Fecal Microbiota and Associated Metabolites are Minimally Affected by Ten Weeks of Resistance Training in Younger and Older Adults

by

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Abstract

Recent evidence suggests that short chained fatty acids (SCFAs) produced by gut microbiota may impact body composition and muscle accretion. Additionally, aging is implicated in negative alterations to the gut microbiome and exercise training has been posited to positively affect gut microbiota. However, limited human evidence indicates that shorter-term resistance training does not appreciably alter the gut microbiome in older adults, and no human study has examined if resistance training differentially alters the gut microbiome and associated SCFAs between younger and older individuals. Therefore, we examined whether 10 weeks of resistance training (RT) differentially altered fecal microbiota composition, fecal and circulating SCFAs, and serum markers associated with gastrointestinal integrity in younger and older adults. Fecal and serum samples were collected from untrained younger (22 ± 2 years, n=12) and older (58 ± 8 years, n=12) participants prior to and following 10-weeks of supervised twice-weekly full body RT. Outcome measures prior to (Pre) and following the training intervention (Post) included body composition measured by dual x-ray absorptiometry, vastus lateralis (VL) thickness measured by ultrasound, fecal microbiome data from 16S rRNA gene sequencing, serum and fecal SCFAs measured by gas chromatography, and serum intestinal fatty acid-binding protein 2 (FABP2), lipopolysaccharide-binding protein (LBP), and leucine-rich alpha-2 glycoprotein (LRG-1) quantified by enzyme-linked immunosorbent assays. Repeated measures two-way (group×time; G×T) ANOVAs were performed for all dependent variables over time, and Spearman correlations were used to explore if changes in training variables were associated with microbiome variables and SCFAs. RT significantly increased VL thickness and lean body mass ($p<0.05$) in both groups, but no GxT interactions were evident. Although differences in beta

diversity were evident between younger and older participants, no significant age, time, or interaction effects were evident. Seven SCFAs were detected in the fecal samples, albeit no significant age, time, or interaction effects were evident. Acetic acid was the only SCFA detected in serum, and no significant age, time, or interaction effects were evident. Serum LRG1 exhibited a significant main effect of time (Pre>Post, $p=0.007$) and age (Younger>Older, $p=0.015$), but this marker nor serum FABP2 or LBP exhibited significant interactions. In all younger, older, and/or all participants combined, there were no significant correlations between RT-induced changes in muscle mass-related outcomes and changes in either metrics of fecal microbiome diversity, total or individual SCFAs, or serum FABP2/LBP/LRG-1 ($p>0.00067$). Our data highlights that 10 weeks of RT largely does not alter the fecal microbiome, associated SCFAs, or select markers of gastrointestinal integrity in untrained younger or older adults.

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Table of Contents

Chapter I: Introduction

The human gut microbiota consists of a diverse array of microorganisms, including bacteria, viruses, fungi, and archaea, which inhabit the gastrointestinal tract. The gut contains the largest and most diverse microbial community in the human body (1). Through symbiotic relationships, the gut microbiota contributes to digestion, vitamin synthesis, immune regulation, and protection against pathogens (2). Environmental factors including diet, exercise, xenobiotics, stress, and trauma have been observed to modify the composition of the gut microbiome (3, 4, 5), thus creating a complex balance between microbes that are beneficial for the host and those that may contribute to disease (6).

In 2007, Bäckhed et al. (7) performed a preclinical study investigating the mechanisms underlying the resistance to diet-induced obesity in germ-free mice and was the first study to suggest the presence of a gut–muscle axis. Others have reported that supplementing mice with the probiotic Lactobacilli plantarum (*L plantarum*) for six weeks significantly increased grip strength, swimming time to exhaustion, normalized muscle weight and the percentage of Type I myofibers (8). Yan and co-workers subsequently provided evidence that the gut microbiome could regulate skeletal muscle fiber-type composition (9), expanding the ways in which skeletal muscle can be influenced by the microbiome. In a more recent study, Fernandez et al. demonstrated that endurance training increased the number of taxa in 8-week-old mice which correlated with physical performance (10). However, neither resistance training nor endurance training altered the phyla and family compositions (10). Cullen et al. (11) reported that resistance training induces mixed changes in the gut microbiome in sedentary young adults. These collective results suggest that the gut microbiota may communicate with skeletal muscle to, in part, regulate the adaptive responses to exercise training.

Short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, are microbial metabolites produced through the fermentation of dietary fibers and resistant starches by gut bacteria (12). Acetate is produced by intestinal bacteria of the genus *Bifidobacteria, Lactobacilli*, *Clostridium,* and *Akkermansia* (13). Propionate is largely produced by intestinal bacteria of the genus *Bacteroides, Ruminococcus,* and *Roseburia* (14). Butyrate is mainly produced by intestinal bacteria of the genus *Faecalibacterium prausnitzii, Eubacterium rectale and Roseburia spp.* (15). SCFAs have been suggested to participate in crosstalk with skeletal muscle, proposing a direct link between microbiome-derived metabolites and skeletal muscle physiology (16, 17). In this regard, Lahiri et al. (18) presented evidence of SCFAs regulating skeletal muscle mass and function in germ-free (GF) mice by treating the GF mice *in vivo* with a cocktail of SCFAs thus partly reversing skeletal muscle impairment. Interestingly, studies in rodents and humans indicate that endurance exercise training (ET) or combined ET+RT training modulates fecal SCFA content (19, 20, 21, 22). However, no study to our knowledge has examined if resistance training alone alters fecal and circulating SCFAs in humans.

Although the aforementioned results are promising, there are human data indicating that resistance training may minimally affect gut microbiome outcomes. For instance, our group recently published a study in older individuals indicating that shorter-term (i.e., six weeks) of resistance training does not affect microbiome diversity or abundances of various taxa (23), and this agrees in principle with other resistance training interventions demonstrating null effects (24, 25). However, our prior null results may have been due to either the shorter-term nature of the intervention and/or only examining older individuals who demonstrate a limited capacity to experience changes in the gut microbiome (26). We previously performed two investigations examining the effects of a peanut protein supplement on training outcomes in younger and older

adults (27, 28), and both studies indicated that training (but not supplementation) promoted increases in measures of skeletal muscle hypertrophy and strength. Given our interest in determining if longer-term training and/or aging affects gut microbiome adaptations, we leveraged banked fecal specimens and remaining blood sera from 12 younger and 12 older participants in these studies to examine if ten weeks of resistance training (RT) either altered the gut microbiome, fecal and serum SCFAs, and/or serum markers of gastrointestinal integrity. A secondary aim was to determine if changes in these outcomes were associated with indices of muscle hypertrophy. We hypothesized that RT would improve microbiome diversity and relative abundance of bacterial taxa and increase microbial metabolites in younger but not older participants. We also hypothesized that RT would alter fecal and serum SCFAs in younger, but not older, participants. However, we adopted the null hypothesis that changes in microbiota and SCFA would not be associated with training outcomes.

Chapter II – Literature Review

Gut microbiome

Composition of the gut microbiome

The gastrointestinal tract harbors the greatest variety of microbes and species in the human body compared to other areas of the human body (29). In this regard, the gut microbiome possesses trillions of microorganisms, including bacteria, viruses, fungi, and other microbes, that reside within the human gastrointestinal tract, particularly in the large intestine. The gut microbiome is highly diverse, with a unique collection of microbial species, and is influenced by various factors, including diet, age, hormones, and environmental exposures (30, 31, 32). The gut is dominated by the microbial phyla *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria*, *Fusobacteria,* and *Verrucomicrobia*, with the two phyla Firmicutes and Bacteroidetes generally representing 90% of gut microbiota (33). The Firmicutes phylum is composed of more than 200 different genera such as Lactobacillus, Bacillus, Clostridium, Enterococcus, and Ruminococcus. Clostridium genera represent 95% of the Firmicutes phyla. Bacteroidetes consists of predominant genera such as Bacteroides and Prevotella. The Actinobacteria phylum is proportionally less abundant and mainly represented by the Bifidobacterium genus (34). The advancement in technology and improvements in metagenomic testing have increased the comprehension of the gut microbiome. In recent years, the 16S rRNA gene sequencing technique, which involves sequencing a specific region of the 16S ribosomal RNA gene to provide information about the taxonomic composition and diversity of the microbial community based on the variation in that region, has increased in popularity and precision (35, 36, 37). The low cost and effectiveness of sequencing have influenced further investigation of the factors that impact the gut microbiome.

Diet and probiotic effects on the gut microbiome

One of the factors that influences diversity of the gut microbiome is diet. A diet rich in fiber, such as fruits, vegetables, whole grains, and legumes, increases gut microbiome diversity and composition (38, 39, 40). Klimenko and co-workers demonstrated that long-term fruit and vegetable consumption, increasing dietary fiber, decreased levels of Bacteroidaceae, Porphyromonadaceae and Rikenellaceae while increasing levels of Methanobrevibacter, Bifidobacterium, Clostridium, and butyrate-producing Lachnospiraceae (41). Additionally, Martinez et al. revealed that the consumption of 60 g whole-grain barley, brown rice, or a mixture of both, increased microbial diversity, the Firmicutes/Bacteroidetes (F/B) ratio, and the abundance of the genus Blautia in fecal samples. Furthermore, whole grain consumption reduced serum plasma inflammatory biomarker interleukin-6 (IL-6) (42). However, a diet high in refined carbohydrates, saturated fats, and processed foods can lead to a less diverse and potentially increase harmful bacteria in the gut (43, 44). For example, David and co-workers showed that a high-fat diet, consisting solely of animal-based foods, significantly shifted composition of the gut microbiota (32). The Western diet, which is high in fat and refined carbohydrate, can lead to endotoxemia, a condition characterized by increased intestinal permeability and dysbiosis (an imbalance of the structure and function of the gut microbiota) (45). Furthermore, the intake of probiotics, enriched with either Lactobacillus or Bifidobacterium, through probiotic-enriched yogurt and milk consumption or supplementation, is beneficial to gut health and influences gut microbiome composition (46, 47). The addition of probiotics in the diet is one of the key solutions to restore the gut microbial balance and prevent diseases and inflammation following antibiotic treatments, which induces gut dysbiosis (48, 49). Moreover, probiotics have been studied for their potential to alleviate symptoms of gastrointestinal disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) (50, 51, 52). For example,

Nobaek et al. administered 400ml/day of probiotic drinks to irritable bowel syndrome (IBS) patients for four weeks and found that the intake of probiotics improved IBS symptoms including abdominal pain and flatulence (53). The collective evidence of the interactions between diet and probiotics in shaping the gut microbiome suggests a therapeutic role in managing gut dysbiosis-related conditions.

Aging and the gut microbiome

Aging is another factor that influences diversity of the gut microbiome. After the age of 65, gut microbiota resilience is generally reduced, and its overall composition is more vulnerable to lifestyle changes, drug treatments such as antibiotics, and disease (54). Evidently, there tends to be a decline in microbial diversity with aging (55, 56), which can lead to imbalances and dysbiosis, thus further exacerbating age-related health complications such as sarcopenia, metabolic disorders, and dementia (57, 58, 59). Sarcopenia is the progressive and generalized loss of muscle mass and function that occurs primarily in the elderly and is strongly associated with frailty (60). Sarcopenia is very common due to factors including reduced physical activity, decreased circulating sex hormone levels, and decreased nutrient digestion and absorption (61, 62). Few studies have shown a correlation between sarcopenia in older adults and changes in the gut microbiome composition and diversity. In comparing the gut composition of the elderly to young adults, Claesson et al. discovered a lower proportion of phylum Firmicutes (40%) and higher proportions of phylum Bacteroidetes (57%) in the elderly than for younger adults (63). The same authors also showed that diversity of the gut microbiome is inversely correlated with physical function and the institutionalization of older adults (54). Similar to the Claesson findings, Zhou and co-workers analyzed fecal samples of sarcopenic patients and found that they

exhibited a significant decrease in the abundance of phylum Firmicutes and a corresponding increase in the abundance of phylum Proteobacteria. The genera Blautia was also significantly decreased in patients with sarcopenia (64). Additionally, Han and co-workers found that the Firmicutes/Bacteroidetes ratio was significantly reduced in elderly adults with low muscle mass, which is a characteristic of sarcopenia (65). Though these changes in the gut microbiome composition may influence muscle atrophy in the elderly, chronic inflammation also plays an important role in sarcopenia (66). The dysregulation of cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), in aging individuals not only perpetuates inflammation but also contributes to tissue damage and impaired cellular repair mechanisms (67, 68, 69). For instance, Buford et al. compared the microbiota profiles of young and older adults, and the results provided evidence of inflammation in the gut with aging. The authors reported a significant abundance of phylum Bacteroidetes in the older adults, which was also positively correlated with inflammatory markers IL-6 and TNF-alpha (70). Furthermore, Sovran et al. discovered that a proportion of goblet cells in the colonic crypts of 19-month-old mice stained positive for cleaved caspase 3 (a crucial protein involved in the execution phase of apoptosis) which may account for the reduced thickness of secreted mucus in the old mice (71). Nonetheless, vitamin deficiencies in the elderly are known to contribute to age-related conditions including sarcopenia and dementia. The microbes in the gut aid in synthesizing essential vitamins including several B vitamins and vitamin K (72, 73, 74). Age-related changes in the gut microbiome may impact the efficiency of key nutrient absorption. Further investigation in this area is warranted.

Gut-muscle axis

The gut-muscle axis represents a bidirectional communication and interaction between the gastrointestinal tract and skeletal muscle function and metabolism. This crosstalk involves various signaling pathways through which the gut microbiota, metabolites, and muscles influence each other's function and health (75, 76). Several rodent studies have demonstrated this bidirectional interaction. For instance, Bäckhed et al. led a pioneering study in 2004 in which they infused germ-free (GF) mice, lacking a microbiome, with the cecal contents of conventionally raised animals, and demonstrated a reciprocal reduction in muscle insulin sensitivity and glucose tolerance. These findings suggest microbiome regulation of muscle metabolic function (77). The same authors also compared skeletal muscle protein activity and gene expression in GF and non-perturbed (control) animals. They reported skeletal muscle from GF mice exhibited greater activity of the AMP-activated protein kinase (AMPK) and carnitine palmitoyl transferase-1 (CPT-1) enzymes (7). Although this is an indication of elevated oxidative capacity, this can also present challenges for muscle mass maintenance given that AMPK overstimulation interferes with signaling cascades (e.g., mTORC1) that promote muscle anabolism (78). Following their work, Yan and co-workers determined that differences in the skeletal muscle properties are transmissible via fecal microbiota transplantation. The GF mice with fecal transplantation from a pig with increased microbial diversity exhibited larger fiber cross-sectional area (fCSA) of the gastrocnemius muscle (9). Alternatively, Kundu et al. revealed that fecal microbiome transplant into GF mice from either old or young mice did not exhibit differences in skeletal muscle mass or function (79). Lahiri and colleagues compared the skeletal muscle of GF mice to the skeletal muscle of a specific pathogen-free (SPF) mice that had gut microbiota. They found that the GF mice skeletal muscle were atrophic compared to SPF mice (18). Additionally, the absence of the gut microbiota induced the degradation of branched-chain

amino acids (BCAA) in muscle, and the enzymes involved in the BCAA catabolic pathway (branched-chain aminotransferase 2 (BCAT2) and branched-chain-keto acid dehydrogenase (BCKDH)) were elevated in GF mice (18). Furthermore, the same authors observed an increased expression of Atrogin-1 and Murf-1 and elevated expression of FoxO3, known to regulate Atrogin-1 and Murf-1. Additionally, MyoD and Myogenin, key genes in skeletal muscle differentiation were decreased in GF mice (18). These muscle molecular markers are indicative of an atrophic phenotype and, again, highlight potential mechanisms in which the gut microbiome may regulate skeletal muscle size and function. These have been a few of the pioneering studies to examine the gut-muscle axis crosstalk. However, the underlying mechanism(s) through which the gut microbiome impacts skeletal muscle mass and function is still complex and not well understood.

