fruge@auburn.edu).

Competing interests

None of the authors has competing interests to declare.

Funding

This study was made possible by a grant provided by the Peanut Institute Foundation (TPIF, Albany, GA, USA) to A.D.F., M.D.R., K.W.H., and K.C.Y. TPIF did not have a role in study design or analysis. None of the authors have financial or other conflicts of interest to report with regard to these data.

Authors' contributions

This experiment was performed at Auburn University's School of Kinesiology in the Molecular and Applied Sciences Laboratory. A.D.F., M.D.R., K.W.H., and K.C.Y were responsible for the conception and design of the experiment. J.H.M., M.D.R., and A.D.F. primarily drafted the manuscript. All authors were involved in different aspects of data collection. All authors read and approved the final manuscript.

Acknowledgements

We thank the participants for kindly agreeing to participate in the study.

Tables and Legends

Table 1. Bacteria that were interrogated from fecal samples prior to and following a 6-week resistance training intervention.

Bacterium	**Interaction**	**Model**	**Reference(s)**
Bacillus Subtilis	Increases gut integrity, increases heat stress resistance, increases dopamine production, increases Strength	Human/Rat/Chicken	6, 18

Lactobacillus Rhamnoses	Increases strength, Reduces reactive oxygen species	Human/Rat	17, 18, 20
Lactobacillus Reuteri	Increases strength	Human/Rat	13, 14, 17, 18
E. Coli	Decreases gut integrity	Human/Rat	12
C. Diff	Decreases gut integrity	Human/Rat	1, 3
C. Scindens	Increases gut integrity, protects against *C. Diff*	Human	1, 3
Lactobacillus Plantarum	Increase strength	Human	2
Bacteroidetes / Firmicutes	Increases gut integrity	Human/Rat	8, 11
Strep. Thermophilus	Increases gut integrity, neurological protection	Mouse	4
Bifidobacterium Breve	Increased gut integrity	Rat	7, 19
Bifidobacterium Longum	Promotes vitamin formation and uptake, SCFA upregulation, neurological repair	Human/Mouse	7, 9, 10, 18
Bifidobacterium Bifidum	Vitamin formation and uptake	Human/Mouse	7
Lactobacillus acidophilus	Increases gut integrity	Human	8

Bifidobacterium animalis	Enhances insulin sensitivity via GLP-2 activity	Chicken	7
Clostridium Symbiosum	SCFA production, neurological protection, reduces inflammation	Mouse	9
Faecalibacterium prausnitzii	SCFA production, neurological protection, reduces inflammation	Mouse	9
Lactobacillus fermentum	SCFA production, neurological protection, reduces inflammation	Mouse	9

Legend: This table was constructed from the following references: 1. (4), 2. (14), 3. (38), 4. (22), 5. (5), 6. (92), 7. (64), 8. (117), 9. (65), 10. (66), 11. (75), 12. (77), 13. (76), 14. (87), 15. (95), 16. (96), 17. (97), 18. (99), 19. (101), 20. (116). Notably, a comprehensive literature search was used to construct this table with the intent of identifying targets that have been shown in various models to be associated with health outcomes. Abbreviation: SCFA, short-chain fatty acid.

Table 2. Training adaptations in older male participants

Training Adaptation	**Values**		**Significance**
FFM LSTM (kg)	PRE POST	58.2 ± 6.0 58.9 ± 6.4	***p=0.003***
DXA Fat Mass (kg)	PRE POST	27.7 ± 7.2 27.2 ± 6.8	***p=0.034***
pQCT mCSA (cm^2)	PRE POST	144 ± 24 147 ± 23	p=0.154

VL thickness (cm)	PRE POST	2.10 ± 0.38 2.21 ± 0.38	***p=0.032***
Leg extensor torque (N*m)	PRE POST	144 ± 57 166 ± 40	***p=0.008***

Legend: means and standard deviations of the pre to post alterations are presented here for DXA lean/soft tissue mass (LSTM), DXA fat mass, mid-thigh muscle cross-sectional area (mCSA) determined by pQCT, vastus lateralis (VL) thickness according to ultrasound, and leg extensor peak torque. Data include all 16 participants for each variable.

Table 3. Self-reported food log data

Variable	**Values**		**Significance**
Calories (per day)	PRE POST	2,121 ± 517 2,113 ± 468	p=0.966
Protein (g/day)	PRE POST	91 ± 31 110 ± 34	***p=0.048***
Carbohydrate (g/day)	PRE POST	241 ± 76 226 ± 58	p=0.452
Fat (g/day)	PRE POST	88 ± 27 84 ± 26	p=0.706
Fiber (g/day)	PRE POST	18 ± 6 23 ± 6	***p=0.023***

Legend: means and standard deviations of the pre to post alterations are presented here for daily calorie and macronutrient intakes. Data include 13 of 16 participants for each variable given that 3 participants did not turn in food logs. Data at POST also consider daily peanut protein supplementation by some participants.

Table 4. Changes in microbiome diversity metrics

Variable	**Abundance values**		**Significance**
Total observed species	PRE POST	156 ± 57 162 ± 59	p=0.458

Whole-tree phylogeny	PRE POST	15.1 ± 4.3 15.4 ± 4.1	p=0.623
Shannon diversity index	PRE POST	5.5 ± 4.4 5.5 ± 4.4	p=0.570
Simpson diversity index	PRE POST	0.95 ± 0.01 0.95 ± 0.01	p=0.974

Legend: means and standard deviations of the pre to post alterations are presented here for variables of interest. Data include all 16 participants.

Table 5. Changes in microbiome taxa/markers of interest identified through systematic literature review

Variable	**Relative abundance values**		**Significance**
Bifidobacterium animalis	PRE POST	0.0003 ± 0.0011 ND	p=0.333
Bifidobacterium longum	PRE POST	0.0061 ± 0.0087 0.0062 ± 0.0111	p=0.966

Faecalibacterium prausnitzii	PRE POST	0.000063 ± 0.000227 0.000054 ± 0.000092	p=0.862
Lactobacillus fermentum	PRE POST	0.000073 ± 0.000294 0.000021 ± 0.000083	p=0.333
Lactobacillus reuteri	PRE POST	0.000045 ± 0.000142 0.000002 ± 0.000010	p=0.257
Strep. thermophilus	PRE POST	0.015 ± 0.027 0.014 ± 0.032	p=0.677
Clostridium symbiosum	PRE POST	0.00034 ± 0.00100 0.00015 ± 0.00039	p=0.255
Escherichia coli	PRE POST	0.0032 ± 0.0077 0.0033 ± 0.0058	p=0.949
Clostridium scindens	PRE POST	0.00060 ± 0.00138 0.00094 ± 0.00170	p=0.329
Bifidobacterium bifidum	PRE POST	0.00059 ± 0.00161 0.00076 ± 0.00210	p=0.321
Bacteroidetes / Firmicutes	PRE POST	0.16 ± 0.17 0.13 ± 0.14	p=0.620

Legend: means and standard deviations of the pre to post alterations are presented here for variables of interest. Data include all 16 participants. ND, not detected.

Table 6. Changes in select pathways according to bioinformatics

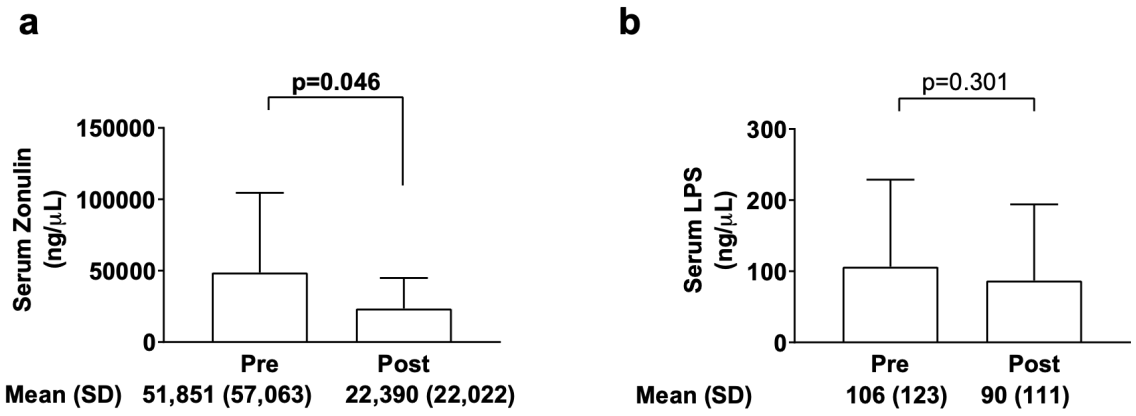
Pathway	**Pathway score**		**Significance**
SCFA production	PRE POST	9,309 ± 3,523 10,567 ± 4,126	p=0.254
Mucin biosynthesis	PRE POST	24,676 ± 11,287 31,424 ± 15,240	***p=0.047***

Mucin degradation	PRE POST	15,354 ± 4,873 18,665 ± 6,987	p=0.082

Legend: means and standard deviations of pre to post alterations are presented here for variables of interest. Data include all 16 participants.

Figures and Legends

Figure 1. Serum gut integrity biomarker changes



Legend: means and standard deviations of pre to post alterations are presented here for variables of interest. Data include all 13-14 participants per biomarker.