Gut permeability, inflammation and muscle damage

Gut barrier integrity and intestinal permeability are likely operative in the gut-muscle axis. The gut barrier plays a crucial role in regulating the passage of bacterial products and metabolites into the systemic circulation. Increased intestinal permeability (leaky gut) can lead to the translocation of bacterial components and trigger inflammation (80), potentially contributing to muscle damage and impaired recovery processes (81). Adding strength to the potential importance of intestinal permeability in maintaining muscle mass, Qi and co-workers found that serum zonulin, a tight junction protein, was high in older adults and associated with systemic inflammation (82). In another study, Cesari and co-workers found that serum concentrations of IL-6 and CRP, measured via enzyme-linked immunosorbent assay (ELISA), were inversely associated with appendicular lean mass (83). Additionally, Haddad and co-workers infused rats

with IL-6 to identify signaling pathways affected by this stimulus. The infusion of IL-6 resulted in a reduction in the amount of phosphorylated S6K1 protein, which is a protein kinase that plays a crucial role in the regulation of protein synthesis (84). Probiotic supplements, specifically Lactobacillus casei LC122 and Bifidobacterium longum BL986, attenuates pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1beta, and upregulates the PGC1-alpha 4 gene to positively affect muscle protein synthesis (85). Despite these promising findings, the field of gut-muscle axis research is relatively nascent, with many questions remaining unanswered. Future studies are needed to elucidate specific mechanisms by which gut microbiota influence muscle physiology in humans.

Gut microbiota and exercise

Emerging evidence suggests that exercise can modulate the gut microbiota composition, promoting the growth of beneficial bacteria and enhancing gut barrier function. This interaction highlights the potential therapeutic implications of targeting the gut-muscle axis in managing conditions related to both systems (86). Endurance exercise is beneficial for human health including physical, mental, and gastrointestinal health (87, 88, 89). Although the relationship between the gut microbiome and exercise has been shown in research to be bidirectional, the mechanism in which exercise influences the composition and diversity of the gut microbiome continues to be investigated. Many rodent studies have provided insight into some of the mechanisms. For instance, Hsu et al. revealed that endurance swimming time was improved for the SPF and Bacteroides fragilis (BF) gnotobiotic mice, but not the GF mice (90). Another study by Fernandez et al. explored the relationship between endurance and resistance training and gut microbiota composition in mice. They found that increased microbiome richness and evenness

were associated with endurance and resistance training, according to Shannon and Simpson indexes, respectively (10). Furthermore, Chen et al. investigated the relationship between the gut microbiome and exercise performance by supplementing mice with probiotics (L. plantarum TWK10) and engaging the animals in strength and endurance exercises. Their results showed an improvement in forelimb grip strength and swimming time to exhaustion (8). While there are limited studies in humans, several studies have shed light on the bidirectional relationship of exercise and the gut microbiome. Huang and co-workers replicated the Chen et al. 2016 study in humans. The authors reported that 6 weeks of L. plantarum TWK10 probiotic supplementation improved the runners' time-to-exhaustion and glucose levels, possibly due to the ability of L. plantarum to regulate blood glucose levels in response to insulin (91). Additionally, Morita and colleagues investigated the effects of aerobic training in improving intestinal microbiota composition in elderly women. They found that moderate-intensity brisk walking increased the relative abundance of Bacteroides and decreased Clostridium subcluster XIVa (92). Walking distance and time spent brisk walking increased, which was positively correlated with the change in the Bacteroides (92). Lastly, Motiani et al. found that short-term moderate-intensity continuous training increased the relative abundance of phylum Bacteroidetes, while decreasing the abundance of genus Blautia and Clostridium. The low abundance of Blautia was associated with better whole-body insulin sensitivity (93). Further, intestinal inflammatory markers LBP, TNF-α, and CRP were reduced because of the training (93).

Although the aforementioned studies highlight the role of endurance training in improving gut microbiome composition in humans, limited effects have been observed with resistance training. Resistance exercises, such as weightlifting or bodyweight exercises, may enhance the abundance of beneficial bacteria in the gut (94, 95). Zhong et al. examined the

effects of aerobic and resistance training on the gut microbiota in sedentary elderly women. The authors reported that resistance training altered beta diversity, but no changes in alpha diversity were observed (96). The relative abundance of Firmicutes was significantly reduced with both resistance and endurance training, but the non-training group experienced a significant increase in the relative abundance of Bacteroidetes (96). Alternatively, Moore et al. found that 6 weeks of resistance training did not significantly alter the composition of gut microbiome. Interestingly, however, the authors revealed that tight junction protein, zonulin, non-significantly decreased with training suggesting that intestinal permeability may have beenmodulated (23). Still, it remains unclear as to whether resistance training can impact the microbiome in either older or young adults.

Short-chain fatty acids (SCFAs)

An important contribution of the gut microbiome-muscle interaction is the production of short chain fatty acids (SCFAs), which are fatty acids with fewer than six carbon atoms. SCFAs are primarily produced through the fermentation of non-digestible carbohydrates and dietary fiber by gut bacteria in the colon (97). The SCFAs mostly reported in research include acetate, propionate, and butyrate, which serve as important metabolites with various physiological roles (98, 99). The production of SCFAs is influenced by factors such as diet composition, gut microbiota composition, and fermentation capacity (100). Acetate is the most abundant SCFA in the human colon (101) and it accounts for more than half of SCFAs content (102). Acetate is produced by intestinal bacteria of the genus *Bifidobacteria, Lactobacilli*, *Clostridium,* and *Akkermansia* (13). Propionate is mainly produced by intestinal bacteria of the genus *Bacteroides, Ruminococcus,* and *Roseburia* (14). Butyrate is mainly produced by intestinal bacteria of the

genus *Faecalibacterium prausnitzii, Eubacterium rectale and Roseburia spp.* (15). Butyrate, although produced in the smallest amount as compared to acetate and propionate, is the main source of energy for colonocytes.

Most SCFAs are absorbed via passive diffusion across the colonic epithelium, facilitated by their small size and lipophilic nature. The absorption rate depends on factors such as luminal pH, SCFA concentration, and the presence of transporter proteins (103, 104). Once absorbed, SCFAs enter the portal circulation and are transported to the liver, where they can be further metabolized or utilized as an energy source. Acetate is primarily metabolized in peripheral tissues, such as skeletal muscle, adipose tissue, and the heart, where it serves as an energy source and contributes to lipid metabolism. Initially, acetate is converted to acetyl-CoA by the enzyme acetyl-CoA synthetase. Acetyl-CoA can then either enter the TCA cycle for energy production or be used for lipid synthesis (105). Propionate is mainly metabolized in the liver, where it plays a role in gluconeogenesis, contributing to the regulation of blood glucose levels. Propionate increases mRNA expression of key genes involved in gluconeogenesis including phosphoenolpyruvate carboxykinase 1 (PCK1), phosphoenolpyruvate carboxykinase 2 (PCK2), and pyruvate carboxylase (PC) (106, 107). Butyrate is the primary energy source for colonic epithelial cells and is essential for maintaining intestinal barrier function and mucosal integrity, reducing inflammation, and regulating immune function (108, 109, 110).

SCFAs can be altered with aging. This phenomenon may be associated with changes in the expression or activity of SCFA transporters and metabolic enzymes involved in SCFA absorption and utilization (111, 112). Dysbiosis may also disrupt the production of SCFAs, especially butyrate, which can negatively impact muscle protein synthesis and insulin sensitivity (16, 113, 114).

SCFAs and exercise

Many of the microbiome effects on skeletal muscle may be modulated through SCFAs, either directly or indirectly. SCFAs may affect muscle metabolism due to their roles in serving as an energy source and acting as signaling mediators. The utilization of SCFAs as an alternate energy source is through the complete oxidation of acetate, butyrate and propionate yielding 10, 27 and 18 ATP/M, respectively (115). SCFAs treatment in GF mice reduced the expression of Atrogin-1 and increased expression of MyoD, a marker associated with myogenesis, in the tibialis anterior muscle (18). Another study by Walsh et al. examined the effects of older mice consuming butyrate, a general histone deacetylase inhibitor, in their diet. The authors found that butyrate improved glucose tolerance and prevented muscle atrophy in 26-month-old mice (116). Furthermore, Huang and colleagues revealed that the SCFAs produced by the gut microbiota enhanced mitochondrial biogenesis, thus improving endurance performance. However, there was no change in grip strength (117). Lastly, Allen et al. found that aerobic exercise increased fecal concentrations of acetate, propionate, and butyrate in sedentary lean individuals (19). Additionally, endurance training also increased butyrate-producing bacteria, Roseburia spp., Faecalibacterium spp., Lachnospira spp., and Clostridiales spp. (19). However, we are unaware of any research studies examining how resistance exercise acutely or chronically alters fecal SCFA concentrations. Hence, further research is needed to investigate the potential effects of resistance training on SCFAs in humans.

Conclusion

The intricate relationship between the gut microbiome and skeletal muscle health, mediated by SCFAs, emphasizes a pivotal intersection of microbiology and human physiology. Through their metabolic activities, human gut bacteria produce SCFAs that exert a variety of documented effects on muscle metabolism, function, and overall health. These SCFAs not only enhance energy utilization and mitochondrial function within muscle cells, but also modulate inflammation, all of which may significantly influence functional and performance outcomes. As research continues to unveil the complexities of this bidirectional relationship, harnessing the potential therapeutic benefits of targeting the gut microbiome and its metabolites presents exciting opportunities for improving strategies in muscle health maintenance, treatment of musculoskeletal disorders such as sarcopenia, and the enhancement of athletic performance with ergogenic aids. Maintaining a balanced gut microbiome and optimizing SCFAs production emerges as a potential avenue to prevent skeletal muscle atrophy and support muscle function and overall well-being.

Chapter III: Methods

Ethical approval and participant eligibility

This study is a secondary analysis of 12 younger adults and 12 older adults that completed 10 weeks of resistance training. Both studies were conducted under a single approved protocol by the Auburn University Institutional Review Board (IRB) (Protocol # 19-249 MR 1907) and both studies were pre-registered clinical trials (NCT04015479; registered July 11, 2019, NCT04707963; registered 13 January 2021). Detailed methods of the original studies have been previously described (27, 28).

Participants were recruited at Auburn University campus and locally via flyers, emails, and direct contact either face-to-face or over the phone. Both studies had identical eligibility criteria aside from age, which included: (i) 18–30 years old, or 50-80 years old, (ii) body mass index (BMI) <35 kg/m^2, (iii) not actively participating in RT more than one time per week in the preceding six months, (iv) no known peanut allergy, (v) free of metal implants that could interfere with X-ray procedures, (vi) no medically necessary radiation exposure for six months prior, (vii) free of obvious cardiovascular or metabolic disease, (viii) free of conditions contraindicating participation in exercise program or donation of muscle biopsy (i.e., blood thinners or blood clotting disorder), (ix) for females, could not be pregnant or trying to become pregnant. Eligible individuals were informed of all study procedures and completed a medical history questionnaire prior to signing informed consent to participate.

Study design

This section serves to provide a brief overview of procedures relevant to the data presented herein. Participants reported to the School of Kinesiology at Auburn University for pre-testing battery (Pre) which included a urine specific gravity (USG) to ensure hydration and a rapid

pregnancy test for female participants to ensure safety for X-ray exposure. Next, height and weight assessments using digital column scales, right leg vastus lateralis (VL) thickness using ultrasound, and body composition using full body dual-energy X-ray absorptiometry (DXA) scan. Venous blood was obtained from an arm vein in serum separator tubes (BD Vacutainer; Franklin Lakes, NJ, USA), and blood was allowed to clot for 30 minutes at room temperature prior to being centrifuged at 3500 g for serum separation. Each participant was provided with a three-day food log and stool specimen collection kit to take home to complete and return them at the next appointment. Participants then performed 10 weeks of supervised full-body RT twice weekly as described by Sexton et al. (27) and Lamb et al. (28). All training sessions were supervised by study personnel to ensure proper form throughout the sessions. After completion of the 10-week RT program, participants were provided with another set of three-day food logs and stool collection kits for post-intervention testing (Post), which was conducted 72 h after their last workout. The post-intervention testing replicated Pre procedures, which included USG, pregnancy test in females, height and weight assessments, right leg VL thickness using ultrasound, a DXA scan, a venous blood draw, and submission of food-logs and stool samples. The following paragraphs provide more in-depth descriptions of testing sessions.

Body composition

During baseline testing and post-intervention testing, participants reported to the School of Kinesiology at Auburn University and submitted a urine sample to assess USG levels using a handheld refractometer (ATAGO; Bellevue, WA, USA). All participants recorded USG values below 1.020 indicating that they were hydrated. Next, height and body weight were then assessed utilizing a laboratory scale (Seca 769; Hanover, MD, USA), with height and body

weight being collected to the nearest 0.5 cm and 0.1 kg. Following these measurements, participants underwent a whole-body DXA scan (Lunar Prodigy; GE Corporation, Fairfield, CT, USA) for determination of total lean soft tissue mass (LSTM) and fat mass (FM). Notably, all scans were performed by the same study personnel during the pre-testing and post-testing.

Resistance training program

Participants completed supervised RT twice weekly for 10 weeks where detailed instructions were provided on proper posture, form, technique, and body positioning to ensure safety. The training protocol required a minimum of 48 hours, in between sessions, to allow for proper recovery. Each training session included a warm-up of 25 jumping jacks and 10 body weight squats, a warm-up of 1 set of 10 reps at 50% of working weight, 1 set of 5 reps at 75% working weight, and 1set of 3 reps at 90% of working weight. Post warm up, younger participants performed either 4 sets of 10 reps (high volume) or 5 sets of 6 reps (high load) at working weight. The older participants performed 4 sets of 10-12 reps at working weight. Following each set, rating of perceived exhaustion (RPE) was recorded to ensure appropriate loads were implemented throughout. An RPE below 7 resulted in a slight increase in working weight on the next set and an RPE at 10 or incomplete set resulted in a slight decrease in working weight on the next set. Furthermore, participants in the PPS group consumed their supplement after workouts under the supervision of study personnel, and participants in the CTL group did not consume any supplement.

Dietary analysis

Participants were instructed to complete a three-day food log, which included nutritional intakes for two weekdays and one weekend day and return them prior to the baseline testing (T1-2) and again the last day of testing (T3). Regular dietary habits were encouraged throughout the duration of the study, besides consumption of protein supplements by individuals in the PPS group. The Nutrition Data System for Research (NDSR; NDSR 2014; University of Minnesota) software was utilized by study personnel to analyze all young participant food logs. The Automated Self-Administered 24-Hour Dietary Assessment tool (ASA24) was utilized by study personnel to analyze all older participant food logs.

Fecal microbiome analysis

Stool samples were collected with a commode specimen collection kit and sterile collection tubes. Participants were instructed to seal and store samples in the freezer prior to submission at the next visit. Upon receipt, samples were stored in a -80 degree Celsius prior to processing. Fecal microbial DNA was isolated using Zymo Research miniprep kits (Irvine, CA, USA). DNA samples were prepared, and polymerase chain reaction (PCR) amplified 250 base pair variable region 4 of the 16S rRNA. The PCR amplicon library was sequenced on the Illumina Miseq (San Diego, CA, USA) (118, 119). Further detailed processing steps have been previously described (13). The Quantitative Insight into Microbial Ecology (QIIME) 2 pipeline, utilizing version 1.8 DADA2 generated amplicon sequence variants (ASVs) to the species level (120, 121, 122). UCLUST clustered sequences into ASVs (previously operational taxonomic units (OTUs)) with a 97% similarity threshold. Thereafter, taxonomic assignments were issued using the SILVA database (123). ASVs with an average abundance >0.005% were further processed and grouped by taxonomy. Microbiota alpha diversity was determined using Observed Species, Shannon

Index, Simpson Index, and Whole Tree Phylogeny. To determine overall microbiota compositional change from baseline to post 10-weeks RT program, beta diversity was measured using Bray Curtis, Unweighted Unifrac, and Weighted Unifrac metrics.

Fecal and serum SCFAs analysis

Short-chain fatty acids (SCFAs) were quantified using gas chromatography (Thermo Trace 1310) coupled to a flame ionization detector (Thermo Fisher Scientific) as previously described (124). Aliquots of stool samples were homogenized using MP Bio FastPrep post suspension in MilliQ-grade water at 4.0 m/s for 1 minute. Next, the samples were acidified by adding 5 M HCL to the fecal suspensions, until it reached a final pH of 2.0. The suspensions were incubated for 10 minutes and then centrifuged at 10,000 revolutions per minute (RPM). Supernatants were spiked with 2-Ethylbturyic acid until a final concentration of 1 mM was reached. SCFAs were detected from the supernatants by direct injection into a Thermo TG-WAXMS A GC Column (30 m, 0.32 mm, 0.25 μm). The injected standard solutions of individual SCFAs were used for calibration. Serum SCFAs were also analyzed in a similar process previously described (125). Gas chromatography-mass spectrometry (GC-MS) is the most common method used for SCFA analysis. Due to their volatile nature, GC is particularly suited for separating and quantifying these compounds from biological samples such as feces, plasma, or urine (126). The chromatogram provides a profile of SCFAs present in a sample, enabling researchers to quantify concentrations and further study metabolic processes linked to gut health and disease states (127).