REFERENCES FOR CHAPTERS 1-4

1. **Ahola AJ, Lassenius MI, Forsblom C, Harjutsalo V, Lehto M, and Groop PH**. Dietary patterns reflecting healthy food choices are associated with lower serum LPS activity. *Sci Rep* 7: 6511, 2017.
2. **Ajamian M, Steer D, Rosella G, and Gibson PR**. Serum zonulin as a marker of intestinal mucosal barrier function: May not be what it seems. *PLoS One* 14: e0210728, 2019.
3. **Allen JM, Mailing LJ, Niemiro GM, Moore R, Cook MD, White BA, Holscher HD, and Woods JA**. Exercise Alters Gut Microbiota Composition and Function in Lean and Obese Humans. *Med Sci Sports Exerc* 50: 747-757, 2018.
4. **Amrane S, Bachar D, Lagier JC, and Raoult D**. Clostridium scindens Is Present in the Gut Microbiota during Clostridium difficile Infection: a Metagenomic and Culturomic Analysis. *J Clin Microbiol* 56: 2018.
5. **Billiauws L, and Joly F**. Emerging treatments for short bowel syndrome in adult patients. *Expert Rev Gastroenterol Hepatol* 13: 241-246, 2019.
6. **Blander JM, Longman RS, Iliev ID, Sonnenberg GF, and Artis D**. Regulation of inflammation by microbiota interactions with the host. *Nat Immunol* 18: 851-860, 2017.
7. **Bycura D, Santos AC, Shiffer A, Kyman S, Winfree K, Sutliffe J, Pearson T, Sonderegger D, Cope E, and Caporaso JG**. Impact of Different Exercise Modalities on the Human Gut Microbiome. *Sports (Basel)* 9: 2021.
8. **Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmee E, Cousin B, Sulpice T, Chamontin B, Ferrieres J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, and Burcelin R**. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56: 1761-1772, 2007.
9. **Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, and Delzenne NM**. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 58: 1091-1103, 2009.
10. **Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, and Knight R**. QIIME allows analysis of high-throughput community sequencing data. *Nat Meth* 7: 335-336, 2010.
11. **Caputi V, Marsilio I, Filpa V, Cerantola S, Orso G, Bistoletti M, Paccagnella N, De Martin S, Montopoli M, Dall'Acqua S, Crema F, Di Gangi IM, Galuppini F, Lante I, Bogialli S, Rugge M, Debetto P, Giaroni C, and Giron MC**. Antibiotic-induced dysbiosis of the microbiota impairs gut neuromuscular function in juvenile mice. *Br J Pharmacol* 174: 3623-3639, 2017.
12. **Caspi R, Altman T, Billington R, Dreher K, Foerster H, Fulcher CA, Holland TA, Keseler IM, Kothari A, Kubo A, Krummenacker M, Latendresse M, Mueller LA, Ong Q, Paley S, Subhraveti P, Weaver DS, Weerasinghe D, Zhang P, and Karp PD**. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases. *Nucleic Acids Research* 42: D459-D471, 2014.

13. **Cerda B, Perez M, Perez-Santiago JD, Tornero-Aguilera JF, Gonzalez-Soltero R, and Larrosa M**. Gut Microbiota Modification: Another Piece in the Puzzle of the Benefits of Physical Exercise in Health? *Front Physiol* 7: 51, 2016.
14. **Chen YM, Wei L, Chiu YS, Hsu YJ, Tsai TY, Wang MF, and Huang CC**. Lactobacillus plantarum TWK10 Supplementation Improves Exercise Performance and Increases Muscle Mass in Mice. *Nutrients* 8: 205, 2016.
15. **Cho I, and Blaser MJ**. The human microbiome: at the interface of health and disease. *Nat Rev Genet* 13: 260-270, 2012.
16. **Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, Hayes P, O'Reilly M, Jeffery IB, Wood-Martin R, Kerins DM, Quigley E, Ross RP, O'Toole PW, Molloy MG, Falvey E, Shanahan F, and Cotter PD**. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* 63: 1913-1920, 2014.
17. **Consitt LA, Dudley C, and Saxena G**. Impact of Endurance and Resistance Training on Skeletal Muscle Glucose Metabolism in Older Adults. *Nutrients* 11: 2019.
18. **Costa RJS, Snipe RMJ, Kitic CM, and Gibson PR**. Systematic review: exercise-induced gastrointestinal syndrome-implications for health and intestinal disease. *Aliment Pharmacol Ther* 46: 246-265, 2017.
19. **Cresci GA, and Bawden E**. Gut Microbiome: What We Do and Don't Know. *Nutr Clin Pract* 30: 734-746, 2015.
20. **Cronin O, Barton W, Skuse P, Penney NC, Garcia-Perez I, Murphy EF, Woods T, Nugent H, Fanning A, Melgar S, Falvey EC, Holmes E, Cotter PD, O'Sullivan O, Molloy MG, and Shanahan F**. A Prospective Metagenomic and Metabolomic Analysis of the Impact of Exercise and/or Whey Protein Supplementation on the Gut Microbiome of Sedentary Adults. *mSystems* 3: 2018.
21. **Daft JG, Ptacek T, Kumar R, Morrow C, and Lorenz RG**. Cross-fostering immediately after birth induces a permanent microbiota shift that is shaped by the nursing mother. *Microbiome* 3: 17, 2015.
22. **Dargahi N, Matsoukas J, and Apostolopoulos V**. Streptococcus thermophilus ST285 Alters Pro-Inflammatory to Anti-Inflammatory Cytokine Secretion against Multiple Sclerosis Peptide in Mice. *Brain Sci* 10: 2020.
23. **David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, and Turnbaugh PJ**. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505: 559-563, 2014.
24. **de Oliveira EP, Burini RC, and Jeukendrup A**. Gastrointestinal complaints during exercise: prevalence, etiology, and nutritional recommendations. *Sports Med* 44 Suppl 1: S79-85, 2014.
25. **Dehghan P, Pourghassem Gargari B, and Asghari Jafar-abadi M**. Oligofructose-enriched inulin improves some inflammatory markers and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized controlled clinical trial. *Nutrition* 30: 418-423, 2014.
26. **den Besten G, Gerding A, van Dijk TH, Ciapaite J, Bleeker A, van Eunen K, Havinga R, Groen AK, Reijngoud DJ, and Bakker BM**. Protection against the Metabolic Syndrome by Guar Gum-Derived Short-Chain Fatty Acids Depends on Peroxisome Proliferator-Activated Receptor gamma and Glucagon-Like Peptide-1. *PLoS One* 10: e0136364, 2015.
27. **den Besten G, Lange K, Havinga R, van Dijk TH, Gerding A, van Eunen K, Muller M, Groen AK, Hooiveld GJ, Bakker BM, and Reijngoud DJ**. Gut-derived short-chain fatty

acids are vividly assimilated into host carbohydrates and lipids. *Am J Physiol Gastrointest Liver Physiol* 305: G900-910, 2013.
28. **den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, and Bakker BM**. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 54: 2325-2340, 2013.
29. **Denou E, Marcinko K, Surette MG, Steinberg GR, and Schertzer JD**. High-intensity exercise training increases the diversity and metabolic capacity of the mouse distal gut microbiota during diet-induced obesity. *Am J Physiol Endocrinol Metab* 310: E982-993, 2016.
30. **Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A, Young VB, Henrissat B, Wilmes P, Stappenbeck TS, Nunez G, and Martens EC**. A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell* 167: 1339-1353 e1321, 2016.
31. **Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, Cox LM, Amir A, Gonzalez A, Bokulich NA, Song SJ, Hoashi M, Rivera-Vinas JI, Mendez K, Knight R, and Clemente JC**. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nat Med* 22: 250-253, 2016.
32. **Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, and Langille MGI**. PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology* 38: 685-688, 2020.
33. **Doyle A, Zhang G, Abdel Fattah EA, Eissa NT, and Li YP**. Toll-like receptor 4 mediates lipopolysaccharide-induced muscle catabolism via coordinate activation of ubiquitin-proteasome and autophagy-lysosome pathways. *FASEB J* 25: 99-110, 2011.
34. **El-Salhy M, Hatlebakk JG, and Hausken T**. Diet in Irritable Bowel Syndrome (IBS): Interaction with Gut Microbiota and Gut Hormones. *Nutrients* 11: 2019.
35. **Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, Blaser MJ, Graham DY, Vacher S, Perez-Perez GI, Yamaoka Y, Megraud F, Otto K, Reichard U, Katzowitsch E, Wang X, Achtman M, and Suerbaum S**. Traces of human migrations in Helicobacter pylori populations. *Science* 299: 1582-1585, 2003.
36. **Fasano A**. All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. *F1000Res* 9: 2020.
37. **Fernandes J, Su W, Rahat-Rozenbloom S, Wolever TM, and Comelli EM**. Adiposity, gut microbiota and faecal short chain fatty acids are linked in adult humans. *Nutr Diabetes* 4: e121, 2014.
38. **Ferrario C, Taverniti V, Milani C, Fiore W, Laureati M, De Noni I, Stuknyte M, Chouaia B, Riso P, and Guglielmetti S**. Modulation of fecal Clostridiales bacteria and butyrate by probiotic intervention with Lactobacillus paracasei DG varies among healthy adults. *J Nutr* 144: 1787-1796, 2014.
39. **Fielding RA, Reeves AR, Jasuja R, Liu C, Barrett BB, and Lustgarten MS**. Muscle strength is increased in mice that are colonized with microbiota from high-functioning older adults. *Exp Gerontol* 127: 110722, 2019.
40. **Frieri M, Kumar K, and Boutin A**. Antibiotic resistance. *J Infect Public Health* 10: 369-378, 2017.
41. **Frost GS, Brynes AE, Dhillo WS, Bloom SR, and McBurney MI**. The effects of fiber enrichment of pasta and fat content on gastric emptying, GLP-1, glucose, and insulin responses to a meal. *Eur J Clin Nutr* 57: 293-298, 2003.