Serum biomarkers

Serum samples were stored and removed from -80C to determine concentrations of intestinal fatty acid-binding protein (FABP), leucine-rich alpha-2-glycoprotein 1 (LRG-1), and lipopolysaccharide-binding protein (LBP) using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (AFG Bioscience, Wood Dale, IL USA; catalog #'s: EK710951, EK710885, EK241481). All three assays followed similar protocols which ended with determining absorbance readings at 450 nm using a microplate spectrophotometer (Biotek Synergy H1 hybrid reader; Agilent, Santa Clara, CA, USA). Sample absorbance readings were compared to standard curve absorbance readings, and values are provided in serum concentration units. The three kits performed sufficiently as determined by unknown absorbance values following within the linear range of the standard curves and coefficient of variation values for duplicates being relatively around or less than 20% (FABP = 22.8%, LBP = 12.3%, LRG-1 = 18.5%).

Statistics

Statistical analysis was conducted using RStudio Team (2023) (Integrated Development for R. RStudio, PBC, Boston, MA, USA). For all dependent variables over time, repeated measures two-way (group × time; GxT) analysis of variance (ANOVA) tests were performed. Kruskal-Wallis ANOVA with false discovery rate (FDR) corrections were conducted to compare relative abundance of all ASVs between both groups at both time points. Alpha diversity was measured using ASV counts (observed species), PD Whole Tree Phylogeny, and Shannon index. Beta-diversity was measured using Bray–Curtis Dissimilarity and Weighted Unifrac distance metrics. Change scores (or delta scores) in all dependent variables were also calculated by subtracting PRE values from POST values, and these scores were compared within each group using

independent samples t-tests. Spearman correlations were conducted on select variables to explore relationships between changes in alpha diversity, changes in SCFAs, and changes in body composition. Spearman's rho and p-values are provided for each group individually and when merged. Statistical significance was established as $p < 0.05$, and relevant p-values are depicted in-text or within figures.

Chapter IV: Manuscript (to be submitted to Journal of Applied Physiology)

Fecal microbiota and associated metabolites are minimally affected by ten weeks of resistance training in younger and older adults.

Running Title Resistance training effects on microbiome

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ABSTRACT

Recent evidence suggests that short chained fatty acids (SCFAs) produced by gut microbiota may impact body composition and muscle accretion. Additionally, aging is implicated in negative alterations to the gut microbiome and exercise training has been posited to positively affect gut microbiota. However, limited human evidence indicates that shorter-term resistance training does not appreciably alter the gut microbiome in older adults, and no human study has examined if resistance training differentially alters the gut microbiome and associated SCFAs between younger and older individuals. Therefore, we examined whether 10 weeks of resistance training (RT) differentially altered fecal microbiota composition, fecal and circulating SCFAs, and serum markers associated with gastrointestinal integrity in younger and older adults. Fecal and serum samples were collected from untrained younger (22 ± 2 years, n=12) and older (58 ± 8 years, n=12) participants prior to and following 10-weeks of supervised twice-weekly full body RT. Outcome measures prior to (Pre) and following the training intervention (Post) included body composition measured by dual x-ray absorptiometry, vastus lateralis (VL) thickness measured by ultrasound, fecal microbiome data from 16S rRNA gene sequencing, serum and fecal SCFAs measured by gas chromatography, and serum intestinal fatty acid-binding protein 2 (FABP2), lipopolysaccharide-binding protein (LBP), and leucine-rich alpha-2 glycoprotein (LRG-1) quantified by enzyme-linked immunosorbent assays. Repeated measures two-way (group×time; G×T) ANOVAs were performed for all dependent variables over time, and Spearman correlations were used to explore if changes in training variables were associated with microbiome variables and SCFAs. RT significantly increased VL thickness and lean body mass ($p<0.05$) in both groups, but no GxT interactions were evident. Although differences in beta diversity were evident between younger and older participants, no significant age, time, or

interaction effects were evident. Seven SCFAs were detected in the fecal samples, albeit no significant age, time, or interaction effects were evident. Acetic acid was the only SCFA detected in serum, and no significant age, time, or interaction effects were evident. Serum LRG1 exhibited a significant main effect of time (Pre>Post, $p=0.007$) and age (Younger>Older, $p=0.015$), but this marker nor serum FABP2 or LBP exhibited significant interactions. In all younger, older, and/or all participants combined, there were no significant correlations between RT-induced changes in muscle mass-related outcomes and changes in either metrics of fecal microbiome diversity, total or individual SCFAs, or serum FABP2/LBP/LRG-1 ($p>0.00067$). Our data highlights that 10 weeks of RT largely does not alter the fecal microbiome, associated SCFAs, or select markers of gastrointestinal integrity in untrained younger or older adults.

Keywords: short chain fatty acids, gut microbiome, resistance training, aging

INTRODUCTION

The human gut microbiota consists of a diverse array of microorganisms, including bacteria, viruses, fungi, and archaea, which inhabit the gastrointestinal tract. The gut microbiome contains the largest and most diverse microbial community in the human body (1). Through symbiotic relationships, the gut microbiota contributes to digestion, vitamin synthesis, immune regulation, and protection against pathogens (2). Environmental factors including diet, exercise, xenobiotics, stress, and trauma have been hypothesized to modify the composition of the gut microbiome (3, 4, 5), thus creating a complex balance between microbes that are beneficial for the host and those that may contribute to disease (6).

In 2007, Bäckhed et al. (7) performed a preclinical study investigating the mechanisms underlying the resistance to diet-induced obesity in germ-free mice was the first study to suggest the presence of a gut–muscle axis. Others have reported that supplementing mice with the probiotic Lactobacilli plantarum (*L plantarum*) for six weeks significantly increased grip strength, swimming time to exhaustion, normalized muscle weight and the percentage of Type I myofibers (8). Yan and co-workers subsequently provided evidence that the gut microbiome could regulate skeletal muscle fiber-type composition (9), expanding the ways in which skeletal muscle can be influenced by the microbiome. In a more recent study, Fernandez et al. demonstrated that endurance training increased the number of taxa in 8-week-old mice which correlated with physical performance (10). However, neither resistance training nor endurance training altered the phyla and family compositions (10). Cullen et al. (11) reported that resistance training induces mixed changes in the gut microbiome in sedentary young adults. These

collective results suggest that the gut microbiota may communicate with skeletal muscle to, in part, regulate the adaptive responses to exercise training.

Short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, are microbial metabolites produced through the fermentation of dietary fibers and resistant starches by gut bacteria (12). Acetate is produced by intestinal bacteria of the genus *Bifidobacteria, Lactobacilli*, *Clostridium,* and *Akkermansia* (13). Propionate is largely produced by intestinal bacteria of the genus *Bacteroides, Ruminococcus,* and *Roseburia* (14). Butyrate is mainly produced by intestinal bacteria of the genus *Faecalibacterium prausnitzii, Eubacterium rectale and Roseburia spp.* (15). SCFAs have been suggested to participate in crosstalk with skeletal muscle, proposing a direct link between microbiome-derived metabolites and skeletal muscle physiology (16, 17). In this regard, Lahiri et al. (18) presented evidence of SCFAs regulating skeletal muscle mass and function in germ-free (GF) mice by treating the GF mice *in vivo* with a cocktail of SCFAs thus partly reversing skeletal muscle impairment. Interestingly, studies in rodents and humans indicate that endurance exercise training (ET) or combined ET+RT training modulates fecal SCFA content (19, 20, 21, 22). However, no study to our knowledge has examined if resistance training alone alters fecal and circulating SCFAs in humans.

Although the aforementioned results are promising, there are human data indicating that resistance training may minimally affect gut microbiome outcomes. For instance, our group recently published a study in older individuals indicating that shorter-term (i.e., six weeks) of resistance training does not affect microbiome diversity metrics or abundances of various taxa (23), and this agrees in principle with other resistance training interventions demonstrating null effects (24, 25). However, our prior results may have been due to either the shorter-term nature

of the intervention and/or only examining older individuals who demonstrate a limited capacity to experience changes in the gut microbiome (26). We previously performed two investigations examining the effects of a peanut protein supplement on training outcomes in younger and older adults (27, 28), and both studies indicated that training (but not supplementation) promoted increases in measures of skeletal muscle hypertrophy and strength. Given our interest in determining if longer-term training and/or aging affects gut microbiome adaptations, we leveraged banked fecal specimens and remaining blood sera from 12 younger and 12 older participants in these studies to examine if ten weeks of resistance training (RT) either altered the gut microbiome, fecal and serum SCFAs, and/or serum markers of gastrointestinal integrity. A secondary aim was to determine if changes these outcomes were associated with indices of muscle hypertrophy. We hypothesized that RT would improve microbiome diversity and relative abundance of bacterial taxa and increase microbial metabolites in younger but not older participants given our laboratory's prior null findings in older participants. We also hypothesized that RT would alter fecal and serum SCFAs in younger, but not older, participants. However, we adopted the null hypothesis that microbiome and SCFA outcomes would not be associated with training outcomes.

METHODS

Ethical approval and participant eligibility

This study is a secondary analysis of 12 younger adults and 12 older adults that completed 10 weeks of resistance training. Both studies were conducted under a single approved protocol by the Auburn University Institutional Review Board (IRB) (Protocol # 19-249 MR 1907) and both studies were pre-registered clinical trials (NCT04015479 registered July 11, 2019; NCT04707963 registered 13 January 2021). This sample size was chosen from the original 47

younger participants and 17 older participants who finished the 10-week interventions due to these 24 total individuals having enough fecal and blood materials to perform the analyses described in the following paragraphs.

Detailed methods of the original studies have been previously described (27, 28). Participants were recruited at Auburn University campus and locally via flyers, emails, and direct contact either face-to-face or over the phone. Both studies had identical eligibility criteria aside from age, which included: (i) 18–30 years old or 50-80 years old, (ii) body mass index (BMI) <35 kg/m^2, (iii) not actively participating in RT more than one time per week in the preceding six months, (iv) no known peanut allergy, (v) free of metal implants that could interfere with X-ray procedures, (vi) no medically necessary radiation exposure for six months prior, (vii) free of obvious cardiovascular or metabolic disease, (viii) free of conditions contraindicating participation in exercise program or donation of muscle biopsy (i.e., blood thinners or blood clotting disorder), (ix) for females, could not be pregnant or trying to become pregnant. Eligible individuals were informed of all study procedures and completed a medical history questionnaire prior to signing informed consent to participate.

Study design

This section serves to provide a brief overview of procedures relevant to the data presented herein. Participants reported to the School of Kinesiology at Auburn University for pre-testing battery (Pre) which included a urine specific gravity (USG) to ensure hydration and a rapid pregnancy test for female participants to ensure safety for X-ray exposure. Next, height and weight assessments using digital column scales, right leg vastus lateralis (VL) thickness using ultrasound, and body composition using full body dual-energy X-ray absorptiometry (DXA)

scan. Venous blood was obtained from an arm vein in serum separator tubes (BD Vacutainer; Franklin Lakes, NJ, USA), and blood was allowed to clot for 30 minutes at room temperature prior to being centrifuged at 3500 g for serum separation. Each participant was provided with a three-day food log and stool specimen collection kit to take home to complete and return them at the next appointment. Participants then performed 10 weeks of supervised full-body RT twice weekly as described by Lamb et al. (28) and Sexton et al. (27). All training sessions were supervised by study personnel to ensure proper form throughout the sessions. After completion of the 10-week RT program, participants were provided another set of three-day food logs and stool collection kits for post-intervention testing (Post), which was conducted 72 h after their last workout. The post-intervention testing replicated Pre procedures, which included USG, pregnancy test in females, height and weight assessments, right leg VL thickness using ultrasound, a DXA scan, a venous blood draw, and submission of food-logs and stool samples. The following paragraphs provide more in-depth descriptions of testing sessions.

Body composition

Participants reported to the School of Kinesiology at Auburn University following an overnight fast and submitted a urine sample to assess USG levels using a handheld refractometer (ATAGO; Bellevue, WA, USA). All participants recorded USG values below 1.020 indicating that they were hydrated. Next, height and body weight were then assessed utilizing a laboratory scale (Seca 769; Hanover, MD, USA), with height and body weight being collected to the nearest 0.5 cm and 0.1 kg. Following these measurements, participants underwent a whole-body DXA scan (Lunar Prodigy; GE Corporation, Fairfield, CT, USA) for determination of total lean soft tissue mass (LSTM) and fat mass (FM). Notably, all scans were performed by the same study personnel during the pre-testing and post-testing.

Ultrasound for vastus lateralis thickness

Real-time B-mode ultrasonography (NextGen LOGIQe R8, GE Healthcare, USA) utilizing a multi-frequency linear-array transducer (L4-12T, 4-12 MHz, GE Healthcare, USA) was used to capture images of the right mid-thigh vastus lateralis (VL). Prior to image acquisition, subjects rested supine on an examination table for a minimum of five minutes with the hip and knee fully extended. For VL thickness at the mid-thigh, images were collected at a depth in which the edge of the femur was visible, and this depth was held constant for post-testing image collection. For all ultrasound images, a generous amount of water-soluble transmission gel was applied to both the skin and probe and care was taken to apply a consistent probe pressure to maximize image quality without compressing the underlying tissue. All ultrasound settings (frequency: 10 MHz, gain: 50 dB, dynamic range: 75) except for depth, were held constant across participants and time points. One image per participant at each time point was obtained. Following the study conclusion, images were analyzed using the freely available ImageJ software (National Institutes of Health, Bethesda, MD, USA). VL thickness was measured using the straight-line function and defined as the distance between the subcutaneous adipose tissue-vastus lateralis interface and deep aponeurosis.

Resistance training program

Participants completed supervised RT twice weekly for 10 weeks where detailed instructions were provided on proper posture, form, technique, and body positioning to ensure safety. The training protocol required a minimum of 48 hours in between sessions to allow for proper recovery. Each training session included a warm-up of 25 jumping jacks and 10 body weight

squats, a warm-up set of 10 reps at 50% of working weight, 1 set of 5 reps at 75% working weight, and 1set of 3 reps at 90% of working weight.

Post warm up, younger participants performed either 4 sets of 10 reps (high volume) or 5 sets of 6 reps (high load) at working weight. The older participants performed 4 sets of 10-12 reps at working weight.

Following each set, rating of perceived exhaustion (RPE) was recorded to ensure appropriate loads were implemented throughout. An RPE below 7 resulted in a slight increase in working weight on the next set and an RPE at 10 or incomplete set resulted in a slight decrease in working weight on the next set.

Dietary analysis

Participants were instructed to complete a three-day food logs prior to and following the 10-week training intervention, which included nutritional intakes for two weekdays and one weekend day. Regular dietary habits were encouraged throughout the duration of the study, besides consumption of protein supplements by individuals in the PPS group. The Nutrition Data System for Research (NDSR; NDSR 2014; University of Minnesota) software was utilized by study personnel to analyze all young participant food logs. The Automated Self-Administered 24-Hour Dietary Assessment tool (ASA24) was utilized by study personnel to analyze all older participant food logs.

Fecal microbiome analysis

Stool samples were collected with a commode specimen collection kit and sterile collection tubes. Participants were instructed to seal and store samples in the freezer prior to submission at the next visit.

Upon receipt, samples were stored at -80°C prior to processing. Fecal microbial DNA was isolated using commercially available kits (Zymo, Irvine, CA, USA). DNA samples were prepared, and polymerase chain reaction (PCR) amplified 250 base pair variable region 4 of the 16S rRNA. The PCR amplicon library was sequenced on the Miseq (Illumina, San Diego, CA, USA) (120, 121). Further detailed processing steps have been previously described (13). The Quantitative Insight into Microbial Ecology (QIIME) 2 pipeline, utilizing version 1.8 DADA2 generated amplicon sequence variants (ASVs) to the species level (122, 123, 124). UCLUST clustered sequences into ASVs (previously operational taxonomic units (OTUs)) with a 97% similarity threshold. Thereafter, taxonomic assignments were issued using the SILVA database (125). ASVs with an average abundance >0.005% were further processed and grouped by taxonomy.