42. **Gao X, Cao Q, Cheng Y, Zhao D, Wang Z, Yang H, Wu Q, You L, Wang Y, Lin Y, Li X, Wang Y, Bian JS, Sun D, Kong L, Birnbaumer L, and Yang Y**. Chronic stress promotes colitis by disturbing the gut microbiota and triggering immune system response. *Proc Natl Acad Sci U S A* 115: E2960-E2969, 2018.
43. **Gibson MK, Crofts TS, and Dantas G**. Antibiotics and the developing infant gut microbiota and resistome. *Curr Opin Microbiol* 27: 51-56, 2015.
44. **Goderska K, Agudo Pena S, and Alarcon T**. Helicobacter pylori treatment: antibiotics or probiotics. *Appl Microbiol Biotechnol* 102: 1-7, 2018.
45. **Gould AL, Zhang V, Lamberti L, Jones EW, Obadia B, Korasidis N, Gavryushkin A, Carlson JM, Beerenwinkel N, and Ludington WB**. Microbiome interactions shape host fitness. *Proc Natl Acad Sci U S A* 115: E11951-E11960, 2018.
46. **Haran PH, Rivas DA, and Fielding RA**. Role and potential mechanisms of anabolic resistance in sarcopenia. *J Cachexia Sarcopenia Muscle* 3: 157-162, 2012.
47. **Hawley JA**. Microbiota and muscle highway - two way traffic. *Nat Rev Endocrinol* 16: 71-72, 2020.
48. **Hawley JA, Lundby C, Cotter JD, and Burke LM**. Maximizing Cellular Adaptation to Endurance Exercise in Skeletal Muscle. *Cell Metab* 27: 962-976, 2018.
49. **Hill CJ, Lynch DB, Murphy K, Ulaszewska M, Jeffery IB, O'Shea CA, Watkins C, Dempsey E, Mattivi F, Tuohy K, Ross RP, Ryan CA, PW OT, and Stanton C**. Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome* 5: 4, 2017.
50. **Hu J, Kyrou I, Tan BK, Dimitriadis GK, Ramanjaneya M, Tripathi G, Patel V, James S, Kawan M, Chen J, and Randeva HS**. Short-Chain Fatty Acid Acetate Stimulates Adipogenesis and Mitochondrial Biogenesis via GPR43 in Brown Adipocytes. *Endocrinology* 157: 1881-1894, 2016.
51. **Human Microbiome Project C**. Structure, function and diversity of the healthy human microbiome. *Nature* 486: 207-214, 2012.
52. **Iizuka K, Machida T, and Hirafuji M**. Skeletal muscle is an endocrine organ. *J Pharmacol Sci* 125: 125-131, 2014.
53. **Jackson MA, Jeffery IB, Beaumont M, Bell JT, Clark AG, Ley RE, O'Toole PW, Spector TD, and Steves CJ**. Signatures of early frailty in the gut microbiota. *Genome Med* 8: 8, 2016.
54. **Johansson ME, and Hansson GC**. Immunological aspects of intestinal mucus and mucins. *Nat Rev Immunol* 16: 639-649, 2016.
55. **Johnston AP, De Lisio M, and Parise G**. Resistance training, sarcopenia, and the mitochondrial theory of aging. *Appl Physiol Nutr Metab* 33: 191-199, 2008.
56. **Karaki S, Tazoe H, Hayashi H, Kashiwabara H, Tooyama K, Suzuki Y, and Kuwahara A**. Expression of the short-chain fatty acid receptor, GPR43, in the human colon. *J Mol Histol* 39: 135-142, 2008.
57. **Kimura I, Inoue D, Hirano K, and Tsujimoto G**. The SCFA Receptor GPR43 and Energy Metabolism. *Front Endocrinol (Lausanne)* 5: 85, 2014.
58. **Kumar R, Eipers P, Little RB, Crowley M, Crossman DK, Lefkowitz EJ, and Morrow CD**. Getting Started with Microbiome Analysis: Sample Acquisition to Bioinformatics. *Current Protocols in Human Genetics* 82: 18.18.11-18.18.29, 2014.
59. **Lahiri S, Kim H, Garcia-Perez I, Reza MM, Martin KA, Kundu P, Cox LM, Selkrig J, Posma JM, Zhang H, Padmanabhan P, Moret C, Gulyas B, Blaser MJ, Auwerx J,**

Holmes E, Nicholson J, Wahli W, and Pettersson S. The gut microbiota influences skeletal muscle mass and function in mice. *Sci Transl Med* 11: 2019.
60. **Lamb DA, Moore JH, Smith MA, Vann CG, Osburn SC, Ruple BA, Fox CD, Smith KS, Altonji OM, Power ZM, Cerovsky AE, Ross CO, Cao AT, Goodlett MD, Huggins KW, Fruge AD, Young KC, and Roberts MD**. The effects of resistance training with or without peanut protein supplementation on skeletal muscle and strength adaptations in older individuals. *J Int Soc Sports Nutr* 17: 66, 2020.
61. **Lamoureux EV, Grandy SA, and Langille MGI**. Moderate Exercise Has Limited but Distinguishable Effects on the Mouse Microbiome. *mSystems* 2: 2017.
62. **Landi F, Marzetti E, Martone AM, Bernabei R, and Onder G**. Exercise as a remedy for sarcopenia. *Curr Opin Clin Nutr Metab Care* 17: 25-31, 2014.
63. **Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RLV, and Knight R**. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature biotechnology* 31: 814-821, 2013.
64. **LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D, and Ventura M**. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr Opin Biotechnol* 24: 160-168, 2013.
65. **Lee J, d'Aigle J, Atadja L, Quaicoe V, Honarpisheh P, Ganesh BP, Hassan A, Graf J, Petrosino J, Putluri N, Zhu L, Durgan DJ, Bryan RM, Jr., McCullough LD, and Venna VR**. Gut Microbiota-Derived Short-Chain Fatty Acids Promote Poststroke Recovery in Aged Mice. *Circ Res* 127: 453-465, 2020.
66. **Ley RE, Turnbaugh PJ, Klein S, and Gordon JI**. Microbial ecology: human gut microbes associated with obesity. *Nature* 444: 1022-1023, 2006.
67. **Lin PW, Nasr TR, and Stoll BJ**. Necrotizing enterocolitis: recent scientific advances in pathophysiology and prevention. *Semin Perinatol* 32: 70-82, 2008.
68. **Liu Z, Liu HY, Zhou H, Zhan Q, Lai W, Zeng Q, Ren H, and Xu D**. Moderate-Intensity Exercise Affects Gut Microbiome Composition and Influences Cardiac Function in Myocardial Infarction Mice. *Front Microbiol* 8: 1687, 2017.
69. **Lopez-Siles M, Duncan SH, Garcia-Gil LJ, and Martinez-Medina M**. Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics. *ISME J* 11: 841-852, 2017.
70. **Macfarlane GT, and Macfarlane S**. Bacteria, colonic fermentation, and gastrointestinal health. *J AOAC Int* 95: 50-60, 2012.
71. **Mailing LJ, Allen JM, Buford TW, Fields CJ, and Woods JA**. Exercise and the Gut Microbiome: A Review of the Evidence, Potential Mechanisms, and Implications for Human Health. *Exerc Sport Sci Rev* 47: 75-85, 2019.
72. **Manickam R, Duszka K, and Wahli W**. PPARs and Microbiota in Skeletal Muscle Health and Wasting. *Int J Mol Sci* 21: 2020.
73. **Manickam R, Oh HYP, Tan CK, Paramalingam E, and Wahli W**. Metronidazole Causes Skeletal Muscle Atrophy and Modulates Muscle Chronometabolism. *Int J Mol Sci* 19: 2018.
74. **Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, Quraishi MN, Kinross J, Smidt H, Tuohy KM, Thomas LV, Zoetendal EG, and Hart A**. The gut microbiota and host health: a new clinical frontier. *Gut* 65: 330-339, 2016.

75. **Mariat D, Firmesse O, Levenez F, Guimaraes V, Sokol H, Dore J, Corthier G, and Furet JP**. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol* 9: 123, 2009.
76. **Marques FZ, Mackay CR, and Kaye DM**. Beyond gut feelings: how the gut microbiota regulates blood pressure. *Nat Rev Cardiol* 15: 20-32, 2018.
77. **Martinez-Medina M, Denizot J, Dreux N, Robin F, Billard E, Bonnet R, Darfeuille-Michaud A, and Barnich N**. Western diet induces dysbiosis with increased E coli in CEABAC10 mice, alters host barrier function favouring AIEC colonisation. *Gut* 63: 116-124, 2014.
78. **Marzetti E, Calvani R, Tosato M, Cesari M, Di Bari M, Cherubini A, Broccatelli M, Savera G, D'Elia M, Pahor M, Bernabei R, Landi F, and Consortium S**. Physical activity and exercise as countermeasures to physical frailty and sarcopenia. *Aging Clin Exp Res* 29: 35-42, 2017.
79. **Marzetti E, Calvani R, Tosato M, Cesari M, Di Bari M, Cherubini A, Collamati A, D'Angelo E, Pahor M, Bernabei R, Landi F, and Consortium S**. Sarcopenia: an overview. *Aging Clin Exp Res* 29: 11-17, 2017.
80. **Mateos J, Estevez O, Gonzalez-Fernandez A, Anibarro L, Pallares A, Reljic R, Mussa T, Gomes-Maueia C, Nguilichane A, Gallardo JM, Medina I, and Carrera M**. Serum proteomics of active tuberculosis patients and contacts reveals unique processes activated during Mycobacterium tuberculosis infection. *Sci Rep* 10: 3844, 2020.
81. **Mayneris-Perxachs J, Arnoriaga-Rodriguez M, Luque-Cordoba D, Priego-Capote F, Perez-Brocal V, Moya A, Burokas A, Maldonado R, and Fernandez-Real JM**. Gut microbiota steroid sexual dimorphism and its impact on gonadal steroids: influences of obesity and menopausal status. *Microbiome* 8: 136, 2020.
82. **Mijnarends DM, Schols JM, Meijers JM, Tan FE, Verlaan S, Luiking YC, Morley JE, and Halfens RJ**. Instruments to assess sarcopenia and physical frailty in older people living in a community (care) setting: similarities and discrepancies. *J Am Med Dir Assoc* 16: 301-308, 2015.
83. **Milani C, Hevia A, Foroni E, Duranti S, Turroni F, Lugli GA, Sanchez B, Martin R, Gueimonde M, van Sinderen D, Margolles A, and Ventura M**. Assessing the fecal microbiota: an optimized ion torrent 16S rRNA gene-based analysis protocol. *PLoS One* 8: e68739, 2013.
84. **Morley JE, Anker SD, and von Haehling S**. Prevalence, incidence, and clinical impact of sarcopenia: facts, numbers, and epidemiology-update 2014. *J Cachexia Sarcopenia Muscle* 5: 253-259, 2014.
85. **Munukka E, Ahtiainen JP, Puigbo P, Jalkanen S, Pahkala K, Keskitalo A, Kujala UM, Pietila S, Hollmen M, Elo L, Huovinen P, D'Auria G, and Pekkala S**. Six-Week Endurance Exercise Alters Gut Metagenome That Is not Reflected in Systemic Metabolism in Over-weight Women. *Front Microbiol* 9: 2323, 2018.
86. **Nay K, Jollet M, Goustard B, Baati N, Vernus B, Pontones M, Lefeuvre-Orfila L, Bendavid C, Rue O, Mariadassou M, Bonnieu A, Ollendorff V, Lepage P, Derbre F, and Koechlin-Ramonatxo C**. Gut bacteria are critical for optimal muscle function: a potential link with glucose homeostasis. *American journal of physiology Endocrinology and metabolism* 317: E158-e171, 2019.