Fecal and serum SCFAs analysis

Fecal and serum samples were shipped on dry ice to a commercial vendor (Microbiome Insights, Richmond, BC, Canada) for SCFA analysis. Briefly, SCFAs were quantified using gas chromatography (Thermo Trace 1310) coupled to a flame ionization detector (Thermo Fisher Scientific, Waltham, MA, USA) as previously described (126). Aliquots of stool samples were homogenized using MP Bio FastPrep post suspension in MilliQ-grade water at 4.0 m/s for 1 minute. Next, the samples were acidified by adding 5M HCl to the fecal suspensions, until it reached a final pH of 2.0. The suspensions were incubated for 10 minutes and then centrifuged at 10,000 rpm. Supernatants were spiked with 2-Ethylbturyic acid until a final concentration of 1 mM was reached. SCFAs were detected from the supernatants by direct injection into a Thermo TG-WAXMS A GC Column (30 m, 0.32 mm, 0.25 μm). The injected standard solutions of

individual SCFAs were used for calibration. Serum SCFAs were also analyzed in a similar process previously described (127).

Serum biomarkers

Serum samples were stored removed from -80C to determine concentrations of intestinal fatty acid-binding protein (FABP), leucine-rich alpha-2-glycoprotein 1 (LRG-1), and lipopolysaccharide-binding protein (LBP) using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (AFG Bioscience, Wood Dale, IL USA; catalog #'s: EK710951, EK710885, EK241481). All three assays followed similar protocols which ended with determining absorbance readings at 450 nm using a microplate spectrophotometer (Biotek Synergy H1 hybrid reader; Agilent, Santa Clara, CA, USA). Sample absorbance readings were compared to standard curve absorbance readings, and values are provided in serum concentration units. The three kits performed sufficiently as determined by unknown absorbance values following within the linear range of the standard curves and coefficient of variation values for duplicates being relatively around or less than 20% (FABP = 22.8%, LBP = 12.3%, LRG-1 = 18.5%).

Statistics

Statistical analysis was conducted on non-microbiome data using Graphpad Prism v10.0, and microbiome data was analyzed using RStudio Team (2023) (Integrated Development for R. RStudio, PBC, Boston, MA, USA). For all dependent variables over time, repeated measures two-way (group × time; GxT) analysis of variance (ANOVA) tests were performed. For microbiome data, Kruskal-Wallis ANOVAs with false discovery rate (FDR) corrections were conducted to compare relative abundance of all ASVs between both groups at both time points.

Alpha diversity was measured using ASV counts, PD Whole Tree Phylogeny, Simpson index, and Shannon index. Beta-diversity was measured using Bray–Curtis Dissimilarity, Unweighted Unifrac distance, and Weighted Unifrac distance metrics. Change scores (or delta scores) for select dependent variables were also calculated by subtracting Pre values from Post values. Pearson correlations were then conducted on select delta scores to explore relationships between changes in muscle hypertrophy outcomes versus changes in fecal microbiome diversity, fecal SCFAs, and serum markers. Correlations were performed for each age cohort individually as well as all participants. Statistical significance for two-way ANOVAs were established as $p<0.05$, and significance was adjusted to $p<0.00067$ for correlation analyses to account for the high number of associations.

RESULTS

Participant characteristics and training outcomes

Table 1 presents participant characteristics and training adaptations in both the younger and older groups. Significant main effects of time but no significant interactions were evident for DXA LSTM and VL thickness indicating that training increased muscle mass regardless of age group. Regarding self-reported food intake, two-way ANOVAs revealed there were no significant main effects or G*T interactions for average caloric, carbohydrate, and fat intake. However, significant main effects of time were evident for protein and fiber intakes whereby these values were higher at Post in both the older and young participants.

INSERT Table 1 HERE

Fecal microbiota between age groups with resistance training

Alpha diversity metrics (observed species and whole tree phylogeny) did not exhibit significant main or interaction effects (Fig. 2a/b). The Firmicutes to Bacteroidetes (F/B) ratio increased exhibited a significant main effect of time (Post>Pre, $p=0.040$), but did not exhibit significant age or interaction effects (Fig. 2c).

INSERT Figure 2 HERE

Beta Diversity between age groups with resistance training

Though alpha diversity did not differ between groups, younger and older samples did exhibit microbial population differences between groups. Beta diversity analyses via Bray Curtis and Weighted Unifrac metrics revealed significant differences between younger and older groups ($p<0.001$ for both), but no differences in composition from Pre to Post ($p>0.05$ for all).

INSERT Figure 3 HERE

Serum and fecal SCFAs between age groups with resistance training

Serum acetic acid was the only SCFA detected in the older and young participants, and analyses revealed no significant main effects or a GxT (Fig. 4a). Seven SCFAs were detected in stool samples and included the three highly enriched acetic acid (Fig. 4c), propionic acid (Fig. 4d), and butyric acid (Fig. 4e) as well as the less enriched isobutyric acid, valeric acid, isovaleric acid, and hexanoic acid (not graphed). No significant main effects or GxT interactions were observed for the seven individual fecal SCFAs or total stool SCFA concentrations (Fig. 4b).

INSERT Figure 4 HERE

Serum biomarkers of intestinal barrier function

No significant main effects or GxT interactions were evident for FABP2 or LBP (Fig. 5a/b). A significant main effect of time was observed for serum LRG1 whereby levels decreased from Pre-to-Post intervention in both groups (Fig. 5c, p=0.007). There was also an observed main effect of group with older participants exhibiting lower values compared to younger individuals (p=0.015); however, there was not a GxT interaction (p=0.551).

INSERT Figure 5 HERE

Correlations among dietary intake, fecal/serum measures and resistance training adaptations

Spearman correlations were performed among POST-PRE change scores for key dependent variables in the younger, older, and all participants (i.e., diet composition, fecal/serum SCFAs, fecal microbiome, and serum biomarkers). Results of the analysis are presented in Figure 6. After adjustment for multiple comparisons, no correlations were statistically significant. However, the following findings are noteworthy. Increasing lean muscle mass was associated with increased alpha diversity, a relationship more strongly observed in young adults compared to old. Dietary fat in younger adults and dietary sugar in older adults had the greatest impacts on alpha diversity. Surprisingly, no relationships were observed between dietary or lean mass changes with serum and stool SCFAs. Serum biomarkers were most strongly associated with measures in older but not younger adults, with inverse relationships observed between LRG-1 and VL thickness, total dietary calories and fat. Dietary carbohydrate, but not fiber, was

inversely associated with LBP, whereas fiber was inversely associated with FABP2 (both in older but not younger adults).

INSERT Figure 6

DISCUSSION

This study explored the effects of 10 weeks of RT on fecal microbiome, serum and fecal SCFAs, and serum markers of gut integrity in younger and older participants. We observed an increase in LBM and VL thickness in both groups after the 10 weeks, which validated the effectiveness of the training program. Our young healthy individuals had greater microbiome diversity than the older healthy individuals which did not change with RT. While many training studies have utilized endurance training interventions, there are several studies that have incorporated resistance training interventions. Taniguchi et al. performed a five-week endurance training intervention in healthy elderly men to examine the effect in gut microbiota. The authors reported that the training program did not change the alpha diversity and beta diversity (37). Alternatively, Bycura et al. examined the effects of the gut microbiome using both 8-weeks of ET and RT interventions in healthy college-aged adults. They found that changes in microbiota differed between training interventions. Beta diversity suggests an increase in microbiota in the first few weeks of ET, but there was a slight shift post-intervention to resemble baseline microbiota composition, though no significant changes in gut microbiota were observed (24). Similarly, a 6-week endurance training intervention in obese and lean individuals conducted by Allen et al. found that beta-diversity changes were dependent on BMI status. Additionally,

following the 6-week sedentary washout period, the authors revealed any changes in the microbiota were reversed (19).

Cullen at al. conducted a 6-week resistance training intervention in healthy young sedentary adults in which they found that RT elicited mixed changes in alpha and beta diversity measures (11). Our data and the previous data utilizing RT interventions collectively suggest that microbiome diversity are not altered with RT. Nonetheless, reasons why ET greatly impacts microbiota composition compared to RT should be further investigated.

We detected seven SCFAs in our fecal samples and only one SCFA in our serum samples. We also discovered that the RT produced minimal changes in SCFAs but not statistically significant. These findings are contrary to preclinical studies suggesting that SCFAs improve performance outcomes in rodents. Huang et al. treated mice with acetate, propionate, and butyrate for four weeks to investigate endurance capacity. They found that higher concentration of SCFAs significantly improved the average running time compared with the control group (38). Furthermore, Okamoto et al. also investigated the effects of SCFAs in endurance training outcomes. The authors administered either a low microbiome-accessible carbohydrate (LMC) diet or a high microbiome-accessible carbohydrate (HMC) diet to 10-week-old mice for 6 weeks. They found that SCFA concentrations were significantly lower in the LMC diet group than the HMC diet group. Additionally, treadmill running time was significantly lower in the LMC diet group than the HMC diet group (39). The differences in diet composition and SCFAs production is consistent with Eveleens Maarse and co-worker's study. They supplemented older adults with dietary fiber mixture of Acacia gum and carrot powder (30g/day) and observed an increase in fecal SCFAs in the intervention group compared to the placebo (40). Estaki et al. investigated

SCFAs effects on aerobic capacity in humans. They concluded that healthy young adults with higher SCFA concentrations had a higher VO_2peak (41).

Rodent studies suggest that there is a correlation with changes in SCFA and exercise performance. However, our training involved resistance exercises rather than endurance exercises, which could explain the differences in the results previously discussed.

Examining intestinal barrier integrity is crucial when studying the gut microbiome because of the intricate relationship between the two. Given our findings of the gut microbiome and SCFAs with RT interventions, it was ideal to determine whether biomarkers of intestinal barrier integrity were altered with training. Although serum LBP levels were not altered, we observed a trend in increased serum FABP2 following the RT intervention. Numao et al reported similar findings as they also observed an increase in FABP after acute low-intensity and moderate-intensity exercise in healthy men. However, this study utilized aerobic training intervention (42). Additionally, we observed a significant decrease in LRG1 levels with the RT intervention. LRG1 Elevated levels of LRG in the bloodstream are often associated with conditions such as inflammatory diseases, cancer, and cardiovascular disorders, making it a potential biomarker for disease activity (43, 44, 45). Our finding suggests that resistance training could improve intestinal barrier integrity. While this study provides an insight into RT and gut health in young and old adults, it does have its limitations. First, our sample was fairly homogeneous, as there were 63% white participants and 75% female participants. It would be valuable to investigate whether race or gender is a factor in gut microbiota composition and SCFAs changes with training. While our sample included participants in an effective RT program, there was no control group that did not train, and our sample (n=24) was relatively small. We did not request that participants only collect stool

samples at a particular time of day, which may have affected fecal microbiome composition. Finally, we had participants self-report their dietary intake for our analysis, which makes false reports a possibility.

This is the first study to investigate the effects of short chain fatty acids on resistance training outcomes in humans. Further, this study also investigates relationships between fecal short chain fatty acids and serum short chain fatty acids. Alpha diversity and beta diversity analyses indicate that 10 weeks of RT did not change overall microbiome composition of untrained adults, though younger and older participant cohorts had differing compositions across time. Fecal and serum SCFAs were not significantly altered after 10 weeks of RT program. Additionally, fecal SCFAs did not translate to serum SCFAs, potentially due to utilization by colonocytes or maintenance of intestinal epithelium integrity.

Competing interests
None of the authors have competing interests regarding these data.

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TABLES

Table 1. Participant characteristics, training outcomes, and self-reported food intakes

Variable	Younger (n=12)	Older (n=12)	Stats outcomes
Age (years)	22±2	59±5	p<0.001
Sex	2 males/10 females	4 males/8 females	
Body mass (kg)			Group p<0.001
Pre	68.0±10.3	96.1±13.6	Time p=0.028
Post	69.1±11.2	97.4±15.1	G*T p=0.807
DXA LSTM (kg)			Group p=0.009
Pre	45.0±7.2	54.8±8.9	Time p<0.001
Post	46.6±7.6	56.0±8.9	G*T p=0.454
VL thickness (cm)			Group p=0.007
Pre	2.32±0.32	1.93±0.50	Time p=0.013
Post	2.54±0.33	2.04±0.34	G*T p=0.364
Energy intake (kcal/d)			Group p=0.146
Pre	1409.8±431.5	1763.7±566.6	Time p=0.804
Post	1438.6±369.2	1705.1±597.5	G*T p=0.470
Fat intake (g/d)			Group p=0.337
Pre	64.9±17.7	73.1±24.2	Time p=0.144
Post	58.4±21.6	67.4±26.1	G*T p=0.921
Protein intake (g/d)			Group p=0.010
Pre	54.2±18.6	87.8±31.8	Time p=0.003
Post	75.7±17.4	107.9±46.3	G*T p=0.909
Carbohydrate intake (g/d)			Group p=0.355
Pre	151.9±75.6	189.4±80.1	Time p=0.331
Post	154.6±43.9	168.6±63.9	G*T p=0.213
Fiber intake (g/d)			Group p=0.621
Pre	13.9±6.9	14.2±5.9	Time p<0.001
Post	21.8±5.5	18.5±10.9	G*T p=0.208
Sugar intake (g/d)			Group p=0.098
Pre	54.4±47.2	75.8±44.4	Time p=0.352
Post	43.3±17.9	71.9±37.4	G*T p=0.647

Legend: Data are mean ± standard deviation values, with significant main effects of age group, time, and the group*time (G*T) interaction p-values being presented; note age was analyzed using an independent samples t-test. Abbreviations: DXA LSTM, lean/soft tissue mass determined by dual-energy x-ray absorptiometry; VL, vastus lateralis