87. **Nilsson AG, Sundh D, Backhed F, and Lorentzon M**. Lactobacillus reuteri reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial. *J Intern Med* 284: 307-317, 2018.
88. **Nohr MK, Pedersen MH, Gille A, Egerod KL, Engelstoft MS, Husted AS, Sichlau RM, Grunddal KV, Poulsen SS, Han S, Jones RM, Offermanns S, and Schwartz TW**. GPR41/FFAR3 and GPR43/FFAR2 as cosensors for short-chain fatty acids in enteroendocrine cells vs FFAR3 in enteric neurons and FFAR2 in enteric leukocytes. *Endocrinology* 154: 3552-3564, 2013.
89. **Otsuka R, Kato Y, Nishita Y, Tange C, Tomida M, Nakamoto M, Imai T, Ando F, and Shimokata H**. Age-related Changes in Energy Intake and Weight in Community-dwelling Middle-aged and Elderly Japanese. *J Nutr Health Aging* 20: 383-390, 2016.
90. **Pannaraj PS, Li F, Cerini C, Bender JM, Yang S, Rollie A, Adisetiyo H, Zabih S, Lincez PJ, Bittinger K, Bailey A, Bushman FD, Sleasman JW, and Aldrovandi GM**. Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. *JAMA Pediatr* 171: 647-654, 2017.
91. **Paone P, and Cani PD**. Mucus barrier, mucins and gut microbiota: the expected slimy partners? *Gut* 69: 2232-2243, 2020.
92. **Park I, Lee Y, Goo D, Zimmerman NP, Smith AH, Rehberger T, and Lillehoj HS**. The effects of dietary Bacillus subtilis supplementation, as an alternative to antibiotics, on growth performance, intestinal immunity, and epithelial barrier integrity in broiler chickens infected with Eimeria maxima. *Poult Sci* 99: 725-733, 2020.
93. **Parks DH, Tyson GW, Hugenholtz P, and Beiko RG**. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* 30: 3123-3124, 2014.
94. **Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, Petersen KF, Kibbey RG, Goodman AL, and Shulman GI**. Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. *Nature* 534: 213-217, 2016.
95. **Picard C, Fioramonti J, Francois A, Robinson T, Neant F, and Matuchansky C**. Review article: bifidobacteria as probiotic agents -- physiological effects and clinical benefits. *Aliment Pharmacol Ther* 22: 495-512, 2005.
96. **Pieper R, Kager L, Weintraub A, Lindberg AA, and Nord CE**. The role of Bacteroides fragilis in the pathogenesis of acute appendicitis. *Acta Chir Scand* 148: 39-44, 1982.
97. **Poutahidis T, Springer A, Levkovich T, Qi P, Varian BJ, Lakritz JR, Ibrahim YM, Chatzigiagkos A, Alm EJ, and Erdman SE**. Probiotic microbes sustain youthful serum testosterone levels and testicular size in aging mice. *PLoS One* 9: e84877, 2014.
98. **Powell N, and MacDonald TT**. Recent advances in gut immunology. *Parasite Immunol* 39: 2017.
99. **Przewlocka K, Folwarski M, Kazmierczak-Siedlecka K, Skonieczna-Zydecka K, and Kaczor JJ**. Gut-Muscle AxisExists and May Affect Skeletal Muscle Adaptation to Training. *Nutrients* 12: 2020.
100. **Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, and Glöckner FO**. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41: D590-D596, 2013.
101. **Raftis EJ, Delday MI, Cowie P, McCluskey SM, Singh MD, Ettorre A, and Mulder IE**. Bifidobacterium breve MRx0004 protects against airway inflammation in a severe asthma model by suppressing both neutrophil and eosinophil lung infiltration. *Sci Rep* 8: 12024, 2018.