FIGURES

Figure 1. Study Flow Diagram

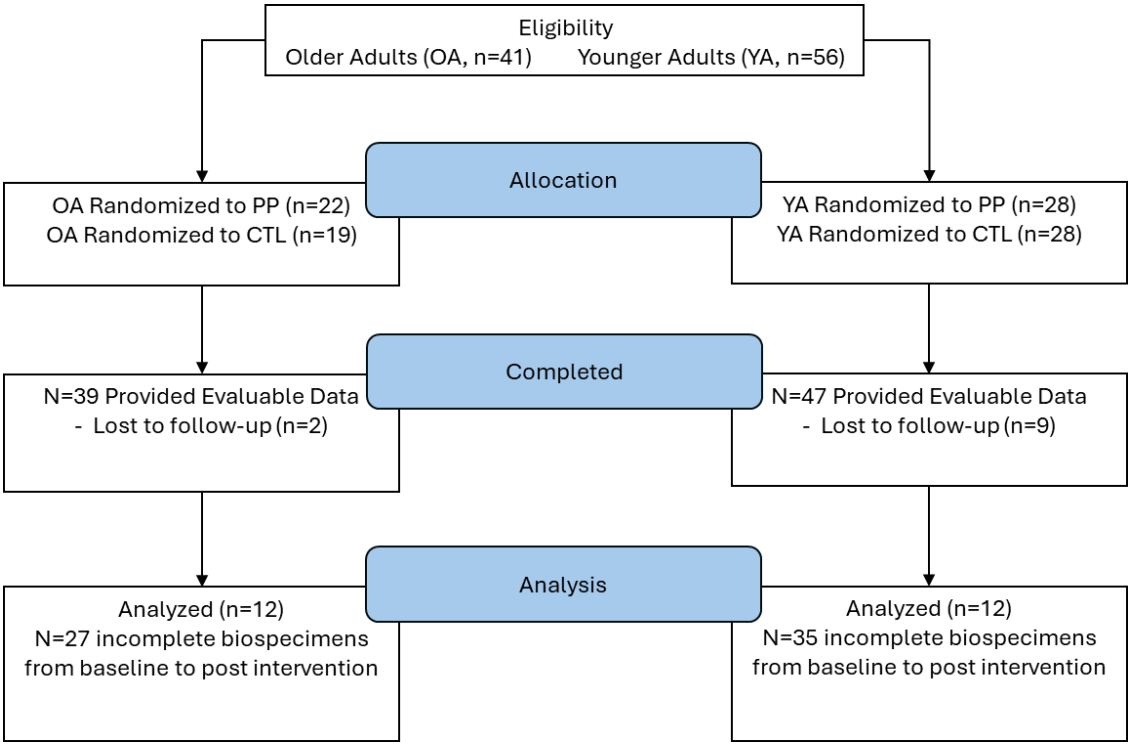
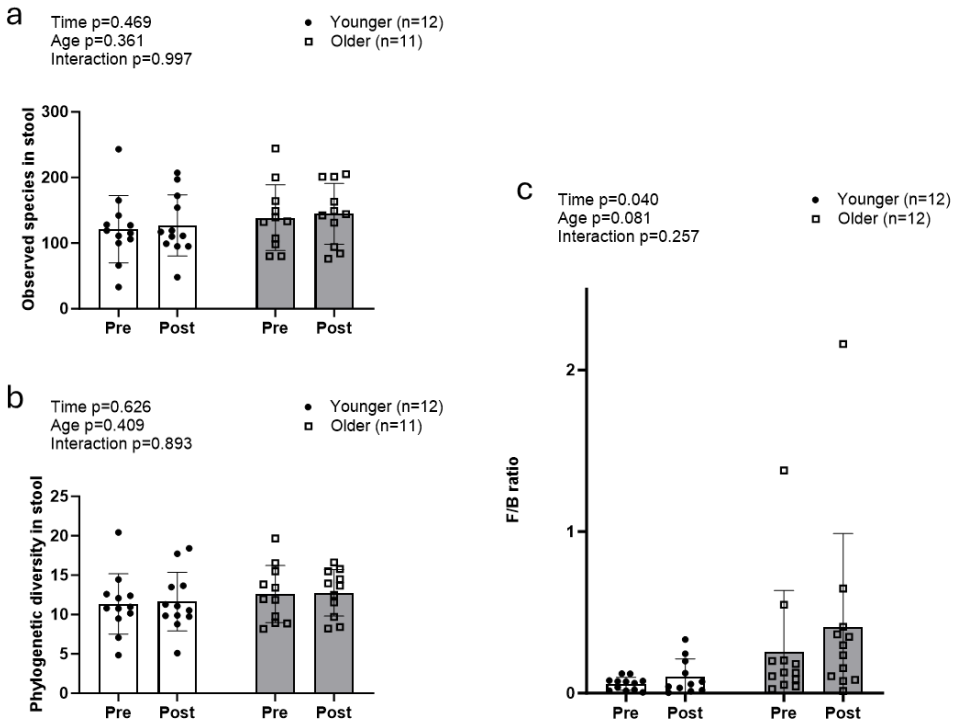
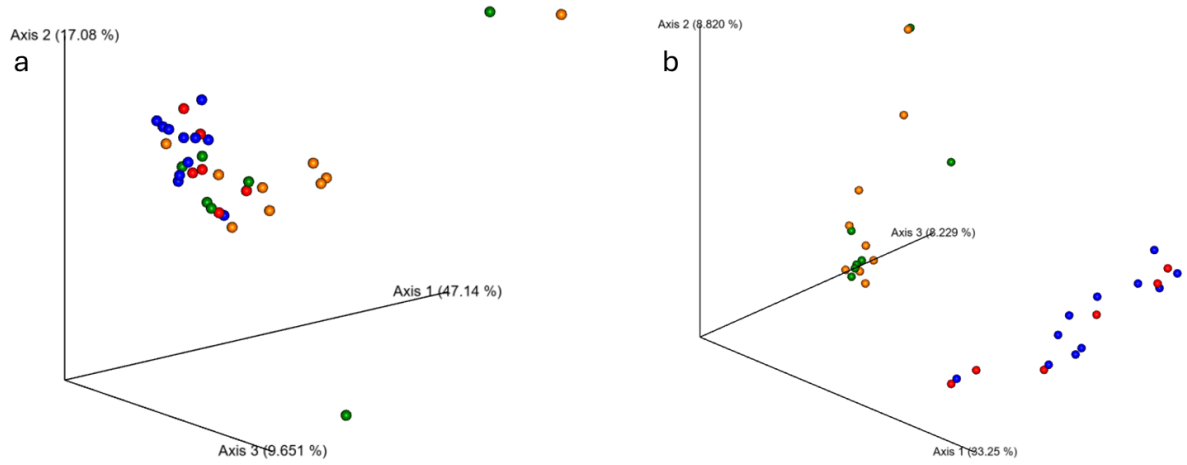


Figure 2. Changes in fecal microbiome diversity with resistance training



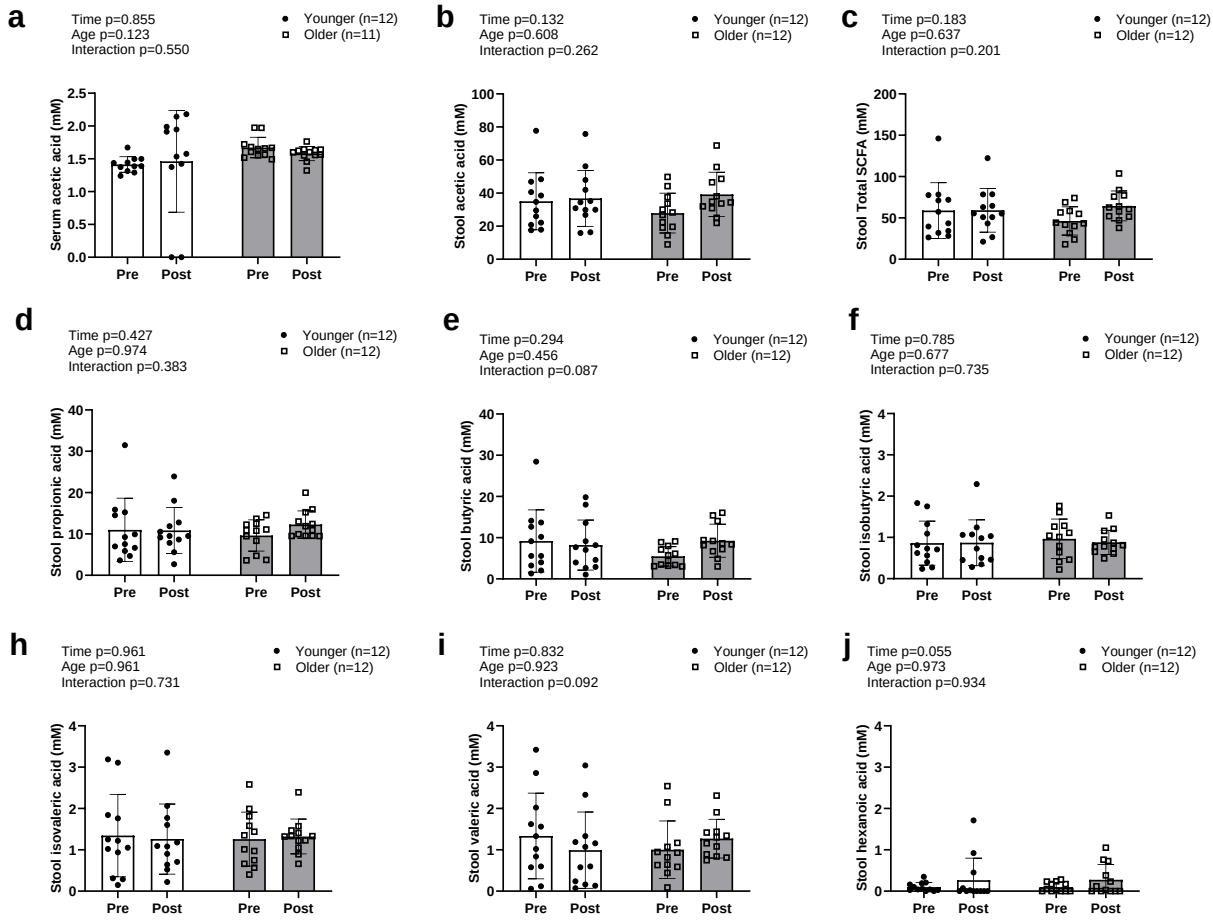
Legend: Data in this figure indicates that there were no significant changes observed with fecal alpha diversity, including observed species **(a)** and whole tree phylogeny **(b)** in both older and younger participants. However, the Firmicutes to Bacteroidetes ratio **(c)** was significantly increased in both groups.

Figure 3. Bray Curtis and Weighted Unifrac scores



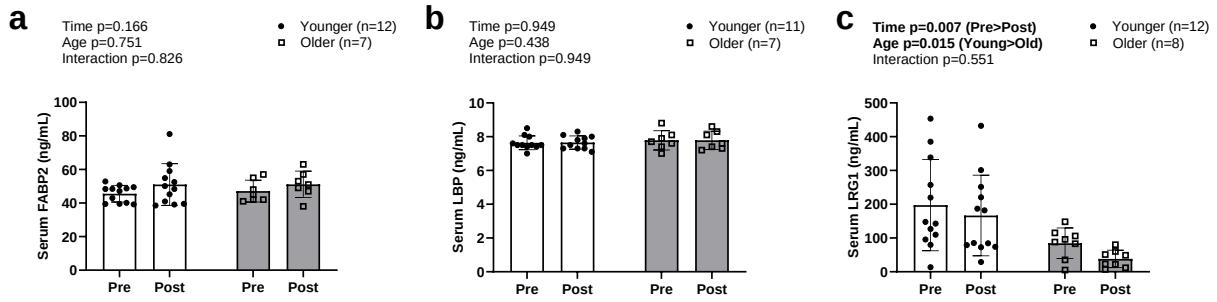
Legend: The average microbiome of young participants was significantly different from the older participants according to **(a)** Bray Curtis ($p<0.001$) and **(b)** Weighted Unifrac ($p<0.001$) metrics. Ten weeks of RT did not alter the microbiome composition in either group. Older Pre: red dots; Older post: blue dots; Younger pre: orange dots; Younger post: green.

Figure 4. Changes in serum and stool short chain fatty acids with resistance training



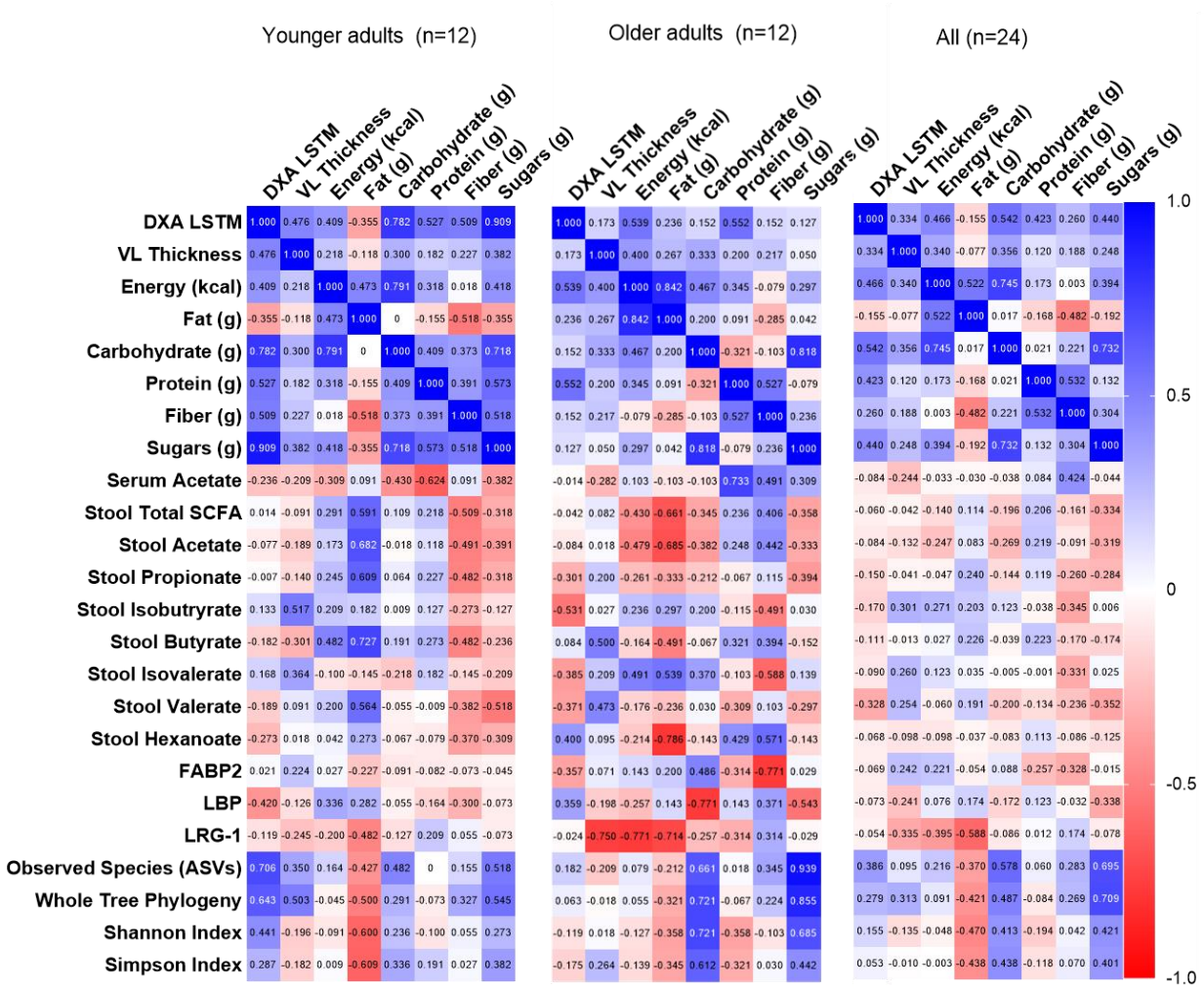
Legend: Data in this figure indicates that only acetic acid **(a)** was found in the serum sample and there was no alteration observed between groups. Total SCFAs in the stool sample **(b)** were also not altered between groups. There were no significant changes in individual stool SCFAs including acetic acid **(c)**, propionic acid **(d)**, butyric acid **(e)**, isobutyric acid **(f)**, isovaleric acid **(g)**, and valeric acid **(h)**. However, there was a trend towards a significant increase in hexanoic acid **(i)** in both groups.

Figure 5. Serum FABP2, LBP, and LRG1 at PRE and POST in older and younger participants



Legend: Data in this figure indicates that serum FABP2 **(a)** and serum LBP **(b)** were not significantly altered in both older and younger participants. However, serum LRG1 **(c)** significantly decreased in both groups. Abbreviations: FABP2, fatty acid binding protein; LBP, lipopolysaccharide binding protein; LRG1, leucine-rich alpha-2-glycoprotein 1.

Figure 6. Correlations among changes in primary and secondary outcomes



Legend: Data are Spearman rho-values. No correlations were significant after adjusting for multiple comparisons; 0.05/576 = p<0.000087. Abbreviations: DXA LSTM, lean/soft tissue mass determined by dual-energy x-ray absorptiometry; obs. species, observed species; SCFA, short chain fatty acids

Chapter V: Conclusion

Short chain fatty acids (SCFAs), byproduct of the gut microbiota, has been suggested to have beneficial effects including increased muscle strength, anti-inflammatory factors, and energy production. Our investigation into the relationship between gut microbiome composition and SCFAs, and resistance training (RT) yields important insights into the complexity of gut health and its responses to physical activity. Despite the well-documented benefits of resistance training on the gut microbiome diversity in rodent, our findings indicate that this form of exercise does not significantly alter gut microbiome diversity or SCFA production in younger and older adult humans. We observed that younger adults had greater diversity in their gut microbiome composition compared to older adults. However, 10 weeks of RT did not significantly influence microbiome composition. We also observed that serum and fecal SCFAs were not altered with the RT program. Given these findings, it was beneficial to determine whether markers of intestinal barrier integrity were altered with RT. Although serum FABP2 and LBP levels were not significantly altered, we observed a significant decrease in serum LRG1 levels following the RT program. LRG1 is a novel biomarker of gastrointestinal inflammation and function and plays a role in health and disease. Therefore, RT could improve intestinal barrier integrity and gut function. The implications of these findings are significant for both researchers and practitioners in the fields of nutrition and exercise science. Understanding that RT may not significantly influence gut microbiome composition or SCFA levels opens avenues for exploring alternative strategies to optimize gut health. Future studies should consider the intensity and duration of resistance training, as well as other forms of exercise, to determine how these variables may collectively impact the gut microbiota and its byproduct. In summary, while resistance training may be a powerful tool for enhancing overall health, its direct effects on gut microbiome composition and SCFA production remain limited.

References

1. **Ogunrinola, G. A., Oyewale, J. O., Oshamika, O. O., & Olasehinde, G. I**. (2020). The Human Microbiome and Its Impacts on Health. *International journal of microbiology*, *2020*, 8045646. https://doi.org/10.1155/2020/8045646
2. **Yoo, J. Y., Groer, M., Dutra, S. V. O., Sarkar, A., & McSkimming, D. I.** (2020). Correction: Yoo, J.Y., et al. Gut Microbiota and Immune System Interactions. *Microorganisms* 2020, *8*, 1587. *Microorganisms*, *8*(12), 2046. https://doi.org/10.3390/microorganisms8122046
3. **Howard, B. M., Kornblith, L. Z., Christie, S. A., Conroy, A. S., Nelson, M. F., Campion, E. M., Callcut, R. A., Calfee, C. S., Lamere, B. J., Fadrosh, D. W., Lynch, S., & Cohen, M. J.** (2017). Characterizing the gut microbiome in trauma: significant changes in microbial diversity occur early after severe injury. *Trauma surgery & acute care open*, *2*(1), e000108. https://doi.org/10.1136/tsaco-2017-000108
4. **Maurice, C. F., Haiser, H. J., & Turnbaugh, P. J.** (2013). Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell*, *152*(1-2), 39–50. https://doi.org/10.1016/j.cell.2012.10.052
5. **Carmody, R. N., Gerber, G. K., Luevano, J. M., Jr, Gatti, D. M., Somes, L., Svenson, K. L., & Turnbaugh, P. J.** (2015). Diet dominates host genotype in shaping the murine gut microbiota. *Cell host & microbe*, *17*(1), 72–84. https://doi.org/10.1016/j.chom.2014.11.010
6. **Sittipo, P., Shim, J. W., & Lee, Y. K.** (2019). Microbial Metabolites Determine Host Health and the Status of Some Diseases. *International journal of molecular sciences*, *20*(21), 5296. https://doi.org/10.3390/ijms20215296
7. **Bäckhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I.** (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(3), 979–984. https://doi.org/10.1073/pnas.0605374104
8. **Chen, Y. M., Wei, L., Chiu, Y. S., Hsu, Y. J., Tsai, T. Y., Wang, M. F., & Huang, C. C.** (2016). Lactobacillus plantarum TWK10 Supplementation Improves Exercise Performance and Increases Muscle Mass in Mice. *Nutrients*, *8*(4), 205. https://doi.org/10.3390/nu8040205
9. **Yan, H., Diao, H., Xiao, Y., Li, W., Yu, B., He, J., Yu, J., Zheng, P., Mao, X., Luo, Y., Zeng, B., Wei, H., & Chen, D.** (2016). Gut microbiota can transfer fiber characteristics and lipid metabolic profiles of skeletal muscle from pigs to germ-free mice. *Scientific reports*, *6*, 31786. https://doi.org/10.1038/srep31786
10. **Fernández, J., Fernández-Sanjurjo, M., Iglesias-Gutiérrez, E., Martínez-Camblor, P., Villar, C. J., Tomás-Zapico, C., Fernández-García, B., & Lombó, F.** (2021). Resistance and Endurance Exercise Training Induce Differential Changes in Gut Microbiota Composition in Murine Models. *Frontiers in physiology*, *12*, 748854. https://doi.org/10.3389/fphys.2021.748854
11. **Cullen, J. M. A., Shahzad, S., Kanaley, J. A., Ericsson, A. C., & Dhillon, J.** (2024). The effects of 6 wk of resistance training on the gut microbiome and cardiometabolic health in young adults with overweight and obesity. *Journal of applied physiology (Bethesda, Md. : 1985)*, *136*(2), 349–361. https://doi.org/10.1152/japplphysiol.00350.2023
12. **Silva, Y. P., Bernardi, A., & Frozza, R. L.** (2020). The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. *Frontiers in endocrinology*, *11*, 25. https://doi.org/10.3389/fendo.2020.00025

13. **Remely, M., Hippe, B., Geretschlaeger, I., Stegmayer, S., Hoefinger, I., & Haslberger, A.** (2015). Increased gut microbiota diversity and abundance of Faecalibacterium prausnitzii and Akkermansia after fasting: a pilot study. *Wiener klinische Wochenschrift*, *127*(9-10), 394–398. https://doi.org/10.1007/s00508-015-0755-1
14. **Louis, P., & Flint, H. J.** (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental microbiology*, *19*(1), 29–41. https://doi.org/10.1111/1462-2920.13589
15. **Liu, H., Wang, J., He, T., Becker, S., Zhang, G., Li, D., & Ma, X**. (2018). Butyrate: A Double-Edged Sword for Health?. *Advances in nutrition (Bethesda, Md.)*, *9*(1), 21–29. https://doi.org/10.1093/advances/nmx009
16. **Tang, G., Du, Y., Guan, H., Jia, J., Zhu, N., Shi, Y., Rong, S., & Yuan, W.** (2022). Butyrate ameliorates skeletal muscle atrophy in diabetic nephropathy by enhancing gut barrier function and FFA2-mediated PI3K/Akt/mTOR signals. *British journal of pharmacology*, *179*(1), 159–178. https://doi.org/10.1111/bph.15693
17. **Frampton, J., Murphy, K. G., Frost, G., & Chambers, E. S**. (2020). Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. *Nature metabolism*, *2*(9), 840–848. https://doi.org/10.1038/s42255-020-0188-7
18. **Lahiri, S., Kim, H., Garcia-Perez, I., Reza, M. M., Martin, K. A., Kundu, P., Cox, L. M., Selkrig, J., Posma, J. M., Zhang, H., Padmanabhan, P., Moret, C., Gulyás, B., Blaser, M. J., Auwerx, J., Holmes, E., Nicholson, J., Wahli, W., & Pettersson, S.** (2019). The gut microbiota influences skeletal muscle mass and function in mice. *Science translational medicine*, *11*(502), eaan5662. https://doi.org/10.1126/scitranslmed.aan5662
19. **Allen, J. M., Mailing, L. J., Niemiro, G. M., Moore, R., Cook, M. D., White, B. A., Holscher, H. D., & Woods, J. A.** (2018). Exercise Alters Gut Microbiota Composition and Function in Lean and Obese Humans. *Medicine and science in sports and exercise*, *50*(4), 747–757. https://doi.org/10.1249/MSS.0000000000001495
20. **Yang, L., Lin, H., Lin, W., & Xu, X.** (2020). Exercise Ameliorates Insulin Resistance of Type 2 Diabetes through Motivating Short-Chain Fatty Acid-Mediated Skeletal Muscle Cell Autophagy. *Biology*, *9*(8), 203. https://doi.org/10.3390/biology9080203
21. **Erlandson, K. M., Liu, J., Johnson, R., Dillon, S., Jankowski, C. M., Kroehl, M., Robertson, C. E., Frank, D. N., Tuncil, Y., Higgins, J., Hamaker, B., & Wilson, C. C.** (2021). An exercise intervention alters stool microbiota and metabolites among older, sedentary adults. *Therapeutic advances in infectious disease*, *8*, 20499361211027067. https://doi.org/10.1177/20499361211027067
22. **Yu, C., Liu, S., Chen, L., Shen, J., Niu, Y., Wang, T., Zhang, W., & Fu, L.** (2019). Effect of exercise and butyrate supplementation on microbiota composition and lipid metabolism. *The Journal of endocrinology*, *243*(2), 125–135. https://doi.org/10.1530/JOE-19-0122
23. **. H., Smith, K. S., Chen, D., Lamb, D. A., Smith, M. A., Osburn, S. C., Ruple, B. A., Morrow, C. D., Huggins, K. W., McDonald, J. R., Brown, M. D., Young, K. C., Roberts, M. D., & Frugé, A. D.** (2022). Exploring the Effects of Six Weeks of Resistance Training on the Fecal Microbiome of Older Adult Males: Secondary Analysis of a Peanut Protein Supplemented Randomized Controlled Trial. *Sports (Basel, Switzerland)*, *10*(5), 65. https://doi.org/10.3390/sports10050065
24. **Bycura, D., Santos, A. C., Shiffer, A., Kyman, S., Winfree, K., Sutliffe, J., Pearson, T., Sonderegger, D., Cope, E., & Caporaso, J. G.** (2021). Impact of Different Exercise

Modalities on the Human Gut Microbiome. *Sports (Basel, Switzerland)*, *9*(2), 14. https://doi.org/10.3390/sports9020014
25. **Cronin, O., Barton, W., Skuse, P., Penney, N. C., Garcia-Perez, I., Murphy, E. F., Woods, T., Nugent, H., Fanning, A., Melgar, S., Falvey, E. C., Holmes, E., Cotter, P. D., O'Sullivan, O., Molloy, M. G., & Shanahan, F.** (2018). 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*(3), e00044-18. https://doi.org/10.1128/mSystems.00044-18
26. **Aya, V., Jimenez, P., Muñoz, E., & Ramírez, J. D.** (2023). Effects of exercise and physical activity on gut microbiota composition and function in older adults: a systematic review. *BMC geriatrics*, *23*(1), 364. https://doi.org/10.1186/s12877-023-04066-y
27. **Sexton, C. L., Smith, M. A., Smith, K. S., Osburn, S. C., Godwin, J. S., Ruple, B. A., Hendricks, A. M., Mobley, C. B., Goodlett, M. D., Frugé, A. D., Young, K. C., & Roberts, M. D.** (2021). Effects of Peanut Protein Supplementation on Resistance Training Adaptations in Younger Adults. *Nutrients*, *13*(11), 3981. https://doi.org/10.3390/nu13113981
28. **Lamb, D. A., Moore, J. H., Smith, M. A., Vann, C. G., Osburn, S. C., Ruple, B. A., Fox, C. D., Smith, K. S., Altonji, O. M., Power, Z. M., Cerovsky, A. E., Ross, C. O., Cao, A. T., Goodlett, M. D., Huggins, K. W., Fruge, A. D., Young, K. C., & Roberts, M. D.** (2020). The effects of resistance training with or without peanut protein supplementation on skeletal muscle and strength adaptations in older individuals. *Journal of the International Society of Sports Nutrition*, *17*(1), 66. https://doi.org/10.1186/s12970-020-00397-y

29. **Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R.** (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, *489*(7415), 220–230. https://doi.org/10.1038/nature11550
30. **Gacesa, R., Kurilshikov, A., Vich Vila, A., Sinha, T., Klaassen, M. A. Y., Bolte, L. A., Andreu-Sánchez, S., Chen, L., Collij, V., Hu, S., Dekens, J. A. M., Lenters, V. C., Björk, J. R., Swarte, J. C., Swertz, M. A., Jansen, B. H., Gelderloos-Arends, J., Jankipersadsing, S., Hofker, M., Vermeulen, R. C. H., … Weersma, R. K.** (2022). Environmental factors shaping the gut microbiome in a Dutch population. *Nature*, *604*(7907), 732–739. https://doi.org/10.1038/s41586-022-04567-7
31. **D'Argenio, V., & Salvatore, F.** (2015). The role of the gut microbiome in the healthy adult status. *Clinica chimica acta; international journal of clinical chemistry*, *451*(Pt A), 97–102. https://doi.org/10.1016/j.cca.2015.01.003
32. **David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A., Biddinger, S. B., Dutton, R. J., & Turnbaugh, P. J.** (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559–563. https://doi.org/10.1038/nature12820
33. **Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., … Wang, J.** (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59–65. https://doi.org/10.1038/nature08821
34. **Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M.,**

Kurokawa, K., … Bork, P. (2011). Enterotypes of the human gut microbiome. *Nature*, *473*(7346), 174–180. https://doi.org/10.1038/nature09944
35. **Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S. R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M.** (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature communications*, *10*(1), 5029. https://doi.org/10.1038/s41467-019-13036-1
36. **Love, C. J., Gubert, C., Kodikara, S., Kong, G., Lê Cao, K. A., & Hannan, A. J.** (2022). Microbiota DNA isolation, 16S rRNA amplicon sequencing, and bioinformatic analysis for bacterial microbiome profiling of rodent fecal samples. *STAR protocols*, *3*(4), 101772. https://doi.org/10.1016/j.xpro.2022.101772
37. **Mizrahi-Man, O., Davenport, E. R., & Gilad, Y.** (2013). Taxonomic classification of bacterial 16S rRNA genes using short sequencing reads: evaluation of effective study designs. *PloS one*, *8*(1), e53608. https://doi.org/10.1371/journal.pone.0053608
38. **Spencer, C. N., McQuade, J. L., Gopalakrishnan, V., McCulloch, J. A., Vetizou, M., Cogdill, A. P., Khan, M. A. W., Zhang, X., White, M. G., Peterson, C. B., Wong, M. C., Morad, G., Rodgers, T., Badger, J. H., Helmink, B. A., Andrews, M. C., Rodrigues, R. R., Morgun, A., Kim, Y. S., Roszik, J., … Wargo, J. A.** (2021). Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. *Science (New York, N.Y.)*, *374*(6575), 1632–1640. https://doi.org/10.1126/science.aaz7015
39. **Tanes, C., Bittinger, K., Gao, Y., Friedman, E. S., Nessel, L., Paladhi, U. R., Chau, L., Panfen, E., Fischbach, M. A., Braun, J., Xavier, R. J., Clish, C. B., Li, H., Bushman, F. D., Lewis, J. D., & Wu, G. D.** (2021). Role of dietary fiber in the recovery of the human gut microbiome and its metabolome. *Cell host & microbe*, *29*(3), 394–407.e5. https://doi.org/10.1016/j.chom.2020.12.012
40. **Dominianni, C., Sinha, R., Goedert, J. J., Pei, Z., Yang, L., Hayes, R. B., & Ahn, J.** (2015). Sex, body mass index, and dietary fiber intake influence the human gut microbiome. *PloS one*, *10*(4), e0124599. https://doi.org/10.1371/journal.pone.0124599
41. **Klimenko, N. S., Tyakht, A. V., Popenko, A. S., Vasiliev, A. S., Altukhov, I. A., Ischenko, D. S., Shashkova, T. I., Efimova, D. A., Nikogosov, D. A., Osipenko, D. A., Musienko, S. V., Selezneva, K. S., Baranova, A., Kurilshikov, A. M., Toshchakov, S. M., Korzhenkov, A. A., Samarov, N. I., Shevchenko, M. A., Tepliuk, A. V., & Alexeev, D. G.** (2018). Microbiome Responses to an Uncontrolled Short-Term Diet Intervention in the Frame of the Citizen Science Project. *Nutrients*, *10*(5), 576. https://doi.org/10.3390/nu10050576
42. **Martínez, I., Lattimer, J. M., Hubach, K. L., Case, J. A., Yang, J., Weber, C. G., Louk, J. A., Rose, D. J., Kyureghian, G., Peterson, D. A., Haub, M. D., & Walter, J.** (2013). Gut microbiome composition is linked to whole grain-induced immunological improvements. *The ISME journal*, *7*(2), 269–280. https://doi.org/10.1038/ismej.2012.104
43. **Wan, Y., Wang, F., Yuan, J., Li, J., Jiang, D., Zhang, J., Li, H., Wang, R., Tang, J., Huang, T., Zheng, J., Sinclair, A. J., Mann, J., & Li, D.** (2019). Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. *Gut*, *68*(8), 1417–1429. https://doi.org/10.1136/gutjnl-2018-317609
44. **Liu, H., Zhu, H., Xia, H., Yang, X., Yang, L., Wang, S., Wen, J., & Sun, G.** (2021). Different effects of high-fat diets rich in different oils on lipids metabolism, oxidative stress and