102. **Rajilic-Stojanovic M, and de Vos WM**. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiol Rev* 38: 996-1047, 2014.
103. **Rampelli S, Candela M, Turroni S, Biagi E, Collino S, Franceschi C, O'Toole PW, and Brigidi P**. Functional metagenomic profiling of intestinal microbiome in extreme ageing. *Aging (Albany NY)* 5: 902-912, 2013.
104. **Rani RP, Anandharaj M, and Ravindran AD**. Characterization of Bile Salt Hydrolase from Lactobacillus gasseri FR4 and Demonstration of Its Substrate Specificity and Inhibitory Mechanism Using Molecular Docking Analysis. *Front Microbiol* 8: 1004, 2017.
105. **Rizzetto L, Fava F, Tuohy KM, and Selmi C**. Connecting the immune system, systemic chronic inflammation and the gut microbiome: The role of sex. *J Autoimmun* 92: 12-34, 2018.
106. **Sardeli AV, Tomeleri CM, Cyrino ES, Fernhall B, Cavaglieri CR, and Chacon-Mikahil MPT**. Effect of resistance training on inflammatory markers of older adults: A meta-analysis. *Exp Gerontol* 111: 188-196, 2018.
107. **Scheiman J, Luber JM, Chavkin TA, MacDonald T, Tung A, Pham LD, Wibowo MC, Wurth RC, Punthambaker S, Tierney BT, Yang Z, Hattab MW, Avila-Pacheco J, Clish CB, Lessard S, Church GM, and Kostic AD**. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. *Nat Med* 25: 1104-1109, 2019.
108. **Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, and Hardt PD**. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)* 18: 190-195, 2010.
109. **Sochocka M, Donskow-Lysoniewska K, Diniz BS, Kurpas D, Brzozowska E, and Leszek J**. The Gut Microbiome Alterations and Inflammation-Driven Pathogenesis of Alzheimer's Disease-a Critical Review. *Mol Neurobiol* 56: 1841-1851, 2019.
110. **Sovran B, Hugenholtz F, Elderman M, Van Beek AA, Graversen K, Huijskes M, Boekschoten MV, Savelkoul HFJ, De Vos P, Dekker J, and Wells JM**. Age-associated Impairment of the Mucus Barrier Function is Associated with Profound Changes in Microbiota and Immunity. *Sci Rep* 9: 1437, 2019.
111. **Swain Ewald HA, and Ewald PW**. Natural Selection, The Microbiome, and Public Health. *Yale J Biol Med* 91: 445-455, 2018.
112. **Tazoe H, Otomo Y, Karaki S, Kato I, Fukami Y, Terasaki M, and Kuwahara A**. Expression of short-chain fatty acid receptor GPR41 in the human colon. *Biomed Res* 30: 149-156, 2009.
113. **Thevaranjan N, Puchta A, Schulz C, Naidoo A, Szamosi JC, Verschoor CP, Loukov D, Schenck LP, Jury J, Foley KP, Schertzer JD, Larche MJ, Davidson DJ, Verdu EF, Surette MG, and Bowdish DME**. Age-Associated Microbial Dysbiosis Promotes Intestinal Permeability, Systemic Inflammation, and Macrophage Dysfunction. *Cell Host Microbe* 21: 455-466 e454, 2017.
114. **Ticinesi A, Lauretani F, Milani C, Nouvenne A, Tana C, Del Rio D, Maggio M, Ventura M, and Meschi T**. Aging Gut Microbiota at the Cross-Road between Nutrition, Physical Frailty, and Sarcopenia: Is There a Gut-Muscle Axis? *Nutrients* 9: 2017.
115. **Ticinesi A, Tana C, and Nouvenne A**. The intestinal microbiome and its relevance for functionality in older persons. *Curr Opin Clin Nutr Metab Care* 22: 4-12, 2019.
116. **Tomosada Y, Chiba E, Zelaya H, Takahashi T, Tsukida K, Kitazawa H, Alvarez S, and Villena J**. Nasally administered Lactobacillus rhamnosus strains differentially modulate

respiratory antiviral immune responses and induce protection against respiratory syncytial virus infection. *BMC Immunol* 14: 40, 2013.
117. **Utzschneider KM, Kratz M, Damman CJ, and Hullar M**. Mechanisms Linking the Gut Microbiome and Glucose Metabolism. *J Clin Endocrinol Metab* 101: 1445-1454, 2016.
118. **Vaiserman AM, Koliada AK, and Marotta F**. Gut microbiota: A player in aging and a target for anti-aging intervention. *Ageing Res Rev* 35: 36-45, 2017.
119. **van Tongeren SP, Slaets JP, Harmsen HJ, and Welling GW**. Fecal microbiota composition and frailty. *Appl Environ Microbiol* 71: 6438-6442, 2005.
120. **Ventura M, Turroni F, Canchaya C, Vaughan EE, O'Toole PW, and van Sinderen D**. Microbial diversity in the human intestine and novel insights from metagenomics. *Front Biosci (Landmark Ed)* 14: 3214-3221, 2009.
121. **Wakimoto P, and Block G**. Dietary intake, dietary patterns, and changes with age: an epidemiological perspective. *J Gerontol A Biol Sci Med Sci* 56 Spec No 2: 65-80, 2001.
122. **Walsh ME, Bhattacharya A, Sataranatarajan K, Qaisar R, Sloane L, Rahman MM, Kinter M, and Van Remmen H**. The histone deacetylase inhibitor butyrate improves metabolism and reduces muscle atrophy during aging. *Aging Cell* 14: 957-970, 2015.
123. **Weiss GA, and Hennet T**. Mechanisms and consequences of intestinal dysbiosis. *Cell Mol Life Sci* 74: 2959-2977, 2017.
124. **Wosinska L, Cotter PD, O'Sullivan O, and Guinane C**. The Potential Impact of Probiotics on the Gut Microbiome of Athletes. *Nutrients* 11: 2019.
125. **Yang Y, Shi Y, Wiklund P, Tan X, Wu N, Zhang X, Tikkanen O, Zhang C, Munukka E, and Cheng S**. The Association between Cardiorespiratory Fitness and Gut Microbiota Composition in Premenopausal Women. *Nutrients* 9: 2017.
126. **Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, and Gordon JI**. Human gut microbiome viewed across age and geography. *Nature* 486: 222-227, 2012.
127. **Yoon MY, and Yoon SS**. Disruption of the Gut Ecosystem by Antibiotics. *Yonsei Med J* 59: 4-12, 2018.
128. **Zhu B, Wang X, and Li L**. Human gut microbiome: the second genome of human body. *Protein Cell* 1: 718-725, 2010.
129. **Zwielehner J, Liszt K, Handschur M, Lassl C, Lapin A, and Haslberger AG**. Combined PCR-DGGE fingerprinting and quantitative-PCR indicates shifts in fecal population sizes and diversity of Bacteroides, bifidobacteria and Clostridium cluster IV in institutionalized elderly. *Exp Gerontol* 44: 440-446, 2009.