gut microbiota. *Food research international (Ottawa, Ont.)*, *141*, 110078. https://doi.org/10.1016/j.foodres.2020.110078
45. **Pendyala, S., Walker, J. M., & Holt, P. R.** (2012). A high-fat diet is associated with endotoxemia that originates from the gut. *Gastroenterology*, *142*(5), 1100–1101.e2. https://doi.org/10.1053/j.gastro.2012.01.034
46. **Wang, W., Xing, W., Wei, S., Gao, Q., Wei, X., Shi, L., Kong, Y., & Su, Z.** (2019). Semi-Rational Screening of Probiotics from the Fecal Flora of Healthy Adults against DSS-Induced Colitis Mice by Enhancing Anti-Inflammatory Activity and Modulating the Gut Microbiota. *Journal of microbiology and biotechnology*, *29*(9), 1478–1487. https://doi.org/10.4014/jmb.1807.06061
47. **Furrie, E., Macfarlane, S., Kennedy, A., Cummings, J. H., Walsh, S. V., O'neil, D. A., & Macfarlane, G. T.** (2005). Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut*, *54*(2), 242–249. https://doi.org/10.1136/gut.2004.044834
48. **Kong, C., Gao, R., Yan, X., Huang, L., & Qin, H.** (2019). Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. *Nutrition (Burbank, Los Angeles County, Calif.)*, *60*, 175–184. https://doi.org/10.1016/j.nut.2018.10.002
49. **Principi, N., Cozzali, R., Farinelli, E., Brusaferro, A., & Esposito, S.** (2018). Gut dysbiosis and irritable bowel syndrome: The potential role of probiotics. *The Journal of infection*, *76*(2), 111–120. https://doi.org/10.1016/j.jinf.2017.12.013
50. **Zhang, X. F., Guan, X. X., Tang, Y. J., Sun, J. F., Wang, X. K., Wang, W. D., & Fan, J. M.** (2021). Clinical effects and gut microbiota changes of using probiotics, prebiotics or synbiotics in inflammatory bowel disease: a systematic review and meta-analysis. *European journal of nutrition*, *60*(5), 2855–2875. https://doi.org/10.1007/s00394-021-02503-5
51. **Pinto-Sanchez, M. I., Hall, G. B., Ghajar, K., Nardelli, A., Bolino, C., Lau, J. T., Martin, F. P., Cominetti, O., Welsh, C., Rieder, A., Traynor, J., Gregory, C., De Palma, G., Pigrau, M., Ford, A. C., Macri, J., Berger, B., Bergonzelli, G., Surette, M. G., Collins, S. M., … Bercik, P.** (2017). Probiotic Bifidobacterium longum NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. *Gastroenterology*, *153*(2), 448–459.e8. https://doi.org/10.1053/j.gastro.2017.05.003
52. **Currò, D., Ianiro, G., Pecere, S., Bibbò, S., & Cammarota, G.** (2017). Probiotics, fibre and herbal medicinal products for functional and inflammatory bowel disorders. *British journal of pharmacology*, *174*(11), 1426–1449. https://doi.org/10.1111/bph.13632
53. **Nobaek, S., Johansson, M. L., Molin, G., Ahrné, S., & Jeppsson, B.** (2000). Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *The American journal of gastroenterology*, *95*(5), 1231–1238. https://doi.org/10.1111/j.1572-0241.2000.02015.x
54. **Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'Connor, E. M., Cusack, S., Harris, H. M., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G. F., Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen, D., Wallace, M., Brennan, L., Stanton, C., … O'Toole, P. W.** (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nature*, *488*(7410), 178–184. https://doi.org/10.1038/nature11319
55. **O'Toole, P. W., & Jeffery, I. B.** (2015). Gut microbiota and aging. *Science (New York, N.Y.)*, *350*(6265), 1214–1215. https://doi.org/10.1126/science.aac8469

56. **Leite, G., Pimentel, M., Barlow, G. M., Chang, C., Hosseini, A., Wang, J., Parodi, G., Sedighi, R., Rezaie, A., & Mathur, R.** (2021). Age and the aging process significantly alter the small bowel microbiome. *Cell reports*, *36*(13), 109765. https://doi.org/10.1016/j.celrep.2021.109765
57. **Kang, L., Li, P., Wang, D., Wang, T., Hao, D., & Qu, X.** (2021). Alterations in intestinal microbiota diversity, composition, and function in patients with sarcopenia. *Scientific reports*, *11*(1), 4628. https://doi.org/10.1038/s41598-021-84031-0
58. **Araos, R., Andreatos, N., Ugalde, J., Mitchell, S., Mylonakis, E., & D'Agata, E. M. C.** (2018). Fecal Microbiome Among Nursing Home Residents with Advanced Dementia and Clostridium difficile. *Digestive diseases and sciences*, *63*(6), 1525–1531. https://doi.org/10.1007/s10620-018-5030-7
59. **Stadlbauer, V., Engertsberger, L., Komarova, I., Feldbacher, N., Leber, B., Pichler, G., Fink, N., Scarpatetti, M., Schippinger, W., Schmidt, R., & Horvath, A.** (2020). Dysbiosis, gut barrier dysfunction and inflammation in dementia: a pilot study. *BMC geriatrics*, *20*(1), 248. https://doi.org/10.1186/s12877-020-01644-2
60. **Kim, G., & Kim, J. H.** (2020). Impact of Skeletal Muscle Mass on Metabolic Health. *Endocrinology and metabolism (Seoul, Korea)*, *35*(1), 1–6. https://doi.org/10.3803/EnM.2020.35.1.1
61. **Malafarina, V., Uriz-Otano, F., Iniesta, R., & Gil-Guerrero, L.** (2012). Sarcopenia in the elderly: diagnosis, physiopathology and treatment. *Maturitas*, *71*(2), 109–114. https://doi.org/10.1016/j.maturitas.2011.11.012
62. **Moctezuma-Velázquez, C., Low, G., Mourtzakis, M., Ma, M., Burak, K. W., Tandon, P., & Montano-Loza, A. J.** (2018). Association between Low Testosterone Levels and Sarcopenia in Cirrhosis: A Cross-sectional Study. *Annals of hepatology*, *17*(4), 615–623. https://doi.org/10.5604/01.3001.0012.0930
63. **Claesson, M. J., Cusack, S., O'Sullivan, O., Greene-Diniz, R., de Weerd, H., Flannery, E., Marchesi, J. R., Falush, D., Dinan, T., Fitzgerald, G., Stanton, C., van Sinderen, D., O'Connor, M., Harnedy, N., O'Connor, K., Henry, C., O'Mahony, D., Fitzgerald, A. P., Shanahan, F., Twomey, C., … O'Toole, P. W.** (2011). Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proceedings of the National Academy of Sciences of the United States of America*, *108 Suppl 1*(Suppl 1), 4586–4591. https://doi.org/10.1073/pnas.1000097107
64. **Zhou, J., Liu, J., Lin, Q., Shi, L., Zeng, Z., Guan, L., Ma, Y., Zeng, Y., Zhong, S., & Xu, L.** (2023). Characteristics of the gut microbiome and metabolic profile in elderly patients with sarcopenia. *Frontiers in pharmacology*, *14*, 1279448. https://doi.org/10.3389/fphar.2023.1279448
65. **Han, D. S., Wu, W. K., Liu, P. Y., Yang, Y. T., Hsu, H. C., Kuo, C. H., Wu, M. S., & Wang, T. G.** (2022). Differences in the gut microbiome and reduced fecal butyrate in elders with low skeletal muscle mass. *Clinical nutrition (Edinburgh, Scotland)*, *41*(7), 1491–1500. https://doi.org/10.1016/j.clnu.2022.05.008
66. **Nardone, O. M., de Sire, R., Petito, V., Testa, A., Villani, G., Scaldaferri, F., & Castiglione, F.** (2021). Inflammatory Bowel Diseases and Sarcopenia: The Role of Inflammation and Gut Microbiota in the Development of Muscle Failure. *Frontiers in immunology*, *12*, 694217. https://doi.org/10.3389/fimmu.2021.694217

67. **Tyrrell, D. J., & Goldstein, D. R.** (2021). Ageing and atherosclerosis: vascular intrinsic and extrinsic factors and potential role of IL-6. *Nature reviews. Cardiology*, *18*(1), 58–68. https://doi.org/10.1038/s41569-020-0431-7
68. **Goldsmith, D. R., Haroon, E., Miller, A. H., Strauss, G. P., Buckley, P. F., & Miller, B. J.** (2018). TNF-α and IL-6 are associated with the deficit syndrome and negative symptoms in patients with chronic schizophrenia. *Schizophrenia research*, *199*, 281–284. https://doi.org/10.1016/j.schres.2018.02.048
69. **Boll, K. M., Noto, C., Bonifácio, K. L., Bortolasci, C. C., Gadelha, A., Bressan, R. A., Barbosa, D. S., Maes, M., & Moreira, E. G.** (2017). Oxidative and nitrosative stress biomarkers in chronic schizophrenia. *Psychiatry research*, *253*, 43–48. https://doi.org/10.1016/j.psychres.2017.03.038
70. **Buford, T. W., Carter, C. S., VanDerPol, W. J., Chen, D., Lefkowitz, E. J., Eipers, P., Morrow, C. D., & Bamman, M. M.** (2018). Composition and richness of the serum microbiome differ by age and link to systemic inflammation. *GeroScience*, *40*(3), 257–268. https://doi.org/10.1007/s11357-018-0026-y
71. **Sovran, B., Hugenholtz, F., Elderman, M., Van Beek, A. A., Graversen, K., Huijskes, M., Boekschoten, M. V., Savelkoul, H. F. J., De Vos, P., Dekker, J., & Wells, J. M.** (2019). Age-associated Impairment of the Mucus Barrier Function is Associated with Profound Changes in Microbiota and Immunity. *Scientific reports*, *9*(1), 1437. https://doi.org/10.1038/s41598-018-35228-3
72. **LeBlanc, J. G., Milani, C., de Giori, G. S., Sesma, F., van Sinderen, D., & Ventura, M.** (2013). Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Current opinion in biotechnology*, *24*(2), 160–168. https://doi.org/10.1016/j.copbio.2012.08.005
73. **Wan, Z., Zheng, J., Zhu, Z., Sang, L., Zhu, J., Luo, S., Zhao, Y., Wang, R., Zhang, Y., Hao, K., Chen, L., Du, J., Kan, J., & He, H.** (2022). Intermediate role of gut microbiota in vitamin B nutrition and its influences on human health. *Frontiers in nutrition*, *9*, 1031502. https://doi.org/10.3389/fnut.2022.1031502
74. **Ellis, J. L., Karl, J. P., Oliverio, A. M., Fu, X., Soares, J. W., Wolfe, B. E., Hernandez, C. J., Mason, J. B., & Booth, S. L.** (2021). Dietary vitamin K is remodeled by gut microbiota and influences community composition. *Gut microbes*, *13*(1), 1–16. https://doi.org/10.1080/19490976.2021.1887721
75. **Ticinesi, A., Lauretani, F., Tana, C., Nouvenne, A., Ridolo, E., & Meschi, T.** (2019). Exercise and immune system as modulators of intestinal microbiome: implications for the gut-muscle axis hypothesis. *Exercise immunology review*, *25*, 84–95.
76. **Bindels, L. B., & Delzenne, N. M.** (2013). Muscle wasting: the gut microbiota as a new therapeutic target?. *The international journal of biochemistry & cell biology*, *45*(10), 2186–2190. https://doi.org/10.1016/j.biocel.2013.06.021
77. **Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., Semenkovich, C. F., & Gordon, J. I.** (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(44), 15718–15723. https://doi.org/10.1073/pnas.0407076101
78. **Roberts, M. D., McCarthy, J. J., Hornberger, T. A., Phillips, S. M., Mackey, A. L., Nader, G. A., Boppart, M. D., Kavazis, A. N., Reidy, P. T., Ogasawara, R., Libardi, C. A., Ugrinowitsch, C., Booth, F. W., & Esser, K. A.** (2023). Mechanisms of mechanical overload-induced skeletal muscle hypertrophy: current understanding and future directions. *Physiological reviews*, *103*(4), 2679–2757. https://doi.org/10.1152/physrev.00039.2022

79. **Kundu, P., Lee, H. U., Garcia-Perez, I., Tay, E. X. Y., Kim, H., Faylon, L. E., Martin, K. A., Purbojati, R., Drautz-Moses, D. I., Ghosh, S., Nicholson, J. K., Schuster, S., Holmes, E., & Pettersson, S.** (2019). Neurogenesis and prolongevity signaling in young germ-free mice transplanted with the gut microbiota of old mice. *Science translational medicine*, *11*(518), eaau4760. https://doi.org/10.1126/scitranslmed.aau4760
80. **Nicoletti C.** (2015). Age-associated changes of the intestinal epithelial barrier: local and systemic implications. *Expert review of gastroenterology & hepatology*, *9*(12), 1467–1469. https://doi.org/10.1586/17474124.2015.1092872
81. **Camilleri M.** (2019). Leaky gut: mechanisms, measurement and clinical implications in humans. *Gut*, *68*(8), 1516–1526. https://doi.org/10.1136/gutjnl-2019-318427
82. **Qi, Y., Goel, R., Kim, S., Richards, E. M., Carter, C. S., Pepine, C. J., Raizada, M. K., & Buford, T. W.** (2017). Intestinal Permeability Biomarker Zonulin is Elevated in Healthy Aging. *Journal of the American Medical Directors Association*, *18*(9), 810.e1–810.e4. https://doi.org/10.1016/j.jamda.2017.05.018
83. **Cesari, M., Kritchevsky, S. B., Baumgartner, R. N., Atkinson, H. H., Penninx, B. W., Lenchik, L., Palla, S. L., Ambrosius, W. T., Tracy, R. P., & Pahor, M.** (2005). Sarcopenia, obesity, and inflammation--results from the Trial of Angiotensin Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors study. *The American journal of clinical nutrition*, *82*(2), 428–434. https://doi.org/10.1093/ajcn.82.2.428
84. **Haddad, F., Zaldivar, F., Cooper, D. M., & Adams, G. R.** (2005). IL-6-induced skeletal muscle atrophy. *Journal of applied physiology (Bethesda, Md. : 1985)*, *98*(3), 911–917. https://doi.org/10.1152/japplphysiol.01026.2004
85. **Ni, Y., Yang, X., Zheng, L., Wang, Z., Wu, L., Jiang, J., Yang, T., Ma, L., & Fu, Z.** (2019). Lactobacillus and Bifidobacterium Improves Physiological Function and Cognitive Ability in Aged Mice by the Regulation of Gut Microbiota. *Molecular nutrition & food research*, *63*(22), e1900603. https://doi.org/10.1002/mnfr.201900603
86. **Bindels, L. B., Beck, R., Schakman, O., Martin, J. C., De Backer, F., Sohet, F. M., Dewulf, E. M., Pachikian, B. D., Neyrinck, A. M., Thissen, J. P., Verrax, J., Calderon, P. B., Pot, B., Grangette, C., Cani, P. D., Scott, K. P., & Delzenne, N. M.** (2012). Restoring specific lactobacilli levels decreases inflammation and muscle atrophy markers in an acute leukemia mouse model. *PloS one*, *7*(6), e37971. https://doi.org/10.1371/journal.pone.0037971
87. **Mikkelsen, K., Stojanovska, L., Polenakovic, M., Bosevski, M., & Apostolopoulos, V.** (2017). Exercise and mental health. *Maturitas*, *106*, 48–56. https://doi.org/10.1016/j.maturitas.2017.09.003
88. **Nystoriak, M. A., & Bhatnagar, A.** (2018). Cardiovascular Effects and Benefits of Exercise. *Frontiers in cardiovascular medicine*, *5*, 135. https://doi.org/10.3389/fcvm.2018.00135
89. **Allen, J. M., Berg Miller, M. E., Pence, B. D., Whitlock, K., Nehra, V., Gaskins, H. R., White, B. A., Fryer, J. D., & Woods, J. A.** (2015). Voluntary and forced exercise differentially alters the gut microbiome in C57BL/6J mice. *Journal of applied physiology (Bethesda, Md. : 1985)*, *118*(8), 1059–1066. https://doi.org/10.1152/japplphysiol.01077.2014
90. **Hsu, Y. J., Chiu, C. C., Li, Y. P., Huang, W. C., Huang, Y. T., Huang, C. C., & Chuang, H. L.** (2015). Effect of intestinal microbiota on exercise performance in mice. *Journal of strength and conditioning research*, *29*(2), 552–558. https://doi.org/10.1519/JSC.0000000000000644
91. **Huang, W. C., Hsu, Y. J., Li, H., Kan, N. W., Chen, Y. M., Lin, J. S., Hsu, T. K., Tsai, T. Y., Chiu, Y. S., & Huang, C. C.** (2018). Effect of Lactobacillus Plantarum TWK10 on

Improving Endurance Performance in Humans. *The Chinese journal of physiology*, *61*(3), 163–170. https://doi.org/10.4077/CJP.2018.BAH587
92. **Morita, E., Yokoyama, H., Imai, D., Takeda, R., Ota, A., Kawai, E., Hisada, T., Emoto, M., Suzuki, Y., & Okazaki, K.** (2019). Aerobic Exercise Training with Brisk Walking Increases Intestinal Bacteroides in Healthy Elderly Women. *Nutrients*, *11*(4), 868. https://doi.org/10.3390/nu11040868
93. **Motiani, K. K., Collado, M. C., Eskelinen, J. J., Virtanen, K. A., Löyttyniemi, E., Salminen, S., Nuutila, P., Kalliokoski, K. K., & Hannukainen, J. C.** (2020). Exercise Training Modulates Gut Microbiota Profile and Improves Endotoxemia. *Medicine and science in sports and exercise*, *52*(1), 94–104. https://doi.org/10.1249/MSS.0000000000002112
94. **Castro, A. P., Silva, K. K. S., Medeiros, C. S. A., Alves, F., Araujo, R. C., & Almeida, J. A.** (2021). Effects of 12 weeks of resistance training on rat gut microbiota composition. *The Journal of experimental biology*, *224*(12), jeb242543. https://doi.org/10.1242/jeb.242543
95. **McKenna, C. F., Salvador, A. F., Hughes, R. L., Scaroni, S. E., Alamilla, R. A., Askow, A. T., Paluska, S. A., Dilger, A. C., Holscher, H. D., De Lisio, M., Khan, N. A., & Burd, N. A.** (2021). Higher protein intake during resistance training does not potentiate strength, but modulates gut microbiota, in middle-aged adults: a randomized control trial. *American journal of physiology. Endocrinology and metabolism*, *320*(5), E900–E913. https://doi.org/10.1152/ajpendo.00574.2020
96. **Zhong, F., Wen, X., Yang, M., Lai, H. Y., Momma, H., Cheng, L., Sun, X., Nagatomi, R., & Huang, C.** (2021). Effect of an 8-week Exercise Training on Gut Microbiota in Physically Inactive Older Women. *International journal of sports medicine*, *42*(7), 610–623. https://doi.org/10.1055/a-1301-7011
97. **Macfarlane, S., & Macfarlane, G. T.** (2003). Regulation of short-chain fatty acid production. *The Proceedings of the Nutrition Society*, *62*(1), 67–72. https://doi.org/10.1079/PNS2002207
98. **Liu, P., Wang, Y., Yang, G., Zhang, Q., Meng, L., Xin, Y., & Jiang, X.** (2021). The role of short-chain fatty acids in intestinal barrier function, inflammation, oxidative stress, and colonic carcinogenesis. *Pharmacological research*, *165*, 105420. https://doi.org/10.1016/j.phrs.2021.105420
99. **den Besten, G., Lange, K., Havinga, R., van Dijk, T. H., Gerding, A., van Eunen, K., Müller, M., Groen, A. K., Hooiveld, G. J., Bakker, B. M., & Reijngoud, D. J.** (2013). Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *American journal of physiology. Gastrointestinal and liver physiology*, *305*(12), G900–G910. https://doi.org/10.1152/ajpgi.00265.2013
100. **Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F.** (2016). From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell*, *165*(6), 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041
101. **Wong, J. M., de Souza, R., Kendall, C. W., Emam, A., & Jenkins, D. J.** (2006). Colonic health: fermentation and short chain fatty acids. *Journal of clinical gastroenterology*, *40*(3), 235–243. https://doi.org/10.1097/00004836-200603000-00015
102. **Schwiertz, A., Taras, D., Schäfer, K., Beijer, S., Bos, N. A., Donus, C., & Hardt, P. D.** (2010). Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring, Md.)*, *18*(1), 190–195. https://doi.org/10.1038/oby.2009.167

103. **Fleming, S. E., Choi, S. Y., & Fitch, M. D.** (1991). Absorption of short-chain fatty acids from the rat cecum in vivo. *The Journal of nutrition*, *121*(11), 1787–1797. https://doi.org/10.1093/jn/121.11.1787
104. **Cummings J. H.** (1984). Colonic absorption: the importance of short chain fatty acids in man. *Scandinavian journal of gastroenterology. Supplement*, *93*, 89–99.
105. **Bose, S., Ramesh, V., & Locasale, J. W.** (2019). Acetate Metabolism in Physiology, Cancer, and Beyond. *Trends in cell biology*, *29*(9), 695–703. https://doi.org/10.1016/j.tcb.2019.05.005
106. **Zhan, K., Yang, T. Y., Chen, Y., Jiang, M. C., & Zhao, G. Q.** (2020). Propionate enhances the expression of key genes involved in the gluconeogenic pathway in bovine intestinal epithelial cells. *Journal of dairy science*, *103*(6), 5514–5524. https://doi.org/10.3168/jds.2019-17309
107. **Zhang, Q., Koser, S. L., Bequette, B. J., & Donkin, S. S.** (2015). Effect of propionate on mRNA expression of key genes for gluconeogenesis in liver of dairy cattle. *Journal of dairy science*, *98*(12), 8698–8709. https://doi.org/10.3168/jds.2015-9590
108. **Zhou, D., Pan, Q., Xin, F. Z., Zhang, R. N., He, C. X., Chen, G. Y., Liu, C., Chen, Y. W., & Fan, J. G.** (2017). Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier. *World journal of gastroenterology*, *23*(1), 60–75. https://doi.org/10.3748/wjg.v23.i1.60
109. **Wang, S., Zhang, C., Yang, J., Wang, X., Wu, K., Zhang, B., Zhang, J., Yang, A., Rajput, S. A., & Qi, D.** (2020). Sodium Butyrate Protects the Intestinal Barrier by Modulating Intestinal Host Defense Peptide Expression and Gut Microbiota after a Challenge with Deoxynivalenol in Weaned Piglets. *Journal of agricultural and food chemistry*, *68*(15), 4515–4527. https://doi.org/10.1021/acs.jafc.0c00791
110. **Han, F., Xu, C., Qi, C., Lin, Z., Li, E., Wang, C., Wang, X., Qin, J. G., & Chen, L.** (2020). Sodium butyrate can improve intestinal integrity and immunity in juvenile Chinese mitten crab (Eriocheir sinensis) fed glycinin. *Fish & shellfish immunology*, *102*, 400–411. https://doi.org/10.1016/j.fsi.2020.04.058
111. **Lee, J., Venna, V. R., Durgan, D. J., Shi, H., Hudobenko, J., Putluri, N., Petrosino, J., McCullough, L. D., & Bryan, R. M.** (2020). Young versus aged microbiota transplants to germ-free mice: increased short-chain fatty acids and improved cognitive performance. *Gut microbes*, *12*(1), 1–14. https://doi.org/10.1080/19490976.2020.1814107
112. **Hildebrand, C. B., Lichatz, R., Pich, A., Mühlfeld, C., Woltemate, S., Vital, M., & Brandenberger, C.** (2023). Short-chain fatty acids improve inflamm-aging and acute lung injury in old mice. *American journal of physiology. Lung cellular and molecular physiology*, *324*(4), L480–L492. https://doi.org/10.1152/ajplung.00296.2022
113. **Gao, Z., Yin, J., Zhang, J., Ward, R. E., Martin, R. J., Lefevre, M., Cefalu, W. T., & Ye, J.** (2009). Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes*, *58*(7), 1509–1517. https://doi.org/10.2337/db08-1637
114. **Meisel, M., Mayassi, T., Fehlner-Peach, H., Koval, J. C., O'Brien, S. L., Hinterleitner, R., Lesko, K., Kim, S., Bouziat, R., Chen, L., Weber, C. R., Mazmanian, S. K., Jabri, B., & Antonopoulos, D. A.** (2017). Interleukin-15 promotes intestinal dysbiosis with butyrate deficiency associated with increased susceptibility to colitis. *The ISME journal*, *11*(1), 15–30. https://doi.org/10.1038/ismej.2016.114

115. **Sukkar, A. H., Lett, A. M., Frost, G., & Chambers, E. S.** (2019). Regulation of energy expenditure and substrate oxidation by short-chain fatty acids. *The Journal of endocrinology*, *242*(2), R1–R8. https://doi.org/10.1530/JOE-19-0098
116. **Walsh, M. E., Bhattacharya, A., Sataranatarajan, K., Qaisar, R., Sloane, L., Rahman, M. M., Kinter, M., & Van Remmen, H.** (2015). The histone deacetylase inhibitor butyrate improves metabolism and reduces muscle atrophy during aging. *Aging cell*, *14*(6), 957–970. https://doi.org/10.1111/acel.12387
117. **Huang, L., Li, T., Zhou, M., Deng, M., Zhang, L., Yi, L., Zhu, J., Zhu, X., & Mi, M.** (2022). Hypoxia Improves Endurance Performance by Enhancing Short Chain Fatty Acids Production *via* Gut Microbiota Remodeling. *Frontiers in microbiology*, *12*, 820691. https://doi.org/10.3389/fmicb.2021.820691
118. **Kumar, R., Eipers, P., Little, R. B., Crowley, M., Crossman, D. K., Lefkowitz, E. J., & Morrow, C. D.** (2014). Getting started with microbiome analysis: sample acquisition to bioinformatics. *Current protocols in human genetics*, *82*, 18.8.1–18.8.29. https://doi.org/10.1002/0471142905.hg1808s82
119. **Frugé, A. D., Van der Pol, W., Rogers, L. Q., Morrow, C. D., Tsuruta, Y., & Demark-Wahnefried, W.** (2020). Fecal Akkermansia muciniphila Is Associated with Body Composition and Microbiota Diversity in Overweight and Obese Women with Breast Cancer Participating in a Presurgical Weight Loss Trial. *Journal of the Academy of Nutrition and Dietetics*, *120*(4), 650–659. https://doi.org/10.1016/j.jand.2018.08.164
120. **Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P.** (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869
121. **Van Der Pol, W. J., Kumar, R., Morrow, C. D., Blanchard, E. E., Taylor, C. M., Martin, D. H., Lefkowitz, E. J., & Muzny, C. A.** (2019). In Silico and Experimental Evaluation of Primer Sets for Species-Level Resolution of the Vaginal Microbiota Using 16S Ribosomal RNA Gene Sequencing. *The Journal of infectious diseases*, *219*(2), 305–314. https://doi.org/10.1093/infdis/jiy508
122. **Estaki, M., Jiang, L., Bokulich, N. A., McDonald, D., González, A., Kosciolek, T., Martino, C., Zhu, Q., Birmingham, A., Vázquez-Baeza, Y., Dillon, M. R., Bolyen, E., Caporaso, J. G., & Knight, R.** (2020). QIIME 2 Enables Comprehensive End-to-End Analysis of Diverse Microbiome Data and Comparative Studies with Publicly Available Data. *Current protocols in bioinformatics*, *70*(1), e100. https://doi.org/10.1002/cpbi.100
123. **Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O.** (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, *41*(Database issue), D590–D596. https://doi.org/10.1093/nar/gks1219
124. **Zhao, G., Nyman, M., & Jönsson, J. A.** (2006). Rapid determination of short-chain fatty acids in colonic contents and faeces of humans and rats by acidified water-extraction and direct-injection gas chromatography. *Biomedical chromatography : BMC*, *20*(8), 674–682. https://doi.org/10.1002/bmc.580
125. **Zhao, G., Liu, J. F., Nyman, M., & Jönsson, J. A.** (2007). Determination of short-chain fatty acids in serum by hollow fiber supported liquid membrane extraction coupled with gas chromatography. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences*, *846*(1-2), 202–208. https://doi.org/10.1016/j.jchromb.2006.09.027

126. **Ribeiro, W. R., Vinolo, M. A. R., Calixto, L. A., & Ferreira, C. M.** (2018). Use of Gas Chromatography to Quantify Short Chain Fatty Acids in the Serum, Colonic Luminal Content and Feces of mice. *Bio-protocol*, *8*(22), e3089. https://doi.org/10.21769/BioProtoc.3089
127. **Zhang, S., Wang, H., & Zhu, M. J.** (2019). A sensitive GC/MS detection method for analyzing microbial metabolites short chain fatty acids in fecal and serum samples. *Talanta*, *196*, 249–254. https://doi.org/10.1016/j.talanta.2018.12.049
128. **Taniguchi, H., Tanisawa, K., Sun, X., Kubo, T., Hoshino, Y., Hosokawa, M., Takeyama, H., & Higuchi, M.** (2018). Effects of short-term endurance exercise on gut microbiota in elderly men. *Physiological reports*, *6*(23), e13935. https://doi.org/10.14814/phy2.13935
129. **Huang, L., Li, T., Zhou, M., Deng, M., Zhang, L., Yi, L., Zhu, J., Zhu, X., & Mi, M.** (2022). Hypoxia Improves Endurance Performance by Enhancing Short Chain Fatty Acids Production *via* Gut Microbiota Remodeling. *Frontiers in microbiology*, *12*, 820691. https://doi.org/10.3389/fmicb.2021.820691
130. **Okamoto, T., Morino, K., Ugi, S., Nakagawa, F., Lemecha, M., Ida, S., Ohashi, N., Sato, D., Fujita, Y., & Maegawa, H.** (2019). Microbiome potentiates endurance exercise through intestinal acetate production. *American journal of physiology. Endocrinology and metabolism*, *316*(5), E956–E966. https://doi.org/10.1152/ajpendo.00510.2018
131. **Eveleens Maarse, B. C., Eggink, H. M., Warnke, I., Bijlsma, S., van den Broek, T. J., Oosterman, J. E., Caspers, M. P. M., Sybesma, W., Gal, P., van Kraaij, S. J. W., Schuren, F. H. J., Moerland, M., & Hoevenaars, F. P. M.** (2024). Impact of fibre supplementation on microbiome and resilience in healthy participants: A randomized, placebo-controlled clinical trial. *Nutrition, metabolism, and cardiovascular diseases : NMCD*, *34*(6), 1416–1426. https://doi.org/10.1016/j.numecd.2024.01.028
132. **Estaki, M., Pither, J., Baumeister, P., Little, J. P., Gill, S. K., Ghosh, S., Ahmadi-Vand, Z., Marsden, K. R., & Gibson, D. L.** (2016). Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions. *Microbiome*, *4*(1), 42. https://doi.org/10.1186/s40168-016-0189-7
133. **Numao, S., Uchida, R., Kurosaki, T., & Nakagaichi, M.** (2023). Correction: Circulating Fatty Acid-binding Protein 4 Response to Acute Aerobic Exercise in Healthy Men. *International journal of sports medicine*, *44*(7), e1. https://doi.org/10.1055/a-2092-0045
134. **Wang, Y., Shan, Q., Hou, G., Zhang, J., Bai, J., Lv, X., Xie, Y., Zhu, H., Su, S., Li, Y., Zi, J., Lin, L., Han, W., Zhao, X., Wang, H., Xu, N., Wu, L., Lou, X., & Liu, S.** (2016). Discovery of potential colorectal cancer serum biomarkers through quantitative proteomics on the colonic tissue interstitial fluids from the AOM-DSS mouse model. *Journal of proteomics*, *132*, 31–40. https://doi.org/10.1016/j.jprot.2015.11.013
135. **Yasutomi, E., Inokuchi, T., Hiraoka, S., Takei, K., Igawa, S., Yamamoto, S., Ohmori, M., Oka, S., Yamasaki, Y., Kinugasa, H., Takahara, M., Harada, K., Furukawa, M., Itoshima, K., Okada, K., Otsuka, F., Tanaka, T., Mitsuhashi, T., Kato, J., & Okada, H.** (2021). Leucine-rich alpha-2 glycoprotein as a marker of mucosal healing in inflammatory bowel disease. *Scientific reports*, *11*(1), 11086. https://doi.org/10.1038/s41598-021-90441-x
136. **Shinzaki, S., Matsuoka, K., Iijima, H., Mizuno, S., Serada, S., Fujimoto, M., Arai, N., Koyama, N., Morii, E., Watanabe, M., Hibi, T., Kanai, T., Takehara, T., & Naka, T.** (2017). Leucine-rich Alpha-2 Glycoprotein is a Serum Biomarker of Mucosal Healing in Ulcerative Colitis. *Journal of Crohn's & colitis*, *11*(1), 84–91. https://doi.org/10.1093/ecco-jcc/jjw132